Open Access
Full Length Article

ORIGINAL ARTICLE

Assessment of Basic Nutritional Profile and Antioxidant Potential of Pakistani Pomegranate (*Punica Granatum* L.) Varieties

Naila Siraj[1]*, Saima Tehseen[1], Muhammad Asim Shabbir[2]

[1]Department of Food Science and Technology, Government College Women University, Faisalabad, 38000, Pakistan.
[2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, 38000, Pakistan.

ABSTRACT

Background: Pomegranate seeds are the remains of industrial processing of pomegranate fruit into various products such as juice, jam, and jelly that cause environmental pollution and a loss of lipid and water-soluble bioactive compounds. The seeds comprise 15-25% of the whole fruit weight. Its lipid fraction has a distinct fatty acid profile with high polyunsaturated fatty acid contents such as punicic acid (PA) (50-80%), tocopherols, and phenolic compounds.

Objective: The objective of the current study was to characterize the three main Pakistani pomegranate varieties (Desi, *Kandhari, and Bedana),* supporting waste exploitation strategies. The physicochemical characteristics, fatty acid profile, antioxidant potential of all three varieties were studied.

Methodology: The extracted oils samples were investigated for their lipid contents, fatty acid composition, physicochemical properties, and antioxidant potential on the varietal base.

Results: Total lipid contents ranged from 8.70-15.9% dry matter with conjugated linolenic acid (68.5-76.7% of total fatty acids) as the predominant fatty acid in all samples, followed by linoleic, oleic acid, stearic acid, and palmitic acid. Further characterization revealed that Desi was superior regarding acidity and oxidation stability over the other two varieties associated with higher total phenolic and tocopherol contents with potent antioxidant activities.

Conclusion: The present results demonstrate a preferable fatty acid pattern in pomegranate seed oil (PSO), which may be potential functional ingredient for product development in food and non-food applications in combination with high antioxidant contents.

Keywords	*Address of Correspondence	Article info.
pomegranate, fatty acids, antioxidant potential, seed oil, waste utilization	*nailaateeq@gcwuf.edu.pk*	*Received: January 26, 2022* *Accepted: December 27, 2022*

Cite this article: *Siraj N, Tehseen S, Shabbir MA. Assessment of Basic Nutritional Profile and Antioxidant Potential of Pakistani Pomegranate (Punica Granatum L.) Varieties. 2022; 13(2):123-133.*

INTRODUCTION

The earliest known fruit plant is Pomegranate (*Punica granatum* L.), *instigating from Ponum granatum, which means seeded or grainy apple, native to Central Asia and Persia[1]. It* is grown in India, Turkmenistan, Pakistan, North Africa, China, Afghanistan, and some Mediterranean countries. USAID (The United States Agency for International Development) estimates global production to be between 3.80 million tons in 2017, with an expected increase in the next years following the risen global awareness of pomegranate fruit's health benefits[2]. In Pakistan, the pomegranate's central cultivation area is in Baluchistan, whereas Punjab and Khyber Pakhtunkhwa

produce pomegranates on a small scale on isolated farms. The best-known varieties in Pakistan are Desi, Kandhari, and Bedana, with the primary harvest season in September but are available around the year when stored under appropriate conditions[3].

The industrial transformation of fruit and vegetables into food products generates enormous by-products, many of which are considered high value due to their high content of different bioactive compounds[4]. Pomegranate seeds are the remains of industrial processing of pomegranate fruit into various products such as juice, jam, and jelly that cause environmental pollution and a loss of lipid and water-soluble bioactive compounds. The seeds comprise 15-25% of the whole fruit weight. Its lipid fraction has a distinct fatty acid profile with high polyunsaturated fatty acid contents such as punicic acid (PA) (50-80%), tocopherols, and phenolic compounds. Omega-6 and omega-3 PUFAs are the two primary groups of PUFAs that are relevant to human health. In most diets, linoleic acid (LA, 18:2-6) and α-linolenic acid (ALA, 18:3-3) are the most abundant PUFAs. LA and ALA are considered animals that cannot produce essential fatty acids since these. Because these PUFAs are produced in plants, LA and ALA are mostly found in high concentrations in plant-based foods. Low essential fatty acid intakes have been linked to dermatitis, renal hypertension, mitochondrial activity problems, cardiovascular disease, type 2 diabetes, impaired brain development, arthritis, depression, and decreased body response to infection. Recent research on pomegranate peels has demonstrated good antioxidant potential and antimicrobial activity. Until recently, PSO considered superior nutritional quality for usage in food applications. However, it has been suggested to have great potential to reduce the risk of cardiovascular disease, inflammation, and cancer, as well as relieve menopausal symptoms and support immune functions[5].

Although the optimization of PSO extraction and characterization of fatty acid profiles have been reported in several studies, there is only limited evidence available on the physicochemical characterization of PSO[6]. Whereas some data were published on phytochemical and antioxidant characteristics of PSO from varieties grown in the USA, Iran, India, Turkey, and China[7], there is a lack of knowledge regarding seed oils from Pakistani pomegranate varieties. Therefore, this study explores the pomegranate seed residues (PSRs) of three Pakistani pomegranate varieties as a material with potential economic value by referring to its lipid yield, fatty acid profile, physicochemical properties, and antioxidant potential.

