

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,

19 arranged in groups of 2 or 3, 4 to 8 cm in length, moderate prickly. The fruits are berry type,
20 globous, measuring up to 12 cm, with numerous seeds surrounded by a rosy juice, full of
21 sweet liquid [1].

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

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

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

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

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

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

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

54

55 **2. MATERIAL AND METHODS**

56

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

62 The orchard has six years of installation, 2.6 ha cultivated with the 'Molar' variety, brought
63 from Europe and propagated on the farm by seeds. The region climate is semi-arid BSh,

64 according to Köppen classification, characterized by temperatures above 25°C and average
65 rainfall below 1000 mm.year⁻¹ distributed in irregular rains. The study area has a dry season
66 from May and may extend to January and rainy season from January to April, with an
67 average annual precipitations of 600 mm. Lithoidal Neosols and Luvisols are the main soil
68 type in the region [9]. The production area is maintained by irrigation with micro-sprinklers.

69 The characterization of the phenological phases of pomegranate (Molar cv.) development
70 was carried out at the beginning of the orchard, in stages prior to the project (Process
71 443989 / 2014-1 MCTI / CNPq / Universal 14/2014 track A). Fruit age was estimated from
72 the anthesis and monitored by the flower marking on the plants in the reproductive phase.

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

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

84 The arils were separated from the fruit and hand pressed in a plastic bag to seeds removal.
85 The seeds were weighed and left in the open air to remove excess water, prior to the oil
86 extraction. To determine the oil amount, the seeds were dehydrated in a convective oven at
87 60 °C until obtain no significant variation in the mass of the material.

88 The samples were ground in a hammer mill, Vieira brand of 35 mesh with a speed of 8000
89 rpm, to obtain particles with an average diameter lower than 1.0 mm, as it increases the
90 efficiency of lipid extraction [10]. The ground seeds were placed in a cartridge, and added to
91 the Soxhlet extractor, using anhydrous ethanol and hydrated ethanol (90 ° GL) as the
92 solvent. The temperature and the solvent/substrate ratio were constant, respectively at 70 °
93 C and 4: 1 (m/m). The reflux lasted 6 hours. The extracted oil was kept in well-closed amber
94 flasks and stored under refrigeration at 4 °C. The oil yield of pomegranate seeds increased
95 continuously with fruit development, starting with 7.77% at 60 days, increasing to 8.70% at
96 70, 9.34% at 80, 12.48% at 90, arriving to 19.34% at 100 days [7].

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

103 The flow of the entrainment gas (Helium) used was 1.5 mL.min⁻¹. The column heating ramp
104 was programmed to start at 70 °C for 1.2 minutes. Then rising to 210 °C at a rate of 12 °C
105 per minute, remaining at this temperature for 2 minutes. Subsequently, the temperature was
106 raised to 300 °C with a heating rate of 5 °C per minute. The injector and detector
107 temperatures were 270 °C and 290 °C, respectively. 1µL of the esterified samples is
108 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 & Ciocalteu, 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 μ L aliquot of the extract was transferred to a tube, the volume was filled with distilled water to 2,125 μ L and added 125 μ L of Folin Ciocalteu reagent. The mixture was allowed to stand for 5 minutes and 250 μ L of 20% sodium carbonate was added, stirring and standing in a water bath at 40 °C 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 μ L) 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 trans isomers. 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

Fatty acids (g/100g)	Fruit age (days)				
	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.

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

149 The ratio SFA/(PUFA + MUFA) showed oscillations between fruit ages starting with 0.67 at
150 60 days of age, increasing at 70 and 80 days to 0.73 and subsequent reduction to 0.69 at 90
151 and 100 days (Table 1). This result is higher than those reported by [4] 0.079 with 'Mollar de
152 Elche', [16] 0.077 for 'Mollar de Elche 16' and [6] 0.395 for cultivating 'Sichuan2'. The higher
153 values may be a result of high SFA values found in this study.

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

158 The behavior of omega 3 and 6 (polyunsaturated fatty acids) showed a reduction at 60 to
159 100 days (Table 1). These fatty acids are important in the daily human diet intake since they
160 build the structure of cell membranes and in metabolic processes. In the last decades,
161 several countries determined that the average intake of fatty acids resulting from Omega
162 6/Omega 3 ratio is in the proportions of 10:1 to 20:1, with records of up to 50:1 occurring,
163 resulting in health benefits. However, concerning Brazil, there is still no information on the
164 values corresponding [19]. Therefore, we verified that in all fruit stages, the oil content is
165 within the parameters established by some countries, and we indicated from the 80 days in
166 which it represents a ratio of 10:1.

