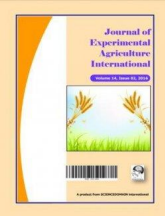

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The Point of Harvest of Pomegranates through the Analysis of Bioactive and Antioxidant Compounds during the Development Stages of the Fruit

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Authors' contributions

This work was conducted under the partnership of all the authors. Authors Elny Alves Onias, TCSF, RHCR and AMFO conducted the lab research, the statistical analysis and the manuscript writing, while authors Eliane Alves Onias, AEMMT and JFL collaborated on the manuscript revision and the improvement of the bibliographic revision based on the gathered data. Authors RHCR, OSS and MPB collaborated on the development of the study and made corrections on the manuscript. In the end, all the authors read and approved the final manuscript.

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ABSTRACT

The knowledge of the biochemical and physical-chemical transformations that happen in each stage of the development of the fruit is important to point the maturation rates and to define the point of harvest. Thus, this work intended to characterize the 'Molar' pomegranate regarding the changes on its bioactive compounds and its antioxidant potential during the phenological stages of its development, as a support to determine its point of harvest. Flowers were marked on the field, in a farm in Várzeas de Sousa, Paraíba, with ribbons of different colors, to register the age of the fruit

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in days after the anthesis (DAA). The harvests supposed to characterize the phenological stages were reaped at 60, 70, 80, 90 and 100 DAA, and those fruits were submitted to the laboratory, where a randomized experimental design was conducted, which treatments were the ages of the fruit, registered in days after the anthesis, with four repetitions and five fruit per parcel. 90-day-old fruit display the best quality indicators for the harvest and the commercialization of raw products. When pomegranate cv. 'Molar' is 90 days old, it reaches the maximum value of soluble solids, SS/TA ratio, total amount of sugars, reducing sugars, ascorbic acid, anthocyanins and antioxidant activity, while it presents a reduction of the titratable acidity, the pH and the phenolic compounds. The ascorbic acid and the anthocyanins were the main bioactive compounds responsible for the antioxidant activity of the pomegranate cv. Molar analyzed through the DPPH and ABTS methods.

Keywords: Maturation; antioxidant potential; Punica granatum L.

1. INTRODUCTION

The cultivation of the pomegranate tree has a great commercial appeal in the whole world, due to the increase in the demand for raw fruit and for products with functional properties. Around 1000 cultivars of *Punica granatum* were identified in the world [1]. Its fruit are spherical, with many seeds in layers that are wrapped in pink or carmine pulpy aril [2]. They're rich in organic acids, sugars, vitamins, polysaccharides, polyphenols and minerals [3]. Pomegranates are classified as nonclimacteric fruit, regarding its breathing pattern. Thus, the harvest of the pomegranate must be reaped when it is fully mature on the plant, which is when it has the highest quality characteristics [4], since its florescence, maturity and senescence. These changes include physical alterations, and structural, biochemical, and physiological changes, and changes on mineral elements, which reflect on the differences of the appearance of each cultivar of the fruit during its maturation [5]. Thus, the harvest time is extremely important, both for its immediate commercialization as a fresh fruit and for its storage, when it is necessary.

The production, commercialization and consumption of pomegranates are growing in the whole world, mainly because of its health-promoting qualities, related to its high concentration of phenolic compounds with antioxidant potential [6,7].

Some of the pomegranate's main phenolic compounds are punicalagins A and B, gallic acid, ellagic acid, flavonoids, vitamin C and anthocyanins that are some of the most important natural antioxidants and that are also responsible for the intense red color of the juice of the pomegranate, which is one of the quality parameters that influence on the sensorial acceptance by the consumers the most [8, 9,10].

The juice of the pomegranate is considered a source of natural antioxidants, with higher antioxidant potential than other natural juices and drinks, such as green tea and red wine [10,11].

In Brazil, the cultivation of pomegranate occurs mainly on the states of Paraíba, Pernambuco, Ceará and São Paulo. Semiarid regions are better for the cultivation of pomegranate, since it is well adapted to regions of tropical and subtropical climates. The spread of the cultivation of pomegranate in Brazil is a fact, but small cultivations of pomegranate as an ornamental or medicinal plant are spread throughout several regions, usually conducted by small producers or by people at home.

The characterization of the transformations that occur during the development of the pomegranate makes it possible to identify the period in which they meet the highest number of characteristics that express the quality, which makes it possible to determine the proper point of harvest for the commercialization of pomegranates, raw or processed.

Thus, this work aimed at characterizing the 'Molar' pomegranate regarding the changes on its bioactive compounds and its antioxidant potential during the phenological stages of the development of the fruit, to support the determination of the point of harvest.

2. MATERIALS AND METHODS

This study was conducted in a commercial orchard of the pomegranate cv. Molar that belongs to Fazenda Águas de Tamanduá, in Várzeas de Sousa, Paraíba (longitude 38°13'41" and latitude 06°45'33") [12], 30 km away from

the municipality of Pombal, Paraíba, which production is certified by the Associação de Certificação Instituto Biodinâmico (IBD), Law no 10,831 [13]. That area is six years old and has 2.6 ha that were cultivated with seedlings produced with seeds. The area of cultivation is characterized as a vertisol with clayey texture, which plants were disposed in a triangle-shaped space of 4 m x 4 m, under an air temperature (°C), a relative humidity and an average precipitation (mm) of 28°C, 68% UR and 250 mm, respectively, collected from a meteorological station near the experiment in the city of São Gonçalo, Paraíba [14].

