

Phenolic Compounds, Fatty Acid Compositions and Antioxidant Activity of Commercial Cold-Pressed Pomegranate (*Punica granatum*) Seed Oils From Turkey

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Abstract— The pomegranate (*Punica granatum*) is a highly nutritious food source with phytochemical dense composition. Pomegranate seed oil has become more popular with punicic acid and polyunsaturated fatty acids (PUFA) content which makes the oil unique. In this study different samples of cold pressed pomegranate seed oil from Turkey have been evaluated for fatty acid compositions, DPPH radical scavenging activity. Phenolic components determined with LC-QTOF-MS. Punicic acid ratio found around 70%. DPPH scavenging activity were between 15.21 ± 0.349 - $49.01 \pm 0.223\%$. Phenolic compounds have identified with Metlin_Metabolomic databases and 16-86 different components were defined.

Index Terms— Cold press, DPPH radical scavenging, Fatty acid composition, LC-QTOF-MS, Phenolics, Pomegranate, *Punica granatum*

1 INTRODUCTION

The pomegranate (*Punica granatum*) belongs to the Puniceae family and is classified as a highly nutritious food source with phytochemical dense composition. Pomegranate trees have been cultivated in the Mediterranean region. The pomegranate fruit is generally preferred to be consumed fresh and recently it has become an ingredient of soft drinks, alcoholic beverages and jams. Pomegranate extracts are used as dietary supplements or herbal medicines. Pomegranate effective parts can be classified as roots, leaves, flowers, bark, seed, oil, juice, peels with interior networks of membranes. Pomegranate by products are researched for different applications and its rind, carpellary membranes, seeds and oil found effective for various food, pharmaceutical and cosmeceuticals and part of traditional medicine in some countries [1], [2], [3], [4].

Recent studies show that each part of the pomegranate has various medicinal properties. The fruit has antibacterial and antiinflammatory effects and with its bark it is effective for dysentery, diarrhea and intestinal parasites. The seeds and juice are used as a tonic for the throat, to stop gum and nose bleeds and hemorrhoids treatments. Pomegranate fruit and seed oil is known for its rich phenolic compounds, tocopherols and antioxidant activity, therefore it is accepted to have anticarcinogenic effects with some cancer types. Tocopherol has been accepted as an important lipid soluble radical-scavenging antioxidant which is in plasma lipoproteins and also in cellular and subcellular membranes. Pomegranate polyphenols include flavonoids such as flavonols, flavanols, an-

thocyanins, condensed tannins like proanthocyanidins, phenolic acid derivatives, flavonoid glycosides, flavan-3-ols, and hydrolyzable tannins such as ellagitannins and gallotannins.

Pomegranate is found with high levels of tocopherols. These tocopherols are determined to be α -tocopherols, γ -tocopherols, and δ -tocopherols and assumed main responsible compounds from the antioxidant activity of the seed oil with phenolic components. Because of the high amount of vitamin E, pomegranate seed oil considered a natural source for medicinal and nutritional applications.

Flavonoids, tannins, anthocyanins with phenolic components and tocopherols are the main group of antioxidants due to their free radical scavenging and biological activities. [4], [5].

Punicic acid (Omega-5) is an important fatty acid found in the pomegranate seed oil more than 70% which inhibits fat peroxidation and makes the oil unique. This fatty acid also stops prostaglandin biosynthesis and cytotoxin for cancer w2a cells and prevents DMBA- and TPA-induced skin cancer also can strengthen and encourage apoptosis. On the contrary, a fermented extract's polyphenols, pomegranate seed oil's polyphenols prevent cyclooxygenase and lipoxygenase eicosanoid enzymes activity hereby the production of prostaglandin or leukotriene is inhibited. Because of these properties pomegranate seed oil can have different external or internal applications or supplementary usages for various diseases such as cardiovascular problems and cancer. Additionally seed oil the isoflavone genistein which is phytoestrogenic compounds. The recent focus on the use of Phytoestrogenic compounds of pomegranate seed oil can be used for the hormone treatment of postmenopausal women as an alternative to hormone replacement therapy (HRT). Organic acids, sterols, fatty acids, alkaloids, phenolic acids, triterpenoids and triglycerides are

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the other phytochemicals of pomegranate seeds. Pomegranate seed oil also comes from one of the only plants in nature which is known to contain the sex steroid estrone [2], [4], [6], [7], [8].

