International journal of Advanced Biological and Biomedical Research
ISSN: 2322 - 4827, Volume 1, Issue 4, 2013: 351-363
Available online at http://www.ijabbr.com

Pomegranate (*Punica granatum* L.) seed: A comparative study on biochemical composition and oil physicochemical characteristics

Biochemical composition of pomegranate seed oil

Saeed Dadashi1[*1], Morad Mousazadeh [2], Zahra Emam-Djomeh [3], Seyed Mohammad Mousavi [3]

[1]PhD Student, Department of Food Science Engineering and Technology, Faculty of Agricultural Engineering & Technology, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran.

[2]MSc Student, Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, College of Agriculture and Natural Resources, University of Tehran, Iran.

[3, 4] Professor, Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, College of Agriculture and Natural Resources, University of Tehran Karaj, Iran.

Abstract

The pomegranate seeds of four Iranian commercial varieties (Abanmahi (AB), Malas (MS), Pust Sefid (PS) and Shahvar (SH)) were evaluated in terms of some quality properties including protein, oil, dietary fiber, mineral contents and fatty acid composition. Physicochemical properties and antioxidant activity of pomegranate seed oils (PSOs) were also determined. The oil antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity. Results showed that PS had the highest oil (16.9%) and crude fiber (42.4%), and nutritional value (460.7Kcal/100g) among selected varieties. PS had the highest level of phosphorus (2766.3 mg/kg) and magnesium (2052.0mg/kg), while the highest calcium (675.3mg/kg) and potassium (3724.6mg/kg) were related to SH. The main fatty acid identified by gas chromatography was punicic acid ranged from 72.07% for SH to 73.31% for MS (p<0.05). The ratios of polyunsaturated/saturated and unsaturated/saturated fatty acids of PSOs were found to be between 9.174 and 9.450, and 10.325 and 10.861, respectively (p<0.05). PSOs obtained presented acid (3.78-8.36% punicic acid), peroxide (0.39-0.48meq O_2/kg), iodine (216.9-220.3g I_2/100g) and saponification (179.3-182.5mg KOH/g) values. Also, refractive index at 25°C, viscosity and density of PSOs varied from 1.461-1.527, 0.036-0.063Pa.s and 0.9202-0.9311g/cm^3, respectively. The oil obtained from MS showed the lowest level of ortho-diphenols (ODC) and DPPH radical scavenging capability. The relationship between percentage of

Corresponding Author: Saeed Dadashi, Email: Dadashis@ut.ac.ir

remaining DPPH and ODC of PSOs also illustrated high correlation among all varieties (R^2 = 0.98, p<0.01).

Key Words: Pomegranate seed, Fatty acid profile, Oil characterization, Ortho-diphenols, DPPH radical scavanging

INTRODUCTION

The pomegranate (*Punica granatum* L.) belonging to the *Punicacea* family, as one of the oldest edible fruits has been used extensively in the folk medicine of many cultures (Schubert *et al*, 1999). This fruit is native to Iran and has been grown widely in that country, India and the USA, and to a lesser level in most near and far eastern countries (Kyralan *et al*, 2009). Total worldwide production of this crop is approximated at 1,500,000 tons and Iran with the highest area under cultivation produces 47% of world production (FAO, 2009). The edible parts of pomegranate fruits are consumed fresh and are also used in the preparation of fresh juice. The fruit contains considerable amounts of seeds, ranging between 40 and 100 g kg^{-1} of fruit weight depending on cultivar (Hernandez et al, 1998, Fadavi et al, 2006). These seeds remain as a waste product after processing, whereas they contained considerable amounts of lipid, protein, sugars and essential minerals (El-Nemr et al, 1990). El-Nemr *et al*, (1992) reported the amount of mineral elements except potassium in seed are higher than juice. The seeds of some pomegranate varities are rich in lipids, which vary between 140-270 g/kg dry matter (Hernandez *et al*, 1998). There is an increasing interest in new sources of industrial oils, in the essential fatty acids of seed oils and in toxicity aspects of edible oils. Pomegranate seed oil (PSO) has several features that make it an attractive nutraceutical ingredient as a consequence of its novelty, good acceptance by the consumers, cheap availability and promising phytochemical composition (Fadavi *et al*, 2006). PSO contains 10-20% of total seed weight, with an ideal highly unsaturated fatty acid content, mainly constituted by the rare conjugate linolenic acid isomer punicic acid, which activities against metabolic syndrome and inflammation have been confirmed by various in vivo experiments (Melgarejo and Artes, 2000, Sassano *et al*, 2009). Moreover, phenolic compounds are other important substances in various parts of the pomegranate fruits specially seed lipid fractions (Al-Maiman and Ahmad, 2002, Abbasi *et al*, 2008). Schubert *et al*, (1999) showed that the antioxidant properties of the cold-pressed PSO due to presence of these compounds were significantly superior to those of red wine, green tea and the synthetic antioxidant butylated hydroxy anisole (BHA). During the past years, many researchers just determined type and amount of the fatty acids and did no study on the physical and chemical characteristics of PSO. For example, El-shaarawy and Nahapetian (1983) showed that 8% of fatty acids of pomegranate seed oil were saturated, 10% monosaturated, 10% disaturated and approximately 70% conjugated acid, most probably punicic acid. El-Nemr *et al*, (1990) showed that 83.6% of the fatty acid was saturated and 16.3% unsaturated. Melgarejo et al, (1995) with study on six cultivars of Spain pomegranate reported 30-33.8% of the fatty acids were saturated and 66.2-69.0% of the fatty acids were unsaturated. In another study, Kyralan et al, (2009) reported fatty acid composition of 15 commercial cultivars of pomegranate that are grown in Turkey. Oil content of studied cultivars and the predominant unsaturated fatty acid were 24.13 to 13.95% and punicic acid (70.42-76.17%) respectively, but they didn't evaluate the properties of seed oil. Physicochemical characteristics of seeds and oils obtained from different varieties of pomegranate fruits depends

