



Determining the Quality and Storage Stability of Pomegranate (*Punica granatum* L.) Seed Oil with Accelerated Shelf-Life Approach

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ABSTRACT

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Punica granatum L.

Pomegranate (*Punica granatum* L.) is a fruit that grows in most tropical and subtropical regions. It has 52% aril, consisting of 78% juice which is used as; juice, molasses, jam, wine, and dried kernels. Potential health benefits increase the demand for the fruit as well as its products. Pomegranate seeds, which consist of approximately 10% of the whole fruit, are a by-product of the juice and juice using products containing nutraceutical functional components such as sterols and puniic acid. Pomegranate seed oil is considered a healthy alternative source of oils, and its production is a valorization process since it is the by-product that usually goes to waste. In the present study, pomegranate seeds were used for oil extraction using the cold solvent extraction method. Oil samples were then taken to the Schaal oven treatment in order to determine changes due to storage. Oil samples were tested for 14 days of total storage at their 1st, 3rd, 7th and 14th days for the oxidation tests, colour, fatty acid composition, and Fourier transform infrared spectra analysis. Data were tested for significance by using statistical analysis. The results indicated that oxidative stability of pomegranate seed oil was decreased by increasing storage time. The studied techniques used in this paper can be valuable processors to monitor the oxidative stability of oils with storage time and evaluate their acceptance on the market.

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Introduction

Pomegranate (*Punica granatum* L.) is known from ancient times and has been picturised in hieroglyph paintings as an edible fruit. The pomegranate trees are found widely in most tropical and subtropical regions (Ali Fadavi et al., 2005). The whole fruit has 52% aril, consisting of 78% juice and 22% seeds (Kulkarni and Aradhya, 2005). Despite the fresh fruit consumption, pomegranate fruits are consumed as various processed products such as juice (Basu and Penugonda, 2009), molasses (Al-Wazni et al., 2018; İncedayi et al., 2010), jam (Abid et al., 2018; Legua et al., 2012), wine (Uzaşçı et al., 2012), dried kernels (Coret et al., 2000). The demand for the fruit is increasing not only due to the wide product array but also to potential health benefits. Pomegranate seeds, which consist of approximately 10% of the whole fruit, are important as a functional product.

Pomegranate seeds are a by-product of the juice and juice using products (including molasses) that contain the nutraceutical functional components as sterols and puniic

acid (Aruna et al., 2018). The literature presents a vast amount of research about these functional components in the pomegranate seed oil, such as puniic acid (Carvalho Filho, 2014; Khajebishak et al., 2019; Vroegrijk et al., 2011), sterols (Fernandes et al., 2015; Verardo et al., 2014), γ -tocopherol (Boroushaki et al., 2016; Verardo et al., 2014), and hydroxyl benzoic (Jing et al., 2012; Kazemi et al., 2016). Pomegranate seeds are a possible functional oil source known to have functional properties due to their high conjugated octadecatrienoic fatty acids, with characteristic fatty acid as puniic acid (9-*cis*, 11-*trans*, 13-*cis*, 18:3) (Mohagheghi et al., 2011). The seeds contain 12-25% crude oil that is considered nutraceutical (Keskin Çavdar et al., 2017). From the health aspect, the most critical content was determined as the puniic acid, which is around three-fourths of the total fatty acid content and gives the functions of antioxidant, antitumor, immunomodulatory, anti-atherosclerotic, and serum lipid-lowering activities (Carvalho et al., 2010; Verardo et al.,

2014). This study aimed to investigate the effect of storage time on the oxidative stability of pomegranate seed oil under accelerated oxidation conditions. Colour, peroxide value, fatty acid composition (GC-MS), and lipid degradation (ATR-FTIR) were investigated parameters to predict lipid oxidation during fourteen days of storage.

Materials and Methods

Materials

Pomegranate (*Punica granatum* L.) seeds were purchased from local farmers' markets in the Osmaniye region, Turkey. Chemicals that were used for the analysis were provided by Merck (Darmstadt, Germany), which were analytical of chromatographic grade.

