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Lipid Contents and Fatty Acids Composition of Seed Oil from Twenty Five Pomegranates Varieties Grown in India

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Abstract: The fatty acids composition of the seed oils of 25 pomegranates varieties obtained from two different regions of India (*Punica granatum* L.) was qualitatively and quantitatively determined by gas chromatography. The seeds contained oil in the range of about 66.3-193 g/kg dry matter, a notably higher content than western pomegranate cultivars. Levels of lipid content probably could be considered insufficient for economic industrial exploitation except for medical usages and specific consumption. The pre-dominant fatty acid was linolenic acid (C18:3) and its content was about 31.8-86.6%, followed by linoleic acid (0.7-24.4%), oleic acid (0.4-17.4%), stearic acid (2.8-16.7%) and palmitic acid (0.3-9.9%). To a lesser extent, the saturated myristic acid (0.1-4.7%) and behenic acid (0.0-3.9%) were also found in some cultivars. We have not confirmed the presence of lauric, arachidic and lignoceric acids previously reported in edible and non-edible pomegranate cultivars. Intervarietal differences in fatty acid compositions were shown and they could be useful to establish chemotaxonomic differences.

Key words: Fatty acids, oilseed, pomegranate, *Punica granatum* L. and total lipids

INTRODUCTION

Punica granatum L., the pomegranate belongs to the Punicacea family (Harde *et al*., 1970) and is one of the oldest edible fruits. It has been cultivated extensively in Mediterranean countries, India and to some extent in the U.S. (California), China, Japan and Russia. India is a native land of the pomegranate which is grown in every area, both coastal and mountainous areas (Parashar *et al*., 2009a, b). The total pomegranate production in India was 665,000 tons in 2005.

The edible parts of pomegranate fruits are consumed fresh (Parashar *et al*., 2008a). They are also used in the preparation of fresh juice, canned beverages, jelly, jam and paste and for flavoring and coloring drinks, etc. (Ewaida, 1987; Hodgson, 1971; Nagy *et al*., 1990).

Pomegranate fruits contain considerable amounts of seeds, ranging between 40 and 100 g/kg of fruit weight depending on cultivar (Parashar *et al*., 2008d).

The seeds of diverse varieties are rich source of total lipids. Data on fatty acid composition in the seed oil of pomegranate also help to establish the chemotaxonomic relationships among the studied varieties. Vegetable oils nowadays are a great source of maintaining oil consumption in families and because of consumers concern with the saturated/unsaturated fatty acid ratio in the diet, the lipid composition of fruit and vegetable has lately received particular attention. Consumers are especially interested in essential fatty acids, with emphasis on the health potential of polyunsaturated fatty acids. It is considered that these fatty acids play a natural preventive role in cardiovascular disease and in alleviation of some other health problem, because they promote the reduction of both total and HDL cholesterol (Melgarejo and Artes, 2000). Also, pomegranate seed oil and extracts might be employed in menopausal women as external and internal phytoestrogen medicaments, as a possible alternative or supplement to conventional Hormone Replacement Therapy (HRT) (Lansky, 1999).

Little attention has been paid to the study of chemical composition, and particularly the fatty acid composition, of ripe pomegranate seed oils from fruit varieties growing in two Indiaian pomegranate research centers, Malagaon and Maharasta. El-shaarawy and Nahapetian (1983) showed that 8% of fatty acids of pomegranate seed oil were saturated, 10% monosaturated, 10% disaturated and approximately 70% conjugated acid, most probably punicic acid. El-Nemr *et al*. (1990) showed that 83.6% of the fatty acid was saturated and 16.3% unsaturated. Melgarejo *et al*. (1995) reported that (among six cultivars of Spain pomegranate) 30-33.8% of the fatty acids were saturated and 66.2–69.0% of the fatty acids were unsaturated. Melgarejo and Artes (2000) studied seven clones of pomegranate from another part of Spain and found that 4.16-26.65% of fatty acids of seed oils were saturated and 73.4-95.8% of fatty acid was unsaturated. The present study reports the total lipid content as well as the nature of the fatty acids of seed oils from 25 pomegranate cultivars which grow in two important regions of India. (Parashar *et al*., 2009a, b).

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MATERIALS AND METHODS

Samples: The seeds were obtained from mature fruits growing in Maharasta province (10 varieties) and Malagaon province (15 varieties) in India (2008) from the agricultural research centers of Maharasta and Malagaon, both cultivating specific varieties (more than 100). These two provinces (Maharasta and Malagaon) besides Khorasan and Fars, represent more than 25% of the total production among 28 provinces. Commercially ripe fresh fruits were harvested during September and November from different mature trees randomly selected to represent the population of the plantation. Fruits were transported by a ventilated car to the laboratory, where pomegranates with defects (sunburns, cracks, cuts and bruises in husk) were discarded (Parashar *et al*., 2008b, c). Physical and chemical parameters at harvest (fruit, seed, skin weights, and titratable acidity, total solid soluble in juice) were analyzed (data are not shown) to characterize the pomegranates. (Parashar *et al*., 2009a, b).