on cultivated status, growth, climate, maturity and storage conditions (Fadavi *et al*, 2006). Considering the effects of environmental conditions, cultivar and cultivation conditions on the physical and chemical properties, the need for further studies in this area is felt. To the best our khowledge, detailed measurements of phsicochemical properties of PSO have not been reported. Therefore, in this research, the seeds of four commercial varieties of Iranian pomegranate were campared by analyzing some chemical composition and oil physicochemical properties.

MATERIALS AND METHODS

Sample preparation: Approximately 15 kg (n=40) of pomegranate fruits with medium size (350-400 g) from four cultivars [Abanmahi (AB), Malas (MS), Pust Sefid (PS) and Shahvar (SH)] in 2011 autumn Season were selected from mature fruits grown in the collection of the Agricultural Research Center of Yazd, Iran. Four fruits located externally and four located internally on each tree were harvested from five randomly selected trees for each pomegranate cultivar. Harvested pomegranate fruits were transported to the laboratory and they were sorted by appearance (i.e., free from sunburn, crack) then maturity seeds were separated from their juice sacs, washed with distilled water, and dried in a vacuum oven at 35°C until a constant weight was reached.

Chemicals:

The solvents and chemicals used in this study were of analytical grade and purchased from Merck Chemical Company (Darmstadt, Germany), Sigma-Aldrich (Oakville, ON, Canada) and Fisher Scientific (Ottawa, Ontario, Canada).

Seed chemical composition:

A dried sample was taken from each variety to be studied. they were later ground and 5g of powdered sample from each was weighted and fat was then extracted with petroleum ether using a soxhlet apparatus according to the AACC method (AACC, 1987). The fat was recovered by petroleum ether distillation in a vapour rotor at 60°C. The samples were then dried in a dessicator for 1 hour, and finally weighed to obtain the grams of fat extracted. Result was expressed as the percentage of lipids in the dry matter of seed powder. The nitrogen content estimated by the Kjeldahl method and was converted to protein content by using the conversion factor 6.25. To remove carbon, about 2g (powdered) of each cultivar in a porcelain container was ignited and incinerated in the muffle furnace at about 550°C for 8 hours. The total ash was expressed as percent of dry weight. The minerals including iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg), and zinc (Zn) concentrations were determined as described by Agte *et al*, (1995) with a minor modification. Absoledaty 1g samples, in triplicate, were dry-ashed in a muffle furnace at 550 ºC for 5 hours until a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20.0 mL of 2.5% HCl, heated in a steam bath to reduce the volume to about 7.0 mL, and then was diluted to volume of 50 mL with deionised water, stored in clean polyethylene bottles and mineral contents determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). Sodium (Na) and potassium (k) contents were determined similar to the method of Champman and Pratt (1961) using flame photometry instrument (JENWAY Limited, Dunmow Essex, UK). The used voltage and power were 230/115 V and 13 VA, respectively. Phosphorus content (P) was determined by the

phosphomolybdate method (AOAC, 1990). Crude fiber was obtained by digesting 2 g of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5 hours (AOAC, 1990). Carbohydrate content was estimated by difference of mean values, from, 100-(Sum of percentages of moisture, ash, protein and lipids).

Oil extraction:

The oil used for the determination of the fatty acid (FA) compositions and other oil indices were extracted according to the method reported by Kornsteiner *et al*, (2006) with minor modifications. 5 gr powdered sample were placed in a conical glass containing around 50 mL of n-hexane and agitated on a magnetic stirrer for 3 hours. Then, the solution was filtered and the remaining solvent was evaporated by using a rotary evaporator at 40°C. Obtained oil was transferred into a small glass vial, flushed with nitrogen and maintained at -18°C until further analysis.

