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RESEARCH ARTICLE — OPEN ACCESS

Oil content, lipid profiling and oxidative stability of "Sefri" Moroccan pomegranate (*Punica granatum* L.) seed oil[☆]

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Abstract – The aim of this study was to determine the chemical composition (fatty acids, tocopherols, and sterols) and evaluate the oxidative stability of Moroccan pomegranate (*Punica granatum* L.) seed oil. The oil content of pomegranate seed was 22.63 g/100g of dry weight. The fatty acid composition showed a dominance of conjugated linolenic acids (CLnAs) (86.96 g/100g). The most dominant fatty acid was punicic acid (75.1 g/100g), followed by catalpic acid (6.7 g/100g) and linoleic acid with amounts of 4.11 g/100g. The seed oil only contained a low level of saturated fatty acids with palmitic (2.64 g/100g) and stearic acids (1.73 g/100g) as main saturated fatty acids. The sterol marker, β-sitosterol, accounted for 404.59 mg/100g of the total sterol content in the seed oil. Total tocopherol content in seed oil was 332.44 mg/100g. γ-tocopherol (190.47 mg/100g oil) is the major constituent, followed by α-tocopherol (74.62 mg/100g oil) and δ-tocopherol (53.3 mg/100g oil). The induction time calculated by the Rancimat accelerated method was found to be of 3.6 h at 120 °C. In terms of oil, pomegranate seed oil may be considered as a valuable source for new multipurpose products with industrial, cosmetic and pharmaceutical uses.

Keywords: Fatty acids / oxidation / *Punica granatum* L. / seed oil / sterols / tocopherols

Résumé – Teneur en huile, profil lipidique et stabilité oxydative de l'huile végétale du grenadier marocain "Sefri" (*Punica granatum* L.). Cette étude a pour but de déterminer la composition en acides gras, tocophérols et stérols, et d'évaluer la stabilité oxydative de l'huile végétale du grenadier marocain (*Punica granatum* L.). Nos résultats montrent que la teneur en huile est de 22,63 g/100g de graines sèches. L'analyse de la composition en acides gras a montré une dominance des acides linoléniques conjugués (86,96 g/100g). En effet, l'acide gras le plus dominant était l'acide punicique (75,1 g/100g), suivi de l'acide catalpique (6,7 g/100g) et de l'acide linoléique (4,11 g/100g). Cependant, l'huile du grenadier contient une faible teneur en acides gras saturés (acides palmitique (2,64 g/100g) et stéarique (1,73 g/100g). L'analyse de la composition en stérols a montré une dominance du β-sitostérol (404,59 mg/100g d'huile). Également, la teneur totale en tocophérol était de 332,44 mg/100g d'huile, avec le γ-tocophérol (190,47 mg/100g d'huile) comme composé majoritaire, suivi de l'α-tocophérol (74,62 mg/100g d'huile) et du δ-tocophérol (53,3 mg/100g d'huile). Du point de vue stabilité oxydative, le temps d'induction calculé par la méthode accélérée Rancimat est de 3,6 h à 120 °C. En termes d'huile, les graines du grenadier peuvent être considérées comme une source précieuse pour de nouveaux produits à usage industriel, cosmétique et pharmaceutique.

Mots clés : Acides gras / huile des graines / oxydation / *Punica granatum* L. / stérols / tocophérols

[☆] Contribution to the Topical Issue "Minor oils from atypical plant sources / Huiles mineures de sources végétales atypiques".
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1 Introduction

Pomegranate (*Punica granatum* L., Punicaceae) is an ancient, beloved plant and delicious fruit consumed worldwide (Jing *et al.*, 2012). They are widely grown in the Mediterranean regions and India, but sparsely cultivated in the USA, China, Japan and Russia (Fadavi *et al.*, 2006). The edible parts of pomegranate fruits are consumed fresh and they are also used for the preparation of fresh juice (Fadavi *et al.*, 2006). The fruits contain considerable amounts of seeds (40–100 g/kg fruit weight) (Syed *et al.*, 2007), that are usually waste products from the fruit processing. Pomegranate seeds a rich source of lipids, which approximately 12 to 20% of total seeds weight, varies according to cultivars (Ky`ralan *et al.*, 2009). Many studies have indicated the pharmaceutical importance of pomegranate seed oil (PSO). For instance, they have been reported to promote epidermal tissue regeneration, boost the immune system *in vivo*, reduce the accumulation of hepatic triglycerides and display chemopreventive activity against hormone-related (prostate and breast) and colon cancers (Melo *et al.*, 2014).