Approximately 2 kg (n = 10) of pomegranates at harvesting maturity was sampled for each variety, from which varietal composites were prepared. The sweet varieties were Bedana (1), Kandhari (2), Alandi (3), Dholka (4), Kabul (5), Vadki (6), Muscat Red (7) and Paper shell (8). The sour sweet varieties were: Poona (9), Ruby (10), and Vellodu (11). The sour varieties were Muscat White (12), Wonderful (13), Mridula (14), Beemo (15), Safee (16), Gorche (17), Malase (18), Porbarij (19), Khoram (20), Mesri (21), Pust Torsh (22), Semnan (23), Mamoli (24) and Alandie (25).

Extraction of seed oils: Three individual 10 g samples of crushed dry seeds of each pomegranate variety were refluxed with 300mL of petroleum benzene in weighed flasks using a Soxhlet apparatus according to the AACC (1987) method. The oils were recovered by distilling the solvent in a rotary evaporator at 45ºC, then dried to constant weight in a vacuum oven at 90ºC for 1 h and weighed.

Fatty acid analysis: Fatty Acid Methyl Esters (FAMEs) were prepared, following the procedure described by AOAC (1990). Three aliquots of 0.2 g lipid extract for each pomegranate variety were esterified with 10mL methanolic NaOH solution by refluxing for 10 min at 85ºC. After addition of internal standard (0.1 mL of 2 g/L, C17:0) and 4.4 mL of BF_3- etherate, the samples were boiled for 2 min. The FAMEs were extracted from a salt saturated mixture with hexane (3.0 mL). For drying FAMEs, anhydride Na_2SO_4 was added and centrifuged (7 min, 2500 rpm, Kubota, 6900 Japan), then the upper part was poured in specific cell.The esters were separated by GC (Unicam Pu 4550, UK) fitted with a capillary column (FFAP, 25 m, 0.25 mm i.d., 0.22 mm film thickness). Helium was used as carrier gas at inlet pressure of 1.2 kg/cm^2. The temperatures of injection port and detector (FID) were maintained at 200 and 240ºC and the temperature programming for the column was applied as follows, 170ºC (4 min), then to 180ºC at 3ºC/min, then to 190ºC at 1ºC/min (25 min). The peaks were identified based on their retention times using authentic standard fatty acids methyl esters and all samples were run in duplicate.

Materials: All solvent/chemicals used were of analytical grade and obtained from Sigma (MO, USA) and Merck (Germany).

Statistical analysis: The statistical examination of the data was performed using MSTATC software package. Mean percentages of each fatty acid in different varieties were compared using the t-test.

RESULTS AND DISCUSSION

The amount of total lipid on dry matter ranged between 66.3 and 193 g/kg in all varieties, but in sweet varieties lipid content was between 66.3 and 134 g/kg, whereas in sour sweet varieties, it was between 124 and 193 g/kg and in sour varieties, between 112 and 176 g/kg (Table 1). The observation on sour and sweet varieties is in agreement with results obtained by Melgarejo *et al*. (1995). Present results on lipid content of sour sweet varieties are in contrast with those reported by Melgarejo *et al*. (1995). Present results on lipid content of sweet varieties are in contrast with those reported by El-shaarawy and Nahapetian (1983). They found a higher lipid content (161g/kg) in a sweet variety grown in India. El-Nemr *et al*. (1990) reported a high level of lipid content (272 g/kg) in an unidentified Egyptian variety which contradicts our results. Our results show that lipid contents of different varieties are as follows: sweetosourosour sweet. Further, an ornamental non-edible sour variety (Nana cv) was shown by Tsuyuki *et al*. (1981) to contain 78 g kg^{-1} of total lipid. Thus it can be seen that lipid content of different pomegranate varieties seed oil can differ from some previously studied varieties. Finally, these lipid contents of pomegranate seeds could probably be considered insufficient for economic industrial exploitation compared with those of conventional oilseeds (Al-Maiman and Dilshad, 2002). Seven fatty acids were identified in seed oils of 25 pomegranates varieties. Individual percentages of each fatty acid are given in Table 1. Linolenic acid was determined to be the pre-dominant fatty acid in 25 pomegranates varieties. Its amounts ranged between 31.8 and 86.6%. Linoleic acid was determined to be the second most abundant in these samples. Percent of linoleic acid was between 0.7 and 24.4. Oleic, stearic, and palmitic acids were determined in all pomegranate varieties. Their amounts ranged between 0.4-17.4, 2.8-16.7 and 0.3-9.9%, respectively. To a lesser extent, the saturated myristic (0.1-4.7%) and behenic acid (0.0-3.9%) were also found in these samples.