Preparation of Fatty Acid Methyl Esters:

Fatty acid methyl esters (FAME) were prepared by transmethylation according to the method 5509 of the ISO (1978), using KOH 2M in methanol and n-heptane medium.

Fatty Acids Profile:

Fatty Acids compositions were determined using a gas chromatography (Agilent 6890, UK) equipped with a flame ionization detector (FID) and a BPX-70 capillary column (120 m, 250 µm i.d., 0.2 µm film thickness). The oven temperature was set at 198°C (Iso thermal) which was held for 6 minutes. Then, it was increased to 180 °C at 20 °C/ min, held for 10 minutes and increased again to 210 °C at 20 °C/min. Injector and detector temperatures were set at 250 and 280°C, respectively. Nitrogen was used as the carrier gas with inlet pressure of 33.3 psi. The FAME was identified by comparing their retention times with those of standard samples. The quantification of fatty acids was made according to their relative area percentages, comparing with their calibration curves.

Oil Indices:

Iodine value **(**IV), acid value (AV), saponification value (SV), peroxide values (PV), ortho-diphenols content (ODC), refreactive index (RI), viscosity (ŋ), and density value of each samples were measured. IV and SV were determined according to the standard methods from AOAC (1990). The AV was determined by method Cd 3d-63 of AOCS Official Methods of Analysis (AOCS, 1998). PV was determined spectrophotometrically based on a method from the International Dairy Federation (Shantha and Decker, 1994). Approximately, 0.25 g of extracted oil was weighed into a glass tube and dissolved in 9.7 mL of a mixture of chloroform:methanol (4:1, v/v). Then, a drop of ammonium thiocyanate solution (30%, w/v) and a drop of ferrous chloride solution (0.35%, w/v) were added to the tube. After standing for 5 minutes, the absorbance of the mixture was read at 500 nm using a spectrophotometer (Cesil, model CE 2502,

UK). A calibration curve was also obtained using ferric chloride solutions containing 5-20 µg of Fe^{3+}. The oils viscosity was determined by using a Brookfield Viscometer (model DV-II+Pro, USA). The refractive index of oil samples was analyzed at room temperature according to the AOAC (1990) by Abbe refractometer (model G manufactured by Carl-Zeiss, Germany). The density of oil samples was determined with a specific gravity hydrometer (Proton, model 72082, Barcelona, Spain) at 20°C. Ortho-diphenols content (ODC) in PSOs was evaluated in methanolic extract following the method described by Gutfinger (1981). The concentration of ortho-diphenols in the methanolic extract was determined with molybdate. The procedure consisted of dilution of 0.2 mL extract to 1 mL with water, addition of 1 mL 0.1 M phosphate buffer (pH=6.5) and 2 ml 5% Na2MoO4 × 2H20 solution. The content was mixed and the extinction was measured using a UV-visible spectrophotometer (DR/4000U-HACH, USA) after 15 min at 350 nm against a reagent blank. Galic acid served as a standard for preparation of a calibration curve.
Radical Scavenging activities of PSO extracts towards the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Martinez and Maestri (2008). Briefly, 100mg of oil in 1mL toluene was vortexed for 20s with 3.9 mL toluene solution (10^{-4} M) of the free stable DPPH (2,2 diphenyl 1-picrylhydrazyl) radical ($DPPH^{\bullet}$). The absorption at 515nm was measured after 1, 30 and 60min of mixing using a UV-visible spectrophotometer (Cesil, model CE 2502, UK) against a blank of pure toluene. The radical scavenging activity toward $DPPH^{\bullet}$ was determined by the following equation:

$$DPPH^{\bullet}_{r} = 1 - \left(\frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100 \right)$$

where A is the absorbance and $DPPH^{\bullet}r$ is the amount of the remained radical in the medium after depletion of antioxidants in the PSO.

Statistical Analysis:

All experiments were performed in triplicate. Comparison among the means was carried out using least significant differences (LSD) at the 95% confidence level using Statistical Analysis System (SAS) release 9.1 (SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Chemical composition: Chemical composition and mineral conentration of the pomegranate seeds obtained from four studied varieties were given in Table 1. As considered in this Table, significant differences were observed among the seeds in their content of oil, crude protein, crude fiber, carbohydrate and ash (p<0.05). Crude fiber was the highest constitute in all samples, ranging from 36.5 % in SH to 42.4 % in PS, whereas ash presented the lowest values; it was higher in SH (1.887 %) and lower in MS (1.592 %). The obtained results of crude fiber and ash

contents has somewhat coordination with the findings of El-Nemr et al, (1990) (35.5 and 2% respectively).

