

Chemical Evaluation of Oils Extracted from Five Different Varieties of Iranian Pomegranate Seeds

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ABSTRACT: Iran is considered number one producer of pomegranate throughout the world. The oil extracted from pomegranate seed is considered to have great medical and nutritional values, therefore the study is concerned with this unique and valuable oil specially its chemical attributes. Five different varieties of pomegranate were obtained from Saveh Pomegranate Research Institute. The seeds were separated, dried and subjected to Soxhlet apparatus to extract the oil. Fatty acid profile, Iodine value, non saponifiable matters, sterols, tocopherols, phosphorus and phospholipids were determined both qualitatively and quantitatively in the extracted oil. The results of this research indicated that the predominant fatty acid (higher than 70%) was puniceic acid. Beta-sitosterol and γ -tocopherol were the predominant sterol and tocopherol present in the extracted oil respectively. The results of this study showed that there are significant differences concerned with the elements under investigation in this research among the varieties tested.

Keywords: Pomegranate Seed Oil, Puniceic Acid, Sterol, Tocopherol .

Introduction

The scientific name of Pomegranate is *Punica Granatum* and it belongs to *Panicaceae* family (Zargari, 1981). Pomegranate was grown in Iran since the ancient time and as the evidences reveal the fruit is indigenous to Iran and the neighboring countries. Currently the tree is cultivated in limited states namely Pakistan, India, Afghanistan, Spain, Greece and United States of America, but Iran has the first rank throughout the world concerning the quality and quantity produced (Hayati, 1995).

Considering many health improving attributes of the fruit, different products and by products are produced from this fruit

such as juice and concentrated paste. The seed of pomegranate up to recent time has been looked at as waste material but now are taken into consideration by different researches, because of their exclusive advantages and properties (Mehta, 2004; Kohno, 2004; Hora, 2003).

The seed of pomegranate is about 20% of fruit's edible part that contains some oil. Pomegranate seed oil contains high amount of unsaturated fatty acids such as puniceic acid that makes up over 70% of the total fatty acids present in the oil.

This has been claimed to have many health improving attributes, especially the link to anti-cancer nature of it. Pomegranate seed oil is a rich source of phytoestrogens (Seeram, 2006). Phytoestrogens are combinations having effects like human

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estrogenic hormones, though they have plant origin. Pomegranate seed oil contains different phytoestrogens and prevents diseases related to hormones such as breast cancer and womb neck cancer (Gilani, 2002). Moreover it has been proved that the pomegranate seed oil contains high amount of antioxidants and can compete with other antioxidant sources such as green tea (Faria, 2010). Many researchers have conducted works on chemical attributes of this oil, concerning the extraordinary nutritional and medical advantages. Fadavi *et al* (2003) in a study on 25 different varieties proved that pomegranate seeds contain 6 to 15.7 % oil and achieved the following results concerning the fatty acid composition:

Behnic acid < Myristic acid < Stearic acid < Palmitic acid < Oleic acid < Linoleic acid < Linolenic acid

In 1983, El-Shaarawy showed that iodine value of pomegranate seed oil is high, therefore it contains high amounts of unsaturated fatty acids. This study also indicated that the amount of non saponifiable matter and volatile fatty acids are low in this oil. Further studies concerned with the oil extracted from pomegranate seed by researchers have indicated different concentrations of linolenic, linoleic, oleic, stearic, palmitic and palmitoleic acids (Melgarejo *et al.*, 1995). Therefore due to the points that pomegranate seed oil might have health improving and medical properties it is the object of this study to evaluate the oil extracted from different varieties of pomegranate seeds.

Materials and Methods

Five different varieties of pomegranate with the names of Red Seed Ardestani, Taft's Tokhm-e Mush, Chatrud's Sour Shahi, Rinzdavar's Dorpaye and Ashkzar's Sour Narak were selected from Saveh Pomegranate Research Centre. The seeds were prepared through the following stages; peeling the fruit, extracting the juice,

separating the seeds, drying the seeds, grinding the seeds, and keeping it at -18 °C.