167 A total of 13 fatty acids were found in pomegranate seed cv. 'Molar', from fruits aged
168 between 60 and 100 days, but only 12 were identified (Table 2). Punicic (C18: 3 (9c, 11t,
169 13c) was the most abundant in the oil of pomegranate seeds at all ages studied. Other acids
170 were present in smaller proportions, such as palmitic (C 16: 0), oleic (C 18: 1 ω -9), linoleic
171 (C 18: 2 ω -6) and stearic 18: 0) had the highest levels 4.14% at 60 days, 4.43% at 100 days,
172 6.32% at 60 days, 1.83% at 60 and 70 days, respectively. The other acids identified
173 accounted for less than 1% of the content.

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

Fatty acid content (%)	Fruit age (days after anthesis)				
	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 Estearico	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 (9c,11t,13c) 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

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

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

182 Puniceic acid is a conjugated linolenic acid (CLnA), claiming to exhibit carcinogenic activity,
183 including tumor cell interference and pharmacological invasion, as well as angiogenesis. In
184 addition to being known as an inhibitor of prostaglandin biosynthesis and may inhibit the
185 promotion of skin cancer. Therefore, pomegranate seed oils can potentially serve as a food
186 source for CLnA to reduce cancer risks, in addition to being a source of compounds that
187 improve skin condition with potential as a topical chemopreventive agent against skin cancer
188 [4].

189 Palmitic acid showed a decreasing behavior up to 80 days, and an increase in growth at 100
190 days. At 80 and 90 days, the content found for this acid was 2.96%, higher than that found
191 by [18], 2.68% of palmitic acid using Italian genotypes, [20] found 2.10 - 2.77% in Turkey,
192 and [21] with lower values of 2.0 - 2.5% using organic solvents.

193 Ingestion of palmitic acid may cause metabolic dysregulation due to excesses of non-
194 esterified fatty acids (NEFAs) leads to the induction of stress in the endoplasmic reticulum
195 causing a lipidic dysregulation that affects calcium signaling and can cause cell death and
196 attenuate the translation of protein. However, in Mediterranean diets [22] found beneficial
197 effects of palmitic acid if the intake of SFA is limited to (7-8%) and a high intake of MUFA
198 (20%).

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

202 The stearic acid decreased at 80 days to 1.56%, a result much lower than those reported by
203 [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 [20].

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.

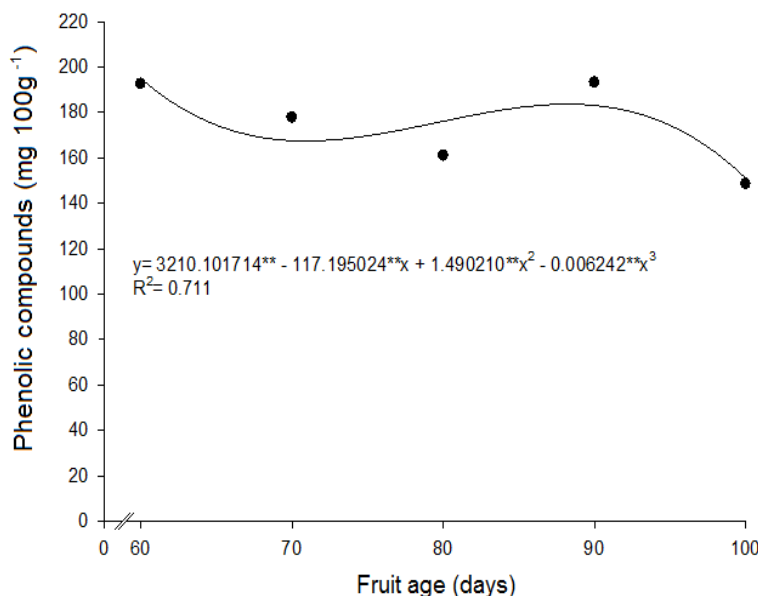
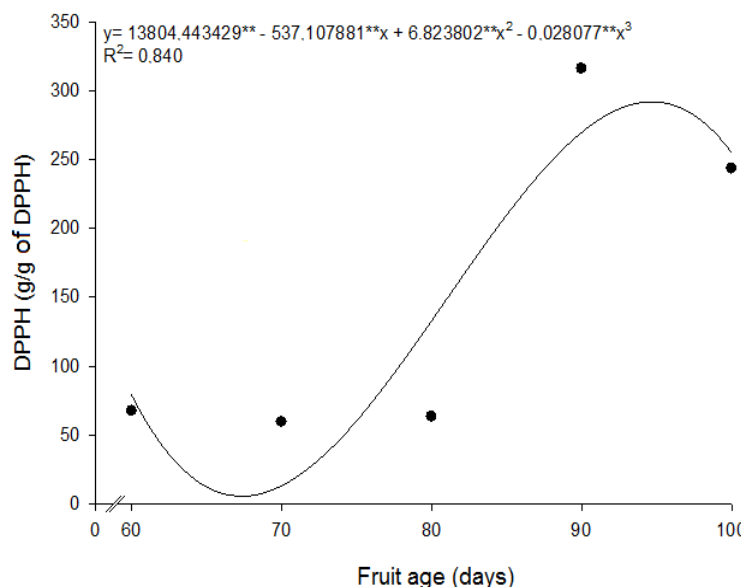


