__Original Research Article__

Composition of fatty acids and antioxidant activity of pomegranate seed oil cv. 'Molar'

ABSTRACT

Aims: Pomegranate has been used since ancient times as a universal therapeutic agent due to the presence of biologically active ingredients in different parts of the plant. Pomegranate seed oil is considered a nutraceutical because of its rich composition. Therefore, this work aimed to study the main changes in the composition of fatty acids and antioxidant activity of pomegranate seed oil (cv. Molar) in different stages of fruit development.
Study design: Completely randomized design. The treatments were the ages (60, 70, 80, 90 and 100 days), counted from the beginning of the anthesis. For each harvest a random sampling of five fruits was used for each repetition, and four replications per stage of fruit development were performed totaling 20 fruits per treatment.
Place and Duration of Study: The research was carried out in partnership with the farm Águas de Tamanduá, located in Várzeas de Sousa, PB, (longitude 38°13'41" and latitude 06°45'33").
Methodology: The characterization of the phenological phases of pomegranate (Molar cv.) development was carried out at the beginning of the orchard. Vigorous and healthy adult plants were selected. Hermaphrodite flowers were marked, evenly distributed in the area, with colored tape resistant to high temperature, sunshine, winds and rains. The marking of the flowers occurred in the early hours of the morning, and at the time of the marking, thinning of flowers was carried out on branches that had two or more flowers at the apex, leaving only a single flower on the branch. Seed oil was extracted from a sample of 20 fruits at different stages of development: 60, 70, 80, 90 and, 100 days counted from the start of the anthesis.
Results: The general composition of the oil of pomegranate seeds cv. Molar, regardless of the stage of fruit development, takes the order of PUFA> SFA> MUFA, with a higher content of polyunsaturated fatty acids (omega 3 and 6), and after saturated and monounsaturated, and low concentrations of total Trans Isomers.
Conclusion: The best periods for the consumption of pomegranate seed oil are between 80 and 90 days due to the higher amount of unsaturated acids and punicic acid, and lower concentrations of palmitic acid, as well as a higher concentration of phenolic compounds. The method of DPPH, with methanol extractor identifies the antioxidant activity of pomegranate seed oil, however not efficiently.

Keywords: Bioactive compounds, phenological phases, Punica granatum L.

1. INTRODUCTION

Pomegranate, family Punicaceae, is native to the Middle East region but spread throughout the Mediterranean region. It is a type of shrub or ravine, with simple leaves, charcoal, arranged in groups of 2 or 3, 4 to 8 cm in length, moderate prickly. The fruits are berry type, globous, measuring up to 12 cm, with numerous seeds surrounded by a rosy juice, full of sweet liquid [1].

Pomegranate (*Punica granatum* L.) is mainly consumed fresh but widely used for juices, jellies, and other nutritional and pharmaceutical purposes. The arillus are the more succulent part, accounting for 50 to 70% of the mass of the fruit, but it includes a woody internal part. The seed, representing 5 to 15%, generally discarded as waste material in many industries of pomegranate processing [2; 3].

Pomegranate seeds are richer in fiber and fats, in addition to other beneficial phytochemicals such as organic acids, sugars, vitamins, polysaccharides, polyphenols and minerals. However, from the economic and environmental point of view, this residues should be used in the production of essential oil [4].

Pomegranate oil is considered a precious nutraceutical, attracting growing interest due to the abundance of punicic acid, a positional and geometric isomer of α-linolenic acid. The structure has two double cis bonds and one double trans bond investigated to understand its role in physiological processes [5]

According to [6], the antioxidant, anticancer and anti-lipidemic properties of pomegranate seed oil make it an auxiliary agent to bring health benefits. The oil concentration increases continuously with fruit growth reaching a maximum of 19.34% at 100 days of age, more than double the value reported to the fruits at 60 days, when the fruit is immature [7].

The role of oils and fats in the human body has been extensively researched in recent decades and evidence shows that not only the amount of fat consumed but also the type of fat, such as fatty acids (Trans, CLAs, CLnAs), are important factors both for health maintenance and for the development of certain diseases [8]. Characterizing each class of dietary lipids is an essential step to develop applications in the food and health industries. Therefore, the lipid profile of several fruits and their seeds have been characterized and several bioactive compounds isolated and identified [5].

