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Research Article

COMPARISON OF QUANTITATIVE AND QUALITATIVE SEED OIL OF POMEGRANATE EXTRACTED BY COLD-PRESS AND HEXANE SOLVENT

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ABSTRACT
Pomegranate seed oil is extremely nutritious and rich, which is used for cosmetic and medicinal purposes. Effect of Solvent extraction (N-hexane solvent) and expression (cold-press) on seed oil quality of pomegranate was investigated. Fatty acids profile determined via gas chromatography apparatus. Iodine value and acid value and peroxide value determined based on AOCS standard while Refractive Index evaluated via AOAC standard method. Higher iodine value and lower viscosity observed for oil, extracted via cold-press. There was a considerable difference between the amounts of polyunsaturated fatty acids of oils extracted via different methods. The higher polyunsaturated fatty acids observed for oil extracted via cold-press. Oil which extracted via hexane solvent contain higher amount of linoleic and linolenic acid while oil obtained via cold-press showed higher punicic acid contents.

Keywords: *Iodine Value, Viscosity, Linolenic Acid, Punicic Acid*

INTRODUCTION
Pomegranate (Punica granatum) is a member of Punicaceawhich is originate from Iran to the Himalayas in northern India and was cultivated and naturalized over the whole Mediterranean region since ancient times (Morton , 1987). Pomegranate fruit is rich in antioxidants like polyphenols, tannin, ellagic acid and anthocyanin (Al-Maiman and Ahmad, 2002). Ellagic acid having the ability to prevent cancer and prevent tumorigenesis (Bell and Hawthorne, 2008). It is rich in folic acid, potassium and iron (Schubert et al., 1999). Pomegranate fruit contains 14% seed. Seeds contain about25% oil content. Pomegranate seed oil is rich in conjugated linolenic acid and a high content of punicic acid (Mukherjee and Bhattacharyya, 2000). These conjugated fatty acids have several potential health benefits including their antioxidant, antitumor, immunomodulatory, anti-atherosclerotic and serum lipid-lowering activities (Carvalho *et al.*, 2010). High content of phytosterols, tocopherols and a unique fatty acid composition, mainly consisting of punicic acid (55 %), were observed during the investigation on pomegranate seed oil (Melo, 2012).
Pomegranate seed oil is good for heart and blood vessels could reduce carotid artery wall thickness, prevent heart attacks and cancers. The oil uses for cosmetic purposes and is useful in reducing joint pain (Kylaran *et al.*, 2007).
Different extracting methods well detailed by researchers but there are very few documents about the effect of different extracting methods on oil chemical and physical characteristics.
Quantitative characteristics are those which related to chromatography profile. It declares the percent of each compound in oil composition (Saha and Ghosh, 2009). Qualitative characteristics related to oil appearance traits such as color, flavor, transparency, refractive index, peroxide index, acidity, saponification value, iodine value, melting point and viscosity (Kaufman and Wiesman, 2007).
Cold-press is using commonly for industrial oil extraction. Nutritional characteristics of oil serve in this method (Gunstone, 2002). The solvent extraction method carried out applying soxhlet apparatus. This method is invented for extracting solid lipids (Soxhlet, 1879).
The effect of different distillation methods on oil content and composition of plants have also been previously reported (Sefikon *et al.*, 2008; Babazadeh, 2014).
The present study carried out to compare two extracting methods and their effects on quantitative and qualitative traits of pomegranate seed oil. The two extracting methods were cold-press and hexane solvent method.

MATERIALS AND METHODS

Pomegranate cultivar used for the study was MALAS which is native to Mashhad-Iran.

Seed Preparing Stages

After washing the fruits, peeling the fruit and extracting the juice performed. Then seeds separated and dried at room temperature for two days and keeping in cotton bags at -18 ℃.

Preprocessing of Seeds

Only the core of each seed contains oil, thus seeds pressed into a fine meshed sieve (5.3 mm) in order to separating pulps and then cleaned with pneumatic device. Then seeds oven dried at 50°C for two hours. The seed humidity reached to 6-7 percent. Finally seeds grinded to 1 mm particles.

Oil Extraction

Oil extracted via cold-press and hexane solvent.

Cold-press Extraction

About 25 gr of seeds was cold-pressed and then, the oil was separated from the crude by centrifugation and finally refined by sedimentation and filtration.

Hexane Extraction

About 25 gr of seeds extracted by soxhlet apparatus for 5 hours. N-hexane solvent used as solvent. The results expressed as the yield of crude extract (Skoog *et al.*, 1999):

$$yield_{extract} = 100 \times \frac{m_{extract}}{m_{seeds}}$$

Where *m* extract is the crude extract mass (gr) and *m* seed is the extracted seed mass (gr).