At first, healthy, vigorous, adult plants were chosen to mark the flowers, using colorful ribbons that resist to high temperatures, insolation, the wind and the rain. By the anthesis time, certified through visual observation, 800 flowers were marked, evenly disposed throughout the orchard. The fruit were reaped and analyzed 60, 70, 80, 90 and 100 days after the anthesis (DAA). In each period of evaluation, 30 fruit were randomly collected among the marked pomegranates and, immediately after the harvest, the fruit were put in a polystyrene box and disposed in layered, wet paper towels, in order to prevent dehydration, and, soon after, they were transported to the Post-Harvest Technology Laboratory of the Federal University of Campina Grande (UFCG), Campus Pombal, Paraíba.

In the laboratory, the pomegranates were washed with neutral detergent at 1%, and, after the rinse, they were sanitized with a solution of sodium hypochlorite at 100 ppm of free chlorine for fifteen minutes and dried under room temperature. Soon after that, the randomized experimental design was established, which treatments were the ages of the fruit, registered in days after the anthesis, with four repetitions and five fruit per parcel. The arils were separated from the seed through manual pressing in polyethylene plastic bags. After that, the amount of juice extracted from the arils was analyzed.

The following analyses were conducted: Titratable acidity (TA, % of citric acid), conducted through titrimetry using a solution of sodium hydroxide (NaOH) 0,1 N for the sample of 1 mL of juice, to which 49 mL of distilled water and 3 drops of alcoholic phenolphthalein at 1% standardized with potassium biphthalate were added [15]; the hydrogen potential (pH) of the juice was determined through a pH meter of the Tecnopon brand (mPA model – 210P/Version 7.1), through the direct insertion of the glass membrane electrode in the juice sample [15]; Soluble solids (SS, %): It was determined directly in the homogenized juice, through the measuring in a digital refractometer (PR model – 100, Palette, Atago Co., LTD., Japan), according to the Association of Official Analytical Chemists [16]. SS/TA Ratio: It was conducted by the ratio between the values of soluble solids and titratable acidity; Total sugars (mL 100 mL^{1}), obtained from the extract prepared through the dissolution of 0.2 mL of juice dissolved in 100 mL of distilled water. Soon after that, an aliquot of 0.2 mL of that extract, to which 0.8 mL of

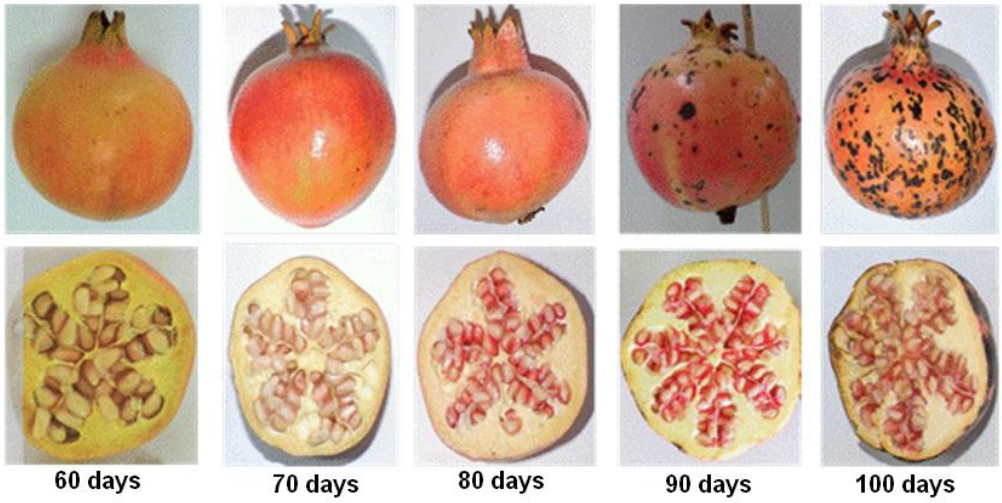

Fig. 1. Pomegranates (cv. Molar) at 60, 70, 80, 90 and 100 days, produced at Fazenda Águas de Tamanduá, Várzeas de Sousa, Paraíba, 2015. Source: Own authorship