Oil extraction

The seeds were grounded in the grinder (Waring 8011 ES blender, NJ, USA). Grounded seeds were sieved for their mash size into fine particle size ($d_1=0.125-0.450$ mm) as previously classified by Keskin Çavdar et al., (2017) and stored undercooled dark conditions until extractions.

For the oil extraction, the cold solvent extraction method was adapted from the method previously applied by Keskin Çavdar et al. (2017). 50 grams of fine-sized seed particles and 500 mL of n-hexane were stirred for 8 h at 25°C with a magnetic stirrer (Isolab 613.01, Wertheim, Germany). The solid residue was separated from the supernatant by centrifuge (EBA 10, Tuttlingen, Germany). Then the hexane was removed from the supernatant with a rotary evaporator at 40°C (Hei-VAP Advantage HL/G1; Heidolph Instrument GmbH & Co. KG, Schwabach, Germany). If not used immediately for analysis, then the oil was stored at -20°C at dark conditions for further analysis.

Schaal Oven Treatment

Extracted pomegranate seed oil was treated with Schaal Oven conditions to observe the shelf-life effect under accelerated conditions. The oil samples were weighed 10 g and placed in transparent glass containers. These containers were placed in an air oven (Mettler UN55, Germany) set to 60°C for 1, 3, 7, and 14 days. Further tests were immediately done on the oil samples for triplicate.

Peroxide Value

As an indicator of oxidation level peroxide value of oil samples was determined according to AOCS methods (Firestone, 1997).

Colour

Hunter Lab spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used for the colour observations of the oil samples. For this purpose, the samples were filled into sample cuvette, and colour properties were assessed for the s L* (lightness), a* (redness), and b* (yellowness) according to the Hunter colour scale at 25°C.

Fatty Acid Composition

The fatty acid composition of pomegranate seed oil was done by gas chromatography (GC7890A, Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector and capillary column that is 100

m in length and 0.25 mm HP-88 column (88% cranopylarly) in diameter. The flow rate of the carrier Helium gas was set to 1 mL per minute at 260°C for the detector and injector temperatures, respectively. The initial (first 10 minutes) column temperature was 175°C and set to increase 5°C rise each minute until reaching 210°C - 230°C. The sample injection volume was 1µL. These analytical conditions were used for fatty acid methyl esters (FAME) extraction with n-heptane after cold methylation with 2N KOH in methanol.

Typical fatty acid standards were used for each fatty acid to identify the fatty acid composition by comparing the retention times with the standards and samples. The area was expressed as percentages of the total fatty acids (IUPAC, 1987).

Fourier Transform Infrared Spectra

Fourier transforms infrared radiation (FTIR) analysis is a quantitative tool that carries out component analyses. The present study involved the assessment of the component analysis where the spectral data were collected on a Perkin-Elmer Spectrum 100 spectrophotometer (Spectrum Two) (Shelton, USA) fitted with a universal attenuated total reflectance (UTAR) sampling device. For this analysis, a drop of oil sample was placed into the Universal diamond ATR crystal. All spectra were measured at room temperature against a background spectrum of air in the wavenumber range from 4000 to 600 cm^{-1} . The cell was cleaned and dried between each sample by aspirating hexane via the cell using a vacuum, and its cleanliness was verified spectrally. Spectra were examined using the instrument's software Spectrum 10 STD (Perkin-Elmer, Shelton, USA) with peak heights and areas computed from the raw spectra.

Statistical Analysis

All experiments and measurements were done in triplicates for each replicate. All collected data were tested (at 95%) with ANOVA and regression analyses using the SPSS v.22 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

In the present study, the storage effect was tested on some analytical properties of pomegranate seed oil extracted with cold solvent extraction. Peroxide value and colour properties are given in Table 1.

