QUALITY, ANTIOXIDANT AND ENZYMATIC ACTIVITIES OF FACHEIRO (*Pilosocereus pachycladus* RITTER) FRUITS DURING MATURATION[1]

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ABSTRACT - The fruit of Cactaceas from northeastern Brazil have been the object of many ethnobotanical and functional quality studies. However, a considerable number of species remain poorly exploited, such as the facheiro (*Pilosocereus pachycladus* Ritter), a native Brazilian plant widely occurring and used in the Caatinga. The objectives of this research were to evaluate the quality, and antioxidant and enzymatic activities of the facheiro fruit during maturation. Fruits were harvested from areas of occurrence of plants in the Paraíba State, Brazil, at three maturity stages, which were determined based on the color of the epicarp (G - green, IP – green with purple traces, and R - completely purple). It was carried out a survey of terms sensorial descriptor terms for fruit appearance and then evaluated the physical, physicochemical, bioactive compounds, antioxidant and peroxidase (POD) activities during maturation. The fruit were described as fleshy berries, with a smooth epicarp; a round, flat shape; opaque purple color; and juicy and soft pulp when ripe. Quality varied during maturation, with the most significant changes being in coloration: the fruit became more purple over time, providing a clear indicator of maturity. With maturation, there was an increase in the content of betalains and total extractable polyphenols, as well as antioxidant activity, by DPPH radical capture method, mainly in the pulp. The pulp of the *Pilosocereus pachycladus* fruit is an important source of betalains, primarily betacyanins. During maturation, POD activity decreased in the pericarp and increased in the pulp and was strongly correlated with the presence of betalains and with antioxidant activity.

Keywords: Cactaceae. Maturity. Bioactive Compounds. DPPH. Peroxidase activity.

QUALIDADE, ATIVIDADE ANTIOXIDANTE E ENZIMÁTICA DE FRUTOS DO FACHEIRO (*Pilosocereus pachycladus* RITTER) DURANTE A MATURAÇÃO

RESUMO - Os frutos de Cactaceas do Nordeste Brasileiro têm sido alvo de muitos estudos etnobotânicos e de qualidade funcional. No entanto, ainda há um número considerável de espécies pouco exploradas, como o facheiro (*Pilosocereus pachycladus* Ritter), planta nativa e de ampla ocorrência e uso na Caatinga. O objetivo desta pesquisa foi avaliar a qualidade, atividade antioxidante e da peroxidase (POD) de frutos do facheiro durante a maturação. Os frutos foram colhidos de áreas de ocorrência de plantas em três estádios de maturação, com base na coloração do epicarpo (V – verde, IP – verde com início de coloração roxa e R – completamente roxo), do município de Pocinhos, Paraíba. Foi realizado um levantamento de termos descritores sensoriais de aparência dos frutos e, em seguida, avaliações físicas, físico-químicas, compostos bioativos, atividade antioxidante e da POD durante a maturação. Os frutos foram descritos como bagas carnosas, com epicarpo liso, formato redondo-achatado, cor roxa opaca, polpa suculenta e macia quando maduros. A qualidade variou durante a maturação, sendo as mudanças mais expressivas as de coloração: tornou-se mais roxa com o avanço da maturação, proporcionando um claro indicador de maturidade. Com a maturação, observou-se um aumento nos conteúdos de betalaínas e polifenóis extraíveis totais, bem como a atividade antioxidante, pela captura do radical DPPH, principalmente na polpa, A polpa do fruto de *Pilosocereus pachycladus* é uma fonte importante de betalaínas, principalmente betacianinas. Durante a maturação, a atividade da POD diminuiu no pericarpo e aumentou na polpa e foi fortemente correlacionada com a presença de betalaínas e com a atividade antioxidante.

Palavras-chave: Cactaceae. Maturidade. Compostos bioativos. DPPH. Atividade da peroxidase.

INTRODUCTION

The Cactaceae family has about 130 genera and 1500 species, with the third largest diversity hotspot occurring in Brazil, particularly in the semi-arid areas of the northeast, where they are well adapted to the adverse climatic and soil conditions (ABUD et al. 2010; SILVA et al., 2009).

In the Northeastern Brazil Cactaceae have been widely used in animal feed during periods of drought and, more restrictively, in human feeding in localized populations (SOUZA et al.,2015). Studies have increased the knowledge of introduced Cactaceae fruit, such as *Opuntia fícus-indica* (SILVA et al., 2009; TORRES et al., 2009; SOUZA et al., 2015), and native fruits, such as *Tacinga inamoena* (DANTAS et al., 2015a; DANTAS et al., 2015c; DANTAS et al., 2016), *Cereus jamacaru* (MELO et al., 2017), *Pilosocereus gounellei* (SILVA et al., 2018; ABUD et al., 2012). However, there are some fruit species which are still underutilized due to the scarcity of nutritional and functional information (SILVA et al., 2009; DANTAS et al., 2015a), including the facheiro (*Pilosocereus pachycladus* Ritter) fruits (SOUZA et al., 2015). In this context, Cactaceae fruits are considered important sources of betalains, which are recognized for their reduction properties and ability to capture free radicals (STINTZING, et al., 2005), as their importance as anti-inflammatories (TESORIERE et al., 2014).

The facheiro fruit is characterized as polyspermic, with numerous seeds and a fleshy pulp (ABUD et al. 2010), that tends to darken after the tissue is cut.

There have been several studies on the sensorial and quality characteristics of other fruits of Caatinga species (DANTAS et al., 2015a; LIMA et al., 2013), as well as on the presence of bioactive compounds of importance and their impacts on the functional potential of the fruit (SILVA et al., 2009; DANTAS et al., 2015b; DANTAS et al., 2015c; DANTAS et al., 2016; SILVA et al., 2018), but more extensive studies are needed to broaden the understanding of the quality features of the facheiro fruit. Also, due to the fast darkening of the pulp following exposure to air, it is needed to evaluate the activity of enzymes of technological relevance, such as peroxidase, which is capable of oxidizing important phenolic compounds, resulting in a reduction in quality (DUARTE; RIVERA; CUENCA, 2005; KOHAICH; BAAZIZ, 2015).