Fig. 1. Phenolic compounds in pomegranate seed oil cv. 'Molar' during the development of the fruit.

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.



232

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

235 [5] reported good results for pomegranate seed oil using DPPH method, with EC50 values of
 236 3.77 mg. However, according to [23], testing the antioxidant activity of essential oils such as
 237 from *Cymbopogon nardus*, *Cinnamomum zeylanicum* and *Zingiber officinale*, the low
 238 solubility of essential oil and its compounds and due to its lipophilic nature, the DPPH test
 239 should not be applied for essential oils, but for hydrophilic compounds such as ascorbic acid.
 240 But, our results demonstrated the existence of antioxidants in pomegranate seed oil during
 241 fruit development using DPPH method.

242 **4. CONCLUSION**

243

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

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

249 **COMPETING INTERESTS**

250

251 Authors have declared that no competing interests exist.

252

253

254 **REFERENCES**

255

256 1. Degáspari CH & Dutra APC. Propriedades fitoterápicas da romã (*Punica granatum* L.).
 257 Visão Acadêmica. 2011;12(1):36-46. Brazilian

258 2. Eikani MH, Golmohammad F, Homami SS. Extraction of pomegranate (*Punica*
 259 *granatum* L.) seed oil using superheated hexane. Food and Bioproducts Processing.
 260 2012;90(1):32-36.