Study the composition of fatty acids and antioxidant potential in pomegranate seed oil, at various stages of development, can generate valuable information for the use as nutraceutical product. Therefore, the harvest of 'Molar' pomegranate, grown in Várzeas de Sousa, PB, can be carried out in the physiological stage of a greater quantity of specific compounds with functional properties to the organism.

The present work aims to investigate the fatty acid profile and antioxidant activity of pomegranate seed oil (cv. Molar) at different stages of fruit development to provide useful information for use as a food functional.

2. MATERIAL AND METHODS

The research was carried out in partnership with the farm Águas de Tamanduá, located in Várzeas de Sousa, PB, (longitude 38°13'41" and latitude 06°45'33"). Águas de Tamanduá farm production is certified by the Biodynamic Institute Certification Association (IBD), using an organic system with no synthetic chemical applied, minimizing risks to the environment and consumers.

The orchard has six years of installation, 2.6 ha cultivated with the 'Molar' variety, brought from Europe and propagated on the farm by seeds. The region climate is semi-arid BSh, according to Köppen classification, characterized by temperatures above 25ºC and average rainfall below 1000 mm.year^{-1} distributed in irregular rains. The study area has a dry season from May and may extend to January and rainy season from January to April, with an average annual precipitations of 600 mm. Lithoidal Neosols and Luvisols are the main soil type in the region [9]. The production area is maintained by irrigation with micro-sprinklers.

The characterization of the phenological phases of pomegranate (Molar cv.) development was carried out at the beginning of the orchard, in stages prior to the project (Process 443989 / 2014-1 MCTI / CNPq / Universal 14/2014 track A). Fruit age was estimated from the anthesis and monitored by the flower marking on the plants in the reproductive phase.

Vigorous and healthy adult plants were selected. Hermaphrodite flowers were marked, evenly distributed in the area, with colored tape resistant to high temperature, sunshine, winds and rains. Hermaphrodite plants were distinguished from the others by presenting a rounded or bell-shaped base. The marking of the flowers occurred in the early hours of the morning, and at the time of the marking, thinning of flowers was carried out on branches that had two or more flowers at the apex, leaving only a single flower on the branch.

The treatments were the ages (60, 70, 80, 90 and 100 days), counted from the beginning of the anthesis. For each harvest a random sampling of five fruits was used for each repetition, and four replications per stage of fruit development were performed totaling 20 fruits per treatment. Immediately after the harvest, the fruits were transported to the Food Analysis Laboratory of the Federal University of Campina Grande (UFCG), Campus of Pombal-PB.

The arils were separated from the fruit and hand pressed in a plastic bag to seeds removal. The seeds were weighed and left in the open air to remove excess water, prior to the oil extraction. To determine the oil amount, the seeds were dehydrated in a convective oven at 60 ° C until obtain no significant variation in the mass of the material.

The samples were ground in a hammer mill, Vieira brand of 35 mesh with a speed of 8000 rpm, to obtain particles with an average diameter lower than 1.0 mm, as it increases the efficiency of lipid extraction [10]. The ground seeds were placed in a cartridge, and added to the Soxhlet extractor, using anhydrous ethanol and hydrated ethanol (90 ° GL) as the solvent. The temperature and the solvent/substrate ratio were constant, respectively at 70 ° C and 4: 1 (m/m). The reflux lasted 6 hours. The extracted oil was kept in well-closed amber flasks and stored under refrigeration at 4 ℃. The oil yield of pomegranate seeds increased continuously with fruit development, starting with 7.77% at 60 days, increasing to 8.70% at 70, 9.34% at 80, 12.48% at 90, arriving to 19.34% at 100 days [7].

We analyzed the composition of fatty acids, phenolic compounds the antioxidant activity. The determination of the fatty acid methyl esters of pomegranate seed oil follows the method of [11]. The gas chromatography was performed in a Varian, model 3900, equipped with an automatic sampler; split injector, 75: 1 ratio; 100 m x 0.25 mm i.d. capillary column, 0.20 µm film (CP-SIL 88, Chrompack); flame ionization detector (FID) and a workstation with Star software.