distilled water and 2 mL of anthron were added, was taken. Those samples were agitated and taken to the double boiler under 100°C for 5 minutes, and the measures were conducted in a spectrophotometer with a wave length of 620 nm [17]; Reducing sugars (mL 100 mL^{-1}); In order to obtain the initial extract, 0.2 mL of the sample that was dissolved in 100 mL of distilled water were taken. From that extract, 0.2 mL of the sample that was mixed with 1.3 mL of water and 1 mL of the 3,5-Dinitrosalicylic (DNS), and measures in a spectrophotometer at 520 nm were posteriorly conducted [18]; Vitamin C (% of ascorbic acid): It was conducted through the titration with 2,6-Dichlorophenolindofenol (DFI), until the obtainment of a permanent clear pink color, using 1 mL of the juice dissolved in 49 mL of oxalic acid at 0.5% [16]; Total phenolic compounds (mL 100 mL^{-1}): The extracts were prepared through the dissolution of 1 mL of juice in 50 mL of distilled water and left to rest for 24 h. An aliquot of 0.2 mL of the juice was transferred to a tube, where 1.925 mL of distilled water and 0.125 mL of the Folin Ciocalteau reagent were added. That mixture rested for 5 minutes and soon after that, 250 µL of sodium carbonate at 20% was added. Then, it was agitated and left to rest in a double boiler at 40°C for 30 minutes. The standard curve was prepared with gallic acid, and the measures were taken in a spectrophotometer at 765 nm according to the methodology proposed by Folin and Ciocalteau, described by Waterhouse [19]; Flavonoids and anthocyanins (mL 100 mL^{-1}): 1 mL of the pomegranate juice to determine the anthocyanins and 0.5 mL for the flavonoids, which were transferred to test tubes enwrapped in aluminum foil. Then, 10 mL of the solution for ethanol extraction at 95% with HCl 1,5 N at the 85:15 (v/v) ratio, respectively, were added. That sample was homogenized in a Vortex agitator. Then, it was left to rest under refrigeration for six hours, with no light. Posteriorly, the measures were conducted in a spectrophotometer, with a wave length of 535 nm, to determine the anthocyanins, calculated through the following formula: dissolution factor x absorbance/76.6 [20]; Carotenoids (µL 100 mL^{-1}): 1mL of juice, 0.2 g of calcium carbonate ($CaCO_3$) and 5 mL of acetone at 80% in a test tube were taken. Then, those samples were centrifuged for 10 minutes at 10°C and 3000 rpm. The measures were conducted with lengths of 470 nm, 646 nm and 663 nm in a spectrophotometer, since the concentration of chlorophyll a and b in the sample is used to determine the concentration of carotenoides [21].

In order to determine the antioxidant potential, two methods were employed: DPPH method (2,2-Dyphenyl-1-picryl-hidrazi) and ABTS method (2,2-Azino-Bis(3-Ethylbenzo-thiazoline-6-sulfonic acid), according to Rufino et al. [22] and [23], respectively, with adaptations. The extract was prepared through the dissolution of 1 g of pomegranate juice in 49 mL of distilled water, left to rest, for, at least, one hour. Then, aliquots of 10 µL, 30 µL and 50 µL were taken from that extract in order to conduct analyses through both methods.

In the DPPH method, the aliquots' volume increased to 100 mL with the distilled water, and 3.9 mL of the solution of DPPH were added. Posteriorly, the tubes were agitated in order to homogenize them. The measures were conducted in a spectrophotometer at 515 nm, and monitored in a span of time ranging from 15 to 20 minutes, which was previously determined according with the treatments. Methanol was used to calibrate the spectrophotometer. All the determinations were made with a third copy and followed by a control (without antioxidant). In order to calculate the values of EC50 (the concentration of the extract needed to reduce 50% of the DPPH radical) of each sample, the antioxidant capacity in different concentrations was calculated, in order to trace a linear curve between the antioxidant capacity of the sample and its concentration. The result was given in g juice g^{-1} of DPPH.

In the ABTS method, at first, the ABTS radical was formed through the reaction of 7 mM of ABTS with 140 mM of potassium persulphate, which were incubated under room temperature, with no light, for 16 hours. After then, the solution was diluted in ethanol until a solution with absorbance of 0.70 (± 0.05) nm at 734 nm was obtained [23]. During the analyses, aliquots of 10 µL, 30 µL and 50 µL were taken from the extract and its volume was completed to 100mL with ethyl alcohol and, then, 3 mL of the ABTS radical were added and homogenized in a tube shaker. Posteriorly, after six minutes of waiting, the measuring with the spectrophotometer was conducted at a wave length of 734 nm using ethyl alcohol as a solvent. The synthetic antioxidant Trolox was used as a standard solution, at the concentrations of 100; 500; 1000; 1500 and 2,000 µM in ethanol. All the measures were conducted with a third copy, and the results were expressed in µM of Trolox mL^{-1} of juice.

Statistical analysis: The data was submitted for analysis of variance and regression (p<0,05) using the SISVAR software version 5.3 [24].

3. RESULTS AND DISCUSSION

According to the Analysis of Variance, there was a significant effect at the level of probability of 1% for all the variables studied, except for the antioxidant activity of the juice through the DPPH method, during the development of the fruit of the pomegranate cv. Molar that did not differ significantly during the growth stages of the fruit (Tables 1 and 2).

Titratable acidity had a value variation between 0.61% and 0.92% of citric acid, at 60 and 80 days, respectively. Posteriorly, a decrease was observed. There were values around 0.66% of citric acid at 100 days old (Fig. 2A), in concordance with the behavior observed by Silva et al. [25] and Onur and Kaska [26]. Those authors classify pomegranates as sweet when the fruit of the cultivar present less than 1% of acidity.

The hydrogen potential (pH) had little variation during the phenological development stages of the fruit, with values ranging from 2.9 to 3.1 (Fig. 2B). A similar behavior was observed in the pomegranate's pH by other authors. Fawole et al. [27], studying the chemical properties of pomegranates cultivated in South Africa, observed pH values ranging from 3.32 to 3.64. Moreira et al. [12] characterized the quality of the 'Molar' pomegranate during the storage of raw fruit under different refrigeration temperatures, and they found out pH values ranging from 3 to 4, throughout the storage period.