Studies show that amongst different extraction techniques such as aqueous enzymatic, cold-pressing, solvent extraction and supercritical CO₂ extraction, cold pressed extracted oils's antioxidant and phytochemicals values are higher than the other extraction methods. The most important reason for this is that during the cold press process there is no heat or chemical applications. Since the customers tend to choose more natural and healthier products, cold pressed oils are preferred more in different forms such as in foods, supplements or personal care products [9],[10],[11] [12], [13], [14], [15].

In this study, mostly preferred cold pressed pomegranate seed oils from Turkish market have been chosen to determine the DPPH radical scavenging activities, fatty acid composition and phenolic profiles. It is possible to analyse the oils with a deeper levels and detect any existing differences between brand names with advanced instruments such as LC-Q-TOF-MS, GC-MS [16] and controlling the authenticity of the products. The present study also used principal component (PCA) analysis to determine the trends of cold pressed pomegranate seed oils.

2 MATERIAL AND METHOD

2.1 Material

Cold pressed pomegranate seed oil samples have been bought from the pharmacies and natural supplements shops. The samples have been chosen from amongst the most preferred brandnames.

2.2 Chemicals

All the chemicals were Sigma-Aldrich and J.T. Baker's analytical grade or chromatographic products. Millipore ultrapure water (Type I) was used for all analysis

2.3 Fatty acid methyl esters (FAME) analysis

Fatty acid methyl esters determination has been done by using COI/T.20/Doc. No 33 for olive oils [17]. A 37 component mixture of FAME (Supelco) has been chosen as standard. The area under the relevant peak determined the results of quantitative analysis. An Agilent 6890 GC-FID system was used. A Supelco 2560 capillary column (100 m x 0.25 mm ID x 0.2 μm) is used and the ratio of split was 1:100, injection temperature was 250°C and detector temperatures was 260°C. The oven

temperature is kept at 140°C for 1 min, later on the temperature increased to 240°C at a rate of 4°C/min and hold for 5 min.

2.4 DPPH Free Radical-Scavenging Assay

The free radical scavenging activity was determined by the DPPH assay spectrophotometrically [18]. In brief, 1.0 mL extract was taken and mixed with DPPH (0.8 mmol/L and 1.0 mL). After shaking vigorously, the mix was left for 30 min. Then, the absorbance was measured at 517 nm against a reagent blank for 5% solution. Gallic acid and BHT used as standards. Analysis have been done triplicate. The inhibition percentage for scavenging DPPH radical was calculated according to the following equation;

$$\text{DPPH radical scavenging effect (\%)} = \frac{(A \text{ control} - A \text{ sample})}{(A \text{ control})} \times 100$$

A Control : The initial concentration of the DPPH

A Sample : The remaining concentration of DPPH's absorbance in the presence of the extract and positive controls.

2.5 Phenolics

An HPLC Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, USA) was used for chromatographic separation. The instrument had automatic sample dispenser, a double pump, degasser. Poroshell 120 EC-C18 (3.0x50 mm, particle size 2.7 μm) (Agilent) column was used for compound separations. Table 1 show the gradient washing (elution) steps. The mixture of 0.1% formic acid and water was mobile phase A, and the acetonitrile was mobile phase B. The column temperature was 35 °C, injection volume was 3 μL and the flow rate was arranged as 0.4 mL/min.

MS analysis was conducted by using the Agilent 6550 iFunnel high resolution Accurate Mass QTOF-MS. The instrument equipped with Agilent Dual Jet Stream which is able to operate in positive ion electrospray ionization (Dual AJS ESI) interface. Mass spectra were recorded in the negative ionization mode in a mass range of 50-1700 m/z. ESI-MS variables are given in Table 2.