Table 1. Proximate composition and mineral content of seeds obtained from four iranian commertial pomegranate varieties

Component (%)	Varieties			
	AB	MS	PS	SH
Oil	14.6 ± 0.08^{c}	15.6 ± 0.09^{b}	16.9 ± 0.11^{a}	13.5 ± 0.08^{d}
Crude protein	8.5 ± 0.029^{d}	11.3 ± 0.064^{a}	10.7 ± 0.052^{b}	10.5 ± 0.034^{c}
Crude fiber	38.9 ± 2.24^{ab}	40.1 ± 3.22^{ab}	42.4 ± 2.80^{a}	36.5 ± 1.99^{b}
Carbohydrate	32.19 ± 1.76^{a}	26.63 ± 1.44^{b}	24.09 ± 1.26^{b}	33.41 ± 2.02^{a}
Ash	1.653 ± 0.004^{c}	1.592 ± 0.006^{d}	1.675 ± 0.006^{b}	1.887 ± 0.007^{a}
Mineral (mg/kg)				
Ca	378.00 ± 10.82^{c}	674.00 ± 7.00^{a}	628.00 ± 5.20^{b}	675.33 ± 8.51^{a}
Cu	5.49 ± 0.86^{b}	6.99 ± 1.12^{b}	9.81 ± 1.00^{a}	6.45 ± 0.95^{b}
Fe	31.227 ± 1.54^{a}	29.017 ± 1.41^{ab}	16.280 ± 1.11^{c}	28.150 ± 1.78^{b}
K	3070.00 ± 6.56^{b}	2677.33 ± 7.51^{c}	2024.67 ± 9.50^{d}	3724.67 ± 10.07^{a}
Mg	1327.67 ± 15.63^{c}	1824.00 ± 22.00^{b}	2052.00 ± 25.00^{a}	1819.67 ± 20.84^{b}
Mn	8.600 ± 1.69^{b}	9.497 ± 1.64^{b}	12.437 ± 1.35^{a}	7.540 ± 1.39^{b}
Na	211.33 ± 3.51^{a}	144.00 ± 2.00^{b}	84.50 ± 0.56^{d}	127.33 ± 3.06^{c}
P	2283.67 ± 19.30^{c}	2416.67 ± 17.01^{b}	2766.33 ± 22.72^{a}	2194.00 ± 18.73^{d}
Zn	29.173 ± 1.44^{c}	31.273 ± 1.66^{bc}	70.957 ± 2.30^{a}	34.540 ± 1.82^{b}

^{a,b,c} letters indicate the statistical difference in rows

The lipid content of the analyzed samples ranged from 13.5 % for SH to 16.9 % for PS. Kyralan et al, (2009) reported the oil content of 15 commercial pomegranate seed varieties in the range of 13.95-24.13%, that was partially consistent with the results obtained in this study. However, the amounts of lipid in this study were in contrast with those of El-Shaarawy and Nahapetian (1983) and Fadavi et al, (2005). These differences could be attributed to the veriety, harvesting year, environmental condition and latitude, with different temperatures, rainfall and light, which can influence the chemical composition of fruits (Parcerisa *et al*, 1995). The crude protein values

were 8.5, 10.5, 10.7 and 11.3%, for AB, SH, PS and MS, respectively. These results were less than those reported by El-Nemr et al, (1990) (13.2%). The Carbohydrate content of samples ranged from 24.09 % to 33.41 %, following the order SH> AB> MS> PS. There were also differences in mineral contents of the pomegranate seed four investigated varieties (p<0.05). Potassium was conspicuously the highest followed by phosphorus, magnesium, calcium and sodium. The potassium content of SH (3724.67 mg/Kg) were higher than those for AB (3070.00 mg/Kg), MS (2677.33 mg/Kg) and PS (2024.67 mg/Kg). In the present work, we found that the presence of potassium in SH could be responsible of the high ash content. In fact, a positive significant correlation (p<0.05) between the ash content and potassium or phosphorus content was established. Potassium is the most important intracellular element; it is required for various physiological functions (Olaniyi et al, 1993). Beside the major mineral metals cited above, substantial amount of some microelements such as Zn, Fe, Mn and Cu were found. Concentrations of these microelements and the ranges of variations for these minerals were 29.173-70.957, 16.280-31.227, 7.540-12.437, and 5.49- 6.9 mg/Kg, respectively. In general, most of the minerals of the AB variety were slightly less than of the other varieties. Chemical composition of pomegranate seeds revealed that this by-product could be valuable. In order to justify the extraction of PSO, it is necessary to study its functional properties.