These studies with facheiro fruits will provide support to future research, breeding programs, production, consumption and use of Cactaceae. In addition, it is important to evaluate the changes resulting from the maturation of these fruit, to establish identity and quality standards, as well as to highlight the quality features of most interest to the fruit producing industry and to reach specific market niches (DANTAS et al., 2016).

Thus, the objective of this research was to evaluate quality, and antioxidant and peroxidase activity during maturation of the facheiro fruit.

MATERIAL AND METHODS

Facheiro (*Pilosocereus pachycladus* Ritter) fruits were harvested from spontaneously occurring plants in rural areas. Thirty plants were previously identified and georeferenced in the municipality of Pocinhos, Agreste mesoregion, state of Paraíba, Brazil. The fruits were harvested before 9:00 AM and stored in closed isothermal boxes and wrapped with bubble plastic for transportation. In the laboratory, the fruits were selected for maturity, uniformity and absence of lesions, washed with tap water, sanitized with 100 ppm sodium hypochlorite solution and dried at room condition on a stainless steel table previously sanitized.

The experiment was carried out in a completely randomized design (CRD), arranged in a simple 3x2 factorial scheme, refering to three maturity stages (G = green, IP = green with purple traces, and R = completely purple, fully ripe fruits) and evaluations in two fruit portions: i) Pericarp (epicarp + mesocarp, P) and ii) Endocarp (pulp + seed, E). For the physical evaluations, 48 fruits per maturity stage were used, each fruit was considered a replication. For the physicochemical characteristics, bioactive compounds, antioxidant and enzymatic activities, 4 replications of 12 fruits/maturity stage were used for each fruit portion, which were homogenized with domestic processor. Physical, physicochemical, sensory and ascorbic acid evaluations were performed on the day of harvesting. For the other, the fruit portions for each maturity stage were immediately frozen after processing for later analyzes.

The descriptive sensory evaluation of fruit appearance was performed through Quantitative Descriptive Analysis (QDA) according to Dantas et al. (2015a). Twenty-four panelists were selected after training to survey the describing terms for the attributes for the internal and external appearances, and texture and firmness of fruits in the three maturity stages, which was performed using the network method (Kelly's Repertory Grid Method) and the basic principles of the QDA method (STONE; SIDEL, 1998). Based on the aspects of appearance and texture, each panelist recorded a list of the main attributes observed. These attributes were put into debate for the final definition of fruit's descriptive terms.

The physical characteristics were: length and diameter, measured with the aid of a caliper; fresh mass (g), obtained with the help of a semi analytical scale (UX 4200H); firmness (N), determined in two points in the equatorial region of each fruit using a

bench penetrometer (Fruit Hardness Tester), and fruit color that was accessed through an objective evaluation, using a Minolta digital colorimeter, which expresses the color in the parameters: *L** (corresponds to the clarity/brightness); *a** (defines the transition from the green color (-*a**) to the red color (+ *a**) and *b** (represents the transition from the blue color (-*b**) to the yellow color (+ *b**), and the farther from the center (= 0), the more saturated is the color.

The physicochemical characteristics were: soluble solids (SS%), determined with a bench refractometer with temperature control (20 °C), titratable acidity (AT%), determined by titration with 0.1 M NaOH up to pH 8.1 (IAL, 2005), SS/AT ratio by the quotient between SS and TA, and pH, determined with digital pHmetro DM-22 according to methods described by Silva et al. (2018).

The ascorbic acid content (mg.100g^{-1}) was measured by titration with DFI (2,6-Dichloroindophenol 0.02%) (STROHECKER; HENNING, 1967). The pulp was extracted by using ethyl alcohol solution (85:15) to decrease the color intensity and homogenized with 50 mL oxalic acid for titration.

The total extractable polyphenols content (TEP) was measured in the methyl phenolic extract (LARRAURI; RUPÉREZ; SAURA-CALIXTO,1997), based on prior testing, using 100 μL aliquots. The oxidation test was performed by adding 1 mL of Folin-Ciocalteu's, 2.0 mL of 20% sodium carbonate, and 2.0 mL of distilled water. The readings were performed at 700 nm and the results expressed in mg of gallic acid per 100 g of fresh weight (mg.100 g^{-1}).

The yellow flavonoids content was determined only in the pericarp. The extract solutions were composed of 95% ethanol and 1.5 N HCl in the ratio of 85:15 (v/v). The readings were performed at 374 nm and the results expressed as mg.100g^{-1} (FRANCIS, 1982).

Betalains content was determined according to Dantas et al. (2015c). Extracts were prepared using ethanol:water (80:20) as the extractor solution. For the determination of betacyanins and betaxanthins, the absorbances of the extracts were measured at 535 and 485 nm, respectively, and the results expressed as mg.100g^{-1}.

The total antioxidant activity of the extracts was determined by 2,2-difenil-1-picrilidrazil (DPPH) free radical scavenging method. Three dilutions were prepared in triplicate of the phenolic extract obtained from the determination TEP. From each dilution, an aliquot of 0.1 mL added to 3.9 mL of DPPH radical (60 μM) was used. The absorbance was monitored at 515 nm, every minute until the readings stabilize to obtain the EC50 (effective extract concentration to induce half (50%) of the maximum effect). The results were expressed as g of pulp per g DPPH^{-1} (DANTAS et al. 2015b).

Peroxidase activity (POD, EC 1.11.1.7) was measured according to Wu et al. (2010). The extract was prepared from 5 g of pulp homogenized with 10 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1% of PVP. The solution was centrifuged at 7600 g for 20 min at 4 °C, and the supernatant was used as enzymatic extract. A standard curve with bovine serum albumine (2.5 a 60 μg mL^{-1}) was used. The protein concentration of the extract was measured at 595 nm (BRADFORD, 1976). The peroxidase activity was assayed based on the guaiacol oxidation using hydrogen peroxide and a extinction coefficient of 26.6 mM^{-1} cm^{-1}. The increase in the absorbance was monitored for 3 minutes at 470 nm. The results were expressed as U g^{-1} protein.