Colour properties are a significant feature, especially for the potential applications to further products (Parker et al., 2003). Hunter colour spectrums emphasize different colour properties as; L value measures the lightness (100 for perfect white to zero for black), a* value measures the redness-greenness for positive and negative, respectively (zero when grey), and finally b* value measures the yellowness and blueness for positive and negative, respectively (zero when grey). Colour properties of the pomegranate seed oil samples within the storage duration are listed in Table 1. Even though the brightness spectrums where very similar fresh seed oil was the lightest where the darkening profile is observable until the 14th day of storage which has the darkest amongst the samples ($P \leq 0.05$). In comparing a* values, the fresh sample had significantly more redness. The green colour change is observable during the increase in the storage period with the greenest colour of the 14th day sample.

Table 1. Effect of storage duration (days) on the colour properties and peroxide value.

Storage (days)	Colour spectrums			Peroxide value (meqO ₂ / kg oil)
	L value	a* value	b* value	
0	59.00 ± 0.09 ^a	-0.47 ± 0.01 ^a	27.03 ± 0.01 ^a	2.41 ± 0.10 ^a
1	58.72 ± 0.02 ^b	-0.19 ± 0.01 ^b	26.37 ± 0.02 ^b	3.41 ± 0.10 ^b
3	58.37 ± 0.03 ^c	0.40 ± 0.02 ^c	26.22 ± 0.01 ^c	3.41 ± 0.20 ^b
7	57.63 ± 0.24 ^d	1.07 ± 0.02 ^d	23.72 ± 0.01 ^d	5.07 ± 0.15 ^c
14	56.97 ± 0.06 ^e	2.35 ± 0.09 ^e	20.11 ± 0.01 ^e	14.2 ± 0.20 ^d

Data are expressed as means±standard deviations (n=5). Values printed in one column, with the different letters (a–e) in superscript are statistically different at the P<0.05 level, 95% confidence limit, according to Duncan's Multiple Range Test.

Table 2. Fatty acid composition of pomegranate seed oil samples in 0, 1,3, 7, and 14 days.

Fatty acid	Fresh oil (%)	1 st day (%)	3 rd day (%)	7 th day (%)	14 th day (%)
C16:0	2.74 ± 0.02 ^{ac}	2.63 ± 0.04 ^b	2.65 ± 0 ^{ab}	2.80 ± 0.01 ^c	2.68 ± 0.03 ^{ab}
C18:0	2.05 ± 0.01 ^a	2.01 ± 0.01 ^b	2.02 ± 0.01 ^{ab}	2.13 ± 0.01 ^c	1.99 ± 0.01 ^b
C18:1	5.15 ± 0.01 ^a	5.05 ± 0.01 ^b	5.03 ± 0.01 ^b	5.35 ± 0.01 ^c	5.25 ± 0.01 ^d
C18:2	4.87 ± 0.01 ^a	4.76 ± 0.01 ^b	4.82 ± 0.01 ^c	4.98 ± 0.02 ^d	4.85 ± 0.01 ^{ac}
C18:3	83.21 ± 0.03 ^a	83.9 ± 0.02 ^b	83.4 ± 0.02 ^c	81.98 ± 0.01 ^d	82.66 ± 0.02 ^e
C20:0	0.31 ± 0.01 ^a	0.32 ± 0.02 ^a	0.32 ± 0.01 ^a	0.40 ± 0.01 ^b	0.33 ± 0.01 ^a
C20:1	0.62 ± 0.01 ^a	0.62 ± 0.01 ^a	0.63 ± 0.0 ^a	0.62 ± 0.01 ^a	0.61 ± 0.01 ^a
C20:3	0.08 ± 0.02 ^a	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.08 ± 0.01 ^a	0.09 ± 0.01 ^a
C20:4	0.22 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.23 ± 0.01 ^a	0.35 ± 0.03 ^b
C24:0	0.75 ± 0.01 ^a	0.42 ± 0.01 ^b	0.84 ± 0.02 ^c	1.42 ± 0.01 ^d	1.19 ± 0.01 ^e

Data are expressed as means±standard deviations (n=3). Values printed in one column, with the different letters (a–e) in superscript are statistically different at the P<0.05 level, 95% confidence limit, according to Duncan's Multiple Range Test