MATERIALS AND METHODS

Materials

Approx. 15 kg fresh, fully ripe, and good-quality pomegranate varieties (Desi, *Kandhari, and Bedana*) were procured from the local market of District Faisalabad, Pakistan. A certified fatty acid methyl ester (FAME) reference standard mixture was obtained from Sigma-Aldrich (Bellefonte, PA, USA). Standards of gallic acid, tocopherols (α (≥96%), β (≥97%), γ (≥96%), δ (≥97%)), Folin-Ciocalteu reagent, 2,2′-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), and 2, 2-diphenyl-1 picrylhydrazyl radical (DPPH) purchased from Sigma-Aldrich (St. Louis, MO, USA). All tests used ultra-pure water generated with < 5 ppb TOC and 18.2 m X resistivity (Merck, MA, USA) by integrated Water Purification System Milli-Q. All other chemicals and solvents were obtained from Fisher Scientific Co. (Nepean, Ontario, Canada) of the American Chemical Society (ACS).

Pomegranate seed oil extraction

Sugars and other clinging components were removed from pomegranate seeds by introducing Ju 2000 Vitae (Moulinex, Barcelona, Spain) in a blender for juice extraction. The pomegranate seed was ground in a regular domestic coffee grinding machine and rinsed and dried in the sun (2-3 days) by passing through 30–40 mesh screens. The pomegranate seed powder was dried at 60ºC for 48 h and kept at -18°C until further use (7±1% moisture content). The oil was extracted from the pomegranate powder using the standard Soxhlet extraction method for 24 h (3 * 8 h) for each variety with hexane as a solvent. A rotary evaporator was used to evaporate the hexane at reduced pressure, followed by drying under nitrogen to remove the remaining hexane. The collected oil samples were preserved at -20ºC in amber glass jars for further research. The extraction yield (%) was determined by

dividing the volume of obtained oil (mL) by the original amount of dry matter (g) multiplied by 100[8].

Fatty acid profile

Fatty acid methyl esters (FAME) of oil samples generated utilizing the KOH-methanol method according to Jing et al. (2012)[7] procedure. A 20 mg oil sample was combined with 2 mL iso-octane and 0.2 mL KOH-methanol. After 5 minutes at room temperature, add 2 mL iso-octane and 3 mL water to the mixture. The supernatant was then drained and thoroughly rinsed with water. The uppermost iso-octane layer was recovered and used for GC-MS (Model: 14-A, Shimadzu, Japan). A DB-5MS column (30 m x 0.25 mm with a 0.25 µm film thickness) used with carrier gas helium flowed at a 1.0 mL/min rate. The sample was injected as 0.5 µL at a split ratio of 5:1. Initially, the temperature was set to 70°C for 5 minutes, up from 10°C/min to 200°C and then to a final temperature of 275°C at the rate of 5°C/min. MS spectra were obtained with an ion source temperature of 200°C at an ionization energy of 70 eV and an interface temperature of 260°C. An integrator was used to quantify fatty acid contents by estimating the area under individual peaks compared to the total area under all peaks.

FAME preparation of PA standard

Following Hashempour *et al.* (2010)[9], the methyl esters of the PA standard were produced independently. Trimethylsilyl diazomethane reagent interacted with the standard. 10 mg of PA were combined with 1 mL of methanol/toluene (1:4, v/v), and then 2 mol/L trimethylsilyl diazomethane (100 L) were added. Following 30 minutes at room temperature, the mixture was added to 2 mL iso-octane and 5 mL water, with the supernatant subjected to GC-MS after phase separation.

Physicochemical analysis

A Sopelem series 3296 refractometer (Sopelem, France) was used to calculate the refractive index (RI) according to the method of Godswill *et al*. (2018)[10] with some modifications. The specific gravity (SG), acid value (AV), iodine value (IV), and saponification value (SV) of recovered oil samples were determined using the AOCS protocols (2006)[11]. The unsaponifiable matter was estimated following the technique outlined in AOAC[12] (2016). The viscosity was measured using a viscometer (model LV DVII-Brookfield, Middleboro, MA, USA). The values recorded as Pa.s. Finally, the melting and flashpoints were determined by following the AOCS (2006) methods[11].

Tocopherol contents

Following Katsanidis and Addis's method (1999)[13], the tocopherol contents were determined using HPLC (Model: Perkin Elmer Series 200, USA). The analysis was carried out using an isocratic mode normal phase HPLC column (250mm x 4.6mm, 5.0m particle size) with a mobile phase of iso-octane/ethyl acetate (96:4 v/v). Simultaneously, the detector was tuned to 290 nm excitation and 400 nm emission wavelengths, with a run time of 30 minutes and a flow rate of 1.0 mL/min. The temperature in the column was 35°C. Individual oil samples or standards were dissolved in hexane, sterile filtered, and injected in a 2.0 µL volume. For every standard (α, β, γ, and δ tocopherol), an external calibration curve was created and utilized to estimate the volume of tocopherol present in the oil samples. After eight (08) injections, the silica column was re-activated using a 10% isopropanol in a hexane solution to increase its efficiency[14]. Duplicate samples were prepared.

Total phenolic content (TPC)

After oil extraction, the residual PSRs from all three types were dried at room temperature overnight. Approximately 1 g of dried defatted PSR was extracted in ultrasonic waves with 10 mL of 80% methanol at room temperature and centrifuged for 10 minutes at 2500 g/s. The supernatants were collected and kept at 4°C in the dark until further examination.