The flow of the entrainment gas (Helium) used was 1.5 mL.min-1. The column heating ramp was programmed to start at 70 ℃ for 1.2 minutes. Then rising to 210 ℃ at a rate of 12 ℃ per minute, remaining at this temperature for 2 minutes. Subsequently, the temperature was raised to 300 ℃ with a heating rate of 5 ℃ per minute. The injector and detector temperatures were 270 ℃ and 290 ℃, respectively. 1µL of the esterified samples is injected. The quantification was performed by normalizing the peak areas, and the peaks were identified by comparing sample retention times with those of fatty acid methyl esters (AccuStandard NHI-003N and NHI-004).

The phenolic compounds were determined using the method of Folin & Ciocalteau, described by [12], with modifications. The extracts were prepared from the weighing of 0.5 g of pomegranate oil diluted in 10 mL of methanol, and resting for an hour. A 200 μL aliquot of the extract was transferred to a tube, the volume was filled with distilled water to 2,125 μL and added 125 μL of Folin Ciocalteau reagent. The mixture was allowed to stand for 5 minutes and 250 μL of 20% sodium carbonate was added, stirring and standing in a water bath at 40 ℃ for 30 minutes. The readings were carried out in a spectrophotometer at 765 nm, and the results were expressed in mg 100 g^{-1}.

The antioxidant activity was determined by the DPPH (2,2-Diphenyl-1-picryl-hydrazide) method according to [13], with adaptations. The extract for the analysis was done by diluting 0.5 g of oil in 10 mL of methanol under constant agitation for 5 min and left two hours to rest. We used three aliquots (50, 70, 100 μL) of each sample with 3.9 mL of the 0.06 mM DPPH radical homogenized on tube shaker and allowed to stand for one hour, according to previously performed kinetics. Control solution of methanol with DPPH solution as standard and methyl alcohol as blank to clear the spectrophotometer were used, the readings were carried out at 515 nm, and the EC50 data expressed in g of oil/g DPPH.

The data related to phenolic compounds and DPPH were analyzed statistically through Variance and Regression Analysis. The regression equations with the coefficients of determination were chosen based on the biological explanation of the phenomenon, simplicity of the equation and test of equation parameters by Student's t test, at 5% probability, using SISVAR software version 5.6, developed by the Federal University of Lavras [14].

3. RESULTS AND DISCUSSION

Table 1 shows the general composition of pomegranate seed oil. Through the analysis of the general composition of the oil of pomegranate seeds cv. 'Molar', we found that, regardless of the stage of fruit development, the oil has the following concentration order PUFA> SFA> MUFA, with a higher content of polyunsaturated fatty acids (PUFA) (omega 3, omega 6 and punic acid), followed by saturated (SFA) and monounsaturated (MUFA), and lower concentrations of total transisomers. This result corroborate other studies carried out with the oils extracted from several cultivars of pomegranates, such as [15] and [4].

Table 1. Determination of fatty acid content (%) in pomegranate seed cv. Molar

	Fruit age (days)				
Fatty acids (g/100g)	60	70	80	90	100
Saturated	7.29	6.84	5.96	6.18	6.16
Monounsaturated	4.18	4.23	3.76	4.42	4.43
Poliinsaturados	6.74	5.09	4.40	4.48	4.56
Omega 3	0.42	0.41	0.41	0.40	0.39
Omega 6	6.32	4.67	3.99	4.08	4.17
Unsaturated	10.92	9.32	8.16	8.90	8.99
Total trans isomers	0.29	0.27	0.23	0.23	0.25
SFA/(PUFA+MUFA) relation	0.67	0.73	0.73	0.69	0.69
*N.I.	77.10	79.18	81.26	80.29	80.21

*(N.I.) - *Not identified.*

We observed a decrease of SFA, MUFA and unsaturated fatty acids with the fruit age. Only monounsaturated fatty acids obtained slightly higher concentrations with increasing fruit age, which may be due to the presence of higher concentrations of oleic acid, one of the main representatives of the MUFA.