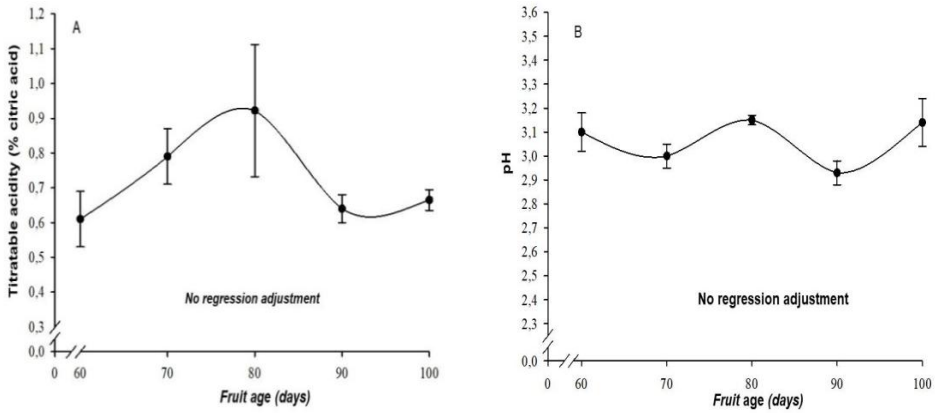

Fig. 2. Titratable acidity (A) and pH (B) in the aril of the 'Molar' pomegranate analyzed during the phenological stages of the development of the fruit

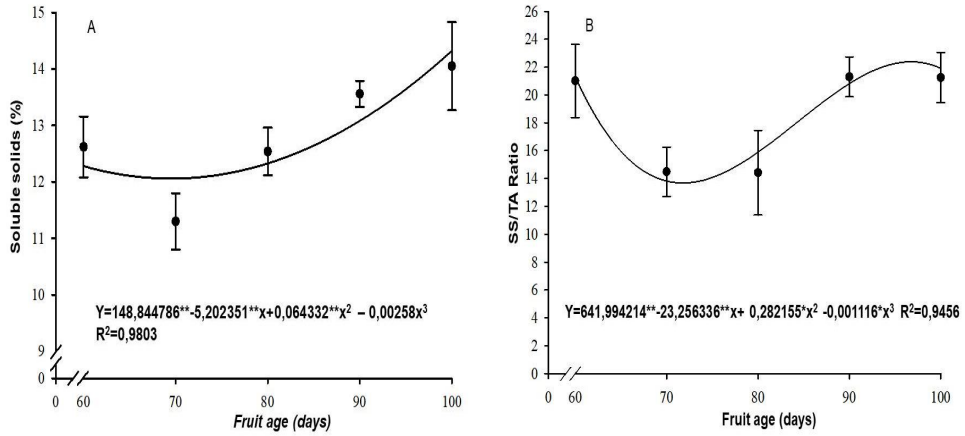

Fig. 3. Soluble solids (A) and SS/TA ratio (B) in the 'Molar' pomegranate aril analyzed during the phenological stages of the development of the fruit

Table 1. Summary of variance analysis for the variables: titratable acidity (AT), pH, soluble solids (SS), SS / AT ratio, total sugar, reducing sugars and vitamin C in 'Molar' pomegranates during the development of the fruit

FV	GL	Mean square						
		AT	PH	SS	SS/AT	Total sugars	Reducing sugars	Vitamin C
Ages of the Fruit	4	0,06728**	0,038832**	4,48583**	54,483632**	15,1890**	0.00070**	18,9810**
Residue	15	0,010065	0,004075	0,275425	4,880303	1,4252	0,000002	0,3955
CV (%)		13,83	2,08	4,1	11,94	8,42	13,78	7,66
Average		0,72	3,06	12,81	18,49	14,18	0,0104	8,20

***Probability of 1%, through the F test*

Table 2. Summary of variance analysis for the variables: phenolic compounds, flavonoids, anthocyanins, carotenoids, DPPH and ABTS in 'Molar' pomegranates during fruit development

FV	GL	Mean square					
		Phenolic compounds	Flavonoids	Anthocyanins	Carotenoids	DPPH	ABTS
Ages of the Fruit	4	38,1648**	68,8261**	4,9612**	0,1630**	3814323,03ns	582076545,62**
Residue	15	34,7142	4,0844	0,6527	0,01119	1701292,9	29357570,45
CV (%)		9,52	14,75	19,61	26,15	51,88	22,58
Average		61,86	13,70	4,11	0,40	2514,32	23992.99

***Probability of 1%, through the F test*

The soluble solids (SS) had an increase between 11% and 14.05%, and between 70 and 100 DAA, respectively, corresponding to an increase of 21.7% (Fig. 3A).

Important compounds responsible for the taste and the consequent acceptance by the consumers can be found among the soluble solids in the pulp of the fruit. The most important are sugars and organic acids, and the soluble solids content is the parameter of greater ease of indirect determination of sucrose [28].