On the other hand, b* values showed that the storage period shifts the seed oil to more yellowish colour with the fresh sample as the least yellow and 14th day as the most yellow colour measurements. Overall, the period of storage significantly alters the colour properties. To be precise, Figure 1 shows the colour observations against the storage for the brightness (L) decreases, red colour spectrum (a*) changes to a greenish colour and samples get higher yellowness properties (b*). Therefore, we can highlight that during the storage, the chlorophyll and carotene content decreased. Notably, usage of the pomegranate seed oil in a food medium and colour kinetics and modelling investigations should be further investigated where it is expected to be effective for the overall appearance of the food medium. During the cold solvent extraction, the colour pigments and colour affecting components such as phospholipids are likely to be extracted along with the oil (Yan et al., 2017). According to the results, these pigments and components are expected to change the color properties throughout the storage period, which is supported in the present study. Recent research was done by Keskin Çavdar et al. (2017) aimed to assess various extraction methods on pomegranate seed oil where colour was selected as one of the physicochemical properties. The findings of this research illustrate very similar L, a*, and b* values as 58.91, -2.45, and 14.44, respectively. The researches also emphasised that cold extraction of the pomegranate seed oils is less efficient than the other methods in terms of colour related compounds such as chlorophyll and carotene.

The pomegranate seed oil samples' peroxide value was measured during the 14 days of storage duration (Table 1). This value is a primary indicator of the oxidation process where lower values indicate better oil quality (Drinić et al., 2020; Özcan, 2009). Moreover, the peroxide value assay determines the hydroperoxide value, which is the primary indicator of the early stages of lipid oxidation (Ramadan,

2013; Ramadan and Mörsel, 2004). In terms of quality assessment of lipids, 9 meqO₂/kg oil indicates oil oxidation (Özcan, 2009). According to our findings, the peroxide value of the pomegranate seed oil samples was significantly increasing through the storage period (P≤0.05) (Figure 2). The peroxide value ranged from 2.41 to 14.2 meqO₂/ kg oil with an increasing trend through the storage, where the highest value was measured at the final storage day (14th day). To be precise, the increase in the peroxide value was almost twice the initial measurement for the first 7 days (2.41 to 5.07), where it was almost three times faster for the second 7 days of the storage (5.07 to 14.02). The present study did not include any antioxidant addition to the seed oil for peroxidation decrease, which would show less oxidation in the case. The literature presents relevant approaches to peroxide measurements for the pomegranate seed oil. Depending on the extraction and environmental conditions (e.g., storage duration, temperature etc.) the value can vary yet fresh measurements of cold solvent extracted pomegranate seed oil was measured as 8 meqO₂/ kg oil where for microwave assisted extraction was 0 meqO₂/ kg oil (Keskin Çavdar et al., 2017). Another research was tested the soxhlet extracted oil and found that fresh oil was not the content of hydroperoxide, which means peroxide value was 0 meqO₂/ kg and was found to be increasing during the 12 days of storage up to 5.75 meqO₂/ kg (Drinić et al., 2020). This finding has similar values with ours in terms of the oxidation speed. In the literature, the majority of the research focuses on the antioxidant added oxidation profile since this is the general approach towards oxidation investigations.

The pomegranate seed oil samples' fatty acid profiles through the storage period are illustrated in Table 2. Findings illustrate that the total unsaturated fatty acid content was 94.15 % right after the extraction and decreased to 93.81 at the end of the 14 days of storage.

