Res. Jr. of Agril. Sci. **11**(6): 1399-1406, Nov-Dec 2020

ISSN: 0976-1675

www.rjas.org

Research Journal of Agricultural Sciences

© Centre for Advanced Research in Agricultural Sciences

Research Paper

DI: 6335-2808-276

Chemical Evaluation for Pomegranate (*Punica granatum* L.) Fruit Peel and Seeds Powders by Products

Roli Tripathi* and Neetu Singh

Department of Food and Nutrition, School for Home Science,
Babasaheb Bhimrao Ambedekar University, Lucknow - 226 025, Uttar Pradesh, India

Received: 28 August 2020; Revised accepted: 01 October 2020

A B S T R A C T

This research was performed to throw the light on the nutritional value indices; proximate chemical composition, indispensable amino acids profile, fatty acids composition, minerals content and vitamins content and polyphenol fractions content of pomegranate fruits peel and seeds powders as by-products of their processing to investigate the ability of using these in food processing and preservation. The present results showed that tested pomegranate fruits by-products; peels and seeds, powder contained a much higher exceptionally content of lysine, isoleucine and amino acids – containing sulpher (Methionine and cysteine), which are usually deficient in the most food stuffs, than the reference protein pattern of FAO/WHO. The current results also illustrated that pomegranate seeds oil contained a high content of W_3, W_{6-} and W_{9-} fatty acids. In addition, the tested pomegranate fruits by-products were characterized with their richness with the most determined minerals and vitamins. Furthermore, pomegranate fruits peel powder had a high content (1.403 g/100g dry matter) of total polyphenols and the predominant polyphenol fractions in pomegranate peels powder were catechins, phenol gallic acid, caffeic acid, ellagic acid, p-coumaric acid and resocenol compounds. Therefore, the current results suggest that it should be directed to incorporate pomegranate fruits seed and peel powders into the food prossing and preservation technology to improve their nutritional quality and to prolong the shelf-life of these food products.

Key words: *Punica granatum* L., Amino acids, Methionine, Food products, Shelf life

The pomegranate (*Punica granatum*) belongs to the Punicaceae family and is a nutrient dense food source rich in phytochemical compounds. Pomegranates are popularly consumed as fresh fruit and juice, beverages, food products (jams and jellies) and extracts wherein they are used as botanical ingredients in herbal medicines and dietary supplements. Several studies reported that phytochemical have been identified from various parts of the pomegranate tree and from pomegranate fruit: peel, juice and seeds (Abdel-Rahim *et al*. 2013). Pomegranate is an important source of bioactive compounds and has been used in folk medicine for many centuries. Most pomegranate fruit parts are known to possess enormous antioxidant activity. In India, pomegranate arils are used as such or are made into juice. Pomegranate juice has been demonstrated to be high in antioxidant activity and is effective in prevention of atherosclerosis, low-density lipoprotein oxidation, prostate cancer, platelet aggregation and various cardiovascular diseases (Adhami and Mukhtar 2006). Antioxidant potential of pomegranate in-vivo and in-vitro has been proved (Ahangari and Sargolzaei 2012). In addition to its antioxidant activity, it has antimicrobial, antibacterial, antiviral, antifungal and antimutagenic properties as well as beneficial effects on the oral and cardiovascular diseases. Besides this, Pomegranate peels have been reported to have pronounced antioxidant activity (Alesón-Carbonell *et al*. 2005). Recently, chemical constituents and their bioactivities in all parts of pomegranate (*Punica granatum* L.), including leaf, seed, juice, husk and peel, have been investigated (Lansky and Newman 2007). Pomegranate seed, the by-product of pomegranate juice processing,

***Corresponding author:** Roli Tripathi, Scholar, Department of Food and Nutrition, School for Home Science, Babasaheb Bhimrao Ambedekar University, Lucknow - 226 025, Uttar Pradesh

e-mail: rolitripathi1491995@gmail.com

contains a range of nutraceutical components such as sterols, γ-tocopherol, punicic acid and hydroxyl benzoic acids (AOAC 2000). Phenyl aliphatic glycosides as phenethyl rutinoside were also found in pomegranate seed (AOAC 2005).

Pomegranate fruits peel is an inedible part obtained during processing of pomegranate juice. Pomegranate peel is a rich source of tannins, flavonoids and other phenolic compounds (Bassaganya–Riera *et al*. 2004). Antioxidant and antibacterial properties of pomegranate peel in in-vitro model systems have been reported (Devatkal and Naveena 2010). However, pomegranate peel has received less attention as natural preservatives in meat (Elfalleh *et al*. 2011). Pomegranate peel extract has both antioxidant and antimutagenic properties and may be exploited as bio-preservative in food applications and neutraceuticals. However, so far, there has been no attempt to investigate the antioxidant properties of pomegranate in meat products (Fadavi *et al*. 2006).