Silva et al. [25] reports an amount of SS ranging from 12% to 15% in 'Molar' pomegranate tree fruit stored under room conditions. Those values are in the range identified by this work. When Gadže et al. [29] evaluated 'Ciparski', 'Konjski zub' and 'Pastun' in Southern Dalmatia, Neretva valley, in Croatia, they described SS values ranging from 13.1% to 15.6%.

The alterations in SS and TA values reflected on the SS/TA ratio (Fig. 3B), which varied from 14.42 to 21.30, and the highest SS/TA ratio happened after 90 days, when TA was low and SS was high, which may present a sweet fruit to the consumer, agreeing with Silva et al. [25] and Onur and Kaska [26]. According to Fawole and Opara [4], the SS/TA ratio is an important indicator of the pomegranate maturation, with values that range from 3.73 to 86.3, depending on the cultivar and the stage of maturation.

Total sugars gradually increased to 90 DAA, and even presented a maximum value of 16.70 mL.100 ml^{-1}, with a posterior reduction to 100 DAA (Fig. 4A), probably due to the natural senescence of the fruit in the plant, intensifying the consumption of carbohydrates as a source of energy. Reducing sugars presented a similar behavior. They also reached a maximum concentration of 0.016 mL.100 mL^{-1} at 90 DAA and consequently a reduction at 100 DAA (Fig. 4B). Generally speaking, reducing sugars in pomegranates were very low. Probably, the Molar cultivar has that characteristic, which can indicate a prevalence of non reducing sugars to the detriment of the reducing sugars.

Li et al. [30] reports that differences on the fruit maturation levels causes distinct sugar concentrations. Ozgen et al. [31] reported an average of 6.4% to 6.8% of fructose and glucose, respectively, in the pomegranate juice in six cultivars ('incekabuk Dikenli', 'Eksi', 'Kan', 'Katirbasi', 'Serife' and 'Tatli') in Turkey. Fawole and Opara [4] reported an increase on the glucose and fructose concentrations during the fruit maturation, with glucose to fructose ratios ranging between 0.67-0.85 and 0.72-0.86 for the 'Bhágwa' and 'Ruby' cultivars, respectively grown in South Africa.

Vitamin C increased from 60 to 90 DAA, leaping from 4.75% to 10.5% of ascorbic acid, respectively (Fig. 5A). From 90 DAA on, as seen with the sugars, there was a reduction, intensifying the possibility of the fruit being in process of senescence at 100 DAA. The phenolic compounds increased from 53.61 mL.100 mL^{-1} to 83.13 mL.100 mL^{-1}, from 60 to 70 DAA, respectively, with a posterior decline to 90 DAA, followed by a small increase to 100 DAA (Fig. 5B), an indicator of the fruit natural senescence. That behavior shows clearly the evolution of the maturation of the fruit, since in the beginning of the development of the pomegranate, up to 80 DAA, agreeing with the phenolic compounds, the highest TA values were observed, but the lowest values were observed for SS, total sugars and reducing sugars. With the evolution of the maturation, the phenolic compounds decreased and reached the lowest values at 90 DAA, when TA decreased, and SS, total sugars, reducing sugars and vitamin C reached the best levels, which might indicate lower astringency and the ideal time for the harvest, regarding the quality characteristics of the pomegranate for it to be consumed raw.

In concordance with the behavior observed on the phenolic compounds, the flavonoids presented the highest values in the beginning of the pomegranate maturation and decreased linearly with the advance of the of age of the fruit (Fig. 6A). Anthocyanins increased in between 60 and 90 DAA, like the color transformations on the aril, which are typical for the Molar cultivar, evident at 90 DAA (Fig. 6B), the red-pink tonality. Values reported for the 'Wonderful' pomegranate where higher than those found in this study, at the point of harvest, 5 mL.100 mL^{-1}, 56-30 mL.100 mL^{-1}, 11 mL.100 mL^{-1} [32] and 226.27 mL.10 mL^{-1} [33], in Iran. According to Borochov-Neori et al. [34], the concentration of anthocyanin can be influenced by the climactic conditions during the fruit development and maturation. Thus, the anthocyanins concentration can increase or decrease according to the climate to which the cultivars are submitted.

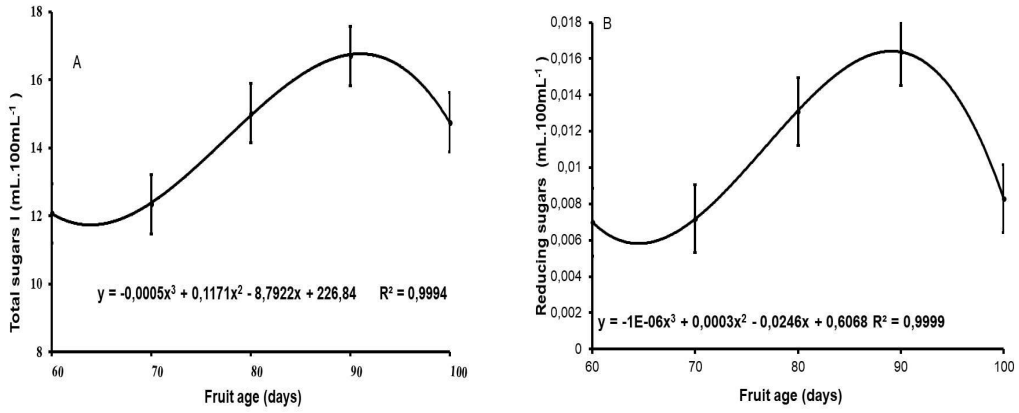

Fig. 4. Total sugars (A) and reducing sugars (B) in the 'Molar' pomegranate aril analyzed during the phenological stages of the development of the fruit

