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Quality evaluation of pomegranate waste and extracted oil

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Abstract

The present study deals with the quality evaluation of pomegranate (*Punica granatum* L.) waste and its extracted oil for their possible utilizations. Amount of moisture, ash, protein, fiber and carbohydrate was found to be 12.46%, 4.38%, 18.34%, 13.78% and 35.44%, respectively. Average oil content in pomegranate waste material was determined to be 15.60%. The extracted oil was analyzed for free fatty acid (FFA), saponification value (SV), iodine value (IV) and peroxide value (PV). GC-MS characterization of fatty acid profile revealed the dominant fatty acid in pomegranate waste oil was punicic acid (84.68%) followed by oleic acid (3.85%), stearic acid (3.57%), palmitic acid (2.88%), linoleic acid (2.67%), arachidic acid (1.22%), and catalpic acid (1.13%). Proximate composition of pomegranate waste material has indicated that it may be used in the manufacturing of poultry and animal feeds. While, physiochemical properties of extracted oil from pomegranate waste revealed that it has great potential to be used as nutraceutical in food industries due to presence of considerable amount of biologically important punicic acid.

Introduction

Pomegranate is member of Punicaceae family, commonly known as *Punica granatum* L. It is very important and delicious fruit consumed throughout the world. The fruit is native to Afghanistan, Iran, China and the Indian sub-continent. The ancient sources of pomegranate are linked to Iran, Pakistan, China and eastern India. From the west of Iran, pomegranate cultivation stretched through the Mediterranean region to the Turkish borders as well as American south west, California and Mexico. In Pakistan, pomegranate fruit is grown in Baluchistan, Waziristan, Kurram agency, Chitral, Dir, Hazara and Azad Kashmir. Pomegranate fruit is harvested in Pakistan from August to October, harvesting also depends on maturity of the fruit and geographical location (Lansky and Newman, 2007; Celik *et al*., 2009).

Recently pomegranate fruit gained more interest due to the exploration of appreciable amount of antioxidants, antimicrobial agents and many bioactive components which are important for human health (Tezcan *et al*., 2009; Tehranifar *et al*., 2010). The pomegranate fruits are consumed as fresh juice, canned beverages, jelly, jam and paste for flavoring and coloring drinks (Nagy *et al*., 1990; Parashar *et al*., 2008). The fruits are rich in arils (ranges from 50 to 70% of total fruit) which generally comprises of 78% juice and 22% seeds (Fadavi *et al*., 2006; Mohagheghi *et al*., 2011). The pomegranate contains seed about 37–143 g/kg of fruit. The pomegranate wastes are byproduct of the pomegranate juice industries, which contains lot of valuable components such as sterols, γ-tocopherol, punicic acid and hydroxyl benzoic acids (El-Nemr *et al*., 1992; Liu *et al*., 2009). Pomegranate seeds contain high quality oil containing high concentration of health beneficial conjugated linolenic acids. According to (Eikani *et al*., 2012), the oil content varies from 12-20% of the seed on dry weight basis. Pomegranate seed oil contains 65–80% conjugated fatty acids (Abbasi *et al*., 2008; Parashar *et al.,* 2010). The conjugated fatty acids have several potential health benefits such as antioxidant, antitumor, anticancer, immune modulatory, anti-atherosclerotic and serum lipid-lowering activities (Arao *et al.,* 2004; Lansky and Newman, 2007; Grossmann *et al*., 2010; Carvalho *et al*., 2010). Proximate composition of pomegranate seed has been reported by some authors (Rowayshed *et al*., 2013; Mirzaee 2014; Khoddami *et al*., 2014) but the data from Pakistan about pomegranate waste is lacking. The aim of present study was to explore the quality of pomegranate waste and its extracted oil

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from mostly consumed and famous Kandhari variety of Pakistan.

Materials and Methods

Reagents and sample collection

All the chemicals and reagents used in the present study were purchased from E-Merck (Darmstadt, Germany). In present study the waste material of pomegranate fruit was collected from fresh juice vendors. The fruit waste were cleaned from adhering material and stored at room temperature till analysis.

Moisture content

The moisture content of pomegranate waste was determined using standard official method Aa 3-38 (AOCS, 2013). A powdered sample (5 g) was weighed into aluminum dish and dried in an oven (Memmert, Schwabach, Germany) at 130±1°C for 3 hours.