According to Mohdaly *et al*. (2011)[15], TPC was measured using the Folin-Ciocalteu reagent. The prepared methanolic pomegranate seed extracts (50 mg) were combined with 0.5 mL Folin reagent and 7.5 mL deionized water, then incubated for 10 minutes at room temperature with 20% sodium carbonate (w/v). The mixture was placed in a water bath at 40°C for 20 minutes before cooling in an ice bath. The absorbance was measured using a spectrophotometer at a wavelength of 765 nm (U-2001, Hitachi Instruments Inc. Tokyo, Japan). The TPC was measured using a 10-100 g/mL gallic acid standard curve (R2 = 0.990). Gallic acid equivalents (GAE) were calculated in mg/g of oil. Both samples were tested three times.

Determination of antioxidant activity

Radical scavenging activity was measured according to Siddiq *et al*. (2013)[16] using a DPPH solution in methanol. DPPH stock solution (2.4 g/l methanol) was diluted with 800 ml/l methanol to create a working solution (A515 w1.1). Blank, standard, or sample (0.6 ml) was combined with 3.0 ml of DPPH working solution, maintained in the dark for 20 minutes, and measured absorbance. Blank (ethanol acetone, 7:3) was utilized to calculate radical scavenging activity. Using 50-250 mmol/l Trolox solution, a standard curve was created.

The Trolox equivalent antioxidant capacity (TEAC) was determined using a modified ABTS radical cation decolorization test[16]. Briefly, 7 mmol/l ABTS solution and 2.45 mmol/l potassium persulfate were combined in a 1:1 ratio and left in the dark for 12-16 hours to form $ABTS^{\cdot+}$ radical cation ($ABTS^{\cdot+}$). This solution was diluted with methanol (800 ml/l) so that its absorbance at 734 nm was between 0.700 and 0.020. The diluted $ABTS^{\cdot+}$ solution (3 ml) was combined with 30 ml of blank, standard, or sample, and the absorbance was measured at 734 nm using a spectrophotometer after a 6-minute reaction. The blank was processed with ethanole: acetone (7:3). Using Trolox solution (0.3-1.5 m mol/l), the standard curve for calculating antioxidant capacity was generated. The experiment indicated the antioxidant capabilities of the samples as m mol Trolox equivalents (TE)/100 g fresh weight (FW).

Oxidative status analysis

The peroxide (POV) and *para*-anisidine (*p*-AV) values were estimated according to the AOCS (2006) method[11]. Conjugated dienes (CD) and conjugated trienes (CT) were determined at 232 and 270 nm, respectively. Oil samples were diluted with iso-octane to change the absorbance within limits, as per the standard IUPAC form.

Statistical analysis and data interpretation

A one-way analysis of variance ANOVA was used in the statistical study, and Duncan's multiple range test was used to assess the significant difference between means. Differences at $p \leq 0.05$ were considered statistically significant. The results are presented as mean±SD of triplicate measurements unless indicated otherwise.

RESULTS AND DISCUSSION

Lipid contents and fatty acid profile

The economically viable PSO exploitation depends on extraction technology, yield, and oil characteristics and quality. That might determine the potential of the oil source for downstream applications, such as food and non-food-based products (Figure **1**).

The total oil content in the current investigation ranged from 8.7-15.9% in the three varieties (Figure **2**), which is comparable to previous studies conducted on PSO from Iranian cultivars (6.6-19.3%)[18], Turkish cultivars (7.6-16.2%)[19] and Indian cultivars (7.0-19.0%)[20]. The kind, number, and placement of fatty acids within the glycerol moiety determine the oil's nutritional and physicochemical properties. Therefore, they can vary considerably in plant oils, depending on their source. While comparing different extraction methods for PSO extraction, the highest oil yield was observed by the Soxhlet method (15.66%)[21]. While six primary fatty acids could be identified in PSO samples (Table **1**), the predominant fatty acid is conjugated linolenic acid (CLnA, C18:3), also known as PA, considered one of the principal and active PSO components responsible for its antioxidant potential. The average PA values of Bedana (68.5%), Kandhari (75.5%), and Desi (76.7%) are following previous studies in cultivars from Georgia (78-83%)[22], China (73-79%)[7], Italy (72.20-84.10%)[19], India (31.80-86.60%), Turkey (70-76%)[20]. Other sources for PA have, on average, a lower PA content, such as seed oils from *Catalpa bignonioides* (28%), *Momordica charantia* (59%), *Prunus mahaleb* (28%), *Trichosanthes kirilowii* (36%), and *Calendula officinalis* (30%)[23].

Figure 1. External view and cross section of the selected Pakistani pomegranate varieties (a) Desi (b) Kandhari (c) Bedana.

