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Original Article

Maturation and antioxidant activity of 'Giombo' and 'Rama Forte' persimmons produced in the Brazilian semiarid

Maturação e atividade antioxidante de caquis 'Giombo' e 'Rama Forte' produzidos no semiárido brasileiro

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Abstract

The objective of this work was characterizing persimmons of the 'Giombo' and 'Rama Forte' cultivars harvested at different ripening stages in the Brazilian semiarid. Fruits were harvested at three ripening stages – green, semi-ripe and ripe – then evaluated for the following characteristics: fruit weight and diameter, skin and pulp color, fruit firmness, pulp pH, soluble solids content, titratable acidity, SSC/TA ratio, total soluble sugars, reducing sugars, astringency index, and the contents of tannin, vitamin C, carotenoid, β-carotene, and total extractable polyphenols. Also, total antioxidant activity by the DPPH and ABTS methods and pectin methylesterase, and polygalacturonase enzyme activities were evaluated. Two experiments were carried out in a completely randomized design, one for each cultivar, with treatments consisting of different stages of maturation, with five replications of three fruits each. Data were submitted to analysis of variance and the differences between the means were compared using the Tukey test at 5% probability. Fruit firmness and soluble solids content did not vary between maturation stages for any of the cultivars. However, the skin color index increased with advancing maturation for both 'Giombo' and 'Rama Forte'. The astringency index, the content of total extractable polyphenols, soluble tannins and the antioxidant capacity were lower in fruits harvested at the ripe stage, for both cultivars. It can be concluded that persimmons of the 'Giombo' and 'Rama Forte' cultivars present better physicochemical quality characteristics when harvested when ripe, with a totally yellow skin.

Keywords: *Diospyros kaki* L., maturation stages, astringency, antioxidant potencial.

Resumo

O objetivo deste trabalho foi caracterizar caquis das variedades 'Giombo' e 'Rama Forte' colhidos em diferentes estádios de maturação, produzidos na região nordeste do Brasil. Os frutos foram colhidos em três estádios de maturação; verde, semimaduro e maduro, e avaliados quanto as seguintes características: diâmetro do fruto, massa fresca, cor da casca e da polpa, firmeza do fruto, pH, acidez titulável, sólidos solúveis, relação sólidos solúveis/acidez, açúcares totais, açúcares redutores, índice de adstringência, teor de taninos solúveis, vitamina C, carotenoides, betacaroteno, polifenóis extraíveis totais, atividade antioxidante total (AAT) pelo método do DPPH e ABTS e atividade das enzimas pectina metil esterase (PME) e poligalacturonase (PG). Foram montados dois experimentos em delineamento inteiramente casualizado, um para cada variedade, sendo os tratamentos constituídos pelos diferentes estádios de maturação. A firmeza do fruto e o teor de sólidos solúveis não variaram entre os estádios de maturação para nenhuma das variedades. No entanto, o índice de cor da casca incrementou com o avanço da maturação tanto para 'Giombo' quanto para 'Rama Forte'. O índice de adstringência, o conteúdo de polifenóis extraíveis totais, taninos solúveis e a capacidade antioxidante foram menores nos frutos colhidos no estádio maduro, para ambas as variedades. Pode-se concluir que caquis das variedades 'Giombo' e 'Rama Forte' apresentam melhores características físico-químicas de qualidade quando colhidos maduros, com casca totalmente amarelada.

Palavras-chave: *Diospyros kaki* L., estádios de maturação, adstringência, potencial antioxidante.

1. Introduction

Persimmon (*Diospyros kaki* L.) is a subtropical fruit from temperate regions originating in Asia. In Brazil, the largest production of the crop is concentrated in the South and Southeast regions, mainly due to favorable climatic conditions for the crop (Brackmann, 2003). In the Northeast region, there is a small production of persimmon in the altitude regions of the state of Bahia (IBGE, 2020). However, the use of technologies combined with research

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has demonstrated the feasibility of growing species from subtropical and temperate climates such as persimmon in a tropical semi-arid condition with high temperature and evapotranspiration rate, in addition to low relative humidity (Lopes and Oliveira, 2010).

Persimmon production in the South and Southeast regions of Brazil is concentrated in the months from February to June. In the other months of the year, the fruit is imported from Spain and Israel, reaching the final consumer with a value up to six times higher. In the semi-arid region, the climatic conditions and the management used, such as flowering induction, make it possible to produce at any time of the year, directing to the months of lower supply, providing better prices for the producer (Lopes and Oliveira, 2010; Lopes et al., 2014). According to Fachinello et al. (2011), the success of this crop is due to its good adaptability, high resistance to pests and diseases, and the possibility of export.

Among the most cultivated persimmon cultivars in Brazil, 'Giombo' and 'Rama Forte' stand out, classified according to tannin content, in the astringent variable pollination group, which contain high levels of soluble tannins, responsible for astringency, and need of treatment to remove this astringency before consumption (Edagi and Kluge, 2009). In experiments carried out in the São Francisco Valley, the 'Rama Forte' and 'Giombo' cultivars have shown the best results in terms of production and fruit quality (Lopes et al., 2014).