Due to the high nutritional and medical values of the samples and to avoid deterioration, common procedure such as soxhlet was not used. Therefore, the oil was extracted through cold press using hexane as the solvent at low temperature. The extracted oil was kept at clean and dark glasses in the refrigerator until required (Ghavami *et al.*, 2008).

Fatty acids composition was determined following the AOCS standard No. GeLe-91 method, using GC apparatus equipped with CPSil 88 capillary column and Flame Ionization Detector. The samples were prepared in the form of their methyl ester derivatives according to the AOAC standard No. 969.33 method.

Iodine value was calculated directly from fatty acid composition of the oil regarding the AOCS standard No. Cd.lc-85 method.

The induction period of the oil was determined according to AOCS standard No. Cd12-57 method using Metrohm Rancimat model 743 apparatus at 110 °C.

The amount of the phosphorus and consequently phospholipids were measured by spectrophotometer at 720 nm in comparison with the standard samples following the IUPAC standard No. IID. 16.20 method.

The non saponifiable matter was isolated and quantified by alcoholic potassium hydroxide saponification of the oil, followed by the extraction of non saponifiable matter with diethyl ether according to AOAC standard No. 933.08 method. The isolated non saponifiable matter was fractionated on a TLC plate into numbers of chemical classes of compounds. The sterol fraction was extracted and identified after spraying with 0.01% Rhodamin 6G in ethanol according to AOAC standard No. 970.151 method.

Tochopherols were identified and quantified by High Performance Liquid

Chromatography (HPLC) following the AOCS standard No. Ce 8-89 method.

The Excel and SPSS software were employed for drawing diagrams and analyzing the results, respectively. Variation test is used and the comparison between variation and significant relationship was made by Duncan test.

Results and Discussion

Pomegranate seed is about 20% of the edible part of the fruit. The amount of oil depends on the type and the variety. Table 1 shows the percentage of oil in different varieties. There is a significant statistical difference between the concentration of the oil in different varieties ($P < 0.05$). The least concentration of the oil belongs to Ashkezar's Sour Narak variety and the highest belongs to Red seed Ardestari variety.

The fatty acid composition of the oil extracted from different varieties of pomegranate seed are presented in Table 2.

Iodine value which indicates the unsaturation of fatty substrate is presented in Figure 1. The highest iodine value belongs to Ashkezar's sour Narak and the lowest iodine value belongs to Chatrood's Sour Shahi pomegranate.

The Induction periods of the samples at 110°C are presented in Table 3. The high concentration of punicic acid which has increased the iodine value of the oil to over 225 g/ 100g has greatly affected the stability of the extract oils.

There is no significant statistical difference between the stability of different varieties of pomegranate examined at 110°C ($P > 0.05$).

Table 4 shows the amount of phosphorus and phospholipid contents of oil samples extracted from pomegranate seeds. There are significant differences between phosphorus and phospholipid contents of the different oil varieties ($P < 0.05$). The highest value belongs to Red Seed Ardestani variety and the lowest value belongs to Taft's Tokhm-e Mush variety.

Table 1. Percentage of oil in different varieties of pomegranate seeds

Varieties	Oil (g/100g)
Red Seed Ardestani	17.06
Taft's Tokhm-e Mush	15.70
Chatrud's Sour Shahi	13.93
Rizdavar's Dorpaye	13.68
Ashkzar's Sour Narak	9.24

Table 2. Fatty acid composition of oils extracted from different varieties of pomegranate seeds