Pomegranate seed oil (PSO) contains predominantly unsaturated fatty acids including oleic, linoleic and in particular, high levels of conjugated linolenic acids (CLnAs), also known as trienoic acids (Lansky and Newman, 2007; Vroegrijk *et al.*, 2011; Melo *et al.*, 2014). The specific trienoic fatty acid found in pomegranate seed oil is referred to as punicic acid, which is a polyunsaturated fatty acid (18:3 n-5), also called trichosanic acid, cis 9, trans 11, cis 13. Punicic acid is referred as a "super CLnA" whose effect is even more potent than that of an ordinary CLnA (Melo *et al.*, 2014; Aruna *et al.*, 2016). It possesses a wide array of biological properties including antidiabetic (Arao *et al.*, 2004a; Arao *et al.*, 2004b), anti-inflammatory (Boussetta *et al.*, 2009), hypolipidemic (Koba *et al.*, 2007a), and anticarcinogenic activity against various forms of cancer (Tsuzuki *et al.*, 2004).

Currently, pomegranate plant is produced throughout the world in tropical and sub-tropical areas. The Mediterranean countries, India, Iran and Californian are considered as the main producers. Argentina, Brazil, Peru, Chile and South Africa are the other important producer countries (Kahramanoglu and Usanmaz, 2016). In 2014, the total production of pomegranate in the world was estimated to be around three million tons. Morocco is one of the biggest producers, the cultivation of pomegranate covers an area of about 4000 ha which gives an estimated production of 46 000 T, an average yield of 12 T/ha (Haddioui, 2012).

Many studies indicate a quantitative and qualitative difference in the chemical composition of pomegranate species oil, growing in different regions. To our knowledge, no study has been done to analyse Moroccan pomegranate seed oil. The aim of this study is to determine the lipid profile and evaluate the oxidative stability of the pomegranate seed oil growing in Morocco.

2 Material and methods

2.1 Sample collection

Pomegranate seeds and press oil samples, from “Sefri” Moroccan variety, analysed to determine the chemical composition and oxidative stability, were collected and prepared in 2017 from Flora cooperative (Boujad city, Morocco, 32°46’10”N 6°23’49”W). The annual average temperature in this region was 29 °C, while the annual average precipitation recorded between October and April was 1 mm.

2.2 Reagents

Petroleum ether (40–60°) was of analytical grade (> 98%; Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol and tocotrienol standard compounds were purchased from Cal Biochem (Darm-stadt, Germany). Sterol standard compounds were obtained from Aldrich (Munich, Germany).

2.3 Oil content

The oil content was determined according to the method ISO standard (ISO 659, 1998). About 2 g of the seeds were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for 6 h. The solvent was removed by a rotary evaporator at 40 °C and 25 Torr. The oil was dried by a stream of nitrogen and stored at −20 °C until used.

2.4 Fatty acid composition

The fatty acid composition was determined following the ISO standard (ISO 12966-2, 2017). Fatty acids (FAs) were converted into fatty acid methyl esters (FAMEs) by shaking a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2 N methanolic potassium hydroxide for 25 min. The fatty acid composition was determined as their corresponding methyl esters by gas chromatography (Varian 5890) coupled with a flame ionization detector (GC-FID). The capillary column CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm) was used. The carrier gas was helium and the total gas flow rate was 1 mL/min. The temperature program was as follows: from 155 °C; heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μl. The peak areas were computed by the integration software, and percentages of fatty acid methyl esters (FAME) were obtained as weight percentage by direct internal normalization method.