Fruit maturation can be defined as the sequence of changes in color, flavor, aroma and texture, culminating in the ideal point for consumption. Most of the fruits reach maximum edible quality when they fully mature in the plant, however they cannot be harvested at this stage due to the inconveniences they present in terms of their higher degree of perishability and sensitivity to handling. For persimmon, the maturity stage for harvest can be determined by evaluating the color of the skin, which will have a direct effect on post-harvest quality and fruit storage potential (Mohammadi et al., 2015).

There is little information about the potential and the ideal stage of maturation for harvesting the fruits produced in the Brazilian semiarid, which guarantee better nutritional quality combined with pleasant taste and longer shelf life. Studies carried out by Itamura et al. (1997) demonstrated that persimmons harvested physiologically immature may have a shorter post-harvest shelf life than those harvested physiologically mature. Krammes et al. (2005) identified that 'Fuyu' persimmons harvested late have a shorter post-harvest shelf life. Albuquerque (2018), studying 'Rama Forte' persimmons produced in a semi-arid region, observed that harvesting with orange skin color resulted in better quality and conservation of physicochemical quality.

In this context, the present work aimed to study the quality and antioxidant capacity of 'Giombo' and 'Rama Forte' persimmons harvested at different stages of maturation, produced in the Brazilian semiarid (Vale do Jaguaribe-CE, Brazil).

2. Material and Methods

2.1. Collection of fruits and installation of the experiment

Persimmon (*Diospyros kaki* L.) fruits of the 'Giombo' and 'Rama forte' cultivars were obtained from an experimental orchard with 10 years of implantation of a commercial company, located in the irrigated (Vale do Jaguaribe-CE), perimeter Tabuleiro de Russas, in the municipality of Russas, CE, Brazil (4°58'03.2"S and 38°03'11.5"W, and altitude of 77 m), Brazilian semiarid. The climate of the region is classified as Bsh, that is, dry, very hot, according to the classification of Köppen (1948). It has a rainfall volume of around 720mm, irregularly distributed throughout the year (wet winter), and an average annual temperature above 18 °C (Brasil, 2019).

According to data from the National Institute of Meteorology (INMET, 2017), the annual persimmon cycle this year presented average temperatures of 28.42 °C and greater precipitation in the first half of the year, a period in which the fruits were still on the plant, with a average precipitation of 62.2 mm (Figure 1). The flowering and fruit development stage comprised the months from May to December.

The fruits were harvested at three stages of maturation (S1-green: around 90% of the green skin color; S2- semi-ripe: 50% of the green skin color and S3-ripe: around 80% of the yellow skin color). The green and semi-mature stages were harvested at 175 days after flowering, and the mature stage was harvested at 193 days after flowering.

Soon after harvest, the fruits were transported to the Laboratory of Physiology and Post-harvest Technology of the Universidade Federal Rural do Semiárido (UFERSA), in Mossoró/RN-Brazil, where the experiments were conducted. The fruits were selected, and those that showed some type of damage, either mechanical or by insect attack, were discarded. Subsequently, they were sanitized in running water and immersed in a chlorinated solution containing 50 ppm of chlorine for 15 minutes, drying naturally.

The fruits of the 'Giombo' and 'Rama Forte' cultivars were separated into five replications of three fruits for each stage. Then, each fruit was submitted to physical

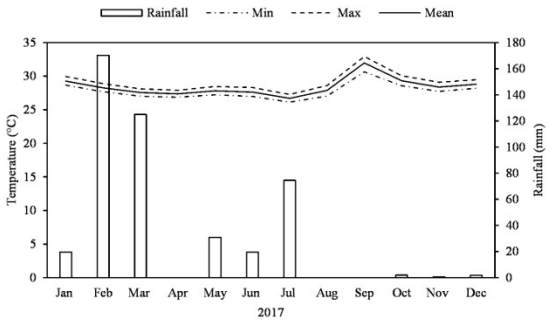

Figure 1. Climate data during the persimmon cycle in the municipality of Russas-Ceará (Vale do Jaguaribe-CE), Brazil. Monthly rainfall and minimum, maximum, and average temperatures in 2017 according to the National Institute of Meteorology (INMET, 2017). The plants began flowering in May, and the fruits were harvested in December.

evaluations. After the longitudinal cut, the fruits were submitted to the determination of the astringency index. Subsequently, the peels and seeds were removed and the fruits were cut with the aid of stainless steel knives and crushed with the aid of a Philips Walita ProMix mixer. Each replicate was placed in two plastic pots protected from light, which were stored in a freezer for physical-chemical and chemical determinations.