Qualitative Characteristics

Fatty acids profile of the oil extracted by both methods, determined using a gas chromatography apparatus (model Acme 6000, South Korea). The column temperature was programmed from 198 °C (6 min) to 210 °C. The injector and detector temperatures were 250 °C and 280 °C respectively. The components of the oils were identified by comparison of their chromatogram with reference compounds (Adams, 2001).

Qualitative Characteristics

Iodine value, acid value, saponification value, peroxide value, refractive index, color, viscosity and density of oil evaluated in order to determining qualitative characteristics of oil. Iodine value, acid value, saponification value and peroxide value determined based on **AOCS (**American Oil Chemist Society**)** procedures (methods coded Cd 1c-85, Cd 3d-63, Cd 3-25 and Cd 8-53 respectively).Oil color determined using a color scale device (LOVIBOND AF 710-3) based on Cd 1c-85 method as described in AOCS.

Refractive index evaluated by an Abbe refractometerat 40 °C based on AOAC (Association of Official Analytical Chemists) procedure (method coded 921/08). Oil viscosity measured using a viscometer device (Brook Field). Oil density evaluated applying a hydrometer device (Proton model).

RESULTS AND DISCUSSION

The physiochemical properties of different extraction methods compared and the results present as tables graphs.

Quantitative Characteristics

The fatty acid composition and the related ratios are summarized in table 1.

Figure 1 and 2 show the chromatogram of oil extracted via hexane solvent and cold-press, respectively. As shown the main constituent of the oil was polyunsaturated fatty acids (PUFA). Up to 70 percent of total PUFA's profile of pomegranate seed oil was punicic acid (table 1, figure 1 and 2). Punicic acid is conjugated acid which prohibit biosynthesis of prostaglandins and shows cancer preventive actions (Mukherjee and Bhattacharyya, 2002). After PUFAs, monounsaturated fatty acid (MUFA) is the main compounds found in pomegranate seed oil (table 1, figure 1 and 2). The amount of punicic acid and total PUFAs was higher in oil gained by cold-press compare with oil extracted via hexane solvent. The amount if other fatty acids were higher at oil extracted via hexane solvent.

There was no difference between the amounts of pentadecanoic acid in different treatments.