Data were submitted to analysis of variance (ANOVA) and the means of stages were compared by the Tukey test and the portions of fruits compared by F test up to 5% probability of error. Pearson correlation among bioactive compounds, enzymatic activity and antioxidant activity was performed. In order to perform these analyzes the SAS® 9.2 was used.

RESULTS AND DISCUSSION

The facheiro is characterized as a fleshy berry, with a thick pericarp and is juicy, dehiscent, and polyspermic, as described by Abud et al. (2010). In the ripe fruit (R), the texture was mucilaginous, and the epicarp, while primarily smooth, was rough in the vicinity of the floral remnant.

Regarding the descriptors of fruit appearance, at the beginning of maturation, the epicarp was green in color, then, at intermediate maturity, the color developed into a light purple color with traces of yellow, advancing towards the intense purple coloration of the fully ripe fruit (Figure 1). The floral remnant tended to be inclined in fruit from the green stage with traces of purple coloration (IP). The endocarp (E – pulp + seeds) of fruit in the IP and intense purple (R) maturity stages had numerous black seeds, while in the green fruit (G), the seeds were pink. The facheiro fruit is polyspermic, containing numerous seeds and a fleshy pulp, which tends to darken after exposure to air. It was recorded dark regions in the fruit epicarp from the IP maturity stage, due to the surface ruptures (cracking) that appear, especially in the more advanced stages, as reported for fruit of xiquexique (SILVA et al., 2018).
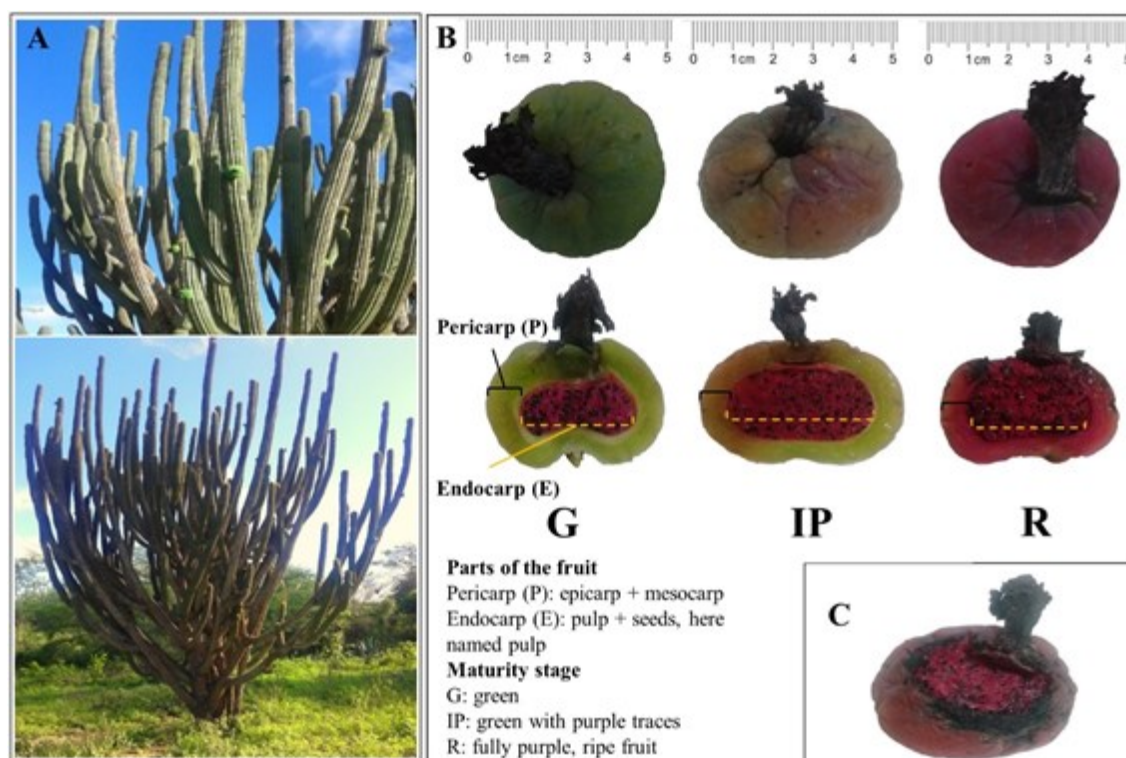
Figure 1. Facheiro plant (*Pilosocereus pachycladus* Ritter) in the productive phase (A), changes in external appearance of fruits according to the evolution of maturity stages (G = green fruit, IP = green with purple traces and R = ripe fruit, with intense and full purple color (B), and cracking of pericarp characteristic of senescent fruits (C).

The ripe fruit is round and smooth, flattened at the ends, with an opaque purple coloration (Table 1). Dantas et al. (2015a) carried out a survey of sensorial descriptors for the appearance of *Tacinga inamoena* fruit during maturation and described the evolution of the epicarp coloration through five maturity stages, varying from green to reddish. The fruit was similar to the facheiro fruit, but with a lower fresh mass (Table 2). In facheiro fruit, the most significant changes were those of color, since pericarp and pulp developed into an increasingly purplish coloration as maturation progressed, clearly delineating the transitions of maturity stages. In this sense, changes in coloration can be an adequate indication of the maturity of facheiro fruit. Facheiro fruit in the advanced maturity, following the R stage, can present dehiscence of the pericarp (Figure 1C), exposing the pulp to the environment and allowing it to be consumed by insects and birds, as a probable seed dispersion strategy (LEAL; WIRTH; TABARELLI, 2007).