Figure 2. Oil recovered from three Pakistani pomegranate varieties (a) Desi (b) Kandhari (c) Bedana

Table 1. Lipid contents and fatty acid composition of pomegranate seed oil

Pomegranate varieties	Lipid contents (g/100g)	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	SFA	UFA	SFA/UFA
Desi	15.90±0.04^{a}	0.51±0.01^{a}	4.60±0.04^{c}	0.75±0.01^{b}	10.61±0.33^{a}	6.73±0.09^{b}	76.66±1.12^{a}	6.00±0.15^{c}	94.00±0.20^{a}	0.06±0.02^{b}
Kandhari	11.00±0.05^{b}	0.34±0.02^{b}	5.30±0.08^{b}	2.92±0.50^{a}	10.40±0.19^{c}	5.42±0.40^{c}	75.50±0.50^{b}	8.50±0.28^{a}	91.50±0.78^{c}	0.09±0.01^{ab}
Bedana	8.70±0.0^{c}	0.15±0.01^{c}	6.18±0.50^{a}	0.67±0.10^{c}	12.75±0.22^{b}	12.00±1.02^{a}	68.52±1.63^{c}	6.80±0.13^{b}	93.20±0.70^{b}	0.07±0.02^{b}

Values in the same row with different letters are significantly different ($p < 0.05$). SFA, saturated fatty acids; USFA, unsaturated fatty acids.

Oleic (C18:1; 10.4-12.8 %) and linoleic acid (C18:2; 5.4-12.0 %) are prominent second and third fatty acids and are somewhat higher compared to other studies investigating PMO-composition growing in various countries (Spain, Israel, Turkey, Iran, Tunisia)[19].

The total saturated fatty acids (SFAs) were between 6.0-8.5%; the main SFAs are palmitic, stearic, and myristic acid, as seen in table **1**, which is significantly lower than other edible oils, such as 15% in soybean oil and pumpkin seed oil[24, 25]. Stearic and palmitic acid values are comparable with Turkish (1.4-2.8%, 1.4-2.0%), Chinese (2.8-3.6%, 1.6-2.8%), and Georgian pomegranate cultivars (2.8-4.8%, 2.1-3.6%)[22, 20]. Consequently, saturated to unsaturated FA (SFA/UFA) ratios align with data on cultivars from Spain, Iran, Turkey, Israel, and Tunisia[19].

Besides cultivar variation, differences in PSO oil content and lipid profiles are most likely due to different climate conditions, local growth, and environmental influence. High

quantities of unsaturated fatty acids, such as linolenic, linoleic acid, and PA, are preferred for human consumption, which may enhance PSO usage in human nutrition.

Physicochemical properties

Physicochemical characteristics are significant in evaluating edible oil's present condition and quality. Therefore, several parameters, such as IV, SV, and AV, are used to monitor oil quality and determine the required level of refinement[26] (table **2**).

The iodine value is useful for determining unsaturation but does not identify the individual fatty acid[27]. A relatively high iodine value was observed in the Desi variety, while other varieties showed a lower iodine value in Kandhari and Bedana, respectively. Due to the high level of punicic acid in oil samples, the higher iodine value indicates a greater number of unsaturated bonds. Besides, the AV, a measure of free fatty acids and inversely proportional to the degree of edibility of an oil[25], was significantly lower in Desi than in the other two varieties. The IV and AV of Iranian pomegranate varieties and cold-pressed oil were 179.4-215.90 and 216.5-220.34g I_2/100g and 3.78-8.36% oleic acid, while own data on AV are more comparable with cold-pressed PSO oil with 0.55-3.78% oleic acid[28]. Short-chain fatty acids have a higher SV than longer-chain fatty acids, with a higher number of carbon atoms in triacylglycerols[29].

The SV was moderately significant; the highest was observed in Bedana following Kandhari and Desi, similar to Dadashi *et al*. (2013)[27] findings. PSO has a substantial percentage of high molecular weight fatty acids, as seen by the greater SV of oil samples, equivalent to other culinary oils used in everyday life. The differences in unsaponifiable materials reported across three cultivars could be due to the distinctive morphology of pomegranate fruit and the different timing between horticultural maturity for commercial harvesting and physiological ripening. The physical characteristics of oils depend on the chemical and functional bodies present with glycerol moiety that ultimately influence the behavior of lipids in food and the procedures for processing and manipulating these lipids. The RI varies significantly with earlier studies conducted on PSO possessing 1.47-1.51 and 1.46-1.52 RI by Elfalleh *et al*. (2007)[31]; Dadashi *et al*. (2013)[27], respectively. SG was observed highest in Bedana, relatively higher than Iranian pomegranate varieties and cold-pressed oil (0.92 to 0.93 and 0.91-0.92 g/cm^3)[27]. Different studies conducted on PSO and evaluated their physicochemical properties agree with our results[27]. Different extraction methods exert no significant effect on the physicochemical attributes of PSO[21].

Tocopherol contents

Tocopherols (α, β, γ, δ) are naturally occurring antioxidants enriched in vegetable & fruit seed oils with varying contents, depending on their source and with a generally more considerable difference between different oils compared to within cultivar variation. Tocopherol contents in seed oils usually range between 0.20-80 mg/100g, and α-tocopherol is the main tocopherol except for β and γ. The total tocopherol content of Pakistani PSO, ranging from 2.5-3.8 µmol/g, is comparable with other natural sources like blueberry, marionberry, onion, cardamom, and milk thistle seed oils[23].