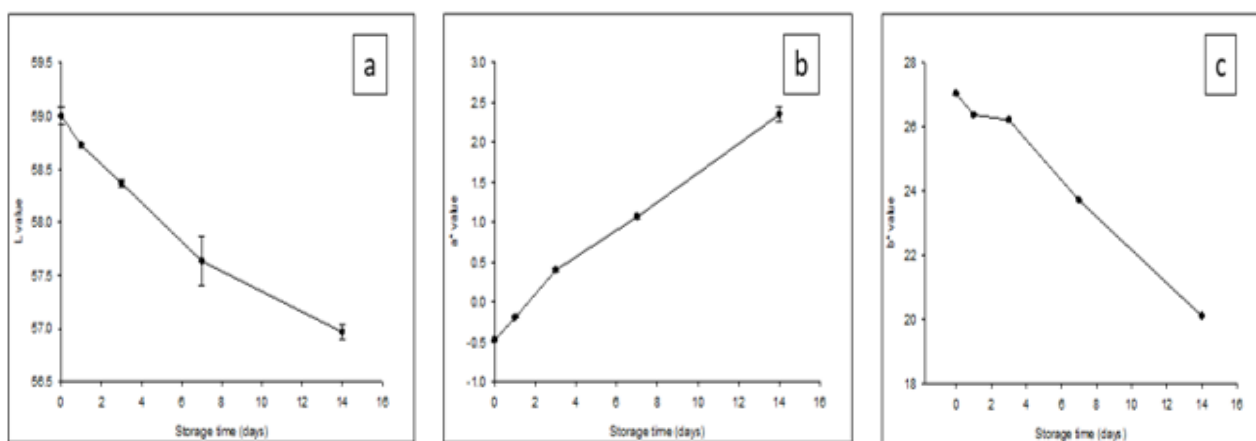


Figure 1. Hunter colour values against storage for; L (a), a* (b), and b* (c)

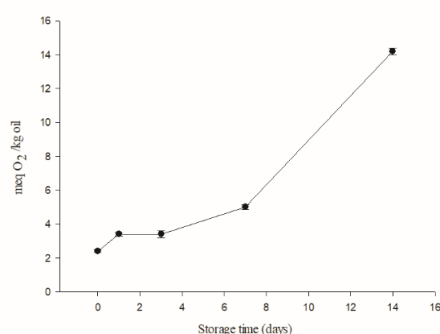


Figure 2. Peroxide value (meq O₂/kg oil) assessment during 14 days of storage

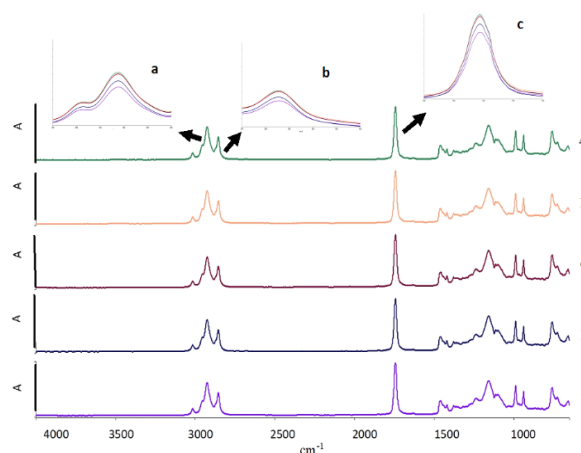


Figure 3. FTIR spectra of cold pressed pomegranate oils at different storage times (from top to bottom, A: initial, B: 1st day, C: 3rd day, D: 7th day, E: 14th day, a: between 2980-2880 cm⁻¹, b: between 2870-2820 cm⁻¹, c: between 1780-1700 cm⁻¹).

The predominant fatty acid was linoleic acid (81.98-83.9%) in the structure which is followed by oleic (5.03-5.35%), linoleic (4.98-4.76%), palmitic (2.63-2.80%), stearic (1.99-2.13%), tetracosanoic acid (0.42-1.42%) unsaturated eicosanoic acid (0.61-0.63%), saturated eicosanoic acid (0.31-0.40%), arachidonic acid (0.35-0.20%), and lastly Dihimo-gamma-linolenic acid (0.08-0.09%) in a descending order. The predominant fatty acid of the pomegranate seed oil was also emphasized to be linolenic in the literature (Carvalho Filho, 2014; Khajebishak et al., 2019; Vroegrijk et al., 2011). Moreover, the pomegranate seeds are addressed as the main source of linolenic acid, highly associated with novel functions for their cytotoxic and antitumor properties (Nagao and Yanagita, 2005). rich phytochemical composition and high linolenic acid content, especially the conjugated α -linolenic acid, creates a functionality to the pomegranate seed and its products when consumed. The total linolenic content of Turkish and Anatolian cultivars of pomegranate fruits seed oil was measured as; 86.53% (Keskin Çavdar et al., 2017), 74.11% (Kıralan et al., 2009), 71.5% (Abolfazl Fadavi et al., 2006), 78.23% (Khoddami et al., 2014). The present study suggests similar findings on the linolenic acid with Keskin Çavdar et al. (2017), and a little higher