Varieties/ Fatty acid (%)	18:3 (Punicic)	18:2 (Linoleic)	18:1 (Oleic)	18:0 (Stearic)	16:0 (Palmitic)	Others
Red Seed Ardestani	79.43	6.95	6.62	2.09	3.16	1.72
Rizdavar's Dorpaye	79.22	6.87	6.30	2.50	3.36	1.73
Ashkzar's Sour Narak	82.40	5.22	5.71	1.99	2.95	1.70
Taft's Tokhm-e Mush	78.25	6.59	7.48	2.54	3.41	1.70
Chatrud's Sour Shahi	78.73	7.08	6.68	2.16	3.57	1.75

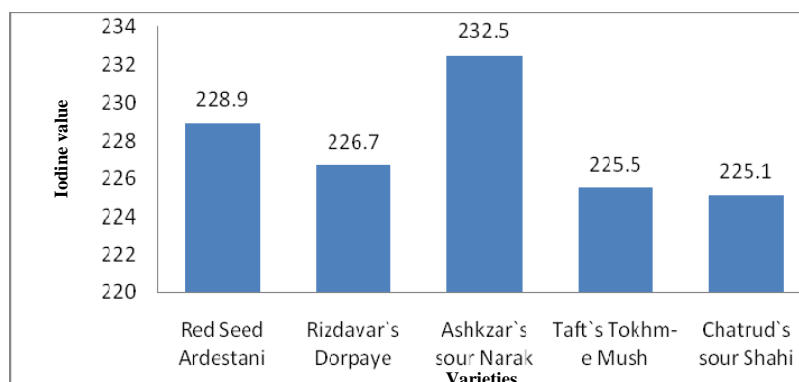


Fig. 1. Iodine value of oils extracted from different varieties of pomegranate seeds

Table 3. Induction period of oil extracted from different varieties of pomegranate seeds at 110°C

Varieties	Induction period(hours)
Red Seed Ardestani	0.87
Taft's Tokhm-e Mush	0.77
Chatrud's Sour Shahi	1.02
Rizdavar's Dorpaye	0.73
Ashkzar's Sour Narak	0.79

Table 4. Phosphorus and phospholipid contents of oils extracted from different varieties of pomegranate seeds

Varieties	Phosphorus(ppm)	Phospholipid(ppm)
Red Seed Ardestani	124.3	3729.0
Taft's Tokhm-e Mush	56.7	1702.8
Chatrud's Sour Shahi	96.9	2907.9
Rizdavar's Dorpaye	80.0	2400.0
Ashkzar's Sour Narak	79.5	2385.9

The concentration of the non saponifiable matters of different pomegranate seed oils are shown in the Figure 2. The results showed that there is a significant statistical difference between percentages of the non saponifiable matters of different varieties ($P < 0.05$). The highest amount of non saponifiable matter is related to Red Seed Ardestani variety and the least amount belongs to Taft's Tokhm-e Moush variety.

Table 5 shows the amount of total Sterols of each oil and Table 6 indicates sterol composition of the oils extracted from different varieties of pomegranate seeds.

There are high concentrations amount of tocopherols in pomegranate seed oil. Table 7 shows tocopherol contents of oils extracted from different varieties of pomegranate seed.

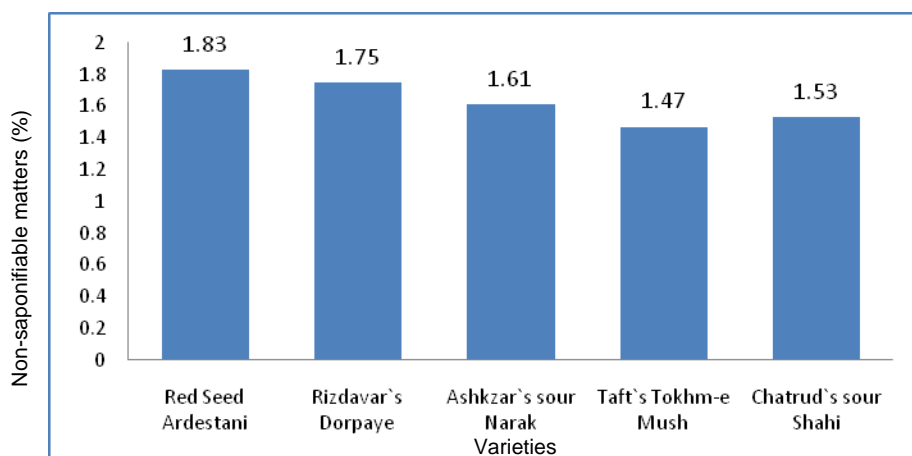


Fig. 2. Percentage of the non-saponifiable matter of oils extracted from different varieties of pomegranate seeds