Table **3** shows that the three cultivars differed in total tocopherol content and composition. Desi has significantly higher overall tocopherol contents than the other cultivars ($p \leq 0.05$). The tocopherol contents observed in different pomegranate cultivars worldwide showed different concentrations in seed oil; Elfalleh *et al*. (2011)[31] observed the following pattern α-tocopherol> γ-tocopherol> δ-tocopherol for Tunisian pomegranate seed oils. Jing *et al*.[7] (2012) indicated the order δ-tocopherol> α-tocopherol> γ-tocopherol for seed oils of Chinese pomegranates, and Caligiani *et al*. (2010)[32] found the δ-tocopherol> α-tocopherol> γ-tocopherol pattern in pomegranate oil samples. The varying tocopherol content of Pakistani pomegranate cultivars could be attributable to genetic differences or environmental factors. The α-tocopherol contents are analogous to 718.70-1388.30 µg/g in China-grown pomegranate cultivars seed oil[7], 1576-1786 µg/g Tunisian cultivars[31], and 1612-1737 µg/g Georgia cultivars[21]. The γ-tocopherol was much lower than studies conducted earlier; 814-928 µg/g in Georgia-grown cultivars and 957-1154.5 µg/g in Tunisian-grown cultivars[31]. Also, δ-tocopherol is comparable with Tunisian cultivars (241.90-299.10 µg/g)[31] and Georgia cultivars (214-238 µg/g)[7]. Results are parallel with Liu et al. (2022), who compare different extraction methods for PSO extraction[21].

Table 2. Physicochemical analysis of pomegranate seed oil

Pomegranate Varieties	Desi	Kandhari	Bedana
Specific gravity	0.98±0.01	0.97±0.03	0.99±0.01
Viscosity (Pa.s; 25ºC)	0.12±0.03	0.13±0.02	0.11±0.01
Refractive index	1.47±0.03^{b}	1.43±0.01^{c}	1.50±0.04^{a}
Density (g/cm^3)	0.91±0.03^{b}	0.90±0.21^{b}	0.92±0.01^{a}
Melting point (ºC)	41.50±0.66^{b}	42.00±0.81^{b}	45.00±0.71^{a}
Flash point (ºC)	100.0±1.61^{c}	103.0±1.19^{b}	106.00±2.36^{a}
Acid value (% oleic acid)	3.77±0.05^{b}	3.98±0.18^{a}	4.00±0.08^{a}
Saponification value (mg KOH/g)	181.10±1.08^{b}	181.50±1.50^{b}	184.30±1.05^{a}
Unsaponifiable matter (%)	1.04±0.04^{a}	0.90±0.12^{b}	0.80±0.07^{bc}
Iodine value (g I_2/100 g oil)	134.90±2.25^{a}	123.50±1.60^{b}	120.40±1.95^{c}
Odor	Characteristics pomegranate odor	Characteristics pomegranate odor	Bland
Color	Dark yellow	Dark Yellow	Light yellow
State at ambient temperature	Liquid	Liquid	Liquid
Vapor properties	Negligible at ambient temperature	Negligible at ambient temperature	Negligible at ambient temperature

Values in the same row with different letters are significantly different ($p < 0.05$).

Table 3. Tocopherol composition of pomegranate seed oil

Pomegranate Varieties	Desi	Kandhari	Bedana
α-Tocopherol (µg/g)	1211.10±18.03^{a}	890.80±10.11^{b}	768.63±20.09^{c}
γ- Tocopherol (µg/g)	50.21±2.30^{a}	42.50±1.45^{b}	35.10±2.39^{c}
δ-Tocopherol (µg/g)	300.51±4.31^{a}	256.90±3.90^{b}	245.71±4.50^{c}
Total tocopherols (µg/g)	1561.82±7.90^{a}	1190.20±10.87^{b}	1049.44±7.01^{c}

Values in the same row with different letters are significantly different ($p < 0.05$).

Antioxidant activity

The natural food sources like fruits and vegetables are enriched with phenolic compounds that function as imperative antioxidants; these exhibit the ability to donate an electron or hydrogen atom to a free radical to form a stable radical and prevent the auto-oxidation chain reactions in biological membranes.

Table **4** shows the TPC range between 3.4-4.9 mg GAE per g DW. Desi is offering the highest TPC, following Kandhari and Bedana. Pakistani pomegranate seeds contain higher TPC than other oils, e.g., soybean, sunflowers, maize, grapes, hemp, flax, and pumpkin[34]. However, Khoddami *et al*. (2014)[35] found higher TPC in cold-pressed PSO of three cultivars of 10.44 mg GAE g^{-1} oil. The DPPH$^{\bullet}$ radical system is frequently used to evaluate antioxidant activity *in vitro* and *in vivo* studies. Rojo-Gutiérrez *et al*. (2021) found 91.29 and 98.28% scavenging activity against DPPH and ABTS radicals, respectively, in PSO extracted through green extraction methods (microwave-assisted and ultrasound-assisted extractions)[36].

Table 4. Antioxidant Potential and oxidative stability of pomegranate seed oils

Pomegranate Varieties	Desi	Kandhari	Bedana
Total phenolic contents (mg GAE/g oil)	4.94±0.58^{a}	4.30±0.83^{b}	3.40±0.38^{c}
DPPH· (µM Trolox/g oil)	67.21±2.82^{a}	56.36±3.06^{b}	52.70±2.59^{c}
ABTS·+ (µM Trolox/g oil)	71.90±3.27^{a}	65.10±3.73^{b}	61.35±2.03^{c}
Peroxide value (meq of O_2/Kg)	1.21±0.05^{c}	1.54±0.30^{b}	1.98±0.10^{a}
Para-anisidine value	0.30±0.01^{c}	0.55±0.15^{b}	0.77±0.08^{a}
Conjugated dienes ($\lambda_{232\ nm}$)	0.22±0.24^{c}	0.75±0.05^{b}	0.99±0.12^{a}
Conjugated trienes ($\lambda_{270\ nm}$)	0.04±0.02^{b}	0.07±0.01^{ab}	0.09±0.02^{a}

Values in the same row with different letters are significantly different ($p < 0.05$).