Due to the large amount of pomegranate seeds as the by-product of juice and concentrate production plants and because of valuable pharmaceutical and nutritional compounds such as unsaturated fatty acids and phenolic compounds in the seed and their antioxidant properties, the seeds can have more beneficial applications in food industries instead of being used as animal feed or in commercial cosmetic products. One way to utilize the seed is to extract the oil and use it in pomegranate juice or other juices and beverages. To meet this, an emulsion of PSO-in-water needs to be prepared that is also kinetically stable for a period of time. Pomegranate seed oil (PSO) consists of approximately 80% conjugated octadecatrienoic fatty acids, with high content of punicic acid (9-cis, 11-trans, 13-cis, 18:3), fatty acid specific to this oil. It has been reported that punicic acid inhibits prostaglandins biosynthesis. The PSO also contains a remarkable array of safe phytoestrogens that are chemically similar to estrogens naturally biosynthesized by the human body. The oil contains not only phytoestrogenic compounds (isoflavones and coumesterol), but also sex steroids (estrone, 17- α-estradiol, estriol and testosterone), sterols (daucosterol, camesterol, stigmasterol and b-sitosterol) and tocopherols (especially γ-tocopherol) (FAO/WHO 1973).

Pomegranate seed oil (PSO) strongly inhibits lipoxygenase and cyclooxygenase, eicosanoid pathway enzyme, which are responsible for transformation of arachidonic acid to prostaglandins, thrombaxane and leukotrienes, respectively, which play important roles in inflammation, atheromatous, plaque formation and platelet aggregation (which are associated with cardiovascular diseases) and also asthma in children. In addition, it was reported that PSO is a safe and effective chemopreventive agent against skin cancer in CD1 mice and inhibits the development of azoxymethane-induced colonic adenocarcinomas in male F344 rats without causing any adverse effects (Food and Nutrition Board 1989).

The main objective of this study was to investigated the ability of using the pomegranate by-products in food processing and preservation technology which having high nutritional value, a better acceptability for consumer and good safe hygienic quality. Therefore, the present study was performed to throw the light on the nutritional value indices; proximate chemical composition, indispensable amino acids profile, fatty acids composition, minerals content and vitamins content and of pomegranate fruits peel and seeds powders as by- products of their processing, as well as on the polyphenol fractions content in pomegranate fruit peel powder.

MATERIALS AND METHODS

Fresh pomegranate fruits (*Punica granatum* L.) were obtained from the local market Goamti nagar Lucknow, Uttar Pradesh, India.

Preparation of pomegranate peels powder

Mature pomegranate fruits were washed and cut manually to separate the seeds and peel. The rind (peels) thus obtained, cut into small pieces using a sharp knife and dried in an air circulatory tray drier at 60±5ºC for 6 hrs. till its moisture content reached 12-14%. Dried pieces were cooled, powdered in a laboratory disc mill (Braun AG Frankfurt Type: KM 32, Germany) to pass through 20 mesh sieve, packed in high density polyethylene bags and stored at ambient temperature (25±5ºC) until use (Hora *et al*. 2013).

Preparation of pomegranate seeds powder

Pomegranate seeds were obtained by manual removing of the pericarp from the rest of the fruit, isolating the endocarp and subsequently carefully splitting the tegumental pulp surrounding the seeds. Then, the obtained pomegranate seeds were washed in water several times and dried in an oven under vaccum at 60±5ºC for 24 hrs. its moisture content reached 5-6%. The dried seeds were ground in a laboratory disc mill (Braun AG Frankfurt Type: KM 32, Germany) and immediately prior to extraction. Ground seeds were sieved into mean particle size distribution of less than 40-mesh measured by the sieve used for the extraction (Huxley and Neil 2003). Then, the pomegranate seeds oil was extracted using petroleum either (B.P. 60- 80ºC) in a Soxhlet apparatus according to the method described in the AOAC (2000). Whereas, the defatted pomegranate seeds powder was dried in vacuum oven at 45±5ºC. Then, dried defatted pomegranate seeds powder was cooled, milled well, packed in polyethylene bags and stored at - 18±2ºC in the deep freezer until used.

Analytical methods

Moisture, crude protein, lipids (Ether extract), ash and crude fiber contents of pomegranate peel and seeds powders were determined according to the procedures of the AOAC (2000), Jaiswal *et al*. (2010). Total carbohydrates were calculated in all samples by subtraction as follows: % Carbohydrates = 100 - the sum of (% moisture + % crude protein + % fat + % ash + % crude fiber).