The length of the facheiro fruit did not differ among maturity stages (green – G, green with purple traces – IP and fully purple –R), with a mean of 33.44 mm. However, the diameter differed, with IP stage fruit having the largest diameter (mean of 50.16 mm) due to the expansion of the pulp at the beginning of maturation (SIDDIQ, 2018). The rate pulp expansion stabilized at the IP stage (Table 2).

The length and diameter of facheiro (*Pilosocereus pachycladus*) fruit were similar to those reported by Abud et al. (2010), with means of 38.13 and 50.53 mm, respectively, and a diameter greater than the length, as described by the sensorial panel. The fruit size of *Pilosocereus catingicola*, evaluated by Medeiros et al. (2015), was close to that reported herein, with a length of 33.61 mm and diameter of 43.51 mm. Fruit of *Pilosocereus gounellei* (ABUD et al., 2012; SILVA et al., 2018) have been reported to have lengths and diameters of 40.67 and 48.09 mm, and 37.76 and 47.95 mm, respectively, and fruit of *Pilosocereuss* sp, have a length of 33.1 and a diameter of 47.9 mm (SILVA et al., 2009).

Table 1. Sensory descriptors of the appearance and texture of facheiro fruit (*Pilosocereus pachycladus* Ritter) in three maturity stages*.

	Maturity Stages		
	G	IP	R
Epicarp	Green with regions slightly dark	Green with purple and yellow traces with dark regions	Fully purple, with dark regions and occasional cracking
Mesocarp	Dark green	Light green with pinkish and yellowish regions	Purple, gelatinous, mucilaginous
Endocarp (pulp + seeds)	Light pink with numerous light pink seeds	Light purple with numerous brown seeds	Intense purple with numerous small black seeds
Texture of the endocarp	Slightly viscous pulp	Viscous and consistent pulp	Very viscous, consistent, juicy and soft pulp
Pericarp (epicarp + mesocarp)	Thick, but slightly firm	Thick, slightly firm, mucilaginous	Thick, soft texture, juicy, very mucilaginous
Floral remnant	Standing, firm and dark in color	Slightly inclined of dark color	Fully inclined and dark color
Shape	Oblong, rounded in diameter and slightly flattened in the extremities	Oblong, rounded in diameter and flattened in the extremities	Oblong, round, very flattened in the extremities
Aspect	Rough, leathery	Slightly smooth in diameter, rough and, leathery near the floral remnant	Smooth in diameter, opaque, rough near to floral remnant
Dehiscence	Not	Not	Eventually

*Sensory panel composed of 24 trained judges.
Maturity stage: G= green, IP= green with purple traces, and R = Fully purple, ripe fruit.

The mean fresh mass of facheiro fruit during maturation was 48.12 g, and there was no significant difference among the maturity stages (Table 2). A lower fresh mass was found by Medeiros et al. (2015) for the fruit of *Pilosocereus catingicola* (33.68 g). However, according to Silva et al. (2009), IP stage fruit of *Pilosocereus* sp. had a fresh mass of 75.37 g, much higher than that verified herein. In addition, xiquexique fruit (*Pilosocereus gounellei*) with a fresh mass of 45.04 g (SILVA et al., 2018), were similar in mass and size to the facheiro in this study.

Table 2. Physical characteristics and color parameters *L*, *a** and *b** of two portions of the fruit pericarp (epicarp + mesocarp, P) and endocarp (pulp + seeds, E) of facheiro fruits (*Pilosocereus pachycladus* Ritter) harvested in three maturity stages.

Characteristic		Maturity Stages		
		G	IP	R
Length (mm)		32.84 ±3.55 a	35.25 ±6.97 a	32.25 ±7.54a
Diameter (mm)		44.90 ± 6.02 b	50.16 ±6.86 a	45.26 ± 8.16 b
Fresh weight (g)		42.82 ±14.12 a	50.96 ± 18,28	50.58 ±20.0 a
Firmness (N)		20.02 ±13.20a	11.76 ±7.27b	13.78 ±10.60ab
Parameters		G	IP	R
L	P	24.64 ± 1.86 aA	24.24 ± 2.90 aA	21.50 ± 1.38 bA
	E	23.63 ± 6.78 aA	23.12 ± 11.39 aA	18.83 ± 2.79 aA
a*	P	-0.16 ± 0.76 cB	2.79 ± 2,65 bB	10.20 ± 1.65 aB
	E	11.75 ± 4.41 bA	13.00 ± 5.16 bA	17.33 ± 7.27 aA
b*	P	23.26 ± 2.10 aA	23.31 ± 2.85 aA	20.36 ± 2.76 bA
	E	20.65 ± 3.13 aA	23.19 ± 4.30 aA	22.60 ± 2.17 aA

Means followed by lower case in the line (maturity stages) and capital letters in the column (portion of the fruit for each color parameter) do not differ, by Tukey and F tests, respectively, at 5% probability. n=24
Maturity Stage: G= Green, IP= Green with purple traces, and R= Ripe fruit with fully and intense purple color.

Firmness declined with maturation, so that the firmer fruit were those at the G stage, with 20.02 N, reaching 13.78 N at maturity stage R (Table 2). Silva et al. (2009) also observed a decrease in firmness during maturation of *Pilosocereus* sp. fruit, with a mean of 8.32 N at beginning of maturation and 4.37 N at the end, i.e., less firm than the fruit in this study.

The coloration of the fruit pericarp and endocarp, measured by the parameters L^*, a^* and b^*, is shown in Table 2. The L^* parameter of the fruit pericarp declined along the maturity stages, with a value of 24.64 for the G stage and 21.50 at the R stage. The L^* value of the pulp did not differ among maturity stages, with an overall mean of 21.86 (Table 2). During maturation of red pitaya (*Hylocereus costaricensis*) the L^* ranged from 27.70 to 33.52 (SATO et al., 2014); in mandacaru fruit, Melo et al. (2017) reported a mean L^* of 28.28, similar to that obtained herein.