2.5 Tocopherol composition

For determination of tocopherols, a solution of 250 mg of press oil in 25 mL of n-heptane was directly used for the HPLC as descripted in Hajib *et al.*, (2018) work. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The samples in the amount of 20 μl were injected by a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt,

Table 1. Comparison of oil yield (g/100g) of "Sefri" Moroccan pomegranate seed oil with literature.

Pomegranate	Morocco (our study)	Turkey (Ky`ralan *et al.*, 2009)	Tunisia (Elfalleh *et al.*, 2011)	China (Jing *et al.*, 2012)	Spain (Fernandes *et al.*, 2015)	Iran (Fadavi *et al.*, 2006)
Oil content	22.63 ± 0.54	13.95–24.13	5.89–21.58	11.4–14.9	4.44–13.7	6.63–19.3

Values are given as means of three replicates ± SD.

Germany) used with a flow rate of 1.3 mL/min. The mobile phase used was n-heptane/tert-butyl methyl ether (99:1, v/v).

2.6 Sterol composition

The sterol composition of the press oil was determined following the ISO standard (ISO 12228-1, 2014). In brief, 250 mg of press oil was saponified with a solution of ethanolic potassium hydroxide (2 N) by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminum oxide column (Merck, Darmstadt, Germany) on which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatography (Merck, Darmstadt, Germany), re-extracted from the TLC material, and afterward, the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on a SE 54 CB (Macherey-Nagel, Düren, Germany; 50 m long, 0.32 mm ID, 0.25 µm film thickness). Further parameters were as follows: helium (1 mL/min) as a carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 245 °C to 260 °C at 5 °C/min. Peaks were identified either by standard compounds (β-sitosterol, campesterol, stigmasterol) by a mixture of sterols isolated from rapeseed oil (brassicasterol) or by a mixture of sterols isolated from sunflower oil (Δ-7-avenasterol, Δ-7-stigmasterol, and Δ-7-campesterol). All other sterols were identified by GC-MS for the first time and afterwards by comparison of the retention time.

2.7 Oxidative stability

The oxidative stability of the press oil was determined by the Rancimat method, according to Gharby *et al.*, (2012) work. All experiments were carried out with a 743 Rancimat (Methrom AG, Herisau, Switzerland). In brief, 3.6 g press oil were weighed into the reaction vessel, which was placed into the heating block kept at 120 °C. Air flow was set at 20 L/h for all determinations. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL of distilled water. The conductivity of this solution was measured and recorded. The resulting curves were evaluated automatically by the software of the Rancimat. All determinations were carried out in triplicate.

3 Results and discussion

3.1 Oil content

The oil content of pomegranate seed oil from Morocco was 22.63 ± 0.54 g/100g (Tab. 1), which is comparable with that from Turkey (13.95–24.13 g/100g) and Tunisia (5.89–21.58 g/100g), as reported by Ky`ralan *et al.*, (2009) and Elfalleh *et al.*, (2011), respectively. Jing *et al.*, (2012) found less oil in the pomegranate seeds from China (11.4–14.9 g/100g). The Pomegranate seeds investigated by Fernandes *et al.*, (2015) (from Spain) and Fadavi *et al.*, (2006) (from Iran) also contained lower amounts (4.44–13.7 g/100g and 6.63–19.3 g/100g, respectively) of oil. According to Taoufik *et al.*, (2015), this variation can be due to the extraction method, cultivars, climatic factors and environmental conditions.