Determination of amino acids composition

The amino acids composition of pomegranate peels and seeds powder was determined by using High Performance Amino Acid Analyzer (Bekman 7300) (Kaufman and Wiesman 2007) according to the method of the AOAC. (2005), Kingsly *et al*. (2006). The oxidation with performic acid was used to protect methionine and cysteine from destruction during acid hydrolysis. Acid hydrolysis was carried out in closed conical flask for determining all amino acids other than tryptophan. Weighed 10 mg of sample in the conical flask and 5 ml of performic acid was added. The flask was closed and placed in an ice water bath for 16 hours 25 ml of Sodium metabisulifite and 6N HCl were added to the oxidized mixture. The flask was placed in an oven at 110°C for 24 hours. after that, the flask was then opened and the solvent removed by evaporating the sample. To reduce the volume to 10-5 ml under vacuum at 40-50°C. A suitable volume of sodium citrate buffer (pH 2.2) was added to the dried film of hydrolyzed sample. After all- soluble materials completely dissolved, the samples are ready for analysis. Indispensable amino acid (IAA) score was calculated in comparison to the reference protein pattern of FAO/WHO (1973), Kohno *et al*. (2004) using the following equation:

$$\text{Amino acid score} = \frac{\text{mg of IAA in 100g tested protein}}{\text{mg of IAA 100 g in reference protein pattern}} \times 100$$

Determination of fatty acids composition

The fatty acids of pomegranate seed oil (PSO) were determined as methyl ester by gas liquid chromatography. The methyl esters of fatty acids in the PSO were prepared using BF3 in methanol (14%) as methylating agent according to the AOAC (2000).

The methyl esters of the fatty acids in the PSO were analyzed with a GCV Hewlett Packard gas chromatography model 5890 equipped with dual flam ionization detector and dual channel recorder. The fractionation of fatty acid methyl esters was conducted using a coiled glass column (1.5m × 4mm) packed with diameter C (100–120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C/min from 70°C to 190°C, then isothermally at 190°C for 25 min with nitrogen at 30 ml /min. Detector, injector temperatures and hydrogen, air flow rates were generally 300°C, 280°C and 33 ml, 330 ml / min; respectively. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of the authentic pure standards of fatty acid methyl esters. All of the quantification was donning by a built-in data-handling program, provided by the manufacturer (Hewlett Packard) of the gas chromatograph. In general, the peak areas were measured by triangulation and the relative percentage of each individual fatty acid was estimated as the ratio of its % partial area to the total area.

Determination of minerals content

Macro elements (calcium and magnesium) and Microelements (iron, zinc, manganese, selenium, aluminum and copper) of pomegranate peel and seed powder were determined according to the method of the AOAC (2000), using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300, Germany), in Central Laboratory for Food and Feed, Agric. Res. Center. Phosphorus was determined by spectrophotometer using molybdovan date method according to the AOAC (2000), while sodium and potassium contents were determined by Flame Photometer (Lansky and Newman 2007).

Determination of vitamin B_1 and B_2 contents

Vitamins B_1 (Thiamin) and B_2 (riboflavin) of pomegranate peel and seed powders were determined according to the method described in the AOAC. (2000) (Jaiswal *et al*. 2010) using High Performance Liquid Chromatography (HPLC) Beckman model equipped by double piston pump 126 with Fluorescence detector LC 240 (Perkin Elmer); pump for reaction (Dioxin); Derivatisering tube 10 m × 0.33mm; Data handling system (Software Gold); Column Supelcosil LC-18-DB, 25Cm × 4.6 mm, 5µm; Injector 20µl (Beckman).

Extraction of samples

The sample is hydrolyzed with hydrochloric acid. If the sample contains of thiamin or riboflavin as phosphate, these forms are converted to thiamin or riboflavin by incubation with an enzyme (phosphatase). 20µl is injected in HPLC equipped with a C18- column and with a fluorescence detector. By a post column reaction thiamin is oxidized to thiochrom with alkaline hexacyanoferrate. For the calculation, concentration of external standard and peak area for external standard and sample are used.

Extraction 5g of the sample (contain minimum 1µg vitamins) in 250 ml conical flask weight. Add 50 ml HCL 0.1N, Shake or use magnetic stirrer and cover the flask with tin foil and sterilized in an autoclave for 30 min at 121°C, then cool in water bath adjust pH to 4.0-4.2 with Sodium acetate 2N. Added 5 ml enzymatic solution (phosphatase) and cover the flasks with tin foil, Incubate at 45°C over night (16-18h) then cooling the flasks. Transfer the solution quantitatively to 100 ml measuring flask with HCL 0.01N. Dilute the solution to concentration of app. 1 µg vitamin/ml with HCl 0.01N. Filtered the extracted samples through 0.45 µ m filter then inject in HPLC system.

Determination of vitamin C (L-Ascorbic acid)

L-Ascorbic acid of pomegranate peels and seeds powder were determined according to the method described in the AOAC. (2000) using High Performance Liquid Chromatography (HPLC) Beckman model equipped by double piston pump126 with UV-detector 166; Data handling system (Software Gold); Column LC-18-RP, 250-150 mm × 4.6 mm, 5µm; Injector 20µl (Beckman). Ascorbic acid separated on a C18 column using ion- pairing chromatography. Quantitave determination by UV absorption at 247 nm, measuring the peak area of external standard and sample and the concentration of external standard.