Because several approaches might produce significantly divergent results, no single method is suitable for measuring the antioxidant potential of foods. Various methods must be utilized, each based on a different mechanism. Table **4** represents the DPPH· Scavenging activities of seed oil of three Pakistani pomegranate cultivars dependent on the concentration by quenching the DPPH• depicting increased contents increased the DPPH· Scavenging activity of oil samples. Desi had considerably higher DPPH• scavenging activities (p≤0.05) than Kandhari and Bedana. Desi variety showed the highest DPPH· Scavenging activity (67.78±2.82%) follow by Kandhari (56.83±3.06%) and Bedana (52.38±2.59%). Table 4 represents the ABTS·+ scavenging activity of oil samples and shows the antioxidant activity comparable with some artificial antioxidants. Compared to other oil samples, the Desi variety oil samples had the highest ABTS•+ intensity (71.48±3.27%). The variation in results may be due to various factors like radical's stereo-selectivity or solubility of the sample in different radical scavenging systems reported to upset natural extracts' ability to scavenge diverse radicals.

Pomegranate cultivars from Sri Lanka showed TPC in the range of 1.19-2.39 mg GAE g^{-1} with a higher 96.70% DPPH, 93.1±2.0% ABTS radical scavenging activity[35]. He *et al*. (2012)[37] extracted the phenolic compounds from pomegranate seed residues and found the TPC in the range of 3.12-27.52 mg GAE g^{-1} with 4265.7 mmol/100 g DW DPPH and 2692.0 mmol/100 g DW ABTS radical scavenging activity. The scavenging assay's consequences recommended that PSO enriched with phenolic contents, gifted to capture the free radicals in a system through hydrogen- or electron-denoting mechanisms. As a result, PSO can scavenge free radicals in vulnerable matrices, such as biological membranes, and prevent fatal free radical arbitrated chain reactions from starting.

These studies confirmed the PSO's ability to capture free radicals in various systems, suggesting that these molecules could be helpful therapeutic mediators for treating free radical-induced pathology. The effect of extraction on PSO using different extraction methods, e.g., Soxhlet, stirring, microwave & ultrasonic irradiation, and supercritical fluid extraction on the phenolic contents, showed significant results with other experimental conditions. The highest phenolic contents were observed in Soxhlet > microwave > stirring > ultrasound with high pressure and lower temperature[38]. Higher TPC with antioxidant activity of Pakistani pomegranate seeds supported by Jing *et al*. (2012)[7].

Oxidative status

The POV, *p*-AV, and CD & CT were used as lipid peroxidation indexes to measure the antioxidant abilities for the present study. Universally, POV is considered a standard method for measuring lipid peroxidation extent in fat/oil-based products. The POV measures the concentration of peroxides and hydro-peroxides generated in the early phases of lipid oxidation. POV is one of the most common methods for determining oxidative rancidity in oils and fats. In our study, the lipid oxidation level of oil samples was determined by placing oil samples at 70°C for 72 h in a hot air oven. Table **4** shows the POV of oil samples with a significant difference (p≤0.05). The results revealed that the POV of the present study samples were higher than 0.39-0.48 meq O_2/kg of four (04) Iranian

pomegranate cultivars[39] while lower than 1.00-4.40 meq O_2/kg oil in seven (07) Turkish pomegranate cultivars oil. The *p*-AV for the three cultivars' oil samples ranged from 0.30±0.01-0.77±0.08% shown in Table **4**. Chemical structures can account for the difference in *p*-AV. The oxidative stability of lipids is improved by the stability of phenoxy radicals, limiting the degree of proliferation[40].

The results showed that the Desi variety has more antioxidant capability than Kandhari and Bedana varieties. The present findings are supported by Carvalho *et al*. (2016)[41]. The CD and CT values of pomegranate oil samples are shown in Table **4**, ranging from 0.22±0.24-0.99±0.12 λ_{232} and 0.04±0.02-0.09±0.02 λ_{270}, respectively. Because the increase in CD and CT is related to the degree of oxidation, the oil sample's OS will be lower[42]. Our study's oxidative status results showed that Pakistani pomegranate seed has more antioxidant potential than tea leaves. The relatively lower POV, AV, and CD & CT values of oil samples showed that Pakistani PSO could categorize as stable oils. The antioxidant activity of vegetable and fruit-based oils is mainly ascribed to the phenolic contents found in plant seed oils.

CONCLUSION

Based on the present study results, the seeds of Pakistani pomegranate cultivars as a by-product after juice extraction can be a potential source of essential fatty acid (CLnAs), biologically active compounds. Findings also showed substantial connections between total phenolic contents and antioxidant activity, representing the influence of phenolic compounds in oil antioxidant activity with higher oxidative stability. The lipid content of pomegranate seeds was probably insufficient for commercial use; on the other hand potential constituent for particular consumption or medical usage. Although, it could be an economical source of a natural antioxidant for different food products due to the antioxidant activity of conjugated linolenic acid. The findings support the use of pomegranate seeds in manufacturing pomegranate oil or developing nutraceutical foods for human nutrition.