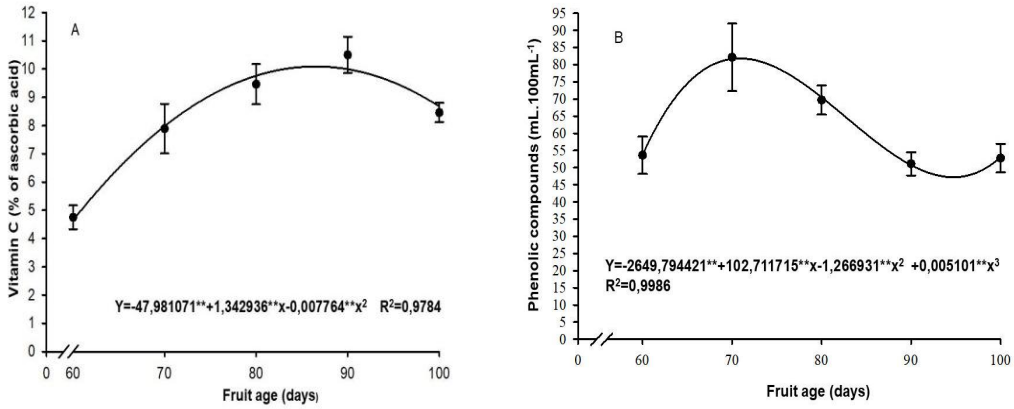

Fig. 5. Vitamin C (A) and phenolic compounds (B) in the 'Molar' pomegranate aril analyzed during the phenologic stages of the development of the fruit

The behavior displayed by carotenoids was similar to the behavior displayed by the phenolic compounds and flavonoids. That is, the highest values were the valued registered for the youngest pomegranates, up to 80 DAA. Then, the highest concentration of that pigment was registered: 0.58 μL.100 mL^{-1} (Fig. 5C). However, Elfalleh et al. [35] reports that anthocyanins are the main water-soluble pigments responsible for the pomegranate juice's bright red color.

According to the Fig. 7A, an increase in the antioxidant capacity until 90 days old was registered. Fruit reaped at 60 days after the anthesis presented a lower antioxidant activity, with an IC50 value of 1407.17 g.juice g^{-1}. DPPH presented the highest antioxidant activity. Regarding the antioxidant capacity of the ABTS method, values of 40,188.13 uM Trolox.mL^{-1} of juice at 60 days and 10,546.52 uM Trolox.mL^{-1} of juice at 90 days, displaying, thus, a higher antioxidant activity at 90 days old (Fig. 7B).

The highest antioxidant activity of the fruit at 90 DAA ratifies with the highest values registered at 90 DAA, for ascorbic acid and anthocyanins, respectively. Kulkarni and Aradhya [36], studying the main chemical alterations, and the antioxidant activity and its importance during the pomegranate development also describe a lower antioxidant activity on the pomegranate fruit at 60 days old. Those authors justify that that low activity can be due to a reduced concentration of phenolic compounds and ascorbic acid in the juice.