3.2 Fatty acid composition

The fatty acid profiles of cold pressed pomegranate (*Punica granatum* L.) seed oil determined by GC are shown in Table 2. The saturated fatty acid (SFA) represented only 4.74 g/100g, palmitic acid (C16:0) was the predominant SFA with 2.64 g/100g followed by stearic acid (C18:0) 1.73 g/100g and arachidic acid (C20:0) 0.37 g/100g. The content of UFA was 95.13 g/100g, in which only 4.06 g/100g was monounsaturated fatty acids (MUFA) and 91.07 g/100g was PUFA. Major PUFA was punicic acid (C18:3 (c9, t11, c13)) which accounted for 75.1 g/100g, followed by catalpic acid (C18:3 (t9, t11, c13)) (6.7 g/100g) and linoleic acid (C18:2) with amounts of 4.11 g/100g. Four conjugated linolenic acids (CLnA) were identified, as different geometric isomers of conjugated linolenic acid, namely punicic acid (C18:3 (c9, t11, c13)), which is the major isomer (75.1 g/100g), followed by catalpic acid (C18:3 (t9, t11, c13)) (6.7 g/100g), α-eleostearic acid (C18:3 (c9, t11, t13)) (3.73 g/100g), and β-eleostearic acid (C18:3 (t9, t11, t13)) (1.43 g/100g). PSO fatty acid composition was found to be in the range of previously published values for pomegranate seed oil from other countries reported in the literature (Fadavi *et al.*, 2006; Sassano *et al.*, 2009; Elfalleh *et al.*, 2011; Hernandez *et al.*, 2011; Jing *et al.*, 2012).

Punicic acid was first isolated from pomegranate seed oil by Toyama and Tsuchiya, (1935). This fact was later confirmed by a reinvestigation of the oil by Farmer and Van den Heuvel (1936) and also by other authors (Ahlers *et al.*, 1954). According to Sassano *et al.*, (2009) in nature, CLnAs are not found to any great extent in animal fat, but they are found in many seed oils as either C18 trienes or C18 tetraenes. The most commonly CLnAs known isomers found in seed oils from important plants are α-eleostearic acid (*Vernicia fordii*), punicic acid (*Punica granatum* L.), calendic acid (*Calendula officinalis* L.), jacaric acid (*Jacaranda mimosifolia*), catalpic acid (*Catalpa ovata*), β-calendic (*Calendula officinalis* L.) and α-parinaric acid (*Parinarium laurinum*) (Sassano *et al.*, 2009; Melo *et al.*, 2014). In general, CLnAs are synthesized from linoleic acid through a specific conjugase enzyme, but they

Table 2. Comparison of fatty acid composition (g/100g) of “Sefri” Moroccan pomegranate seed oil with literature.

Compound	Our results (g/100g oil)	(Jing *et al.*, 2012)	(Fadavi *et al.*, 2006)	(Sassano *et al.*, 2009)	(Hernandez *et al.*, 2011)	(Elfalleh *et al.*, 2011)
Miristic acid (C14:0)	–	–	0.1–4.7	–	–	Tr-0.85
Palmitic acid (C16:0)	2.64 ± 0.24	2.82–3.61	2.8–16.7	2.87–3.06	2.99–4.3	3.13–11.82
Stearic acid (C18:0)	1.73 ± 0.17	1.6–2.81	0.3–9.9	2.21–2.37	1.6–2.38	2.28–15.64
Oleic acid (C18:1)	3.64 ± 0.29	3.37–6.01	4.8–17.4	6.82–7.17	5.23–6.85	3.03–12.88
Linoleic acid (C18:2)	4.11 ± 0.57	3.74–5.13	0.7–24.4	6.46–6.84	4.98–8.54	3.57–13.92
Linolenic acid (C18:3)	–	7.43–11.71	–	–	–	–
Arachidic acid (C20:0)	0.37 ± 0.09	0.25–0.35	–	0.43–0.49	–	Tr-1.7
Gadoleic acid (C20:1)	–	–	–	–	–	0.6–9.94
Ecosoneic acid (C20:1)	0.42 ± 0.11		–	0.64–0.66	–	–
Behenic acid (C22:0)	–	–	0–3.9	–	–	–
Punicic acid (C18:3)	75.1 ± 1.62	73.45–78.80	55.8–86.6	64.9–71.76	80.41–91.03	12.45–55.45
α–Eleostearic acid (C18:3)	3.73 ± 0.47	–	–	4.6–6.15	–	8.63–22.53
Catalpic acid (C18:3)	6.7 ± 0.35	–	–	2.64–4.59	–	6.17–17.09
β–Eleostearic acid (C18:3)	1.43 ± 0.11	–	–	0.49–2.39	–	0–17.82
Lignoceric acid (C24:0)	–	–	–	0.04–0.51	–	0–8.1
Nervonic acid (C24:1)	–	–	–	0.15–0.24	–	–
SFA	4.74					
MUFA	4.06					
PUFA	91.07					
UFA	95.13					
CLnA	86.96					
Total	99.87					