Fatty Acid Composition:

The fatty acid composition of the seed oils of the pomegranate varieties studied consisted of fifteen fatty acids (Table 2). There were small variations in the contents of fatty acids among the oils studied, leading to likeness in total saturated (SFA), total unsaturated (USFA), monounsaturated (MUFA) and polyunsaturated (PUFAs) fatty acids. The main SFA among the four varieties studied here was palmitic acid ranging from 4.04% (in PS) to 4.46% (in MS). Stearic and arachidic acids, other SFAs that were found in the oils, were within 2.81-3.00 and 0.60-0.64 %, respectively. Other saturated fatty acids such as lauric, myristic and margaric acids identified in level of insignificant and their ranges were 0.01% for lauric, 0.03-0.04% for myristic and 0.01% for margaric. Results of this study on the SFAs levels in the PSO were in good agreement with those reported by Melgarejo and Artes (2000). But our results do not correspond with the studies of Kyralan et al, (2009). All oil samples had high amount of total unsaturated fatty acids. Major mono-unsaturated fatty acid (MUFA) among all studied varieties was oleic acid, ranging from 8.31% (for MS) to 9.77% (for PS). Also, oleic acid contents in AB and SH were 9.06 and 9.36%, respectively. Eicosenoic acid (C20:1) as a MUFA was identified in PSOs and its ranges were 0.90 to 1.08%. MS was allocated highest value (1.08%) and follow that were AB (0.98%), PS (0.98%) and SH (0.9%). Besides MUFAs mentioned above, a number of other MUFAs, such as palmitoleic (C16:1) (0.06-0.09%), margaroleic (C17:1) (0.01-0.02%) and elaidic (C18:1t) (0.06-0.07%) were also identified that their amounts was not significant. These results of MUFAs did not consistent with a number of previous studies, which can relate to variety, maturity level, soil composition and latitude (Parcerisa et al, 1995). Melgarejo and Artes (2000) reported 3.81-20.25% and 0.92-2.88% for oleic and palmitoleic acids, respectively. Reported values by Sassano *et al*, (2009) for oleic (6.82- 7.17%) and eicosenoic (0.64 - 0.66%) acids were less than amounts that obtained in this study.

Table 2. Fatty acids composition of seed oil obtaned from four Iranian commercial pomegranate varieties (%, w/w)

Fatty acids	Varieties			
	AB	MS	PS	SH
Lauric $C_{12:0}$	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Myristic $C_{14:0}$	0.03 ± 0.00[b]	0.03 ± 0.00[b]	0.03 ± 0.00[b]	0.04 ± 0.00[a]
Palmitic $C_{16:0}$	4.35 ± 0.2[a]	4.46 ± 0.3[a]	4.04 ± 0.1[a]	4.41 ± 0.2[a]
Palmitoleic $C_{16:1}$	0.06 ± 0.00[d]	0.07 ± 0.00[c]	0.09 ± 0.00[a]	0.08 ± 0.00[b]
Margaric $C_{17:0}$	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Margaroleic $C_{17:1}$	0.02 ± 0.00[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.02 ± 0.00[a]
Stearic $C_{18:0}$	2.99 ± 0.1[a]	2.88 ± 0.2[a]	3.00 ± 0.4[a]	2.81 ± 0.1[a]
Oleic $C_{18:1c}$	9.06 ± 0.3[b]	8.31 ± 0.3 [c]	9.77 ± 0.3[a]	9.36 ± 0.3[ab]
Elaidic $C_{18:1t}$	0.07 ± 0.00[a]	0.06 ± 0.00[b]	0.06 ± 0.00[b]	0.07 ± 0.00[a]
Linoleic $C_{18:2c}$	8.11 ± 0.5[b]	8.53 ± 0.4[ab]	8.13 ± 0.1[b]	9.03 ± 0.3[a]
Linoelaidic $C_{18:2t}$	0.33 ± 0.02[ab]	0.35 ± 0.02[a]	0.30 ± 0.01[b]	0.31 ± 0.01[b]
α-Linolenic $C_{18:3}$	0.04 ± 0.00[b]	0.04 ± 0.00[b]	0.04 ± 0.00[b]	0.1 ± 0.00[a]
Punicic $C_{18:3}$	73.15 ± 0.4[a]	73.31 ± 0.5[a]	72.71 ± 0.4[ab]	72.07 ± 0.3[b]
Arachidic $C_{20:0}$	0.62 ± 0.08[a]	0.60 ± 0.03[a]	0.64 ± 0.07[a]	0.62 ± 0.06[a]
Eicosenoic $C_{20:1}$	0.98 ± 0.01[b]	1.08 ± 0.02[a]	0.98 ± 0.01[b]	0.90 ± 0.01[c]
SFA**	8.01 ± 0.18 [a]	7.99 ± 0.07 [a]	7.73 ± 0.37 [a]	7.90 ± 0.16 [a]
USFA**	91.82 ± 1.19[a]	91.76 ±0.24[a]	92.09 ± 0.8[a]	91.94 ± 0.3[a]
MUFA/PUFA**	0.123 ± 0.00[ab]	0.117± 0.00[b]	0.133 ±0.00[a]	0.127±0.00 [ab]

** SFA saturated fatty acid, USFA unsaturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid, [a,b,c] letters indicate the statistical difference in rows.