- 261 3. Silva IMBR, Rocha RHC, Silva HdeS, Moreira IdosS, Sousa FdeAde, Paiva EP. Quality
262 and post-harvest life organic pomegranate 'Mollar' produced in Paraíba semiarid.
263 Semina: Ciências Agrárias. 2015;36(4):2555-2564.
- 264 4. Fernandes L, Pereira JA, López-Cortés I, Salazar DM, Ramalhosa E, Casal S. Lipid
265 composition of seed oils of different pomegranate (*Punica granatum* L.) cultivars from
266 Spain. International Journal of Food Studies. 2015;4:95-103. Espain.
- 267 5. Melo ILP, Carvalho EBTD, Yoshime LT, Sattler JAG, Pavan RT, Mancini-Filho J.
268 Characterization of constituents, quality and stability of pomegranate seed oil (*Punica*
269 *granatum* L.). Food Science and Technology, 2016;36(1):132-139.
- 270 6. Elfalleh W, Ying M, Nasri N, Sheng-Hua H, Guasmi F, Ferchichi A. Fatty acids from
271 Tunisian and Chinese pomegranate (*Punica granatum* L.) seeds. International journal of
272 food sciences and nutrition. 2011;62(3):200-206.
- 273 7. Oliveira AMFde, Furtunato TCdeS, Araújo RHCR, Lima JFde, Onias EA. Rendimento
274 de óleo e amido da semente de romã durante o desenvolvimento do fruto. In: Dantas
275 CdeO, Silva Filho CRMda, Santiago Neto JF, Medeiros JA. (Org.). Encontro nacional
276 da Agroindústria: desafios da agroindústria no Brasil. Bananeiras: Universidade Federal
277 da Paraíba. 2016. 1556p.
- 278 8. Pande G & Akoh CC. Antioxidant capacity and lipid characterization of six Georgia-
279 grown pomegranate cultivars. Journal of agricultural and food chemistry.
280 2009;57(20):9427-9436.
- 281 9. Embrapa. Sistema brasileiro de classificação de solos. Brasília: Centro Nacional de
282 Pesquisas de Solos, 2006. 412p.
- 283 10. Silva ICC. Uso de processos combinados para o aumento do rendimento da extração e
284 da qualidade do óleo de macaúba, 2009. 99p. Dissertação (Mestrado em Tecnologia
285 de Processos Químicos e Bioquímicos) – Escola de Química, Universidade Federal do
286 Rio de Janeiro, Rio de Janeiro. 2009.
- 287 11. Hartman L & Lago RCA. Rapid preparation of fatty acid methyl esters from lipids. Lab.
288 Pract. 1973;(22)8:494-495.
- 289 12. Waterhouse A. Folin-ciocalteau micro method for total phenol in wine. American Journal
290 of Enology and Viticulture. 2006:3-5. Available in: <[http://](http://waterhouse.ucdavis.edu/faqs/folinciocalteaumicromethod-for-total-phenol-in-wine)
291 waterhouse.ucdavis.edu/faqs/folinciocalteaumicromethod-for-total-phenol-in-wine>.
292 Accessed on: August 03, 2017.
- 293 13. Rufino MS, Alves RE, Brito ES, Moraes SM, Sampaio CG, Jiménez JP, Calixto FDS.
294 Metodologia científica: determinação da atividade antioxidante total em frutos pela
295 captura do radical livre DPPH. Fortaleza, Embrapa Agroindústria Tropical. 2007. 4p.
296 (Comunicado Técnico, N. 127).
- 297 14. Ferreira DF. Sisvar: um sistema computacional de análise estatística. Ciência e
298 Agrotecnologia. 2011;35(6):1039-1042.
- 299 15. Jing P, Ye T, Shi H, Sheng Y, Slavin M, Gao, B, Liu L, Yu L. Antioxidant properties and
300 phytochemical composition of China-grown pomegranate seeds. Food Chemistry.
301 2012;132:1457–1464.

- 302 16. Hernández F, Melgarejo P, Martínez JJ, Martínez R, Legua P. Fatty acid composition of
303 seed oils from important Spanish pomegranate cultivars. *Journal of Food Biosciences*
304 and *Technology*. 2011;2:35–40.
- 305 17. Fadavi A, Barzegar M, Azizi MH. Determination of fatty acids and total lipid content in
306 oilseed of 25 pomegranate varieties grown in Iran. *Journal of Food Composition*
307 *Analysis*. 2006;19:676–680.
- 308 18. Ferrara G, Cavoski I, Pacifico A, Tedone L, Mondelli D. Morpho-pomological and
309 chemical characterization of pomegranate (*Punica granatum* L.) genotypes in Apulia
310 region, Southeastern Italy. *Scientia horticultrae*. 2011;130(3):599-606.
- 311 19. Martin CA, de Almeida VV, Ruiz MR, Visentainer JEL, Matshushita M, de Souza NE,
312 Visentainer JV. Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e
313 ocorrência em alimentos. *Revista de Nutrição*. 2006;19(6):761-770. Brazilian.
- 314 20. Kýralan M, Gölükcü M, Tokgöz H. Oil and conjugated linolenic acid contents of seeds
315 from important pomegranate cultivars (*Punica granatum* L.) grown in Turkey. *Journal of*
316 *the American Oil Chemists' Society (JAOCS)*. 2009;86:985-990.
- 317 21. Abbasi H, Rezaei K, Rashidi L. Extraction of essential oils from the seeds of
318 pomegranate using organic solvents and supercritical CO₂. *J. Am. Oil Chem. Soc.* v.
319 2008;85:83–89.
- 320 22. Palomer X, Pizarro-Delgado J, Barroso E, Vázquez-Carrera M. Palmitic and Oleic Acid:
321 The Yin and Yang of Fatty Acids in Type 2 Diabetes Mellitus. *Trends in Endocrinology &*
322 *Metabolism*. 2017.
- 323 23. Andrade MA, Cardoso MG, Batista LR, Mallet ACT, Machado SMF. Óleos essenciais
324 de *Cymbopogon nardus*, *Cinnamomum zeylanicum* e *Zingiber officinale*: composição,
325 atividades antioxidante e antibacteriana. *Revista Ciência Agronômica*. 2012;43(2):399-
326 408.