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SUMMARY – Pomegranate seeds are mainly consumed fresh, although in recent years it is used as an ingredient for jam, soft and alcoholic drinks, etc. New applications of pomegranate seeds for fresh consumption and possible industrial use of oil seeds should be sought. Consumers are particularly concerned with the saturated to unsaturated fatty acid ratio, as well as essential fatty acids (linoleic, linolenic and arachidic), with special emphasis on polyunsaturated fatty acids. The main aim of the work was to determine the total lipid content and the fatty acid composition of three commercial pomegranate cultivars, grown under homogeneous conditions. In the seed oils of all varieties studied, only five fatty acids have been identified and the profile of the fatty acid composition was quite similar. The highest levels were those of polyunsaturated fatty acids, with about 90%, and between 60-80% were $C_{18:3}$ fatty acids with double bonds in position 9, 11 and 13, called punicic acids.

Key words: Pomegranate, lipids, fatty acid, punicic acids.

RESUME – *"Composition en acides gras et teneur totale en lipides de l'huile des graines de trois cultivars commerciaux de grenades". Les graines de grenade sont principalement consommées en frais, bien que lors des dernières années elles soient utilisées comme ingrédient pour la confiture, pour des boissons rafraîchissantes et alcoolisées, etc. Il faudrait rechercher de nouvelles applications pour les graines de grenade pour une consommation en frais et une utilisation industrielle possible des graines oléagineuses. Les consommateurs se préoccupent en particulier du ratio acides gras saturés et insaturés, ainsi que des acides gras essentiels (linoléique, linolénique et arachidique), avec une attention spéciale portée aux acides gras polyinsaturés. Le principal objectif de ce travail était de déterminer la teneur totale en lipides et la composition en acides gras de trois cultivars commerciaux de grenades, cultivés en conditions homogènes. Dans les huiles des graines de toutes les variétés étudiées, uniquement cinq acides gras ont été identifiés et le profil de la composition en acides gras a été tout à fait semblable. Les plus hauts niveaux ont été ceux des acides gras polyinsaturés, avec environ 90%, et les acides gras $C_{18:3}$ avec doubles liaisons en position 9, 11 et 13, appelés acides puniques, représentaient entre 60-80%.*

Mots-clés : *Grenade, lipides, acides gras, acides puniques.*

Introduction

The pomegranate is a fruit that is mainly consumed fresh, although lately it is being used in the preparation of soft drinks, alcoholic drinks and jams, etc. However the pomegranate is not widely used. New forms should be sought in order to present the consumer with pomegranate seeds to be consumed fresh as well as their oil.

The pomegranate is a fruit rich in seeds, the percentage of which range from 50 to 70% of the rest of the fruit (carpellary membranes + rind). Of this percentage, from 5% to 15% corresponds to the woody part of the fruit. Hereafter, the term seed will refer to the woody part (rich in fibre and fat) and will not include the juicier part (aril).

Studies carried out on the seed content of the pomegranate show average contents of about 37-143 g/kg of fruit (Melgarejo *et al.* 1995).

The seeds of some pomegranate cultivars are rich in lipids, which vary between 140-270 g/kg DM (El-Shaarawy and Nahapetian,1983; El-Nemr *et al.*, 1990; Melgarejo and Martínez, 1992). One quality

parameter for the consumer of today is the fat content and fatty acid composition and in particular, the saturated fatty acid/unsaturated fatty acid ratio. The fat composition in fruit and vegetables in recent years has had a great repercussion, particularly on the essential fatty acids (linoleic, linolenic and arachidonic) with greater emphasis on the polyunsaturates. This is because they play a very important role in the prevention of cardiovascular disease and other heart problems, as polyunsaturated fatty acids reduce the levels of HDL cholesterol (De Hoya and Mata, 1989).

It is important to know the fatty acid composition of the pomegranate seeds as this may help us to establish chemical and taxonomic relationships between the varieties studied (Sunder Rao and Sino, 1992). In recent years studies are being carried out in order to examine the chemical composition of the pomegranate, in particular, work is focusing on the study of fatty acids in the pomegranate seed, both by researchers of the Mediterranean area and from eastern countries. The large group of researchers are finding significant differences regarding the fatty acid content. El-Shaarawy and Nahapetian (1983) report that 8% of fatty acids are saturated, 10% are monounsaturated, 10% di-unsaturated and approximately 70% would probably be punicic acid. El-Nemr *et al.* (1990) report that 83.6% of the fatty acids of the pomegranate are saturated and only 16.3% are unsaturated, whereas Melgarejo *et al.* (1995), in their studies conducted in 6 pomegranate cultivars of the Mediterranean area, point out that 30-35% of the fatty acids present in the pomegranate are saturated, 25-37% monounsaturates, 25-39% di-unsaturates, 1-10% polyunsaturates and approximately 67.6% would correspond to punicic acid.