Samples preparation

L-ascorbic acid extracted with metaphosphoric acid with addition of ethylene diamintetra acetic acid. Sample extracted by weight a 100 ml beaker with 40-50 ml

extraction solvent (metaphosphric acid), add 5-10 g of sample according to conc. of sample, transfer the solution to a warning blender by extraction solvent, blow the solution with nitrogen throw glass tube for 3 min, remove the oxygen from the surface by blowing with nitrogen for 1 min extra, place the lid on the blender and mix for exactly 5 min, transfer the solution quantitatively to 250 ml measuring flask, if any foam appear added anti foam, fill to volume with distilled water then mix well, transfer to a centrifuge glass and centrifugation for 10 min 4000 r.p.m.

Determination of vitamin E (α- tocopherol)

Vitamin *E* (α-tocopherol) was measured by using high pressure liquid chromatography (HPLC) method (Leth and Sondergaro 1983). After saponification with alcoholic potassium hydroxide in the presence of ascorbate as antioxidant, the unsaponifiable fraction was extracted with diethyl ether. The ether was evaporated and the unsaponifiable was diluted in n-heptan. This solution was centrifuged and injected in HPLC equipped with a Kieselgedl-columm. The tocopherols and tocotrinol were separated by elution with isoprpanol in n- heptan.

Determination of vitamin A (Retinol)

Vitamin A (Retinol) was measured in pomegranate peels and seeds powder by using high pressure liquid chromatographic (HPLC), according to the method of Leth and Jacobsen (1993). After saponification of tested. Liquid extraction with petrolium ether (B.p. 40-60°C). The unsaponifiable fraction was taken up in a suitable amount of n-heptan and retinol acetate was added as internal standard. The solvent (n-heptan) solution was centrifuged and 15 ul was injected in high pressure liquid chromatography equipped with a Kieselgedl-columm. All trans retinol and retinol acetate were separated by elution with iso-propanol in an n-heptan as mobile phase. Spectrophotometric detection at 325nm for all trans retinol and vitamin A acetate from the area all the peaks. The amount of vitamin A in sample was calculated.

Determination of total phenolics

The measurement of total phenolics (TPs) content was conducted according to the modified Folin– Ciocalteu colorimetric method (Leth and Sondergaro 1983). Each sample (0.5 g) of pomegranate peel powder or pomegranate seed popwder was extracted with 50 mL methanol for 1 h and then the methanolic extract (ME) was diluted 1:5 (v/v) with distilled deionized (DI) water. 125 μL of the diluted extract was mixed with 0.5 mL of DI water in a test tube followed by addition of 125 μL of Folin–Ciocalteu reagent (FCR) and allowed to stand for 6 min. Then, 1.25 mL of 7% sodium carbonate solution was added and the final volume was made up to 3 mL with DI water. Each sample was allowed to stand for 90 min at room temperature and measured at 760 nm using an UV/Vis spectrophotometer. Gallic acid was used as a standard and results were expressed as gallic acid equivalents (GAE) per 100 g DM. The linear reading of the standard curve was from 0 to 600 μg of gallic acid permilliliter.

HPLC analysis for phenolic compounds of pomegranate fruit peels powder

The phenolic compounds of pomegranate fruit peels powder (ppp) were extracted according to the method of Duke *et al*. (2003) in which a known weight (0.5 g) of dried ppp was extracted with 50 ml. methanol for 3 hrs. Identification of individual phenolic compounds of investigated pomegranate fruit peels powder was performed on a JASCO HPLC, using a hypersil C_{18} reversed-phase column (250 × 4.6 mm) with 5 µm particle size. Injection by means of a Rheodyne injection valve (Model 7125) with 50 pj fixed loop was used. A constant flow rate of 1ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.62; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 245 nm. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standard mixtures chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, then convert to µg phenolic g^{-1} dry weight.

RESULTS AND DISCUSSION

Gross chemical composition for produced pomegranate fruit peel and seed powders

The gross chemical components; namely moisture, crude protein, crude fat, ash, crude fibers and carbohydrates, content of pomegranate fruits peel and seed powders are represented as in (Table 1).

Table 1 Gross chemical composition (%, on dry weight basis) of pomegranate peel and seed powders

Component (%)	Gross Chemical Component (%)* Pomegranate waste	
	Peel powder	Seed powder
Moisture	13.70	5.82
Protein	3.10	13.66
Fat	1.73	29.60
Ash	3.30	1.49
Fiber	11.22	39.36
Carbohydrate	80.50	13.12
Total phenolic	27.92	0.25

As shown in Table (1), the moisture content of produced pomegranate fruits peel and seed powders was found to be 13.7 and 5.82 %; respectively. In addition, crude protein, crude fat, ash, crude fibers and carbohydrates contents for pomegranate fruits peel powder were 3.10, 1.73, 3.30, 11.22 and 80.50%, versus, 13.66, 29.60, 1.49, 39.36 and 13.12% for pomegranate seeds powder, on dry weight basis; respectively. Thereupon, the pomegranate fruits peel powder is considered a good source of crude fibers, ash and carbohydrates, while pomegranate seeds powder is considered a good source of crude protein, crude fat and crude fibers. So, pomegranate fruit peels and seeds powders should be utilized in fortification of foodstuffs. These results are nearly in according with those found by Liu *et al*. (2009), Melgarejo and Artes (2000).