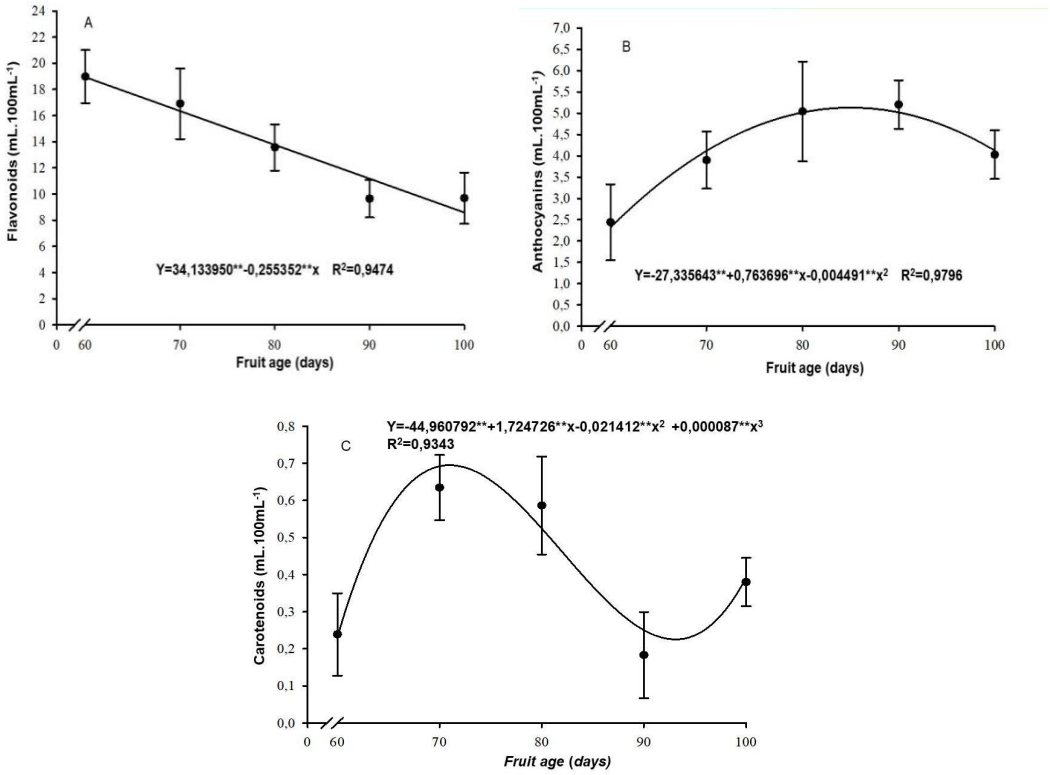

Fig. 6. Flavonoids (A), anthocyanins (B) and carotenoids (C) in the 'Molar' pomegranate aril analyzed during the phenologic stages of the development of the fruit

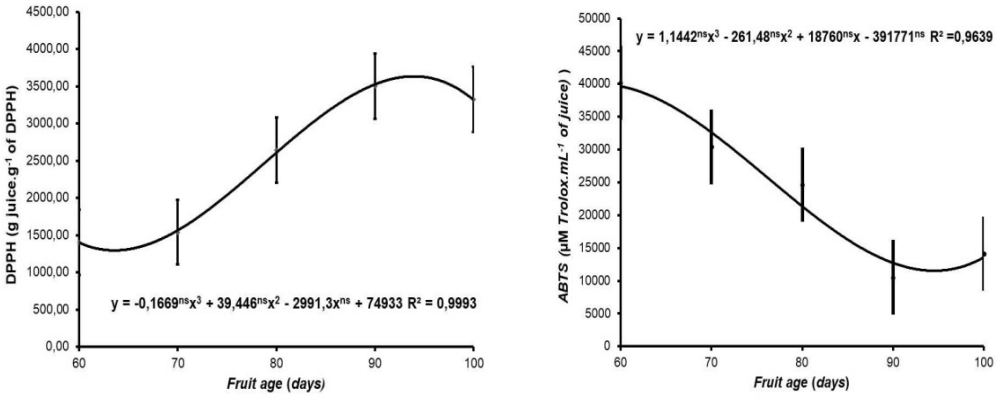

Fig. 7. Antioxidant activity, DPPH (A) and ABTS (B) methods for the 'Molar' pomegranate aril analyzed during the phenologic stages of the development of the fruit

4. CONCLUSIONS

The 'Molar' pomegranate produced in Várzeas de Sousa, Paraíba, reaches its best quality indicators for harvest at 90 DAA. In that period, it presents the highest rates of soluble solids, total sugars, reducing sugars, vitamin C and anthocyanins, and the highest antioxidant activity.

Ascorbic acid and anthocyanins are the main bioactive compounds responsible for the antioxidant activity in the pomegranate cv. Molar analyzed through the DPPH and ABTS methods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Santiago MCPA. Avaliação de processos para obtenção de produtos ricos em antocianinas utilizando suco de romã (*Punica granatum* L.). Tese (Doutorado) - Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro; 2014.
2. Werkman C, Granato DC, Kerbauy WD, Sampaio FC, Brandão AAH, Rode SM. Aplicações terapeuticas da (*Punica granatum* L.) romã. Revista Brasileira de Plantas Medicinais. 2008;10(3):104-111.
3. Al-Maiman SA, Ahmad D. Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chemistry. 2002;76:437–441.
4. Fawole OA, Opara UL. Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. Ruby) fruit at five maturity stages. Scientia Horticulturae. 2013;150:37–46.
5. Holland D, Hatib K, Bar-Ya'Akov I. Pomegranate: Botany, horticultivation, breeding. In: Janick J. (Ed.), Horticultural Reviews. 2009;35:127–191.
6. Salgado JM, Ferreira TRB, Biazotto FO, Dias CTS. Increased antioxidant content in juice with dried extract of Pomegranate (*Punica granatum*) peel. Plants Food for Human Nutrition. 2012;67(1):39-43.
7. Erkan M, Dogan A. Pomegranate/Roma—*Punica granatum.* Exotic Fruits. 2018;355-361.
   Available:https://doi.org/10.1016/B978-0-12-803138-4.00049-6
8. Patras A, Brunton NP, O'Donnell C, Tiwari BK. Effect of thermal processing on anthocyanin stability in foods; mechánisms and kinetics of degradation. Trends in Food Science and Technology. 2010; 21(1):3–11.
9. Qu W, Breksa AP, Pan Z, Ma H, Mchugh TH. Storage stability of sterilized liquid extracts from Pomergranate pell. Journal of Food Science. 2012;77(7):765-772.
10. Mphahlele RR, Fawole OA, Stander MA, Opara UL. Preharvest and postharvest factors influencing bioactive compounds in pomegranate (*Punica granatum* L.) — A review. Scientia Horticulturae. 2014;178: 114-123.
11. Furtunato TC de. Propriedades físicas, químicas e capacidade antioxidante da romã (cv. Molar) durante o desenvolvimento do fruto. Dissertação (Mestrado em Horticultura Tropical) – Universidade Federal de Campina Grande, Pombal; 2016.
12. Moreira IS, Rocha RHC, Paiva EP, Silva HS, Sousa FA. Biometria e componentes físico-químicos de romã armazenada sob refrigeração. Pesquisa Agropecuária. 2015;45(2):209-215.
13. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Lei Nº 10831, de 23 de dezembro de 2003. Ministério da Agricultura, Pecuária e Abastecimento. Diário Oficial da União, Brasília, Seção 1, p. 8, 24 dez; 2003.
14. INMET-Instituto Nacional de Meteorologia-Banco de Dados Meteorológicos para Ensino e Pesquisa.
   Available:http://www.inmet.gov.br/projetos/rede/pesquisa/gera_serie_txt_mensal.php?&mRelEstacao=82689
   (Acesso em 12 de Jan 2017)
15. IAL – Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. 4ª Ed. São Paulo: Instituto Adolfo Lutz; 2008.
16. AOAC – Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists. 18ª ed. Gaithersburg, Maryland; 2006.
17. Ymn EW, Wills AJ. The estimathion of carbohydrate in plant estracts by anthrone. The Biochemical Journal. 1954;57(3):508-514.
18. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426-428.
19. Waterhouse A. Folin-ciocalteau micro method for total phenol in wine. American Journal of Enology and Viticultivation. 2006;3-5.
   Available:http://waterhouse.ucdavis.edu/faqs/folin-ciocalteau-micromethod- for-total-phenol-in-wine
   (Acesso em: mai. 2018)
20. Francis FJ. Analysis of anthocyanins. In: Markakis P.(Ed.). Anthocyanins as Food Colors, New York: Academic Press. 1982;181-207.
21. Lichtenthäler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer L, Douce, R.