According to Table 2, PSOs were rich in punicic acid (C18:3) as a PUFA. Punicic acid was the highest level among all fatty acids and found at 73.31% for MS, 73.15% for AB, 72.71% for PS and 72.07% for SH. Similarly, Kyralan et al, (2009) reported high levels of punicic acid (70.42 – 76.17%) in some Turkish varieties, while Melgarejo and Artes (2000) and Fadavi *et al*, (2006) found higher and lower levels of punicic acid than amounts detected in this study, respectively. Dietary PSO due to high level of punicic acid has been shown to have an effect on immune function and lipid metabolism in mice (Yamasaki et al, 2005). Therefore, not only pomegranate juice has properties of pragmatic, its oil is also considered as a functional food and has many healthy effects. The levels of linoleic (C18:2c) and linoelaidic (C18:2t) acids ranged from 8.11% for AB to 9.03% for SH, and 0.30% for PS to 0.35% for MS, respectively. α-linolenic acid (C18:3) were also present but in much lesser amounts. The highest amount of α-linolenic acid was in SH (0.1%), whereas its content in other varieties was 0.04%. The USFA amount in the oil extracted from PS seeds was about 92.09%, which was greater than that of the MS oil at a level of 91.76 % and the AB oil of 91.82 % and the SH oil of 91.94%. Moreover, the ratio of MUFA to PUFA is an important parameter for oil stability in highly unsaturated oils (Kodad and Socias, 2008). Concerning the ratio of MUFA to PUFA, this is similar in the four varieties studied, varying between 0.117 (MS) and 0.133 (PS). The corresponding values for this ratio were 0.123 and 0.127 for AB and SH, respectively. This ratio indicates that these varieties have a high content of PUFA, which may make them more attractive for the consumer who wishes to use this type of acids.

Physicochemical properties of oils:

The physical and chemical properties of the oils extracted by cold press method were compared and the results are presented in Table 3. The states of oils were liquid at room temperature and the colors of oils were golden. The IR for oil of all of studied varities at 25°C were close: 1.461 for MS, 1.495 for SH, 1.500 for AB and 1.527 for PS. These results were in good agreement with previous study (1.518) that performed by El-Nemr *et al*, (1990). El-shaarawy and Nahapetian (1983) reported 1.477-1.518 for IR in PSO, that was consistent with the results of this study.

Table 3. Some physicochemical characteristics of pomegranate seed oils obtained from four studied varieties

Sample	IV	AV	PV	SV	RI	(n_D^{20})	ŋ
AB	220.34 ± 4.9[a]	8.36 ± 0.06[a]	0.48 ± 0.11[a]	181.1± 0.08[a]	1.500 ± 0.028[a]	0.9245±0.0015[bc]	0.054± 0.003[ab]
MS	219.89 ± 6.4[a]	3.78 ± 0.11[d]	0.44 ± 0.05[a]	182.5± 0.32[a]	1.461 ± 0.048[a]	0.9311±0.0009[a]	0.036± 0.002[b]
PS	217.25 ± 4.2[a]	5.25 ± 0.07[c]	0.50 ± 0.11[a]	179.3± 0.26[a]	1.527 ± 0.016[a]	0.9202±0.0031[c]	0.063± 0.004[a]
SH	216.95 ± 8.5[a]	5.84 ± 0.08 [b]	0.39 ± 0.07[a]	182.0± 0.12[a]	1.495 ± 0.035[a]	0.9288±0.0021[b]	0.054± 0.001[ab]