Protein content

Total protein content of the pomegranate waste was determined by Kjeldahl method Aa 3-38 (AOCS, 2013) by using a nitrogen conversion factor of 6.25. Data were expressed as percentage on a weight basis.

Ash content

About 2 g of powdered pomegranate waste sample was ignited and incinerated in a muffle furnace (Amalgam, Sheffield, England) at 600±15°C for 2 hours, (method Ba 5a-49) (AOCS, 2013). The total ash was expressed as a percentage on a weight basis.

Fiber content

Fiber content in the waste of pomegranate fruit was determined by decomposing starch and protein with dilute acid, while fatty material with dilute base and then filtering and igniting in the muffle furnace at 600°C using AOCS method Ba 6-84 (AOCS, 2013).

Carbohydrate content

The carbohydrate content of the pomegranate waste was estimated by the difference between the total percentage and the sum of the mean values for the content of protein, lipid, ash, fiber and moisture; i.e.
[100 – (Protein + lipids + Ash +Moisture + Fiber)] as reported by (Khoddami *et al.*, 2014).

Oil extraction

Oil from pomegranate waste was extracted by AOCS method Aa 4-38 (AOCS, 2013) using hexane as a solvent. About 10 g of sample were put into cellulose thimble and placed inside the extractor, then 300 mL of hexane was added to round bottom flask. Temperature was set at 80°C and the process continued for about 4 hours. After complete extraction, hexane was evaporated using rotary evaporator (Buchi, Switzerland). And extracted oil was kept in refrigerator for further analysis.

Physico-chemical properties of extracted pomegranate seed and peel oil

The refractive index (RI) of the pomegranate waste oil was determined at 25°C by refractometer (ATAGO hand refractometer, N-3E, Japan) according to AOCS method Cc 7-25 (AOCS, 2013). The viscosity of the oil was evaluated using an AR G2 rheometer (TA Instrument, New Castle, UK) with 60mm diameter cone plate geometry at 25°C AOCS method Ja 11-87 (AOCS, 2013). For the chemical characteristics, official methods of AOCS were used for the determination of PV method C d 8-53, IV method Cd 1-25, SV method Cd 3-25, and FFA content method Aa 6-38 (AOCS, 2013).

Determination of fatty acid composition

For the determination of fatty acid composition, fatty acids methyl esters (FAMEs) were prepared using standard IUPAC method 2.301 (IUPAC, 1979). The instruments used in the study included Agilent 6890 N gas chromatograph instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent auto sampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA), a capillary column HP-5MS (5% phenyl methyl siloxane), column length 30 m, i.d 250 µm, film thickness 0.25 mm. Temperature programming and other conditions were as follows: initial temperature 150°C maintained for 2 min, final temperature 260°C Kept for 5 min, ramp rate 4°C/min, carrier gas (helium) flow rate 0.8 mL/min, injector temperature 240°C, detector temperature 270°C, electron impact (EI) mode 70 eV, scan range 50-550 m/z. GC-MS chromatograms were compared with two libraries (NIST and Wily) which provided much information about major and minor fatty acids in pomegranate oil.

Statistical analysis

Values of different parameters were expressed as the mean ± standard deviation (x ± SD) whereas x is the mean of three replications. The data were put into Origin 7 program and reported as the means (n = 2×3 ±standard deviation).

Result and Discussion

Proximate analysis

Generally food waste materials are considered to be cheap sources of many useful components. Therefore, present study was carried out on pomegranate waste material after extraction of juice from Kandhari variety. Table 1 shows the proximate analysis of pomegranate waste containing moisture content (12.46%), oil content (15.60%), ash content (4.38%), protein content (18.34%), fiber content (13.78%), carbohydrate content (35.44%), dry matter (87.54%), energy (355.52 Kcal/100g of pomegranate waste) and nitrogen free extract (35.44%).