CONTRIBUTOR STATEMENT

All authors contributed equally in preparation and evaluation of the manuscript.

CONFLICTS OF INTEREST

No conflict of interest.

FUNDING SOURCE

The current research projected was funded by Higher Education Commission, Islamabad, Pakistan.

ACKNOWLEDGEMENTS

NS is grateful for the stipend provided by the Higher Education Authorities.

LIST OF ABBREVIATIONS

None

REFERENCES

1. Verma N, Mohanty A, Lal A. Pomegranate genetic resources and germplasm conservation: A review. Fruit, Vegetable and Cereal Science and Biotechnology. 2010;4:120–5.
2. Kahramanoglu I. Trends in pomegranate sector: production, postharvest handling and marketing. International Journal of Agriculture Forestry and Life Sciences. 2019;3(2):239–46.
3. Nafees M, Jaskani MJ, Ahmed S, Awan FS. Morpho-molecular characterization and phylogenetic relationship in pomegranate germplasm of pakistan. Pakistan Journal of Agricultural Sciences. 2015;52(1).
4. Hrelia S, Angeloni C, Barbalace MC. Agri-food wastes as natural source of bioactive antioxidants. Antioxidants (Basel) . 2023;12(2). Available from: http://dx.doi.org/10.3390/antiox12020351
5. Tehranifar A, Selahvarzi Y, Kharrazi M, Bakhsh VJ. High potential of agro-industrial by-products of pomegranate (punica granatum l.) as the powerful antifungal and antioxidant substances. industrial crops and products. 2011;34:1523–7.
6. Tian Y, Xu Z, Zheng B, Martin Lo Y. Optimization of ultrasonic-assisted extraction of pomegranate (Punica granatum L.) seed oil. Ultrason Sonochem . 2013;20(1):202–8. Available from: http://dx.doi.org/10.1016/j.ultsonch.2012.07.010
7. Jing P, Ye T, Shi H, Sheng Y, Slavin M, Gao B, et al. Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. Food Chem . 2012;132(3):1457–64. Available from: http://dx.doi.org/10.1016/j.foodchem.2011.12.002

8. Bimakr M, Rahman RA, Taip FS, Adzahan NM, Sarker MZI, Ganjloo A. Optimization of ultrasound-assisted extraction of crude oil from winter melon (Benincasa hispida) seed using response surface methodology and evaluation of its antioxidant activity, total phenolic content and fatty acid composition. Molecules . 2012;17(10):11748–62. Available from: http://dx.doi.org/10.3390/molecules171011748
9. Hashempour A, Ghazvini RF, Bakhshi D, Sanam SA. Fatty acids composition and pigments changing of virgin olive oil (Olea europea L.) in five cultivars grown in Iran. Australian Journal of Crop Science. 2010;4(4):258–63.
10. Godswill AC, Amagwula IO, Igwe VS, Gonzaga A. Effects of repeated deep frying on refractive index and peroxide value of selected vegetable oils. 2018.
11. Official methods and recommended practices of the American Oil Chemists' Society. AOCS. 2006;
12. Official Method of Analysis. Association of Official Analytical Cereal Chemists. AOAC. 2016;
13. Katsanidis E, Addis PB. Novel hplc analysis of tocopherols, tocotrienols, and cholesterol in tissue. Free Radical Biology and Medicine. 1999;27(11):1137–40.
14. Fernandes L, Pereira JA, Lopéz-Cortés I, Salazar DM, Ramalhosa E, Casal S. Fatty acid vitamin E and sterols composition of seed oils from nine different pomegranate (Punica granatum L.) cultivars grown in Spain. Journal of Food Composition and Analysis. 2015;39:13–22.
15. Mohdaly AA, Smetanska I Ramadan MF, Sarhan MA, Mahmoud A. Antioxidant potential of sesame (Sesamum indicum) cake extract in stabilization of sunflower and soybean oils. Industrial Crops and Products. 2011;34(1):952–9.
16. Siddiq M, Sogi DS, Dolan KD. Antioxidant properties total phenolics, and quality of fresh-cut 'Tommy Atkins' mangoes as affected by different pre-treatments. LWT-Food Science and Technology. 2013;53:156–62.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26(9):1231–7.
18. Fadavi A, Barzegar M, Azizi MH. Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in iran. Journal of Food Composition and Analysis. 2006;19(6):676–80.
19. Verardo V, Garcia-Salas P, Baldi E, Segura-Carretero A, Fernandez-Gutierrez A, Caboni MF. Pomegranate seeds as a source of nutraceutical oil naturally rich in bioactive lipids. Food Research International. 2014;65:445–52.
20. Parashar A, Sinha N, Singh P. Lipid contents and fatty acids composition of seed oil from twenty five pomegranates varieties grown in india. Advance Journal of Food Science and Technology. 2010;2(1):12–5.
21. Liu N, Ren G, Faiza M, Li D, Cui J, Zhang K, et al. Comparison of conventional and green extraction methods on oil yield, physicochemical properties, and lipid compositions of pomegranate seed oil. Journal of Food Composition and Analysis. 2022;114.
22. Pande G, Akoh CC. Antioxidant capacity and lipid characterization of six georgia-grown pomegranate cultivars. Journal of Agricultural and Food Chemistry. 2009;57(20):9427–36.
23. Król B, Paszko T. Harvest date as a factor affecting crop yield, oil content and fatty acid composition of the seeds of calendula (Calendula officinalis L.) cultivars. Industrial crops and products. 2017;97:242–51.
24. Hernández-Santos B, Rodríguez-Miranda J, Herman-Lara E, Torruco-Uco JG, Carmona-García R, Juárez-Barrientos JM, et al. Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (Cucurbita pepo). Ultrasonics Sonochemistry. 2016;31:429–36.
25. Harris WS. Stearidonic acid-enhanced soybean oil: a plant-based source of (n-3) fatty acids for foods. The Journal of nutrition. 2012;142(3):600S-604S.
26. Roshandel-Hesari N, Mokaber-Esfahani M, Taleghani A, Akbari R. Investigation of physicochemical properties, antimicrobial and antioxidant activity of edible films based on chitosan/casein containing Origanum vulgare L. essential oil and its effect on quality maintenance of cherry tomato. Food Chemistry. 2022;396.
27. Samek O, Jonáš A, Pilát Z, Zemánek P, Nedbal L, Tříska J, et al. Raman microspectroscopy of individual algal cells: sensing unsaturation of storage lipids in vivo. Sensors. 2010;10(9):8635–51.
28. Verma N. Mohanty A. and Lal A. 2010. Pomegranate genetic resources and germplasm conservation: A review. Fruit, Vegetable and Cereal Science and Biotechnology. 4(S2): 120-125.
29. Dadashi S, Mousazadeh M, Emam-Djomeh Z, Mousavi SM. Pomegranate (punica granatum l.) seed: A comparative study on biochemical composition and oil physicochemical characteristics. Int J Adv Biol Biom Res. 2013;1:351–63.
30. Nehdi IA, Sbihi H, Tan CP, Zarrouk H, Khalil MI, Al-Resayes SI. Characteristics, composition and thermal stability of acacia senegal (l.) willd. Seed oil. industrial crops and products. 2012;36:54–8.