The parameter a^*, for both epicarp and pulp, differed among the three maturity stages. For the epicarp at the G stage, it was observed a negative mean value (-0.16), indicating that the fruit presented a green color, which evolved during the IP stage to a pink color, reaching a positive value of 10.20 in the fully ripe fruit (maturity stage R). For the pulp, the highest value for the a^* parameter was also found in R stage fruit (17.33), but the values of a^* did not differ between the G and IP stages, with means of 11.75 and 13.00, respectively (Table 2). For mandacaru (*Cereus jamacaru*) fruit, which express red coloration when ripe, Melo et al. (2017) reported a^* varying from 3.70 to 23.98 during maturation, indicating that these lower a^* values for facheiro fruit characterize the purplish color.

For the epicarp, the mean values of the b^* parameter were lower for R stage fruit (20.36), than for the G and IP stages, which had similar values. In contrast, the b^* values of the pulp did not differ among maturity stages, averaging at 22.14 (Table 2). Melo et al. (2017) found mandacaru fruit's epicarp b^* values to be 20.45, similar to the values reported herein.

The soluble solids (SS) content of the ripe facheiro fruit (3.73%) was much lower, compared to those of other cacti fruit, such as full ripe mandacaru fruit with 11.44% SS (MELO et al., 2017). In facheiro fruit, the SS content differed among the maturity stages, with a higher content in both pulp and pericarp of the fully ripe stage (R) of 3.73% and 3.53%, respectively (Table 3), that did not differ between these two portions. However, fruit of *Pilosocereus* sp. had a higher SS content, with 5.96% in green fruit and 9.95% when ripe (SILVA et al., 2009). Fruit of the xiquexique (*Pilosocereus gounellei*) had an SS content of 3.97% (SILVA et al., 2018), close to that of facheiro fruit. In addition, Dantas et al. (2015a) reported an SS of 14.07% in ripe fruit of *Tacinga inamoena* (K. Schum), another semiarid cactus. Thus, there are considerable differences among the SS contents reported for *Pilosocereus* fruit, as well as those for fruit of other Cactaceae, such as mandacaru and *Tacinga inamoena* (K. Schum).

As maturation progressed the titratable acidity (TA) of facheiro fruit increased, with the highest content in the R stage fruit for both the pericarp (epicarp + mesocarp - P) and the pulp (endocarp + seeds - E). However, when comparing the P and E portions, the TA differed to higher contents only for the R fruits, with of 0.21 and 0.14 g citric acid. $100g^{-1}$, respectively (Table 3).

Table 3. Quality characteristics in the pericarp (epicarp + mesocarp, P) and endocarp (pulp + seeds, E) of facheiro fruits (*Pilosocereus pachycladus Ritter) harvested at three* maturity stages.

Characteristic		Maturity Stages		
		G	IP	R
SS (%)	P	2.17 ± 0.17bA	2.76 ± 0.05 bA	3.53 ± 0.05 aA
	E	2.63 ± 0.15 bA	2.60 ± 0.10 bA	3.73 ± 0.11 aA
TA	P	0.11 ± 0.032 bA	0.12 ± 0.009 abA	0.14 ± 0.009 aB
(% citric acid)	E	0.12 ± 0.036 bA	0.17 ± 0.041 abA	0.21 ± 0.045 aA
Ratio SS/AT	P	23.60 ± 5.08 aA	23.65 ± 7.22 aA	26.19 ±10.30 aA
	E	21.14 ± 2.85 aB	15.28 ± 0.40 aB	18.03 ± 1.32 aB
pH	P	4.50 ± 0.37 aB	4.47 ± 0.10 aA	4.60 ± 0.0030 aA
	E	5.10 ± 0.0060 aA	4.47 ± 0.10 aA	4.35 ± 0.08 bA

Means followed by lower case in the line (maturity stages) and capital letters in the column (portion of the fruit for each characteristic) do not differ, by the Tukey and F tests, respectively, at 5% probability. n=4
Maturity Stage: G= Green, IP= Green with purple traces, and R= Ripe fruit with fully and intense purple color.

In facheiro fruit at physiological maturity, the TA was 0.35 g citric acid.100g^{-1} (SOUZA et al. 2015), higher than for the fruit studied herein, however, the TA values found were similar to the fruit of *Pilosocereus* sp., which ranged from 0.22 to 0.14 g citric acid.100g^{-1} (SILVA et al., 2009). Thus, facheiro fruit can be classified as low acidity (SIDDIQ, 2018) in relation to other fruit, such as mango (1.0 g citric acid.100g^{-1}) (AZERÊDO et al., 2016).

The SS/AT ratio did not differ among maturity stages (Table 3) but differed between the fruit's portions, with the pericarp having the highest ratio. This difference may be due to specific physiological responses of the fruit (SIDDIQ, 2018), as previously reported for other Cactaceae, which contents and compound profiles vary during maturation and even between the different portions of the fruit (DANTAS et al., 2015b; SILVA et al., 2018). Notably, even with low SS content, the SS/AT ratio is high due to the very low acidity of the fruit.

G stage fruit of *Pilosocereus* sp., evaluated by Silva et al. (2009), presented mean SS/AT ratio (27.09), similar to the present study. In fruit of *Tacinga inamoena* (K. Schum), the SS/AT ratios were similar to those found herein, i.e., 23.71 (DANTAS et al., 2015a). However, mandacaru fruit from two different plant populations presented mean SS/AT ratios of 35.81 and 78.96 (MELO et al., 2017). In general, high SS/AT ratios are related to a sweet taste perception, while low values are associated with an acidic taste (SIDDIQ, 2018; DAMODARAN; PARKIN, 2019).

With maturation, only the pulp of the R fruit changed in terms of pH, with lower values (pH 4.35), but no difference was observed between other two stages. In turn, the pH of the fruit portions differed only with the G fruit, with a pH of 5.10 in the pulp and 4.50 in the pericarp (Table 3).