The moisture content of the pomegranate waste in current study was higher than the moisture reported in the literature 4.0-5.82% (Ullah *et al.*, 2012; Middha *et al.*, 2013), while lower than 13.20-13.70% (Rowayshed *et al.*, 2013; Khoddami *et al.*, 2014). Oil content (15.60%) in the waste of Pakistani Kandhari pomegranate fruit was higher than the reported literature (1.73-9.4%) (Ullah *et al.*, 2012; Rowayshed *et al.*, 2013). Higher amount of oil is clear indication that extraction of oil from pomegranate waste is more feasible. Ash content (4.38%) of pomegranate waste was higher than the reported values of ash in the range of 1.46-4.0% (Tahar-Maddah *et al.*, 2012; Middha *et al.*, 2013; Rowayshed *et al.*, 2013; Khoddami *et al.,* 2014; Mirzaee 2014), except reported (5%) by Ullah *et al.*, (2012). Ash contents are directly related to mineral concentration. Protein content (18.34%) was almost higher than the reported values except quoted (19.53%) by (Khoddami *et al.*, 2014), while fiber content (13.78%) was found to be lower as compared to the reported studies (Tahar-Maddah *et al.*, 2012; Middha *et al.*, 2013; Rowayshed *et al.*, 2013; Khoddami *et al.*, 2014; Mirzaee 2014) except one (11.22%) (Rowayshed *et al.*, 2013). Kandhari variety from Pakistan showed slightly higher results (35.44%) of carbohydrate with the reported values (32.19-33.41%) (Mirzaee 2014), except one (54.56%) reported by (Khoddami *et al.*, 2014). These results indicated that pomegranate waste contains a good combination of minerals, oil, protein and carbohydrates.

Table 1. Proximate composition of pomegranate waste.

Proximate Analysis	Mean ± S.D	CV (%)
Moisture (%)	12.46 ± 0.22	1.76
Oil (%)	15.60 ± 0.31	1.98
Ash (%)	4.38 ± 0.12	2.73
Protein (%)	18.34 ± 0.31	1.69
Fiber (%)	13.78 ± 0.21	1.52
Carbohydrate (by difference)*	35.44 ± 1.02	2.87
Dry Matter (%)	87.54±2.32	2.65
Energy (Kcal/100 g of sample)**	355.52±4.02	1.13
Nitrogen free extract (NFE %) ***	35.44±1.02	2.87

*Carbohydrate = 100- (H_2O + Ash + CP + EE+CF)
**Energy (kcal) = 4 (g protein + g carbohydrate) + 9 (g lipid)
***NFE = 100 - (H_2O + CP + CF + lipids + Ash)
CV% = S.D/mean × 100

Table 2. Physico-chemical characteristics of pomegranate waste oil.

Physico-chemical parameter	Mean± S.D	CV %
Specific gravity at 28°C (g/cm^3)	0.9300±0.02	2.15
Viscosity at 25°C (*m* Pas. s)	0.037±0.001	2.70
RI at 25°C	1.372±0.02	1.45
IV gI$_2$/100g	212±2.31	1.08
PV meq/Kg	2.60±0.1	3.84
SV mg KOH/g	156±2.01	1.28
FFA (%)	0.96±0.01	1.04

Physico-chemical characteristics of waste oil

Table 2 shows important physiochemical characteristics of pomegranate waste oil. The specific gravity of pomegranate waste oil was found to be 0.9300g/ cm^3, while viscosity and RI were determined as 0.037mPa.s and 1.372, respectively. Parameters such as IV, PV and SV were determined as 212 gI$_2$/100g, 2.60 mEq/Kg, and 156 mg KOH/g, respectively.

In current study specific gravity, viscosity, RI, IV, SV and PV were found to be different than reported values (0.0217-0.054g/cm^3, 1.5088-1.461, 220.34-221.24%, 194.23-181.1% and 4.4-4.67% (Basiri *et al.*, 2013; Khoddami *et al.*, 2014; Mirzaee 2014). The iodine value (212 gI$_2$/100g of oil) places the extracted oil from pomegranate waste in a in a group of drying oils. Through implication, the oil can be also used for the production of paints and vanish or the lubricant industry in addition to food applications. The FFA is very important and main quality index of the oil. Generally FFA is calculated as percentage of oleic acid except some oils such as palm oil. In present study pomegranate waste oil showed a little greater % FFA than the reported values in Iranian varieties 0.28-0.65% (Basiri *et al.*, 2013; Khoddami *et al.*, 2014). The higher IV indicated that pomegranate waste oil contain high level of unsaturation in the form of punicic acid. The higher SV showed that the oils have smaller molecular weight than other edible oils (Dadashi *et al.*, 2013). Very low percentage of FFA and PV represents the high quality of oil present in the waste of pomegranate fruit.

Fatty acid profile of pomegranate waste oil

Table 3 shows the fatty acid profile of the oil

Table 3. Fatty acid profile of pomegranate waste oil (%).