Values are given as means of three replicates ± SD.

Table 3. Comparison of sterol composition (mg/100g) of “Sefri” Moroccan pomegranate seed oil with literature.

Compound	Our results (mg/100g)	(Fernandes *et al.*, 2015)	(Pande and Akoh, 2009)
Cholesterol	0.39 ± 0.01	–	–
Brassicasterol	–	–	0–2.2
Campesterol	38.18 ± 1.91	25.1–36.3	17.9–39.3
Stigmasterol	15.53 ± 1.28	11.8–17.0	27.8–46.3
β-Sitosterol	404.59 ± 4.72	220.1–354.2	243.5–338.3
Δ-5-Avenasterol	17.86 ± 0.29	–	–
Δ-7-Stigmasterol	2.67 ± 0.17	–	–
Sitostanol	–	14.8–25.9	–
Δ-7-Avenasterol	0.76 ± 0.08	–	–
Others	–	89.8–122.3	–

Values are given as means of three replicates ± SD.

may also be produced during the processing of vegetable oils, as a result of isomerization and dehydration of secondary oxidation products of linoleic and α-linolenic acids (Koba *et al.*, 2007b; Hennessy *et al.*, 2011).

3.3 Sterol composition

Sterols are very useful parameters for detecting adulterations or to check authenticity, since it can be considered as a fingerprint (Gharby *et al.*, 2017, 2018). Besides, their determination is of major interest due to their antioxidant activity and impact on health. Table 3 lists the sterol levels obtained from Moroccan pomegranate seed oil.

The total sterol contents of pomegranate seed oil were 494.61 mg/100g, which was found to be in the range of previously published values for pomegranate seed oil from other countries reported in the literature (408.9–620.5 mg/100g) (Kaufman and Wiesman, 2007). In pomegranate seed oil, β-sitosterol was also the most abundant sterol which constituted about 404.59 mg/100g. This sterol is also abundantly found in

Table 4. Comparison of tocopherol composition (mg/100g) of "Sefri" Moroccan pomegranate seed oil with literature.

Compounds	Our results (mg/100g oil)	(Fernandes *et al.*, 2015)	(Pande and Akoh, 2009)	(Jing *et al.*, 2012)
α-Tocopherol	74.62 ± 1.77	7.3–17.9	161.2–173.7	71.87–138.83
γ-Tocopherol	190.47 ± 3.52	123.0–449.7	80.2–92.8	3.42–5.49
Plastochromanol 8	10.56 ± 0.29	–	–	–
γ-Tocotrienol	3.49 ± 0.16	–	–	–
δ-Tocopherol	53.3 ± 1.22	4.9–15.2	20.3–23.8	141.42–351.32

Values are given as means of three replicates ± SD.

Table 5. Comparison of oxidative stability (h) of "Sefri" Moroccan pomegranate with argan and olive seed oils.