In accordance with the present study, Souza et al. (2015) recorded a pH of 4.76 in fruit pulp, and Silva et al. (2009) recorded a pH of 4.70 and 4.96 in green and red-purplish maturity stages of *Pilosocereus* sp. fruit, respectively. On the other hand, Dantas et al. (2015a) reported a lower pH (4.33) in fruit of *Tacinga inamoena* (K. Schum). The pH value is a good indication of the postharvesting processing conditions required, as it influences the time, processing temperature, packaging selection, type of cleaning and disinfection procedures, equipment, and additives used in the industry (DAMODARAN, PARKIN, 2019).

The ascorbic acid (AA) content differed only between the pulp and pericarp of G, IP, and R fruit, with 4.10; 4.35, and 3.84 mg.100 g^{-1} in the pulp, and 4.84; 4.61, and 4.92 mg.100 g^{-1} in the pericarp, respectively, indicating that a higher AA content is present in the outermost portions of the fruit (Table 4). However, the levels of AA in facheiro fruit were found to be low (Table 4), and considerably lower than those reported by Torres et al. (2009) for fruit of *Opuntia fícus-indica* (AA of 9.5 mg.100 g^{-1} in the mesocarp and 34.5 mg.100 g^{-1} in the pulp), and by Silva et al. (2009), in fruit of *Opuntia inamoena* (with 44.18 mg.100 g^{-1}) at the end of maturation). The fruit of *Tacinga inamoena* (K. Schum) had an AA content of 42.01 mg.100 g^{-1} (DANTAS et al., 2015a). Thus, it is clear that the AA content of fruit varies considerably among the Cactaceaes.

The total extractable polyphenols (TEP) content of the facheiro fruit differed only between the pulps of G fruit, with 147.40 mg.100 g^{-1}. However, the content of TEP did not differ for the other maturity stages and for the pericarp. In turn, TEP content in the pulp (E) and pericarp (P) differed from the other stages in IP fruit only, with 173.80 and 324.80 mg.100 g^{-1}, respectively (Table 4).

The results of the TEP levels analyzed in two portions of the fruit with methyl extraction herein were higher than for facheiro fruit in the physiological maturity study analyzed by Souza et al. (2015), which had TEP contents of 82.23 and 107.67 mg.100 g^{-1} in water and ethanol extracts, respectively.

When Dantas et al. (2015b) compared four stages of fruit maturity, the TEP content in the green stages was 33.9 mg.100 g^{-1}, increasing to 74.02 mg.100 g^{-1} by the mature stage. However, these values are much lower than for the fruit studied herein, although in *O. stricta* fruit the authors also observed an increase in the TEP content with maturation.

The yellow flavonoid content of the facheiro fruit's pericarp differed among the maturity stages (Table 4), showing that those phenolic compounds accumulated as maturation progressed: the content in G, IP, and R fruit was 0.74; 0.72, and 1.3 mg.100 g^{-1}, respectively. Lima et al. (2013) reported a yellow flavonoid content of 6.03 mg.100 g^{-1} in pitayas (*H. costaricensis*), which, according to the authors, was 6-fold higher than in *S. megalanthus* fruit (0.88 mg.100 g^{-1}). Thus, the yellow flavonoid content found in the facheiro pericarp in the present study is similar to that of *S. megalanthus*, but much lower than the other pitaya species, as evaluated by Lima et al. (2013). However, Dantas et al. (2016) found that yellow flavonoids in *T. inamoena* increased from 1.51 to 5.21 mg.100 g^{-1} in the pulp and 1.14 to 9.11 mg.100 g^{-1} in the epicarp, during maturation. These contents are much higher than in the facheiro fruit but there was a similar pattern of increasing content with maturation.

Table 4. Bioactive compounds in the pericarp (epicarp + mesocarp, P) and endocarp (pulp + seeds, E) of facheiro fruits (*Pilosocereus pachycladus* Ritter) harvested at three maturity stages.

Characteristic		Maturity Stages		
		G	IP	R
Ascorbic acid	P	4.84 ± 0.43 aA	4.61 ± 0.42 aA	4.92 ±0.49aA
(mg.100 g^{-1})	E	4.10 ± 0.23aB	4.35 ± 0.25 aB	3.84 ± 0.26 aB
TEP	P	196.6 ± 5.03 aA	173.8 ± 5.65 aB	221.9 ± 3.77 aA
(mg.100 g^{-1})	E	147.4 ± 7.37 bA	324.9 ± 6.80 aA	269.3 ± 5.10aA
Yellow Flavonoids	P	0.74 ± 0.05 b	0.72 ± 0.06b	1.3 ± 0.03 a
(mg.100 g^{-1})	E	nd	nd	nd
Betalains	P	14.33 ± 0.49 abB	12.92 ± 1.19 bB	35.91± 0.68 aB
(mg.100 g^{-1})	E	34.13 ± 2.78 cA	152.25 ± 5.51 bA	418.79 ± 3.18 aA
Betacyanins	P	7.06 ± 0.63 aB	5.75 ± 0.36 aB	19.77 ± 0.57aB
(mg.100 g^{-1})	E	23.19 ±1.00 cA	119.89 ±1.48 bA	335.54 ±0.89 aA
Betaxanthins	P	7.27± 0.97 bB	7.16 ± 0.93 bB	16.14 ± 1.53aB
(mg.100 g^{-1})	E	10.97 ± 0.84 cA	32.36 ± 2.44 bA	83.25 ± 2.16aA

Means followed by lower case in the line (maturity stages) and capital letters in the column (portion of the fruit for each characteristic) do not differ, by the Tukey and F tests, respectively, at 5% probability. n=4.
Maturity Stage: G= Green, IP= Green with purple traces, and R= Ripe fruit with fully and intense purple color.
TEP = Total Extratable Polyphenols. nd= Not determined.

The contents of betalains were higher in the pulp, with much higher amounts of betacyanins (BTcs) than betaxanthins (BTxs). The BTcs and BTxs contents differed among maturity stages, both in the pulp and pericarp, with the highest contents in R fruit. The BTcs differed among the stages in the pulp only and were much more abundant in R stage fruit (335.54 mg.100 g^{-1}). It was observed, though, a clear distinction in BTcs and BTxs among maturity stages, especially between G and R fruit (Table 4), however the BTxs contents were much lower than BTcs.