In this concern, pomegranate fruits peel can be used as functional ingredient as a good source of crude fibers which provide numerous health benefits such as their ability to decrease serum LDL-Cholesterol level, improve glucose tolerance and the insulin response, reduce hyperlipidemia and hypertension, contribute to gastrointestinal health and the prevention of certain cancers such as colon cancer (Lansky and Newman 2007, Viuda-Martos *et al*. 2010a,b). On the other hand, fruits' fibers can be considered as potential ingredients of foods; especially of meat products because of their ability to reduce the residual nitrite level, thus avoiding the possible formation of nitrosamines and nitrosamides (Viuda *et al*. 2009b) and they have been used in meat products processing as fat replacer, reducing agent of fat absorption during frying, volume enhancer, binder, bulking agent and stabilizer (Metche *et al*. 1996).

The nutritional protein quality of produced pomegranate fruits peel and seeds' powders

The nutritional protein quality of pomegranate fruits peel powder (PPP) and pomegranate seeds powder (PSP) was evaluated according to their content of the indispensable amino acids (IAAs), in comparison to the reference protein pattern of FAO/WHO (1973) as evident in (Table 2).

Table 2 Amino acids composition of pomegranate peel and seed powders; compared with the reference protein pattern of FAO/WHO

Amino acids	Pomegranate waste				FAO/WHO g/100g protein	Amino Acid Score %	
	Peel powder		Seed powder				
	g/100g sample	g/100g protein	g/100g sample	g/100g protein		Peel	Seed
			+ I.A.As				
Lysine	0.19	7.08	0.28	2.04	5.5	128.7	37.09
Meth + Cyst	0.09	3.35	0.54	4.68	3.5	95.7	133.7
Isoleucine	0.1	3.73	0.58	4.24	4.0	93.2	106.0
Leucine	0.19	7.08	1.08	7.90	7.0	101.1	112.8
Phen + Tyro	0.23	8.57	1.09	7.98	6.0	142.8	133.0
Valine	0.14	5.22	0.62	4.53	35.0	104.4	90.0
Total I.A.As	0.94	35.03	4.29	30.41			
			++ D.A.As				
Histidine	0.22	8.20	0.46	3.36			
Aspartic	0.30	11.19	1.21	8.85			
Glutamic	0.52	19.4	3.51	25.6			
Serine	0.11	4.10	0.61	4.46			
Glycine	0.41	15.20	0.97	7.10			
Arginie	0.23	8.58	1.47	10.76			
alanine	0.19	7.08	0.61	4.46			
Proline	0.14	5.22	0.71	5.19			
Total D.A.As	2.12	78.97	9.55	69.78			

+I.A.As: indispensable amino acids; ++D.A.As: Dispensable amino acids; Meth+ Cyst: Methionine + Cysteine (amino acids containing sulfur); Phen + Tyro: Phenylalanine + Tyrosine (aromatic amino acids); Tryptophan was not determined

From the obtained results (Table 2), it could be illustrated that pomegranate fruits peel powder (PPP) protein contained a much higher content from lysine, leucine, aromatic fatty acids (phenylalanine and tyrosine), threonine and valine than the reference protein pattern and therefore the amino acid score of these IAAs was higher than 100. On the other hand, the PPP had slight lower contents of amino acids containing sulphur (methionine & cysteine) and isoleucine which having amino acid score of 95.7 and 93.2; respectively.

As also shown in (Table 2), it could be noted that pomegranate seeds powder (PSP) protein contained a much higher contents of amino acid containing sulphur (methionine & cysteine), aromatic fatty acids (phenylalanine and tyrosine), leucine and isoleucine than the corresponding contents in the reference protein pattern of FAO/WHO (1973). On the other hand, the PSP protein was found to be deficient in lysine and threonine. Therefore, the incorporation of available inexpensive pomegranate by-products; peel and seeds, powder in Egypt into the meat products and the other foodstuffs; especially which deficient in amino acids containing sulphur, aromatic amino acids, leucine and isoleucine has a great economic value and a good standpoint in food technology and human nutrition.

Fatty acids profile of produced pomegranate seeds oil

Fatty acids composition of pomegranate seeds oil was determined and the obtained data was recorded as in (Table 3). It is worth to note that tested pomegranate seeds powder contained the crude oil at level of 29.6 % from its dry weight as illustrated before in Table (1).

From the obtained results of Table (3), it could be observed that the sum of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) percentages in pomegranate seeds oil (PSO) were 8.1, 8.6 and 82.0%; respectively. Therefore, the percentage of the total saturated fatty acids in the PSO was very low (8.1%) that explained the beneficial for the health and nutrition by incorporating of the PSO into food technology and human nutrition. The obtained results of

Table (3) also indicated that the PSO contained a high exceptional content (59.4%) from punicic acid (9, 11, 13; $C_{18:3}$), therefore, the percent of punicic acid represented 72.4% of the polyunsaturated fatty acids content.