31. Alhibshi EA, Ibraheim JA, Hadad AS. Effect of heat processing and storage on characteristic and stability of some edible oils. In: Proceedings of the 6th Int'l Conference on Agriculture, Environment and Biological Sciences (ICAEBS'16). 2016. p. 105–9.
32. Elfalleh W, Tlili N, Nasri N, Yahia Y, Hannachi H, Chaira N, et al. Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (Punica granatum) fruits. J Food Sci . 2011;76(5):C707-13. Available from: http://dx.doi.org/10.1111/j.1750-3841.2011.02179.x
33. Caligiani A, Bonzanini F, Palla G, Cirlini M, Bruni R. Characterization of a potential nutraceutical ingredient: Pomegranate (punica granatum l.) seed oil unsaponifiable fraction. Plant foods for human nutrition. 2010;65:277–83.
34. Wu G, Chang C, Hong C, Zhang H, Huang J, Jin Q, et al. Phenolic compounds as stabilizers of oils and antioxidative mechanisms under frying conditions: A comprehensive review. Trends Food Sci Technol . 2019;92:33–45. Available from: http://dx.doi.org/10.1016/j.tifs.2019.07.043
35. Khoddami A, Man YBC, Roberts TH. Physico-chemical properties and fatty acid profile of seed oils from pomegranate (punica granatum l.) extracted by cold pressing. European journal of lipid science and technology. 2014;116:553–62.
36. Bopitiya D, Madhujith T. Antioxidant potential of pomegranate (punica granatum l.) cultivars grown in sri lanka. Tropical Agricultural Research. 2015;24(1).
37. Rojo-Gutiérrez E, Carrasco-Molinar O, Tirado-Gallegos JM, Levario-Gómez A, Chávez-González ML, Baeza-Jiménez R, et al. Evaluation of green extraction processes, lipid composition and antioxidant activity of pomegranate seed oil. J Food Meas Charact . 2021;15(2):2098–107. Available from: http://dx.doi.org/10.1007/s11694-020-00804-7
38. He L, Zhang X, Xu H, Xu C, Yuan F, Knez Ž, et al. Subcritical water extraction of phenolic compounds from pomegranate (punica granatum l.) seed residues and investigation into their antioxidant activities with HPLC-ABTS+ assay. Food and Bioproducts Processing. 2012;90:215–23.
39. Vernès L, Vian M, Chemat F. Ultrasound and microwave as green tools for solid-liquid extraction. In: Liquid-Phase Extraction. Elsevier; 2020. p. 355–74.
40. Mirzaee S. Studying seed and oil physicochemical characteristics of four iranian pomegranate (Punica granatum l.) varieties. International Journal of Biosciences (IJB). 2014;4(8):78–86.
41. Zhang Q, Saleh ASM, Chen J, Shen Q. Chemical alterations taken place during deep-fat frying based on certain reaction products: a review. Chem Phys Lipids . 2012;165(6):662–81. Available from: http://dx.doi.org/10.1016/j.chemphyslip.2012.07.002
42. Carvalho E, Yoshime LT, Sattler JAG, Pavan RT, Mancini-Filho J. Characterization of constituents, quality and stability of pomegranate seed oil (punica granatum l). In: Food Science and Technology. Campinas; 2016. p. 0–0.
43. Ramadan MF. Healthy blends of high linoleic sunflower oil with selected cold pressed oils: Functionality, stability and antioxidative characteristics. Ind Crops Prod. 2013;43:65–72. Available from: http://dx.doi.org/10.1016/j.indcrop.2012.07.013