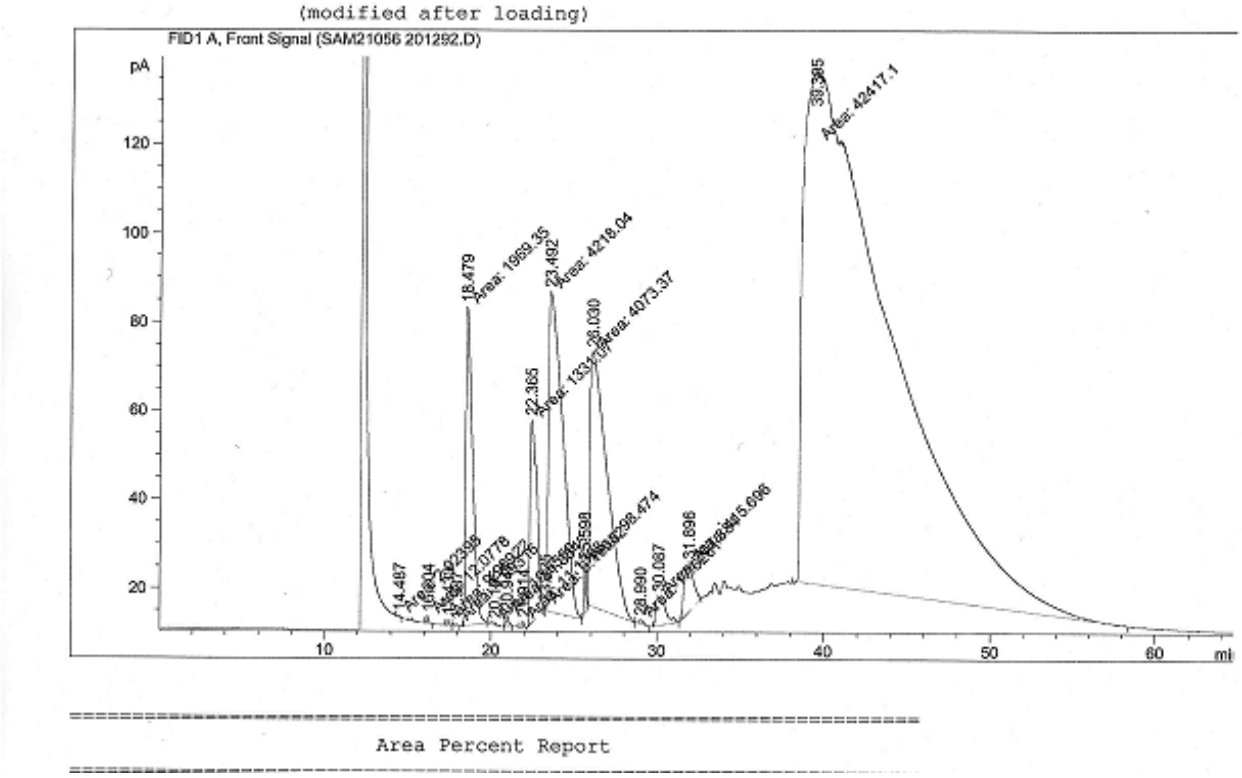

Figure 1: GC Chromatogram for hexane solvent extracting method

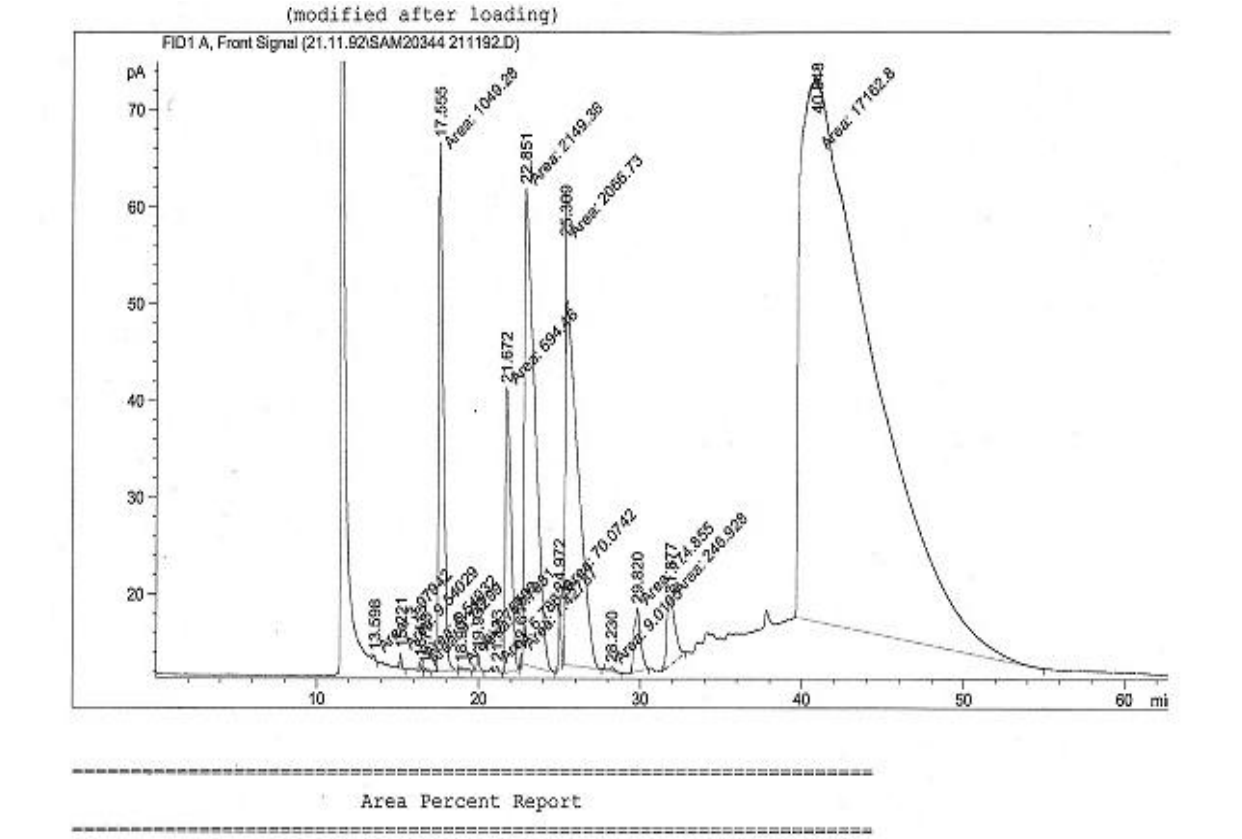

Figure 2: GC Chromatogram for cold-press extracting method

Comparative characteristics of seed oil, extracted via different methods shows at table 1.