Table 3 Fatty acids composition of pomegranate fruit seeds oil (PSO)

Fatty acids		Fatty acids content (%)
Saturated fatty acids		
Myristic	C 14:0	-
Palmitic	C 16:0	4.2
Srearic	C18:0	3.2
Arachidic	C20:0	0.4
Behenic	C22:0	0.3
Total saturated fatty acids		8.1
Monounsaturated fatty acids		
Oleic ω-9	C18:1	6.5
Stearoleic	C18:1	0.5
Gadoleic	C20:1	0.7
Erucic	C22:1	0.9
Total monounsaturated fatty acids		8.6
Polyunsaturated fatty acids		
Linoleic ω-6	C18:2	9.4
Punicic (9, 11, 13) all cis C18:3		59.4
Arachedonic w6 (3, 8, 11, 14) all cis C 20:4		8.5
Ecosapentaenoic C20:5		3.0
Docosatetraenoic C22:4		1.7
Total polyunsaturated fatty acids		82.0
Others		1.3

Furthermore, the PSO contained the essential fatty acids; oleic W_9 ($C_{18:1}$), linoleic W_6 ($C_{18:2}$) and arachedonic w6 ($C_{20:4}$), at adequate amounts of 6.5, 9.4 and 8.5%; respectively. In addition, the PSO contained a considerable content from myristic (14:0), palmitic (16:0), ecosapentaenoic (20:5) and docosatetraenoic (22:4) fatty acids. The current results are in nearly agreement with those found by Kaufman and Wiesman (2007), Sassano *et al*. (2009), Vroegrijk *et al*. (2011).

In this concern, consumers are especially interested in essential fatty acids, with emphasis on the health potential of polyunsaturated (n-3) fatty acids which play a natural preventive role in cardiovascular disease and in the alleviation of some other health problems, basically because they promote the reduction of both total and LDL cholesterol (Melgarejo and Artes 2000). It has been also reported that conjugated fatty acids such as conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA) have recently attached significant attention because of their healthy benefits in a variety of models of metabolic and chronic inflammatory disease. Various conjugated fatty acids have been shown to inhibit the growth of transplanted cancer cells or to exert cancer cell killing activity, as well as to sensitize the prostaglandin for protection from many diseases, inflammation and to have vital role in nerves transport through the nervous cells. In addition, the w-fatty acids, naturally present in pomegranate seeds oil, have essential role in human nutrition, prevention the cardiac diseases, protection the human body from different cancers (anti-cancer) and in dissolving the saturated fatty acids in the body (Mohagheghi *et al*. 2011, Mori-Okamoto *et al*. 2004, Naveena *et al*. 2008).

In this rank, pomegranate seeds oil (PSO) has been reported to chemoprevent the skin tumor development in mice (Naveena *et al*. 2007) and prostate cancer in human (Negi and Jayaprakasha 2003). In addition, the PSO can decrease the leukotriene production from arachidonic acid which plays a major role in the accuracy of asthma in children, skin inflammation and palte let aggregation association with cardiovascular diseases (Negi *et al*. 2003).

Minerals content of produced pomegranate peel and seed powders

The nutritional quality of pomegranate peel powder (PPP) and pomegranate seeds powder (PSP) with regards their minerals content was evaluated and the obtained results are recorded as in (Table 4). From the obtained data (Table 4), it could be showed that the pomegranate fruits peel powder (PPP) and pomegranate seeds powder (PSP) contained all tested minerals, with the exception of Mg which was not detected in them. The PPP contained the most determined minerals at adequate concentration and the predominant minerals in it were found to be Ca, K, P and Na at level of 338.5, 146.4, 117.9 and 66.4 mg/100g dry matter; respectively. In addition, the PPP contained a considerable content of Fe, Zn, Cu and Se at level of 5.93, 1.01, 0.60 and 1.02 mg/100g dry matter; respectively. The obtained results (Table 4) also showed that the major macro-elements in the PSP were P, K and Ca which were found at the level of 481.1, 434.4 and 229.2 mg/100g dry matter; respectively. Also, the PSP contained a considerable content of Na, Fe, Zn, Mn, Cu and Se at level of 33.03, 10.88, 5.54, 2.26, 3.82 and 0.23 mg/100g dry matter respectively. In general, it could be concluded that pomegranate fruits peel and seed powders were characterized with their richness with the most determined nutritious minerals and they are considered a good source of macro and micro elements. Therefore, they should be utilized in food fortification.

Table 4 Minerals content (mg/100g dry matter) of pomegranate peel and seed powders

Mineral	Pomegranate waste		RDA* (mg/day)	
(mg/100g)	Peel powder	Seed powder	Children	Adults
Ca	338.50	229.20	800	(800-200)
Mg	--	--	80-170	280-350
K	146.40	434.40	--	--
Na	66.43	33.03	--	--
P	117.90	481.10	800	800-1200
Fe	5.93	10.88	10	10-15
Zn	1.01	5.54	10	12-15
Mn	0.80	2.26	--	--
Cu	0.60	3.82	--	--
Se	1.03	0.23	--	--

Vitamins content of produced pomegranate peel and seed powders

The determined vitamins; including Vitamin B_1 (thiamine), Vitamin B_2 (riboflavin), Vitamin C (L-ascorbic acid) Vitamin E (α-tochoferol) and Vitamin A (retinol) content of produced pomegranate peels powder (PPP) and pomegranate seeds powder (PSP) was evaluated and the obtained results were recorded as in (Table 5).