(Eds). Methods in Enzymology. 1987;148: 350-382.
22. Rufino MS, Alves RE, Brito ES, Morais SM, Sampaio CG, Jiménez JP, Calixto FDS. Metodologia científica: determinação da atividade antioxidante total em frutos pela captura do radical livre DPPH. Fortaleza, Embrapa Agroindústria Tropical, (Comunicado Técnico, N. 127). 2007a;4.
23. Rufino MS, Alves RE, Brito ES, Morais SM, Sampaio CG, Jiménez JP, Calixto FDS. Metodologia científica: Determinação da atividade antioxidante total em frutos pela captura do radical livre ABTS. Fortaleza, Embrapa Agroindústria Tropical, (Comunicado Técnico, N. 128). 2007b;4.
24. Ferreira DF. Sisvar: um sistema computacional de análise estatística. Ciência e Agrotecnologia. 2011;35:1039-1042.
25. Silva IMBR, Rocha RHC, Silva HS, Moreira IS, Sousa FA, Paiva EP. Quality and post-harvest life organic pomegranate 'Molar' produced in Paraiba semiarid. Semina: Ciências Agrárias. 2015;36(4): 2555-2564.
26. Onur C, Kaska N. Akdeniz bölgesi narlarının (*Punica granatum* L.) seleksiyonu (Selection of Pomegranate of Mediterranean region). Turkish Journal of Agricultivation and Forestry. 1985;9(1):25-33.
27. Fawole OA, Opara UL, Theron KI. Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. Food and Bioprocess Technology. 2012;5:2934-2940.
28. Waclawovsky AJ, Sato PM, Lembke CG, Moore PH, Souza GM. Sugarcane for bioenergy production: An assessment of yield and regulation of sucrose content. Plant Biotechnol J. 2011;8(3):263-76.
29. Gadže J, Voća S, Čmelik Z, Mustać I, Ercisli S, Radunić M. Physicochemical cháracteristics of main pomegranate (*Punica granatum* L.) cultivars grown in Dalmatia region of Croatia. Journal of Applied Botany and Food Quality. 2012;85:(2):202–206.
30. Li X, Wasila H, Liu L, Yuan T, Gao Z, Zháo B, Ahmad I. Physicochemical cháracteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. Food Chemistry. 2015;175(1):575-584.
31. Ozgen M, Durgac C, Serce S, Kaya C. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chemistry. 2008;111(5):703-706.
32. Tehranifar A, Zarei M, Nemati Z, Esfandiyari B, Vazifeshenas MR. Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. Scientia Horticulturae. 2010; 126(2):180-185.
33. Arendse E, Fawole OA, Opara UL. Discrimination of pomegranate fruit quality by instrumental and sensory measurements during storage at three temperature regimes. Journal of Food Processing and Preservation. 2015;39: 1867–1877.
34. Borochov-Neori H, Judeinstein S, Hárari M, Bar-Ya'Akov I, Patil BS, Lurie S, Holland D. Climate effects on anthocyanin accumulation and composition in the pomegranate (*Punica granatum* L.) fruit arils. Journal Agricultural Food Chemistry. 2011;59:5325–5334.
35. Elfalleh W, Ying, Nasri N, Sheng-Hua, Guasmi F, Ferchichi A. Fatty acids from Tunisian and Chinese pomegranate (*Punica granatum* L.) seeds. International Journal of Food Sciences and Nutrition. 2011;62:200-206.
36. Kulkarni AP, Aradhya SM. Chemical chánges and antioxidant activity in pomegranate arils during fruit development. Food Chemistry. 2005;93(2): 319-324.

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