This work has studied the fat and fatty acid contents in 3 pomegranate varieties, for a period of two consecutive growing seasons (95/96-96/97), all cultivated in homogeneous conditions in the experimental plot of the Higher Polytechnical School of Orihuela (EPSO), created in 1992 (Melgarejo, 1993).

Materials and methods

The pomegranates used in this work were obtained from the experimental plot of the EPSO of Orihuela. The varieties studied were: VA1 (Valencia No. 1), ME16 (Mollar de Elche 16), and BA1 (Borde Albatera No. 1).

The fruit was harvested by hand in its optimum state of ripeness for two consecutive seasons. After a morphological and chemical characterisation the samples were prepared for the determination of fat and fatty acids.

Preparation of the sample for the determination of total fat content

3 samples were taken from each variety to be studied, each sample weighing 100 g. They were dried for 2 days in an air oven at 60°C. They were later ground and fat was then extracted with petroleum ether using a Soxhlet apparatus according to the AACC method (1987). The fat was recovered by petroleum ether distillation in a vapour rotor at 60°C. The samples were then dried in a dessicator for 1 hour, and finally weighed to obtain the grams of fat extracted.

The fat extracted was later dissolved with ethyl ether and kept to be used later when determining fatty acid composition.

Preparation of the sample for the determination of fatty acids

The fat composition in fatty acids was determined using methyl esters. Before GLC and GLC–ME, analysis, all samples were subjected to a purification process consisting of: (i) purification of the triglyceride fraction; (ii) esterification with alcohol KOH; and (iii) derivation of the methyl esters to fix the positions of the insaturations (oxazole derivatives).

The *purification of the triglyceride fraction* was carried out by carefully evaporating the samples until they were dry, and using a nitrogen current, they were redisolved in ether hexane 90:10. In a

silica column of 20×0.9 cm with a hexane:ether 90:10 tap, the samples are deposited and eluted at ml/min with 50 ml of hexane:ether 90:10.

Esterification with alcohol KOH was carried out by taking an aliquot from each sample, and methylating them by transesterification with alcohol KOH 2N. After the esterification the methyl ester compositions are analysed using GLC, injecting 1 microlitre of organic phase. The column of melted silica DB-WAX (J&W scientific) of 25 m × 0.25 mm and 25 micras phase thickness. The initial temperature of 140°C is maintained for 2 minutes and then increased at a rate of 4°C up to 200°C for 20 minutes. Nitrogen carrier-gas at 1 ml/min and a split ratio of 1:70.

The derivitization of methyl esters to fix the position of the insaturations was carried out by obtaining the oxazoline derivates (DMOX) through reaction with 2 amino-2 methyl 1 propanol. For this purpose an aliquot of methyl esters was taken and made to react with 2 amino-2 methyl 1 propanol, following the procedure described by Yu *et al.* (1989).

The samples (methyl esters and DMOX) were analysed by GLC-MS under the aforementioned conditions of the DB-WAX column:

(i) Methyl esters: 180°C initial temperature 5 min. Rate of 2°/min up to 220°C for 20 min. Injector at 250°C.

(ii) DMOX derivates: 180°C initial temperature. Rate of 2°/min up to 240°C for 20 min. Injector at 250°C.

The equipment used was GLC-FID for quantification: HP-5890 Series II and GLC-EM: HP-5890 Series II coupled by direct insertion Interphase to high-resolution mass spectrometer Finnigan MAT95's.

The mass spectrometries were done by ionization with electronic impact at 70 eV, resolution 2300. Sweep range 50-600 amu at 2 sec/dec.

Results and discussion

(i) The seed content in the varieties studied varies within a range of 30-45 g/kg of fruit in the ME16 and VA1 (sweet) varieties, and around 65 g/kg in the BA1 (bitter) variety. These values fall within the range described by Melgarejo *et al.* (1995), who reported the seed content between 37 and 143 g/kg of fruit.

(ii) The lipid content, expressed in g/kg of dry matter varies between 70-80 g/kg in the sweet varieties (ME16 and VA1), and is about 105 g/kg in the bitter variety (BA1) (Table 1).

It is to be pointed out that these values are concordant with those determined by Melgarejo *et al.* (1995) for sweet and bitter varieties. These values differ from those found by El-Nemr *et al*. (1990) who report approximately 272 g/kg total fat.