Table 5 Vitamins content (mg/100g) of pomegranate peel and seed powders

Vitamin (mg/100g)	Pomegranate waste	
	Peel powder	Seed powder
B_1 (Thiamine)	0.123	0.930
B_2 (Riboflavin)	0.07	0.146
C (*L*-Ascorbic acid)	12.90	3.02
E (α-Tochoferol)	3.99	1.35
A (Retinol)	0.164	0.089

As shown in (Table 5), pomegranate peel powder contained adequate content of Vitamin C, Vitamin E, Vitamin A, thiamine and riboflavin at the level of 12.90, 3.99, 0.146, 0.123 and 0.07 mg/100g dry matter, versus 3.02, 1.35, 0.089, 0.930 and 0.146 mg/100g dry matter for pomegranate seed powder; respectively. The determined vitamins naturally occurred in pomegranate peels and seeds are considered one of the most important phytochemicals having the anti-oxidant, anti-microbial and chemo preventive cancer properties and good standpoint in human nutrition [34]. From the above discussion, it could be concluded that produced powders of pomegranate by-products; peels and seeds, are considered a good source of the determined vitamins.

Polyphenol fractions content of produced pomegranate fruits peel powder

The polyphenol fractions naturally occurred in the produced pomegranate fruits peel powder were identified and determined quantitatively by using the HPLC technique. The obtained results were recorded as in (Table 6).

From the obtained results (Table 6), it could be seen that produced pomegranate fruits peel powder contained a high content (~1.403%, on dry weight basis) of total polyphenols. The major polyphenol fractions in pomegranate peels powder were catechins, phenol, gallic acid, caffeic acid, ellagic acid, p-coumaric acid and resocenol which were found at the level of 868.4, 242.7, 125.8, 60.46, 44.19, 17.64 and 12.50 mg/100g dry matter; respectively. In addition, the pomegranate peels powder also contained adequate amounts from protocatechol, *p*-hydroxy benzoic acid, vanilline and ferulic acid at concentrations of 4.17, 9.02, 3.19 and 5.89 mg/100g dry matter; respectively. The current results are nearly in accordance with those obtained by Wang *et al*. (2004), Wang *et al*. (2004a,b).

Table 6 Polyphenol fractions content of produced pomegranate fruits peel powder

Phenolic fraction	Content (mg/100g dry matter)
Ellagic acid	44.19
Catechins	868.40
Gallic acid	125.80
Resocenol	12.50
Protocatechol	4.17
Parahydroxy benzoic acid	9.02
Phenol	242.70
Vanilline	3.91
Caffeic acid	60.46
Ferulic acid	5.89
P-coumaric acid	17.64
Others	8.20
Total	1402.88

The present findings showed that tested by-products of pomegranate fruits; peels and seeds, powder produced a much higher content of sulpher-containing lysine, isoleucine and amino acids (methionine and cysteine), which is normally deficient in most food products, than the FAO / WHO reference protein pattern. Current findings have also shown that pomegranate seed oil has a high fatty acid content of W3, W6- and W9-. In addition, the pomegranate fruit by-products tested with the most specified minerals and vitamins were characterized by their richness. In addition, pomegranate fruit peel powder had a high total polyphenol content (1.403 g/100 g dry matter) and catechins, phenol gallic acid, caffeic acid, ellagic acid, p-coumaric acid and resocenol compounds were the predominant polyphenol fractions in pomegranate peel powder. Therefore, current findings indicate that the incorporation of pomegranate fruit seeds and peel powders into food printing and preservation technology should be aimed at enhancing their nutritional quality and prolonging the shelf life of these food items.

LITERATURE CITED

Abdel-Rahim E A, El-Beltagi H S and Romela R M. 2013. White bean seeds and pomegranat peel and fruit seeds as hypercholesterolmic and hypolipidemic agents in albino rats. *Grasas Y. Aceites* **64**(1): 50-58.

Adhami, V.M. and H. Mukhtar, 2006. Polyphenols from green tea and pomegtanate for prevention of prostate cancer. *Free Radical Research* **40**: 1095-1104.

Ahangari B and Sargolzaei L. 2012. Extraction of pomegranate seed oil using subcritical propane and superitical carbon dioxide. *Theoretical Foundations of Cheical Engineering* **46**(3): 258-265.

Alesón-Carbonell L, Fernández-López J, Pérez-Alvarez J A and Kuri V. 2005. Characteristics of beef burger as influenced by various types of lemon albedo. *Innovative Food Science and Emerging Technology* **6**: 247-255.

AOAC. 2000. Association of Official Analytical Chemists. Official Methods of Analysis of the AOAC International, 17th ed. Gaithersburg, Marylad, USA.