Table 5. Total sterol contents of oils extracted from different varieties of pomegranate seeds

Varieties	Total sterol(ppm)
Red Seed Ardestani	5239.44
Taft's Tokhm-e Mush	5270.59
Chatrud's Sour Shahi	5580.36
Rizdavar's Dorpaye	5757.62
Ashkzar's Sour Narak	5750.44

Table 6. Sterol composition of oils extracted from different varieties of pomegranate seeds

Varieties Sterol (%)	Cholesterol	Campesterol	Stigmasterol	Beta-sitosterol
Red Seed Ardestani	0.39	8.76	3.14	87.71
Taft's Tokhm-e Mush	0.37	8.18	3.57	87.21
Chatrud's Sour Shahi	0.37	8.21	5.93	85.49
Rizdavar's Dorpaye	0.37	7.56	5.09	86.89
Ashkzar's Sour Narak	0.40	8.83	4.06	86.71

Table 7. Tocopherol contents of oils extracted from different varieties of pomegranate seeds

Varieties	Alpha tocopherol(ppm)	Gamma tocopherol(ppm)
Red Seed Ardestani	1038.6	3283.7
Taft's Tokhm-e Mush	1134.6	6930.8
Chatrud's Sour Shahi	894.8	7106.1
Rizdavar's Dorpaye	989.2	3572.6
Ashkzar's Sour Narak	543.6	1856.6

The results derived from this study indicate that the extracted oil is quite unsaturated and the predominant fatty acid is punicic acid. Punicic acid which is an 18-carbon fatty acid with 3 unsaturated double bond in the form of conjugated makes up over 78 percent of the existing fatty acid in

the oils examined. Punicic acid has been claimed to have nutritional and health improving effects. The high concentration of punicic acid has affected the oil iodine value, therefore iodine value of the pomegranate seed oil is higher than other existing vegetable oils. Due to the

unsaturation, oxidation might create problem and affect the stability (Deman, 1999). The stability of the oil against oxidation is measured by Rancimat apparatus, which is the method based on measuring the secondary products, derived from oxidation of oils and fats (Ghavami *et al.*, 2008). As it is obvious, the induction periods of the oils studied are shorter than other edible oils, that is due to high concentration of unsaturated fatty acids, namely punicic acid. Therefore the extracted oil should be kept at dry, cold place, away from light and in closed vessels.

Phospholipids or phosphatids are among saponifiable lipids which only existed as a minor fraction in the oils studied. Separation of the non saponifiable matters on TLC plate into a number of chemical classes of compounds namely sterols and tocopherols indicated that β -sitosterol, the predominant sterol followed by campesterol and stigmasterol constituted the sterol fraction. Cholesterol was present in trace quantity.

Tocopherols such as γ -tocopherol and α -tocopherol, the former an active antioxidant and the latter a potent vitamin E were present in an unexpected concentrations.

Conclusion

Studies and investigations carried out in the past and present, suggest that the oil extracted from pomegranate seed contains a high concentration of punicic acid, a precious fatty acid known to have beneficial effect on human health. The oil has high concentrations of tocopherols, both γ -tocopherol a potent antioxidant and α -tocopherol which has high vitamin E activity. Although the oil itself is quite susceptible to oxidation due to high unsaturation, it might be employed and consumed as a health food or used as an ingredient in formulated foods.

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