than cited even though the cultivar used were the same. However, as mentioned previously by Kıralan et al. (2009), the genotype, location, climatic conditions, and harvest maturity alter the oil content and fatty acid composition.

FTIR results are illustrated in Figure 3. The spectral regions were selected to determine the fingerprint regions according to the previous literature findings (Quiñones-Islas et al., 2013). Our FTIR results are well-resolved to determine the corresponding regions of the functional groups of the pomegranate seed oil samples. We can observe the dominating peaks at 3016, 2925, 2854, 1742, 1457, 1419, 1377, 1236, 1156, 1113, 987, 937, 759, and 658 cm⁻¹. The FTIR literature and standards illustrate that region between 3016 and 2854 cm⁻¹ are due to the bands of CH₂ stretching vibrations. The high value of this band is an indicator of polyunsaturated acyl groups (Ozen and Mauer, 2002; Quiñones-Islas et al., 2013). Previous oil studies show that linseed oil has the highest frequency in polyunsaturated acyl groups, whereas olive oil near 3005, rapeseed oil around 3007, and corn oil at 3008 cm⁻¹ (Guillén et al., 2003). The frequency of the pomegranate seed oil during the 14 days of storage is higher than that of corn oil, which illustrates the richness of polyunsaturated acyl groups. Another two significant frequencies are valid at 1744 cm⁻¹ and 1457, 1419

and 1377 cm^{-1} where the former indicates the C=O stretching vibrations of aldehydes and ketones and the latter are associated with C-O stretching vibrations, respectively (Ozen and Mauer, 2002; Quiñones-Islas et al., 2013). The very strong bands at 2924 and 2853 cm^{-1} are associated, respectively, with the asymmetric and symmetric stretching of the aliphatic CH_2 functional group. The very strong band at 1743 cm^{-1} is related to an ester carbonyl bond associated with triglycerides. The medium band at 1467 cm^{-1} is related to the aliphatic stretching of the CH_2 and CH_3 functional groups. The medium bands at 1239 and 1164 cm^{-1} are associated with ester stretching and CH_2 bending vibrations. The medium band at 1097 cm^{-1} is associated with ester stretching, while the medium band at 723 cm^{-1} is associated with a CH_2 rocking vibration and cis-disubstituted olefins.

In the focused part of Figure 3 (a), the absorbance value of oils was decreased over time due to the production of peroxides between 2980-2880 cm^{-1} . Gedikoğlu et al. (2021) examined the lipid oxidation in ground beef meatballs by FTIR spectra. They found that the absorbance decreased over time for the same band region. Figure 3 (b) displays the FTIR spectra of pomegranate seed oil between 2870-2820 cm^{-1} . The absorbance of the band decreased during the accelerated storage conditions. Valdés et al. (2015) monitored the oxidative stability of different processed almonds, and they found a decrease in absorbance for fried almond spectra during aging. FTIR spectra of the oil between 1780 and 1700 cm^{-1} were presented in Figure 3 (c). The results showed a slight widening in the band from day 1 to 14. The shift in this band is related to the production of some oxidation products (Beltrán Sanahuja et al., 2009).

Conclusion

In this study, pomegranate seed oil was extracted according to the cold solvent extraction protocol. The oil samples were investigated for some characteristic features such as; colour, oxidation, fatty acid composition, and component analysis for the 14 days of shelf-life.

The obtained results showed the suitability of the investigated methods to be used in food analysis in order to monitor the oxidative stability of oils. Besides, similar results could be obtained using both GC and FTIR data. FTIR analysis requires less time to get results that could be used in order to examine the oxidative stability of oils.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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