AOAC. 2005. Association of Official Analytical Chemists. Official Methods of Analysis of the AOAC International, 18th ed.

Gaithersburg, Marylad, 20877- 2417, USA.

Bassaganya–Riera J, Reynolds K, Martino-Catt S, Cui Y, Hennighausen L, Gonzalez F, Rohrer J, Benninghoff A U and Hontecillas R. 2004. Activation of PPAR gama and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenteralogy* **127**: 777-791.

Devatkal S K and Naveena B M. 2010. Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science* **85**: 306-311.

Elfalleh W, Tlili N, Nasri N, Yahia Y, Hannachi H, Chaira N, Ying M and Ferchichi A. 2011. Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits. *Journal of Food Science* **76**(5): 707-713.

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

FAO/WHO. 1973. Energy and Protein Requirements. Report of a Joint FAO/WHO Expert Committee. WHO Tech. Report Series 522. *FAO Nutrition Meetings Report Series* **52**: 57-62.

Food and Nutrition Board. 1989. United States Recommended Dietary Allowances (USRA). National Research Councils. National Academy of Science, Washington, DC.

Hora J J, Maydem E R, Lansky E P and Dwivedi C. 2003. Chemopreventive effect of pomegranate seed oil on skin tumor development in CD1 mice. *Journal of Med. Food* **6**: 157-161.

Huxley R R and Neil H. 2003. The relationship between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition* **57**: 904-908.

Jaiswal V, DerMarderosian A and Porter J R. 2010. Anthocyanins and polyphenoloxidase from dried arils of pomegranate (*Punica granatum* L). *Food Chemistry* **118**: 11-16.

Kaufman M and Wiesman Z. 2007. Pomegranate oil analysis with emphasis on MALDI-TOF/MS triacylglycerol fingerprinting. *Journal of Agriculture and Food Chemistry* **55**: 10405-10413.

Kingsly A R P, Singh D B, Manikantan M R and Jain R K. 2006. Moisture dependent physical properties of dried pomegranate seeds (Anardana). *Journal of Food Engineering* **75**(4): 492-496.

Kohno H, Yasui Y, Suzuki R, Hosokawa M, Miyashita K and Tanaka T. 2004b. Dietary seed oil rich in conjugated linolenic acid from bitter melon inhibits azoxymethane-induced rat colon carcinogenesis through elevation of colonic PPAR gamma expression and alteration of lipid composition. *International Journal of Cancer* **110**: 896-901.

Lansky E P and Newman R A. 2007. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmac* **109**: 177-206.

Leth T and Sondergaro H. 1983. Biological activity of all-trance tocopherol determined by three different rat bioassays. *International Journal of Vit. Nutrition Research* **53**: 297-311.

Leth T and Jacobsen J S. 1993. Vitamin A in danish pig, calf and liver. *Journal of Food Comp Analysis* **6**: 3-9.

Li Y, Guo C, Yang J, Wei J, Xu J and Cheng S. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry* **96**: 254-260.

Liu G, Xu X, Hao Q and Gao Y. 2009. Supercritical CO_2 extraction optimization of pomegranate (*Punica granatum* L.) seed oil using response surface methodology. *LWT-Food Science and Technology* **42**: 1491-1495.

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

Metche M, Nasr C B and Ayed N. 1996. Quantitative determination of the polyphenolic content of pomegranate peel. *Zeitschrzfi fur Lebensmittel Unterschung and Forschung* **203**: 374-378.

Mohagheghi M, Rezaei K, Labbafi M and Seyyed M. 2011. Pomegranate seed oil as a functional ingredient in beverages. *European Journal of Lipid Science and Technology* **113**(6): 730-736.

Mori-Okamoto J, Otawara-Hamamoto Y, Yamato H and Yoshimura H. 2004. Pomegranate extract improves a depressive state and bone properties in menopausal syndrome model ovariectomized mice. *Journal of Ethnopharmacy* **92**: 93-101.

Naveena B M, Sen A R, Kingsly R P, Singh D B and Kondaiah N. 2008. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. *International Journal of Food Science and Technology* **43**:1807-1812.

Naveena B M, Sen A R, Vaithiyanathan S, Muthukumar M and Babji Y. 2007. Microwave cooking properties of chicken patties containing honey and vitamin-C. *Journal of Food Science and Technology* **44**: 505-508.

Negi P S and Jayaprakasha G K. 2003. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of Food Science* **68**(4): 1473-1477.

Negi P S, Jayaprakasha G K and Jena B S. 2003. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry* **80**: 393-397.

Wang M L, Wang J T and Choong Y M. 2004a. A rapid and accurate method for determination of methanol in alcoholic beverage by direct injection capillary gas chromatography. *Journal of Food Composition and Analysis* **17**: 187-196.

Wang R F, Xie W D, Zhang Z, Xing D M, Ding Y, Wang W, Ma C and Du L J. 2004b. Bioactive compounds from the seeds of *Punica granatum* (Pomegranate). *Journal of Natural Products* **67**: 2096-2098.