The ratio SFA/(PUFA + MUFA) showed oscillations between fruit ages starting with 0.67 at 60 days of age, increasing at 70 and 80 days to 0.73 and subsequent reduction to 0.69 at 90 and 100 days (Table 1). This result is higher than those reported by [4] 0.079 with 'Mollar de Elche', [16] 0.077 for 'Mollar de Elche 16' and [6] 0.395 for cultivating 'Sichuan2'. The higher values may be a result of high SFA values found in this study.

Due to the high proportion of unsaturated fatty acids (Σ Usant), pomegranate seed oils cv. 'Molar' studied here is highly recommended for human consumption, having a fatty acid profile more favorable than other vegetable oils rich in SFA. This result confirms the found by other studies with different pomegranate genotypes reported by [17; 18].

The behavior of omega 3 and 6 (polyunsaturated fatty acids) showed a reduction at 60 to 100 days (Table 1). These fatty acids are important in the daily human diet intake since they build the structure of cell membranes and in metabolic processes. In the last decades, several countries determined that the average intake of fatty acids resulting from Omega 6/Omega 3 ratio is in the proportions of 10:1 to 20:1, with records of up to 50:1 occurring, resulting in health benefits. However, concerning Brazil, there is still no information on the values corresponding [19]. Therefore, we verified that in all fruit stages, the oil content is within the parameters established by some countries, and we indicated from the 80 days in which it represents a ratio of 10:1.

A total of 13 fatty acids were found in pomegranate seed cv. 'Molar', from fruits aged between 60 and 100 days, but only 12 were identified (Table 2). Punicic (C18: 3 (9c, 11t, 13c) was the most abundant in the oil of pomegranate seeds at all ages studied. Other acids were present in smaller proportions, such as palmitic (C 16: 0), oleic (C 18: 1 ω-9), linoleic (C 18: 2 ω-6) and stearic 18: 0) had the highest levels 4.14% at 60 days, 4.43% at 100 days, 6.32% at 60 days, 1.83% at 60 and 70 days, respectively. The other acids identified accounted for less than 1% of the content.

Table 2. Fatty acid content (%) in pomegranate seed cv. Molar (n = 20).

	Fruit age (days after anthesis)				
Fatty acid content (%)	60	70	80	90	100
C 15:1 Cis-10- pentadecanoic acid	0.14	*	*	*	*
C 16:0 Palmitic	4.14	3.55	2.96	2.96	3.08
C 18:0 Esteárico	1.83	1.83	1.56	1.66	1.68
C 18:1 ω-9 Oleic	4.03	4.23	3.76	4.42	4.43
C 18:2 ω-6 Trans Linoleic	0.29	0.27	0.23	0.23	0.25
C 18:2 ω-6 Linoleic	6.32	4.67	3.99	4.08	4.17
C 20:0 Arachidonic	0.48	0.43	0.43	0.42	0.39
C 18:3 ω-3 α- Linolenic	0.42	0.41	0.41	0.40	0.39
C 22:0 Beenic	0.13	0.11	0.11	0.11	0.11
C 23:0 Tricosanoic	*	0.27	0.14	0.28	0.21
C 18:3 (9*c*,11*t*,13*c*) Punicic	69.57	72.65	73.11	72.03	73.74
C 24:0 Lignoceric	0.72	0.66	0.75	0.75	0.69
*N.I.	7.53	6.53	8.15	8.26	6.47

*(N.I.) - *Not identified.*

Our results were similar to those reported by other authors who indicated punicic acid as the most abundant in the oils of pomegranate seeds. The punicic acid in percentage terms ranged from 69.57 to 73.74% of the total acids present in the oils (Table 2). These results are similar to those obtained in studies using pomegranate seed oil produced in Europe, such as [16], who reported that the cultivar "Mollar de Elche 16" varied from 66.7 to 79.2%, indicating a better adaptation of the pomegranate in the semi-arid region.

Punicic acid is a conjugated linolenic acid (CLnA), which has shown: Carcinogenic activity, including interference in tumor cell growth, pharmacological invasion, angiogenesis. Known as an inhibitor of prostaglandin biosynthesis, this compound may inhibit the incidence of skin cancer and still be used as a promising source of human food [4]. observing the results; it is verified that the highest amount of Punicic acid was produced during the phenological stages of the fruit, where the highest peak of this compound occurred at 60 days after the anthesis, a value of 69.57% increasing to 72.65 at 70 days, 73.11% at 80 days, and a small reduction at 90 days was 72.03%, again increasing to 100 days at 73.74%. Punicic acid is an isomer with the highest predominance in the oil of pomegranate seeds [20]. The volume of this isomer represents 72% of the total fatty acids produced in the pomegranate seeds, values also confirmed by [21; 22].