The pulp of facheiro fruit at physiological maturity was found to contain 53 mg.100 g^{-1} of betalains (SOUZA et al., 2015). Dantas et al. (2015c) found the fruit of *O. ficus-indica* to contain BTxs at contents of between 13.09 and 37.36 mg.100g^{-1}, similar to the contents in the pericarp of facheiro fruit during maturation and the pulp of G stage fruit. The pulp of fully mature *Opuntia stricta*, was found to contain BTcs and BTxs concentrations of 182.27 and 46.90 mg.100 g^{-1}, respectively, with a total betalains content of about 240 mg.100g^{-1} (DANTAS et al., 2015b), well below that found in the ripe fruit (R) in this study. According to Souza et al. (2015),

the levels of betalains in the fruit provide the regions where facheiro plants grow a strategic opportunity. This underutilized fruit could become a prime source of these important pigments for the control of chronic diseases (TESORIERE et al., 2014).

The total antioxidant activity (TAA) in the pulp, measured with DPPH free radical capture method, markedly increased with maturation and, therefore, was much higher in R stage, once for fruit with the highest TAA, a smaller amount of the sample was required for DPPH radical capture (Figure 2A). In addition, among the portions of the fruit evaluated, the highest antioxidant activity was found in the endocarp (pulp + seeds), which increased with maturation.

In *Tacinga inamoena* and *Opuntia stricta* pulp, Dantas et al. (2015c) observed antioxidant activity (with the DPPH method) of 2153 and 1730 g.DPPH.g^{-1}, respectively. In the pulp of *Tacinga inamoena* fruit, at six maturity stages, Dantas et al. (2016) found lower antioxidant activity at the beginning of maturation, of approximately 2000 g.DPPH.g^{-1}, and approximately 500 g.DPPH.g^{-1} ($\approx$ 4-fold higher) at the end, similar to the levels found in the pericarp of facheiro fruit in here.
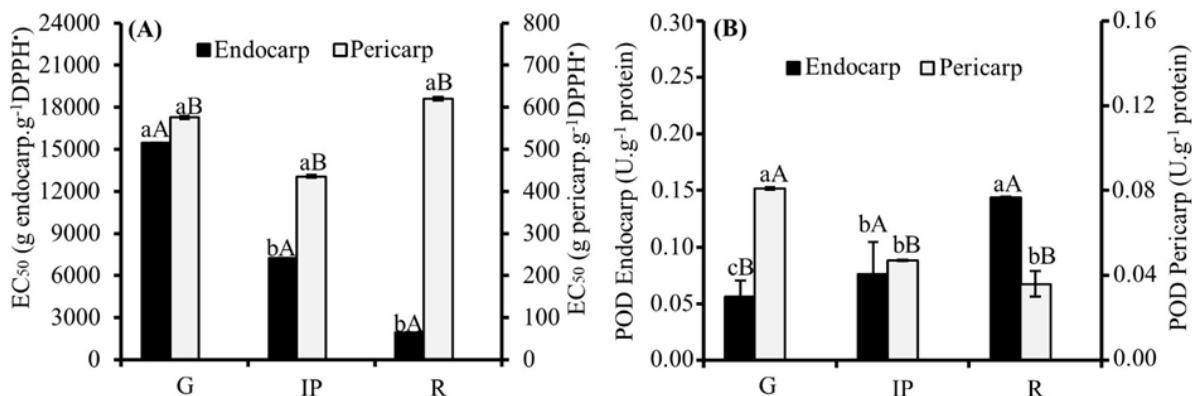
Figure 2. Total antioxidant activity by the DPPH (A) radical capture method and peroxidase activity (POD, EC 1.11.1.7) (B), in the pericarp (epicarp + mesocarp) and endocarp (pulp + seeds) of facheiro fruits harvested at three maturity stages. Bars followed by the same lower case letters among maturity stages and capital letters between the portions of the fruit do not differ by the Tukey and F tests, respectively, at 5% probability. n = 4
Maturity Stage: G= Green, IP= Green with purple traces, and R= Ripe fruit with fully and intense purple color.

Studies have highlighted the antioxidant potential of Cactaceae fruit, which is mainly due to the presence of betalains (DANTAS et al., 2015c); together with phenolic compounds, betalains inhibit various oxidative (STINTZING et al., 2005) and inflammatory processes (TESORIERE et al., 2014). In addition, Yahia and Jacobo (2011) reported that the high antioxidant capacity of fruit of *Opuntia* spp. is due to the presence of betalains, vitamin C, and phenolics, in that order, which was confirmed using the DPPH analyzes of the hydrophilic extracts.

Therefore, the ripe facheiro fruit at stage R, evaluated herein, had higher levels of bioactive compounds, such as TEP, betalains, BTc, and BTx, as maturation progressed, and the mature stage contained higher antioxidant activity. These bioactive compounds may be responsible for the increased antioxidant activity, as suggested by Dantas et al. (2015c), Yahia and Jacobo (2011) and Stintzing et al. (2005).

The activity of peroxidase (POD, EC 1.11.1.7) differed between the maturity stages G, and IP and R, and POD activity decreased in pericarp and increased in pulp during maturation (Figure 2B). The POD activities observed herein were superior to those described for other Cactaceae, such as yellow pitayas (*Acanthocereus pitajaya*) that ranged from 0.014 to 0.026 U.g^{-1} protein over 12 days at 24 °C (DUARTE; RIVERA; CUENCA, 2005), and fruit of three *Opuntia* species, with activities ranging from 0.00068 to 0.0010 U.g^{-1} protein (TALEMI; SEDAGHATHOOR, 2017). Cladodes of 10 ecotypes of *Opuntia ficus-indica* showed POD activity ranging from 0.0038 to 0.044 U.g^{-1} protein (KOHAICH; BAAZIZ, 2015), with the highest values being similar to those recorded for the pericarp of facheiro fruit at the IP (0.047) and R (0.036 U.g^{-1} protein) maturity stages. Therefore, the activity of POD in facheiro fruit is much higher than in pitaya and *Opuntias* and this is an important technological indicator for the darkening potential of this fruit.