Integration and data detailing were completed using the station MassHunter Workstation software. Identification of analytes has been completed with Agilent METLIN Metabolomix database, and full mass personal composite database and library (METLIN_AM_PCDL). Positive and negative modes are conducted in the same conditions.

TABLE 1
ELUTION STEPS

Time (min)	Mobile phase
0	% 5 B
8	% 15 B
10	% 20 B
13	% 25 B
18	% 30 B
20	% 45 B
24	% 60 B
27	% 80 B
30	% 90 B
32	% 5 B
3	Conditioning cycle

TABLE 2
ESI-MS VARIABLES

Desiccant gas flow	14.0 L/min
Desiccant gas temperature	35 psi
Nebuliser gas pressure	290 °C
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min

2.6 Data Analysis

The multivariate data matrix consists samples' results of fatty acid methyl esters, DPPH radical scavenging activity and phenolics compounds. Results are given for triplicates as means±SD. Classification and characterization of the pomegranate seeds oil samples completed by using chemometric methods, PCA (Ward's algo-rithmic method). Minitab 15 Statistical Software is preferred for the multivariate analyses. Data and auto scaled variables were standardized prior to the chemometric analysis. Loading plots and scores are used for visualization of PCA results. Score plots and loading plots provided a clear relevance between principal groupings and observations and indicated the significance of each variable for the results. The plots are used to explain the correlation between variables and cluster observations also. The relation between observations and principal groupings is seen clearer with the score plots. Loadings plot also indicate the significance of each variable for the results. The plots are used to explain the correlation between variables and cluster observations in the score plots.

3 RESULTS AND DISCUSSION

3.1 Fatty Acid Composition

Fatty acid (FAME) analyses show that puni-cic acid is the main fatty acids of pomegranate seed oil with the amount of 67.16-76.52% for sample C and A respectively. Linoleic acid, oleic acid, stearic acid and palmitic acid found as the other fatty acids which have high amounts amongst the other fatty acids. Sample A found with the highest rate of puni-cic acid, essential fatty acids (EFAs), polyunsaturated fatty acids (PUFAs) and PUFAs/SFAs (saturated fatty acids) while it has the lowest value of SFAs. Sample B was with the lowest puni-cic acid but had the highest palmitic acid and SFAs amount. The analysed samples showed high variation in fatty acids values. Different from the other studies eicosapentaenoic acid level found as the second fatty acid after the puni-cic acid for sample A and C. The level of puni-cic acid found very low for sample and B. The reasons behind this might be the storage conditions or the mixing the oil with other oil(s). The results were generally in accordance with the other studies with slight differences. In a study Hicaz, Mayhos, Silifke asisi, Deve dişi, Nizip and Erdemli Asisi pomegranate varieties' seed oils from Turkey have been evaluated. Puni-cic acid content found the highest rate between 71.17-77.62 %. Linoleic, oleic, stearic and palmitic acid found in higher content also [5]. Hernandez et al. [1] found the puni-cic acid content between 66.76-79.29% for three different cultivars. The amount of the other fatty acids were similar with the present study. Similar results found also in other studies with additional fatty acids such as α -eleostearic, β -eleostearic, catalpic, gadoleic [8], [19]. The differences between this study and the other studies probably based on pomegranate varieties, climatic factor, harvesting times, oil extraction and storage conditions [20], [5]. High level of puni-cic acid considered important due to anticarcinogenic effects for some cancer types [2].