Several results show that Punicic acid is synthesized in a higher concentration by the plant, in the stages of development of the fruit, its greater volume is produced after finishing the anthesis, and extends until the fruit matures. The results suggest that the initial peak of higher production of this compound was detected at 60 days after the anthesis completion, and these values continued to increase until the end of the monitoring at 100 days, with some small oscillations in the percentages produced The results obtained in this study show that the concentrations of the Punicic acid isomer (PA) represent the largest fraction contained in the oil of seeds of Punica granatum L. as described by [23; 24]

the results from the accumulation of fatty acids in pomegranate seeds depend on factors such as type of soil nutrient, spot light, temperature and many other factors that directly influence the quantity and quality of the synthesized acids, according to results obtained by [25; 26] in different countries.

Palmitic acid showed a decreasing behavior up to 80 days, and an increase in growth at 100 days. At 80 and 90 days, the content found for this acid was 2.96%, higher than that found by [18], 2.68% of palmitic acid using Italian genotypes, [27] found 2.10 - 2.77% in Turkey, and [28] with lower values of 2.0 - 2.5% using organic solvents.

Ingestion of palmitic acid may cause metabolic dysregulation due to excesses of non-esterified fatty acids (NEFAs) leads to the induction of stress in the endoplasmic reticulum causing a lipidic dysregulation that affects calcium signaling and can cause cell death and attenuate the translation of protein. However, in Mediterranean diets [29] found beneficial effects of palmitic acid if the intake of SFA is limited to (7-8%) and a high intake of MUFA (20%).

According to our results, fruits of 80 and 90 days are the best for oil consumption, due to the lower concentrations of palmitic acid, mainly at 90 days. At 90 days, besides the lower content of palmitic acid, there is a high oleic acid content, enhancing its use.

The stearic acid decreased at 80 days to 1.56%, a result much lower than those reported by [18] in Italy of 2.865% and in Iran of 1.8 - 2.2% [17].

Arachidic acid was also detected in smaller quantities, and remained with small decreases, with some stability between 70 (0.43%), 80 (0.43%), and 90 days (0.42%). Behenic acid accounts for 0.11% of concentration from 70 days of age, similar values were found in Iran genotypes (0.33-0.48% for arachidic acid and 0.16-0.21% for behenic) reported by [27].

Phenolic compounds are primary antioxidants; therefore, important in the antioxidant activity of pomegranate fruits, they are easier to donate hydrogen to the free radical, preventing the oxidative process. The phenolic compounds are significantly affected by fruit age, at 1% of probability having a cubic behavior (figure 1). At 60 days, the oil presented a value of 192.43 $mg.100g^{-1}$, a reduction at 70 and 80 days, and another increase at 90 days of 193.14 $mg.100g^{-1}$ and again reduction and lower value among all ages of 148.51 $mg.100g^{-1}$.