Plants under stress conditions produce large amounts of peroxidase and regardless of the substrates used or the stress applied, this enzyme is often the first to alter its activity after stimulation (SIEGEL, 1993). This author also emphasized that there is considerable versatility and heterogeneity among peroxidases and this allows this enzyme to act in different regulatory processes in the plants, from germination to senescence.

According to Kohaich and Baaziz (2015), studies on peroxidases in Cactaceae have not been well designed, however, the authors observed that the activity of this enzyme on guaiacol was negatively affected by ascorbic acid in *Opuntia ficus-indica* cladodes.

The results in here showed strong correlations between POD activity and levels of betalains, betacyanins, and betaxanthines, in the two fruit portions evaluated (Figure 3).

However, these correlations were negative for the pericarp and positive for the pulp, that is, the higher the levels of pigments, the higher the POD activity in the pulp and the lower the activity in the pericarp.
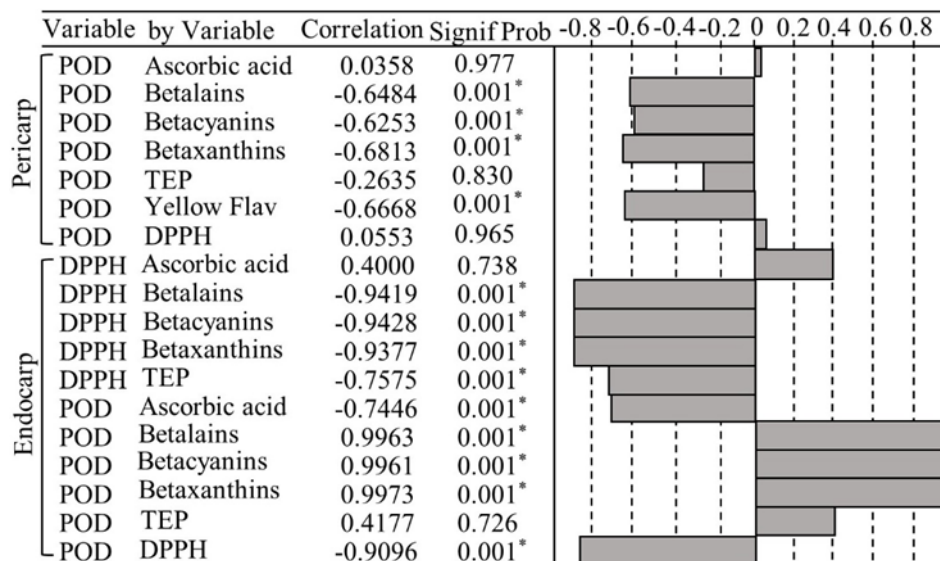
	Variable	by Variable	Correlation	Signif Prob	-0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8
Pericarp	POD	Ascorbic acid	0.0358	0.977	
	POD	Betalains	-0.6484	0.001*	
	POD	Betacyanins	-0.6253	0.001*	
	POD	Betaxanthins	-0.6813	0.001*	
	POD	TEP	-0.2635	0.830	
	POD	Yellow Flav	-0.6668	0.001*	
	POD	DPPH	0.0553	0.965	
Endocarp	DPPH	Ascorbic acid	0.4000	0.738	
	DPPH	Betalains	-0.9419	0.001*	
	DPPH	Betacyanins	-0.9428	0.001*	
	DPPH	Betaxanthins	-0.9377	0.001*	
	DPPH	TEP	-0.7575	0.001*	
	POD	Ascorbic acid	-0.7446	0.001*	
	POD	Betalains	0.9963	0.001*	
	POD	Betacyanins	0.9961	0.001*	
	POD	Betaxanthins	0.9973	0.001*	
	POD	TEP	0.4177	0.726	
	POD	DPPH	-0.9096	0.001*	

Figure 3. Correlation of peroxidase (POD) activity and total antioxidant activity (DPPH) with the bioactive compounds in the pericarp and endocarp of facheiro (*Pilosocereus pachycladus* Ritter) fruits.

In the pulp, the antioxidant activity was strongly and negatively correlated with POD (r = -0.9096), as well as with BTcs and BTxs (r = -0.9428 and r = -0.9377, respectively), indicating that the higher the levels of these pigments and the higher the enzymatic activity, the greater the antioxidant activity, and the fewer grams of pulp needed to eliminate the DPPH free radical. This suggests that the betalains are primarily responsible for antioxidant activity in facheiro fruit. It was also highlighted a strong negative correlation between ascorbic acid (AA) and POD activity (r = -0.7446), showing that POD activity decreases under the influence of higher AA levels, as was also observed by Kohaich and Baaziz (2015).

CONCLUSION

The physical and physicochemical aspects of facheiro (*Pilosocereus pachycladus* Ritter) fruit changed during maturation. In addition, the bioactive compounds content varied between the portions of the fruit and were highest in the pulp.

The most noticeable changes in the fruit were of coloration: with maturation the pericarp and pulp became increasingly purple, parallel to the increase in the levels of betalains and total extractable polyphenols.

Facheiro fruit, and in particular the pulp, are important sources of betalains mainly betacyanins.

The antioxidant activity of the fruit also increased with maturation and was higher in the fruit pulp (endocarp) at all maturity stages.

The activity of POD in facheiro fruit is much higher than in pitaya and *Opuntias* and this is an important indicator for the darkening potential of this fruit.

The peroxidase activity of facheiro fruit strongly correlated with the presence of betalains, and the highest antioxidant activity was found in the pulp during maturation. These results will aid in future studies exploring strategies aiming at adding value to the facheiro fruit and to exploit this important Caatinga plant.

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