TABLE 3
FATTY ACID COMPOSITIONS

FATTY ACIDS	SAMPLES			
	A	B	C	D
Palmitic Acid	2.57±0.005	6.38±0.002	2.27±0.001	5.08±0.001
Stearic Acid	1.97±0.008	3.23±0.002	1.62±0.001	2.26±2.001
Oleic Acid	4.35±0.012	28.18±0.001	4.20±0.001	51.55±0.000
Linoleic Acid	4.72±0.048	45.84±0.003	3.83±0.001	22.87±0.002
Punicic Acid	76.52±0.021	0.05±0.001	67.16±0.003	8.94±0.001
Eicosapentaenoic Acid	6.80±0.016	0.24±0.001	14.34±0.001	1.85±0.001
EFAs	81,88	52,75	71,19	35,77
PUFAs	88,94	53,89	86,27	37,97
MUFAs	4,95	34,07	4,94	53,04
SFA	5,82	12,28	8,80	9,00
PUFAs/SFAs	15,27	4,45	10,19	4,22

3.2 DPPH Radical Scavenging Activity

DPPH Radical Scavenging Activity method has been chosen due to the stable properties of the method in determination of antioxidant's radical scavenging capacities since this capacity may be influenced by the radical systems and testing conditions [28]. In this study, Sample C found with the highest capacity (49.01±0.223%) and followed by Sample A (40.38±0.582 %), Sample B (35.86±1.37 %) and Sample D (15.21±0.349 %). Different studies about antioxidant capacity of pomegranate seed oil found different results with different methods such as, FRAP values between 5.38 - 10.04 mmol/100 g, ORAC values between 5.55-7.65 mmol/100 g [4], antioxidant activity 17.56-22.93% [5], DPPH radical scavenging activity 38.21-14.91 mg/mL and 6.2g/g [27]. Antioxidant activity of pomegranate fruit and its derivatives has been attributed to the presence of some components like tocopherols, phenolic compounds and ascorbic acid [2], [4] and studies show that seed oil shows three times greater antioxidant activity than green tea extract [7]. These properties make seed oil of pomegranate an important ingredient for medical usages and nutraceuticals.

TABLE 4
DPPH RADICAL SCAVENGING ACTIVITY

Sample	Antioxidant Activity (%)
A	40.38±0.582
B	35.86±1.37
C	49.01±0.223
D	15.21±0.349

3.3 Phenolics

Phenolic compounds in plant extracts are related to their antioxidant activity potential significantly [6]. These compounds have an important role on protection of seeds from oxidation and effecting the stability of oil, quality parameters and their nutritional properties, colour and taste [13], [21], [22], [23], [24]. In this study phenolic compounds have been identified with Metlin_Metabolomic database. This identification is the widest range for pomegranate oil in our knowledge. According to the results Sample A found with the high level components which is 86. Other samples B,C and D contain 16, 48 and 60 different compounds respectively including Δ2-cis-Hexadecenoic Acid, Urocanic acid, α-Linolenic Acid, α-Linolenic Acid, α-Linolenic Acid, Traumatic Acid, trans-EKODE-(E)-Ib, Totarol-19-Carboxylic Acid, Stearidonic Acid, Stearic acid, Sinapyl aldehyde, R-(-)-Mandelic acid, Pinolenic Acid, Pinocembrin, Petroselinic acid, Methylnorlichexanthone, Methyl N-(a-methylbutyryl)glycine, MEDICA 16, Kaempferol, Hexadecanedioic acid, Ferulic acid, Eupatorin, Elaidic Acid, cholesterol sulfate, Corosolic acid, 4-Hydroxybenzaldehyde, 18α-Glycyrrhetic Acid, 18α-Glycyrrhetic Acid, Stearidonic Acid, Stearamide, Sinapyl aldehyde, PGJ2, PGF2α methyl ester, Lariciresinol, Linoleoyl Ethanolamide, 8-iso-PGF2β, 4-Hydroxy-3-methoxycinnamaldehyde, 3-methoxy Prostaglandin F1α, 2-Linoleoyl Glycerol. Other studies mentioned some phenolics in pomegranate structure such as anthocyanins, ellagic acid, punicalagin [7] flavonoids, anthocyanidins such as cyaniding, pelargonidin and delphinidin [25] ellagitannins, gallic acid, punicalagin, ellagic acid, punicalin, and anthocyanins [2]. Various studies show different values for total phenolic content (TPC) of pomegranate seed oil such as 7.8-72.1 mg/g [26], 19.17 mg gallic acid equivalent/g (Mayhoş) 7.69 mg gallic acid equivalent/g (Devediş) and between the range of 23.56-28.75% generally [5], 11.5% total phenol bearing 22.9% antioxidant content [27]. Oil polyphenols show free radical scavenging activity also inhibit eicosanoid and cyclooxygenase enzymes' activity, and human breast cancer. Also these components are beneficial for the prevention of inflammatory, cardiovascular, and other diseases. Additionally phenolic compounds facilitate skin repair and promote regeneration of dermis and epidermis [25].