[a,b,c,d] letters indicate the statistical difference in columns

Iodine value (g I_2/100g oil); acid value (% oleic acid); peroxide value (meq O_2 /kg oil); saponification value (mg KOH/g); ortho-diphenols content (mM ortho-diphenols/kg oil);refractive index (at 25°C); (n_D^{20}) density at 20°C (g/cm^3); viscosity (Pa.s). Flow behavior analysis of the samples to determine oils viscosity showed a Newtonian rheological behavior (Figure 1). The results indicated that SH had the highest level of oil viscosity (0.063 Pa.s), while the lowest viscosity of the walnut oil was related to MS (0.036 Pa.s) (Table 3). The oils density significantly varied from 0.9202 to 0.9311 g/cm^3 among different varieties (p<0.05). The measured chemical properties of oils were IV, AV, SV, PV, ODC and DPPH radical scavenging. IV (g I_2/ 100g oil) demonstrates the levels of unsaturation and potential oxidative sensitivities of the oils (Moodley *et al*, 2007). The IV of the analyzed samples ranged from 216.95 for SH to 220.34 for AB. Also, this factor for PS and MS were 217.25 and 219.89, respectively. These high values of IV are due to the high level of punicic acid (72.07-73.31%) in PSOs. Therefore, PSO is susceptible to oxidative degradation. The AV of the samples ranged from 3.78 (MS) to 8.36 (AB). AV of AB was significantly higher (p<0.05) than that of the other seeds. This parameter for oil of SH, PS and MS varities were 5.84, 5.25 and 3.78, repectively. The values obtained for the IV and AV had significant differences with the results of El-shaarawy and Nahapetian (1983) and El-Nemr et al, (1990). The high SV showed that the oils have smaller molecular weight than other edible oils. This property of the oil depends on the presence of higher fatty acids and its unsaturation level. No significant (p>0.05) difference was observed for the values of SV (179.3-182.5mg KOH/g) among the PSOs. The PV of oil is a valuable index to determine oil quality. If the PV becomes higher than 9 meq/1000g oil, it indicates oxidative corruption in oil (Ozcan, 2009). As can be seen in Table 3, the amount of PV for studied varieties is in the range of 0.39 for SH to 0.50 for PS, which represents good extraction and maintenance condition. No significant difference was obtained in the PV of oils from samples at the 95% confidence level. The PV depends on a number of factors such as the state of oxidation (quantity of oxygen consumed), the method of extraction used and the type of fatty acids present in the oil. We did not find any report for PV of PSO among previous papers for comparison. As demonstrated by the data depicted in Figure 2a, significant differences (p<0.05) were obtained in the amounts ODC quantified in samples extracted from different pomegranate varieties. Generally, the highest ODC was measured in extracted oil from SH (58.7 µg/g oil), while the lowest ODC (34.3 µg/g oil) of the PSO was attributed to MS. A kinetic assay recorded by means of spectrophotometric absorbance to evaluate percentage of DPPH radical inhibition of the oils (Figure 2b). The results of oil antioxidant activity test showed that the extracted oil from SH had the highest most efficiency and activity to scavenge DPPH radical followed by the oil obtained from AB, PS and MS. Moreover, a high positively correlation was found between ODC and percentage of DPPH radical scavanging (R^2 = 0.98, p<0.01). This fact indicated that antioxidant activities of PSOs were attributed to the presence of phenolic compounds specially ortho-diphenols.

Conclusion

The observations made in this study reveal that the pomegranate seeds are rich in crude protein, crude lipids, dietry fiber and minerals such as K, P, Mg and Ca. The utilization of pomegranate seeds for oil and protein production could alleviate food shortages and provide extra income to the rural farmers where the plant grows. The results of this study also showed that the PSOs obtained from four Iranian varieties were quite similar in their physicochemical characteristics, with only a few significant variations. However, SH in the respect of antioxidant activity and phenolic compounds such as ortho-diphenols was the best of pomegranate among all varieties. Noting the increasingly popularity of PSOs as a source on PUFAs and natural antioxidants, this investigation supports a view of the characteristic and quality of PSOs in Iran and could also serve as a starting point for developing quality standards since there are no specifications available for PSO.

REFERENCES

AACC. (1987) Method 46-11. Approved methods of the AACC. *American Association of Cereal Chemists*, Inc., St Pau, Minn.

Abbasi, H., Rezaei, K., Emamdjomeh, Z., Ebrahimzadeh Mousavi, S.M. (2008) Effect of various extraction conditions on the phenolic contents of pomegranate seed oil. European *Journal of Lipid Science and Technology*, 110: 435-440.

Agte, V.V., Gokhale, M.K., Paknikar, K.M., Cheplonkar, S. (1995) Assessment of peral millet versus rice-based diets for bioavailability of four trace metals. *Plant Foods Human and Nutrition*, 48: 149-158.

Al-Maiman, S.A., Ahmad, D. (2002) Change in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation. *Food Chemistry*, 76: 437–441.

AOAC. (1990) Official methods of analysis of the Association of Official Analytical Chemists., Washington D.C.

AOCS. (1998) Official Methods and Recommended Practices of the American Oil Chemists' Society, AOCS Press, Champaign, USA.

Chapman, D.H., Pratt, P.F. (1961) Methods of analysis of soils, plants and water. University of California, Riverside: Division of Agriculture Science.

El-Nemr, S.E., Ismail, I.A., Ragab, M. (1990) Chemical composition of juice and seed of pomegranate fruit. *Die Nahrung*, 34: 601–606.