Fatty acid Composition	Current study	India[a]	Iran[b]	Turkey[c]
Myristic $C_{14:0}$	-	0.3 ± 0.02	-	-
Palmitic; $C_{16:0}$	2.88 ± 0.10	5.3 ± 0.80	4.61 ± 0.23	2.77 ± 0.03
Stearic; $C_{18:0}$	3.57 ± 0.05	2.9 ± 0.00	2.64 ± 0.06	1.52 ± 0.00
Oleic; $C_{18:1\ n9}$	3.85 ± 0.10	10.4 ± 0.90	7.62 ± 0.14	4.87 ± 0.02
Linoleic; $C_{18:2\ n9c,12c}$	2.67 ± 0.08	5.4 ± 0.40	8.20 ± 0.16	5.27 ± 0.04
Punicic; $C_{18:3\ n9c,11t,13c}$	84.68 ± 2.31	75.7 ± 3.50	76.10 ± 0.62	70.83 ± 1.17
Catalpic; $C_{18:3\ n9t,11t,13c}$	1.13 ± 0.04	-	-	4.40 ± 0.57
β-eleostearic; $C_{18:3\ n9t,11t,13t}$	-	-	-	1.35 ± 0.14
α-eleostearic; $C_{18:3\ n9c,11t,13t}$	-			6.72 ± 0.50
Arachidic; $C_{20:0}$	1.22 ± 0.03	-	0.20 ± 0.02	0.35 ± 0.01
Gadoleic; $C_{20:1}$	-	-	-	0.42 ± 0.01
Behenic; $C_{22:0}$	-	-	-	0.21 ± 0.01
Saturated fatty acids (SFA)	7.67	8.5	7.45	4.85
Unsaturated fatty acids (UFA)	92.33	91.5	91.92	93.86

[a] Parashar *et al*., 2010; [b] Khoddami *et al*., 2014; [c] Kýralan *et al*., 2009

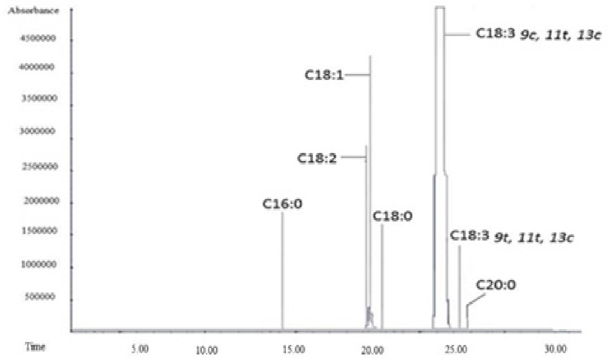

Figure 1. Representative GC-MS chromatogram of pomegranate waste oil.

extracted from waste of pomegranate determined by GC-MS along with the data reported in the literature. Total seven fatty acids (three saturated and four unsaturated) including palmitic, stearic, oleic, linoleic, punicic, catalpic and arachidic acid were identified. Figure 1 is the representative GC-MS chromatogram of pomegranate waste oil. The total contents of saturated fatty acids were determined as 7.67%, which include stearic acid (3.57%), palmitic acid (2.88%) and arachidic acid (1.22%). Unsaturated fatty acids were found to be dominant (92.33%) over saturated fatty acids in the following order: punicic > oleic > linoleic >catalpic acid. Punicic and catalpic are the isomers of linolenic acid and generally known as conjugated linolenic acid. Most important and main fatty acid was punicic. In current study, higher percentages of punicic acid (84.68%) and total unsaturated fatty acids (92.33%) in the waste oil of Kandhari pomegranate variety were observed as compared to Indian Kandhari variety (75.7% and 91.5%, respectively) reported by (Parashar *et al.*, 2010).

Conclusion

Many wastes materials are considered to be sources of various valuable components which are pharmaceutical and biologically active and could be used in food applications. Pomegranate waste after extraction of juice which is thrown without proper utilization is one of them. The proximate analysis indicated that it contains reasonable amount of oil, protein, carbohydrates, fiber and ash. So it could be used for the manufacturing of animal feed. Pomegranate waste contained reasonable amount (15.60%) of good quality oil. Therefore, commercial extraction on pilot or industrial scale may be feasible. Waste of pomegranate could be used a potential source for the extraction of high quality oil with unique composition in which health beneficial conjugated linolenic acids (CLA) i.e. punicic acid (84.68%) and catalpic acid (1.13%) are dominant.

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Conflict of interest

Authors have no any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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