3.4 Principal Component (PCA) Analysis

The data obtained from the positive and negative mod library results of LC QTOF M are analysed by using MINITAB programme. Score and loading plots graphics are given in figures below. According to score plot graphics, effect of PC1 was 49.26% and effect of PC2 was 34.72%. It can be seen that total effect of PC1 and PC2 was very high. While Sample A and Sample C were clustering Sample D and Sample B separated in their own area.

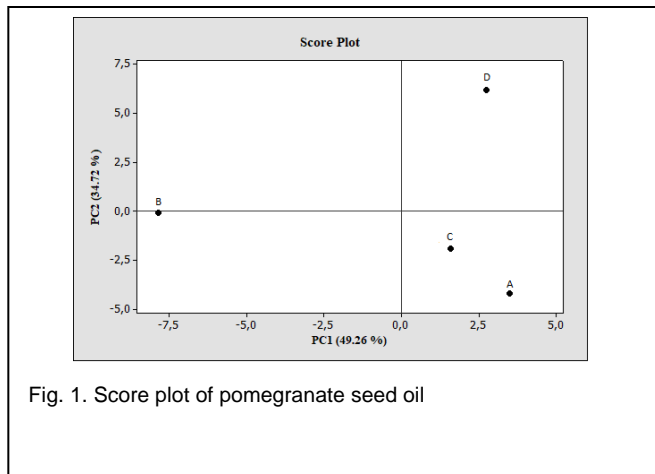


Fig. 1. Score plot of pomegranate seed oil

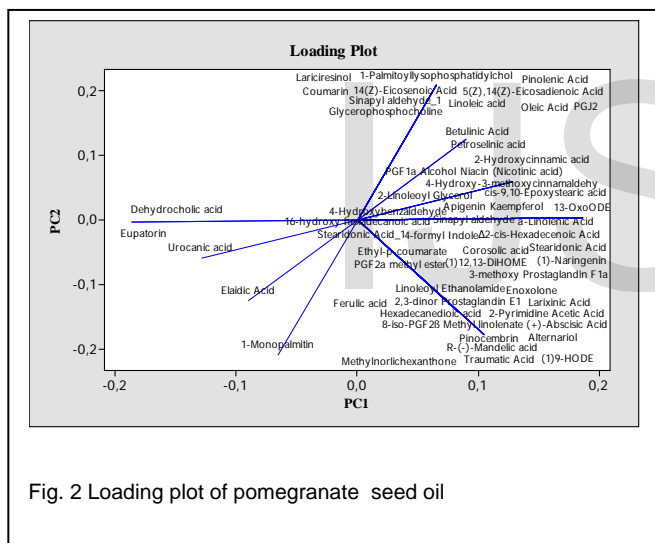


Fig. 2 Loading plot of pomegranate seed oil

4 CONCLUSION

In this study, the most preferred cold pressed pomegranate seed oils in the Turkish market have been evaluated for their fatty acid composition, antioxidant capacity and phenolic components. According to the fatty acid compositions, the cold pressed pomegranate seed oil samples were found rich with Punicic acid, PUFA. Therefore it can be used in many applications especially cancer treatments and nutraceuticals. Phenolic compounds also were found rich in the pomegranate seed oil which makes the oil important for supplements and cosmeceuticals formulations with its antioxidant effect. DPPH radical scavenging activity analysis results also support the antioxidant characteristics of pomegranate seed oil for

health studies. The detailed data of this study could be used for further researches about pomegranate seed oil and its alternative usages in different nutraceutical and pharmaceutical applications.

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