El-Nemr, S., Ismail, I., Ragab, M. (1992) The chemical composition of the juice and seeds of pomegranate fruits. *Journal of Fruits Vegetables and Nuts*, 11: 162-164.

El-shaarawy, M.I., Nahapetian, A. (1983) Studies on pomegranate seed oil. *Fette Seifen Anstrichm*, 85: 123–126.

Fadavi, A., Barzegar, M., Azizi, M.H., (2005) Bayat, M. Physicochemical Composition of Ten Pomegranate Cultivars (Punica granatum L.) Grown in Iran. *Food Science and Technology International*, 11(2): 113-119.

Fadavi, A., Barzegar, M., Azizi, M.H. (2006) Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. *Journal of Food Compostion and Analysis*, 19: 676–680.

FAO. (2009) Food Agriculture Organization of the United Nations, Project document for a regional standard for Pomegranate, Rome, Italy.

Gutfinger, T. (1981) Polyphenols in olive oils. *Journal of the American Oil Chemists' Society*, 58: 966-968.

Hernandez, F., Melgarejo, P., Olias, J.M., Artes, F. (1998) Fatty acid composition and total lipid content of seed oil from three commercial pomegranate cultivars. Symposium on production, processing and marketing of pomegranate in The Mediterranean Region: advances in research and technology. CIHEAM-IAMZ Zaragoza, Spain 15–17 October, pp 205–209.

ISO. (1978) Animal and vegetable fats and oils - preparation of methyl esters of fatty acids. Method ISO 5509,.

Kodad, O., Socias, I.C.R. (2008) Variability of oil content and of major fatty acid composition in almond (Prunus amygdalus Batsch) and its relationship with kernel quality. *Journal of Agricultural and Food Chemistry*, 56: 4096–4101.

Kyralan, M., Golukcu, M., and Tokgoz, H. (2009) Oil and conjugated linolenic acid contents of seeds from important pomegranate cultivars (Punica granatum L.) grown in Turkey. *Journal of the American Oil Chemists' Society*, 86: 985–990.

Kornsteiner, M., Wagner, K.H., Elmadfa, I. (2006) Tocopherols and total phenolics in 10 different nut types. *Food Chemistry*, 98: 381–387.

Martinez, M.L., Maestri, D.M. (2008) Oil chemical variation in walnut (Juglans regia L.) genotypes grown in Argentina. *European Journal of Lipid Science and Technology*, 110: 1183–1189.

Melgarejo, P., Salazar, D.M., Amoros, A., Artes, F. (1995) Lipid and fatty acid composition of seed oil from six pomegranate cultivars. *Journal of the Science of Food and Agriculture*, 69: 253–256.

Melgarejo, P., Artes, F. (2000) Total lipid content and fatty acid composition of oil seed from lesser known sweet pomegranate clones. *Journal of the Science of Food and Agriculture*, 80: 1452–1454.

Moodley, R., Kindness, A., Jonnalagadda, S.B. (2007) Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa. *Journal of Environmental Science and Health, Part B*, 42: 585–591.

Olaniyi, A.A., Ayin, J.S.K., Ogundaini, A.O., Olugbade, T.A. (1993) Essential Inorganic and Organic Pharmaceutical Chemistry. Shaneson C.I. Ltd., Ibadan, Nigeria.

Ozcan, M.M. (2009) Some Nutritional Characteristics of Fruit and Oil of Walnut (Juglans regia L.) Growing in Turkey. *Iranian Journal of Chemistry & Chemical Engineering*, 28: No. 1.

Parcerisa, J., Rafecas, M., Castellote, A., Codony, R., Farran, A., Garcia, J., Gonzalez, C., Lopez, A., Romero, A., Boatella, J. (1995) Influence of variety and geographical origin on the lipid fraction of hazelnut (Corylus avellana L.) from Spain: (III) oli stability, tocopherol content and some mineral contents (Mn, Fe, Cu). *Food Chemistry*, 53: 71-74.

Sassano, G., Sanderson, P., Franx, J., Groot, P., Straalen, V.J., Bassaganya-Rierac, J. (2009) Analysis of pomegranate seed oil for the presence of jacaric acid. *Journal of the Science of Food and Agriculture*, 89: 1046-1052.

Schubert, S.Y., Lansky, E.P., Neman, I. (1999) Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of Ethnopharmacology*, 66: 11–17.

Shantha, N.C., Decker, E.A. (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International*, 77(2): 421–424.

Yamasaki, M., Kitagawa, T., Koyanagi, N., Chujo, H., Maeda, H., Kohno- Murase, J., Imamura, H., Tachibana, K., Yamada, R. (2005) Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. *Nutrition*, 22: 54–59.