Table 1: Lipid 1 content (g/kg dry matter) and fatty acid composition2 of oil (fatty esters as % (w/w) total fatty acid esters) from seeds of 25 varieties of pomegranate

No.	Varieties	Lipids	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C22:0	ϵ Sat.	ϵ Unsat.	Sat./Unsat.
Sweet Ruby(10), and Vellodu (11). The sour varieties were Muscat White(12), Wonderful(13), Mridula(14), Beemo(15), Safee(16), Gorche (17), Malase (18), Porbarij (19), Khoram (20), Mesri (21),Pust Torsh (22), Semnan (23), Mamoli (24) Alandie (25)												
1	Bedana	82.0±2.5	0.1±0.01	6.1±1.0	0.6±0.1	12.7±1.2	12.0±0.5	68.5±2.5	ND	6.8±1.0	93.2±2.8	0.07±0.01
2	Kandhari	123±1.5	0.3±0.02	5.3±0.8	2.9±0.0	10.4±0.9	5.4±0.4	75.7±3.5	ND	8.5±0.8	91.5±3.6	0.09±0.01
3	Alandi	66.3±1.8	1.2±0.01	6.0±0.5	2.7±0.2	6.3±0.5	0.7±0.2	83.1±3.7	ND	9.9±0.5	90.1±3.7	0.11±0.01
4	Dholka	84.3±3.0	3.4±0.04	2.8±0.2	1.1±0.1	9.0±2.0	10.4±0.8	69.4±2.0	3.9	11.2±0.2	88.8±2.9	0.13±0.00
5	Kabul	134±2.8	0.2±0.00	12.3±0.3	0.8±0.1	16.2±2.1	14.6±1.2	55.8±2.1	ND	13.3±0.3	86.7±3.2	0.15±0.01
6	Vadki	72.3±1.1	0.4±0.05	5.2±0.5	2.4±0.3	10.3±1.5	10.9±1.5	70.8±1.9	ND	8.0±0.6	92.0±2.8	0.09±0.01
7	Muscat Red	95.0±0.9	0.3±0.02	5.1±0.4	2.0±0.4	9.0±1.0	8.9±0.9	74.6±1.3	ND	7.4±0.6	92.6±1.9	0.08±0.01
8	Paper shell	122±2.4	0.8±0.03	5.5±0.3	2.2±0.1	10.1±0.8	9.8±1.0	71.6±0.9	ND	8.5±0.3	91.5±1.6	0.10±0.00
	Average	97.4±25.6a	0.8±1.0a	6.0±2.±a	1.8±0.9a	10.5±2.9a	9.1±4.3a	71.2±7.7a	0.5a	9.2±2.2a	90.8±2.1a	0.10±0.03a
Sour sweet												
9	Poona	127±3.1	0.5±0.06	5.9±0.4	5.7±0.5	6.9±0.6	9.0±0.5	72.0±1.0	0.03	12.1±0.6	87.9±1.3	0.10±0.01
10	Ruby	124±2.2	0.8±0.01	5.3±0.2	0.3±0.1	1.5±0.	8.2±0.5	84.0±1.5	ND	6.4±0.2	93.6±1.7	0.08±0.00
11	Vellodu	193±1.5	1.0±0.05	7.5±0.4	0.7±0.1	10.7±1.0	13.8±2.0	66.3±0.8	ND	9.2±0.4	90.8±2.4	0.10±0.01
	Average	148±29.0b	0.76±0.3a	6.2±1.1a	2.2±3.0a	6.4±4.6a	10.3±3.0a	74.1±9.0a	0.01a	9.2±2.9a	90.8±2.8a	0.10±0.01a
Sour												
12	Wonderful	176±4.2	0.1±0.02	5.8±0.7	3.2±0.5	10.1±1.2	9.4±1.3	70.8±0.5	0.7	9.8±0.9	90.2±1.8	0.11±0.01
13	Mridula	122±1.2	0.8±0.02	4.8±0.6	0.5±0.2	4.8±0.4	4.7±0.2	84.5±1.6	ND	6.1±0.6	93.9±1.7	0.06±0.01
14	Beemo	107±1.3	0.3±0.01	6.0±0.5	2.1±0.1	10.5±0.5	10.4±0.5	70.7±1.5	ND	8.4±0.5	91.6±1.7	0.09±0.01
15	Beemo	151±2.7	0.4±0.04	4.9±0.8	2.3±0.2	8.1±0.6	8.8±1.0	75.5±2.1	ND	7.6±0.8	92.4±2.4	0.08±0.01
16	Safee	127±2.0	4.7±0.08	4.0±0.3	0.3±0.07	11.7±0.3	13.2±1.2	65.6±2.3	0.5	9.5±0.3	90.5±2.6	0.10±0.00
17	Gorche	112±1.3	0.2±0.04	16.7±1.2	9.9±0.6	17.4±2.5	24.4±1.5	31.8±2.4	ND	26.8±1.3	73.2±3.8	0.37±0.03
18	Malase	126±1.5	0.3±0.06	6.1±1.0	3.9±0.3	16.1±1.7	21.0±1.5	52.4±3.5	0.2	10.5±1.0	89.5±4.2	0.12±0.01
19	Porbarij	135±2.6	0.5±0.02	4.6±0.8	0.6±0.1	0.4±0.1	7.3±0.9	86.6±1.0	ND	5.7±0.8	94.3±1.3	0.06±0.01
20	Khoram	153±3.2	0.4±0.01	4.0±0.2	0.3±0.04	4.6±0.2	8.3±0.5	82.3±4.1	0.1	4.8±0.2	95.2±4.1	0.05±0.00
21	Mesri	152±2.4	0.6±0.05	4.5±0.3	1.6±0.2	8.7±1.1	7.7±0.2	76.8±3.2	ND	6.7±0.4	93.3±3.4	0.07±0.00
22	Pust Torsh	134±2.1	0.2±0.03	6.0±0.3	0.5±0.05	11.3±1.4	11.8±1.0	70.0±1.5	0.1	6.8±0.3	93.2±2.3	0.07±0.00
23	Semnan	142±1.8	0.1±0.00	3.8±0.5	1.7±0.3	7.6±0.8	8.0±0.5	78.8±1.1	ND	5.6±0.6	94.4±1.5	0.06±0.01
24	Mamoli	111±1.5	0.8±0.07	5.2±0.6	2.7±0.6	8.8±0.6	9.5±0.3	73.1±1.0	ND	8.7±0.9	91.3±1.2	0.09±0.01
25	Alandie	125±3.0	0.8±0.05	3.7±0.4	0.4±0.03	6.4±0.5	6.1±0.7	82.6±2.0	ND	4.9±0.4	95.1±2.2	0.05±0.00
	Average	134±19.4b	0.7±1.5a	5.7±4.1a	2.1±3.1a	9.0±5.6a	10.8±6.9a	71.5±17.9a	0.1a	8.7±6.6a	91.3±6.8a	0.09±0.01a