(iii) As the ultimate aim of this study is to determine the total fat content and fatty acid composition, in 2 pomegranate varieties in homogeneous conditions, to study if there are significant differences between such varieties and to be able to establish a biochemical classification of them, we will point out that the trials conducted during the 95/96 and 96/97 seasons show that the greatest fat content corresponds to the bitter variety (BA1), whereas the sweet varieties (ME16 and VA1), have much lower contents of total fat, almost half that of the bitter variety (Table 1).

(iv) As for the fatty acid composition of the pomegranate seeds, in the 3 varieties 5 fatty acids have been identified (Table 1).

The profile of the fatty acid composition of the triglycerides is very similar (but not identical) in all varieties. The mean content of polyunsaturated fatty acids is almost 90%, of which between 60 and 80% are tri-unsaturated fatty acids: $C_{18:3}$ in position 9,11,13, called punicic acids, which appear at different retention times, this would indicate different geometric isomeres as the insaturation positions are invariable.

Table 1. Total fat content (g/kg dry matter) and its fatty acid composition in 3 varieties of pomegranates

	ME16	VA1	BA1
Fat† (g/kg)	80.92±18.2	68.97±11.49	104.9±7.31
% Palmitic ($C_{16:0}$)	3.83±0.96	3.63±0.33	2.99±0.14
% Estearic ($C_{18:0}$)	2.38±0.89	1.6±0	1.64±0.16
% Oleic ($C_{18:1}$) (9)	4.82±1.97	4.39±1.13	4.09±0.10
% Oleic ($C_{18:1}$)(10)	1.09±0.53	0.84±0.21	0.61±0.049
% Linoleic ($C_{18:2}$)	7.74±3.63	7.3±2.07	4.98±0.13
% Punicic†† ($C_{18:3}$)	66.76±15.4	78.5±1.96	79.29±0.077
Saturates	6.21	5.23	4.63
Mono-unsaturates	5.91	5.23	4.7
Unsaturates	7.74	7.3	4.98
Tri-unsaturates	66.76	78.5	79.29
Unsaturates	80.41	91.03	88.97
Saturates/unsaturates	0.077	0.057	0.052

†The values are the average of a triple determination over 2 consecutive seasons (1995/96-1996/97).
††Punicic acid $C_{18:3}$ at position 9,11,13, without determining geometric configuration.

The punicic acid values determined in this study lie within the same range as those determined by El-Shaarawy and Nahapetian (1983) who talk about a punicic acid percentage of 67.6%.

As for the existence of "punicic acid", we should point out that in 1935, this acid was isolated and identified as such by Toyama and Tsuchiga in pomegranate seeds. Later studies, carried out by Chisholm and Hopkins (1966) on Cucurbitae ssp. affirmed the presence of this acid, and in the same year Takagi, studying punicic acid, reports $C_{18:3}$ at the position 9-cis, 11-trans, 13-cis octadecatrienoic acid. Years later, in studies conducted on this acid, Yong-Goe *et al.* (1995) corroborate that punicic acid is that described by Takagi, and furthermore, in the same studies the existence of other $C_{18:3}$ isomeres is shown as well as geometric isomeres $C_{18:3}$ at position 9-cis, 11-trans, 13-trans as alpha-eleostearic acid and $C_{18:3}$ in position 9-trans, 11-trans, 13-cis as catalpic acid. Toyama and Tsuchiga (1936) indicate the existence of the alpha-eleostearic isomere and even identify the $C_{18:3}$ isomere at position 9-cis, 11-cis, 13-trans as trycosanic acid.

In the 3 varieties studied the acids that are by far predominant over the rest of the punicic acids (we include all the isomeres as punicic acids, given that in mass spectrometry it is not possible to determine its configuration) (66.7-80%), followed by linoleic acid (5-8%) and oleic acid (4-5%). The less predominant acids are palmitic acid (2.5-4%) and stearic acid (1.5-2.5%).

We have found some differences between the varieties studied, regarding the content in fatty acids. BA1 and VA1 show the greatest percentage of punicic acid (80%). BA1 has the lowest linoleic acid content in comparison with the rest of the varieties.

The 3 varieties present very few differences in palmitic, oleic and estearic acid content.

Concerning the saturated fatty acid/unsaturated fatty acid ratio (Table 1), this is similar in the 3 varieties studied, varying between 0.052 (BA1) and 0.077 (ME16). This ratio indicates that the 3 varieties have a high content in unsaturated fatty acids, which may make them more attractive for the consumer who wishes to ingest this type of acid.

The results obtained here are of great interest since the 3 varieties studied are under the same cultivation conditions.

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