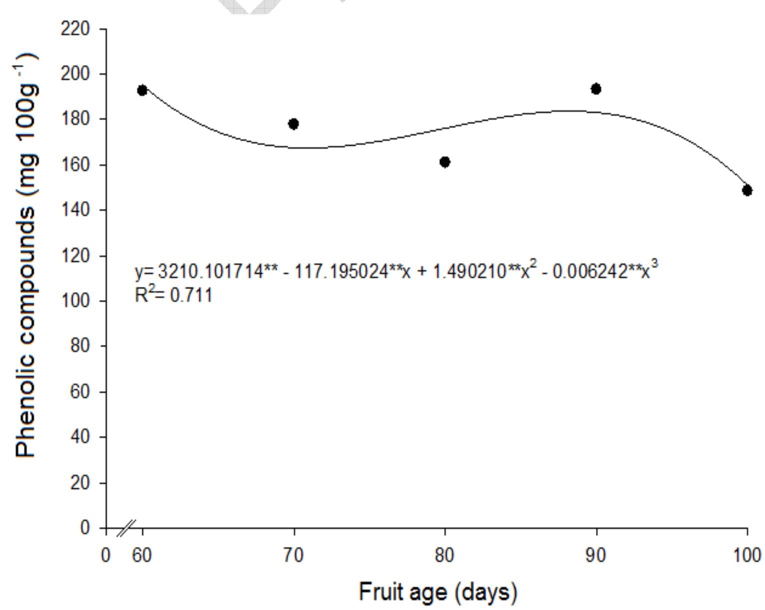

Fig. 1. Phenolic compounds in pomegranate seed oil cv. 'Molar' during the development of the fruit.

During fruits ripening, phenolics are associated with taste (acidity, astringency, bitterness). Therefore, we observed that, according to the maturation and increase of days, the phenolic values tend to reduce. However, concerning pomegranate seed oil there is an increase up to 90 days, which can be explained by the fact that these compounds are consumed during ripening and are mainly stored in the bark, because are produced as a form of defense of the plant, and in smaller quantity in the seed. There are no reports of phenolic compounds on the oil of pomegranate seed in the literature.

We adopted the DPPH method to identify the antioxidant potential of pomegranate seed oil. 'Molar', which was significantly affected by fruit age at 1% of probability (Figure 2). We observed a cubic behavior, ranging from 60 to 80 days, and the higher antioxidant capacity at 70 days with EC50 value of 59.54 g/g DPPH. However, during the maturation of the fruit, its antioxidant potential is reduced, with a lower capacity verified at 90 days in which the EC50 values show 315.96 g/g of DPPH, that is, it is needed a greater amount of oil to reduce 50% of DPPH.

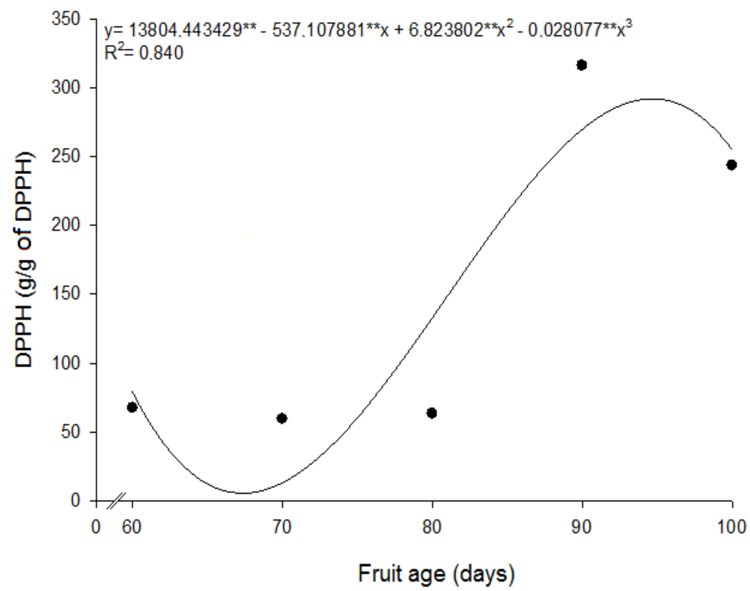

Fig. 2. Antioxidant activity by DPPH method in pomegranate seed oil cv. 'Molar' during fruit development.

[5] reported good results for pomegranate seed oil using DPPH method, with EC50 values of 3.77 mg. However, according to [30], testing the antioxidant activity of essential oils such as from *Cymbopogon nardus*, *Cinnamomum zeylanicum* and *Zingiber officinale*, the low solubility of essential oil and its compounds and due to its lipophilic nature, the DPPH test should not be applied for essential oils, but for hydrophilic compounds such as ascorbic acid. But, our results demonstrated the existence of antioxidants in pomegranate seed oil during fruit development using DPPH method.

4. CONCLUSION

The best periods for the consumption of pomegranate seed oil are between 80 and 90 days due to the higher amount of unsaturated acids and punicic acid, and lower concentrations of palmitic acid, as well as a higher concentration of phenolic compounds.

The method of DPPH, with methanol extractor identifies the antioxidant activity of pomegranate seed oil, however not efficiently.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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