a,b means in a column with the same letter are not significantly different, $p<0.05$.

1,2 Values are average of three individual samples each analyzed in duplicate, ± standard deviation.

It has been confirmed that the unsaturated fatty acids were pre-dominant in all varieties (Parashar *et al.*, 2009a, b). However, linolenic acid was the main fatty acid in the present study. We could not confirm caprylic and stearic acids as the major fatty acids reported in a sweet Egyptian pomegranate cultivar (El-Nemr *et al.*, 1990). Also, our results did not confirm the presence of caproic, capric, myristoleic acids as reported by El-Nemr *et al.* (1990). Neither could we confirm the presence of C12:1, C15:0, C14:1, C20:1, C20:2 and C22:1 found by Tsuyuki *et al.* (1981) in the ornamental Nana pomegranate. However, our results confirm the presence of linolenic (C18:3) and linoleic (C18:2) acid in all seed oils of pomegranates as previously reported (Melgarejo and Artes, 2000). The saturated/unsaturated acid ratio was generally very low (Table 1). The lowest and highest are 0.05 (varieties No. 20 and 25) and 0.37 (variety No. 17), respectively. These ratios were the same as previously reported (Melgarejo and Artes, 2000) and considerably lower than those reported by these researchers earlier (Melgarejo *et al.*, 1995).

CONCLUSION

Based on our study, the seeds of all 25 varieties so far had similar (but not identical) fatty acid compositions and contained low amounts of saturated fatty acids (except for variety Gorche (No. 17). Intervarietal differences in fatty acids composition were found and they can be used to establish chemotaxonomic differences, which have also been shown in some other species (Melgarejo *et al.*, 1995; Sundar and Sino, 1992). The lowest saturated/unsaturated acid ratio was found in varieties No. 20 and 25, whilst variety No. 17 showed the highest (0.37). These ratios were considerably lower than those reported by Melgarejo *et al.* (1995), because saturated lipids were lower and unsaturated acids were higher in the present study. The saturated/unsaturated acid ratios were in strong contrast with those (5.1) reported earlier (El-Nemr *et al.*, 1990).

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