Oil	Pomegranate	Argan (Matthäus and Brühl, 2015)	Olive (Mateos *et al.*, 2006)
Induction time (h)	3.6 ± 0.93	6.1	5.5

Values are given as means of three replicates ± SD.

sesame, cactus and olive oil (Gharby *et al.*, 2012, 2017). Among the different sterols, β-sitosterol has been most intensively investigated with respect to its beneficial and physiological effects on human health. Besides, β-sitosterol lowers cholesterol levels, enhances immunity, and has anti-inflammatory, antipyretic and anti-carcinogenic effects (prostate essentially) (Gupta *et al.*, 1980; Villaseñor *et al.*, 2002). The next major component was campesterol where it reaches about 38.18 mg/100g of the total sterols. Δ5-avenasterol and stigmasterol accounted for about 17.86 and 15.53 mg/100g respectively in this oil. Minor sterols were also detected (Δ7-stigmasterol and cholesterol). The sterol content of pomegranate seed oil from Morocco was similar to that from Georgia and Spain, as reported by Pande and Akoh, (2009) and Fernandes *et al.*, (2015), respectively.

3.4 Tocopherol composition

In addition to the fatty acid composition and sterol profile, the composition of vitamin E active compounds is an important characteristic feature used to describe the identity of vegetable oils. These compounds have some nutritional importance because they are known to have an antioxidative activity, which protects the polyunsaturated fatty acids against oxidative deterioration; additionally, a biological activity exists, which protects cells against oxidative stress (Bieri and Evarts, 1974; Blumberg and Block, 1994; Tucker and Townsend, 2005).

The total tocopherol contents of pomegranate seed oil were 332.44 mg/100g, which is higher than that of argan (85 mg/100g), olive (22 mg/100g), sesame (44.6 mg/100g) and cactus (94.6 mg/100g) oils (Gharby *et al.*, 2017). Only α-, γ-, δ-tocopherols, P8 and γ-tocotrienol were present in pomegranate seed oil (Tab. 4). γ-tocopherol was the main component and represented about 190.47 mg/100g of total tocopherols, followed by α-tocopherol (74.62 mg/100g), δ-tocopherol (53.3 mg/100g), plastochromanol 8 (10.56 mg/100g) and γ-tocotrienol (3.49 mg/100g).

Our tocopherol profile was similar to the tocopherol composition reported by Fernandes *et al.*, (2015), who revealed that the major tocopherol in pomegranate seed oil is γ–tocopherol (123.0–449.7 mg/100g). However, this result contrasts with that reported by Pande and Akoh, (2009) study, where the α-tocopherol was the major tocopherol in pomegranate seeds oil (161.2–173.7 mg/100g). In addition, Jing *et al.*, (2012) found that the main tocopherol in pomegranate seed oil is the δ-tocopherol (141.42–351.32 mg/100g).

3.5 Oxidative stability

The preservation of edible or cosmetic oil is an important economic parameter (Matthäus *et al.*, 2010). In fact, oxidation of lipid is a major cause of deterioration in the quality of oils. It is the cause of important deteriorative changes in their chemical, sensory and nutritional properties (Gray, 1978; Frankel, 1980). The oxidative stability of pomegranate seed oil is expressed as the induction period determined by the Rancimat method at 120 °C. The induction time of pomegranate seed oil, as evaluated by the Rancimat accelerated method, was found to be 3.6 ± 0.93 h at 120 °C. At the same temperature, the Rancimat induction time is 6.1 and 5.5 h for argan and olive, respectively (Mateos *et al.*, 2006; Matthäus and Brühl, 2015). Therefore, the oxidation sensitivity of pomegranate seed oil, that is much higher than that of argan and olive oils, could be likely attributed to high content of CLnAs; molecules that do oxidize easily (Tab. 5).

4 Conclusion

The study of pomegranate (*Punica granatum* L.) seed oil growing in Morocco revealed high oil content, with conjugated linolenic acids (punicic acid, catalpic acid) as the predominant fatty acid, beside considerable amounts of tocopherols and sterols. This study shows also that Moroccan pomegranate seed oil is particularly sentitive to oxidation, which could be explain by the high content of CLnAs. Thus, special care such as refrigeration should be considered for oil prolonged storage. If enough precautions are taken, pomegranate seed oil deserve to find its place in the cosmetics and food industry, as a potent functional and/or nutraceutical ingredient.

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