Table 1: Comparative characteristics of seed oil, extracted via different methods

No.	Fatty acids	Cold-press	Hexane solvent	Variance
1	Polyunsaturated fatty acidsΣPUFA	85.36	82.19	3.17
2	Punicic C18:3	77.72	77.45	0.27
3	Monounsaturated fatty acid ΣMUFA	10.23	8.51	1.72
4	Stearic C18:0	2.42	2.93	1.51
5	Saturated fatty acid ΣSFA	6.14	7.54	1.40
6	Cis oleic acid C18:1c	7.68	9.07	1.39
7	Cis linoleic acid C18:2c	7.42	8.72	1.30
8	Palmetic acidC16:0	3.59	4.43	0.84
9	Gadoleic acid C20:1	0.76	1.04	0.28
10	Linolenic acid C18:3	0.49	0.73	0.24
11	trans fatty acids	0.19	0.32	0.13
12	Trans linoleic acid C18:2t	0.18	0.29	0.11
13	Trans oleic C18:1t	0.01	0.03	0.02
14	Myristic acid C14:1	0.02	0.04	0.02
15	Arachidic acid C20:0	0.03	0.04	0.01
16	Heptadecanoic acid C17:0	0.02	0.03	0.01
17	Margaric acid C17:0	0.06	0.07	0.01
18	Palmitoleic acid C16:1	0.03	0.03	0.01
19	Myristoleic acid C10:1	0.02	0.03	0.01
20	Lauric acid C12:0	0.01	0.02	0.01
21	Pentadecanoic acid C15:0	0.01	0.01	0.0

Qualitative Characteristics

A comparison was carried out between Iodine value, acid value, saponification value, peroxide value, refractive index, color, viscosity and densityof oil extracted via hexane solvent and cold-press method. Acid value, iodine value and the amounts of unsaponifiable material was higher in cold-press method compare with hexane solvent method (table 2). The higher amount of peroxide value, saponification value observed for oil extracted via hexane solvent (table 2).

Oil extracted via hexane solvent showed higher viscosity and lower density (table 2).

Table 2: Qualitative characteristics of oil extracted via cold-press and hexane solvent

Qualitative characteristic	Cold-press	Hexane solvent	Variation
Iodine value (g I2/100 gr oil)	215.9±1.87	179.4±0.16	36.5
Acid value(mg KOH/gr oil)	3.78±0.01	0.559±0.01	3.22
Peroxide value(meqO_2/ kg oil)	0.41±0.19	0.791±0.19	0.38
Saponification value (mg KOH/ g oil)	180.12±0.57	181.1±0.08	0.98
Unsaponifiable materials	2.1±0.0	1.26±0.0	0.84
Color (yellow/red)	4.6±0.019	6.71±0.16	2.11
Viscosity (cP)	36.2±4.0	155.57±3.0	119.37
Density (gr/cm^3)	0.9202±0.011	0.919±0.019	0.0012
Refractive Index	1.4647±0.0	1.5095±0.0	0.044

The highest variance seen for oil viscosity and iodine value values respectively (table 2). Color diversity was observable too (table 2). The variance between acid value, peroxide value, amounts of Unsaponifiable materials, density and refractive index was not significant (table 2).
There was significant difference between hexane solvent and col-press methods in respect of viscosity and iodine values. No noticeable difference observed for other qualitative characteristics (table 2).

Oil Appearance

The oil extracted via cold-press was transparent, clear with a bland odor and natural taste. The taste did not affected by oxidation. It was pure with no additives. The oil extracted via hexane solvent was not as pure as cold-press due to trace of residual concentrations of hexane in it. The oil extracted via hexane solvent was liquid but converted to semi-solid in temperatures lower than room temperature. It was not as clear and transparent as cold-press oil was. The taste was natural but the odor was not pleasant.

Conclusion

Physiochemical characteristics of pomegranate seed oil extracted via extraction and expression compared. Results showed that the oil extracted via hexane solvent was suitable for cosmetic and medicine purposes because it was rich in necessary fatty acids such as linoleic and linolenic acids compare with oil extracted via cold-press. These two fatty acids are important in cosmetic and medicine industry for producing skin care productions and remedies. Higher amount of punicic acid observed in oil extracted via cold-press method. Thus the oil is proper for preparingmenopausal symptoms remedies and heart disease preventing medicines. It has potential in combating breast and prostate cancer and preventing diabetes. Higher amount of PUFAs observed for oil extracted via cold-press which results in lower blood cholesterol. Thus the oil extracted via cold-press is proper for medicine purposes more than oil extracted via hexane solvent. The higher iodine value for cold-press oil resulted in its fluidity at room temperature. Oil extracted via cold-press had a more pleasant odor and taste because no additives used in its processing. Oil extracted via hexane solvent was not as pure as oil extracted via cold-press dut to trace of residual concentrations of hexane. Differences between fatty acid contents, and qualitative traits resulted in varioususes of oil extracted via different methods.

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