2.2. Physical, chemical and biochemical analysis

The fresh weight of the fruits was determined with the aid of a semi-analytical balance, and the result was expressed in grams (g). The fruit shape was obtained by the relationship between the longitudinal diameter and the transversal diameter of the fruits. According to the values obtained, the fruits were classified as: tablet (RF< 0.9); spherical (0.9 < RF < 1.1); oblong (1.1 < RF < 1.7) and cylindrical (RF >1.7), according to the scale adapted from Lopes (1982). The longitudinal and transversal diameters were determined with the aid of a Lotus Plus digital caliper, with a scale ranging from 0 to 150 millimeters (mm), and the result was expressed in mm.

Fruit firmness was determined using a Texture Analyzer® texturometer, model TA.XTExpress/TA.XT2icon (Stable Micro Systems Ltd). A 6 mm diameter cylindrical tip was used (model P/6). The pre-test, test and post-test speeds were 2 mm s^{-1}, 2 mm s^{-1} and 10 mm s^{-1}, respectively, and the penetration distance was 10 mm. In each fruit, two readings were performed, in the equatorial region and at equidistant points. The result was expressed in Newton (N).

The skin and pulp color were determined using a benchtop digital colorimeter (CR-410, Minolta®). Two readings were performed at equidistant points on each fruit and the results were expressed according to the CIE lab coordinates, which include the variables L* (luminosity), a* (variation between green and red colors) and b* (variation between blue and yellow colors) (Minolta Corp, 2007). The values of a* and b* were converted into Hue (hue) and Chroma (saturation) angles, which are the variables that best represent the evolution of the color of the persimmon peel during the ripening process.

The soluble solids (SS) content was determined using a Palette Atago Co model PR-100 digital refractometer, with automatic temperature correction according to the methodology recommended by Zenebon et al. (2008), and the results were expressed in °Brix. The samples were ground in a domestic mixer and, at the time of reading, they were filtered through filter paper.

Total sugars were determined by the Antrona method (9,10-dihiro-9-oxoanthracene), according to Yemn and Willis (1954). The reading was performed in a Pró-Anais® spectrophotometer, model UV-1600, at 620 nm, and the results were expressed in g of glucose 100 g^{-1} of pulp. For reducing sugars, the extraction was performed with distilled water and determined according to Miller (1959). The readings were made in a Pró-Anais® spectrophotometer, model UV-1600, at 540 nm and the results were expressed in g of glucose 100 g^{-1} of pulp.

The pH was determined with the aid of a Tecnal® direct reading potentiometer, model mPA-210, duly standardized with pH 7.0 and pH 4.0 buffer solutions. The reading was performed directly on the crushed samples, the measured data were expressed in real pH values (Zenebon et al., 2008). Titratable acidity (TTA) was determined by volumetric titration with NaOH solution, according to Zenebon et al. (2008). Results were expressed as % malic acid. The SS/AT ratio was determined by the quotient between the values of soluble solids and titratable acidity.

Vitamin C was determined by titration with Tilman solution (DFI – 2,6 dichlorophenol-indophenol 0.02%), taking 5 g of the samples and diluting in a 50 mL volumetric flask with 0.5% oxalic acid, as methodology proposed by Zenebon et al. (2008), and the results expressed in mg of ascorbic acid 100 g^{-1} of pulp.

Soluble tannin contents were determined spectrophotometrically using the Follin-Ciocalteau reagent (25%), according to the technique recommended by Taira and Ono (1996), in which 1g of crushed pulp was used for a final volume of 100 mL of extract, from the from which an aliquot of up to 1 mL was withdrawn. Results were expressed in mg 100 g^{-1} of pulp.

Carotenoids were determined according to the methodology of Higby (1962). For extraction, 2.5 g of the samples were weighed, 15 mL of isopropyl alcohol and 5 mL of hexane were added. Readings were taken in a Pró-Anais® spectrophotometer, model UV-1600, at a wavelength of 450 nm, using 1.5 cm wide glass cuvettes.

The β–Carotene content was determined according to the methodology of Nagata and Yamashita (1992), in which 1g of the sample was dissolved in 6mL of Hexane and 4mL of acetone, homogenized and centrifuged immediately afterwards. The reading of the supernatant was performed in a Pró-Análise® spectrophotometer, model UV-1600 at 663, 645, 505 and 453 nm.

The astringency index was determined using the method proposed by Campo-Dall'Orto et al. (1996), in which the impression of one of the sides of the fruits cut in filter paper, previously prepared with a solution of ferric chloride (FeCl3) at 5%, is evaluated. The tannin, in the soluble form, reacts with the ferric chloride, becoming darkened. The impressions were evaluated using the rating scale: 1- not tannin; 2- slightly tannin; 3- moderately tannin; 4- tannin; 5- very tannin.

For extraction of pectin methyl esterase (PME), 5 g of pulp with 20 mL of 0.2N NaCl solution were homogenized in LUCADEMA TURRATEC turrax for 1 min at speed 4, and then filtered, this entire step of the procedure was performed. at low temperature to inhibit enzymatic activity (Jen and Robinson, 1984). To determine the PME activity, a mixture of 30 mL of 1% citrus pectin, diluted in NaCl at pH 7, with 5 mL of the enzymatic extract, kept under constant agitation, was titrated with 0.01N NaOH solution. PME activity was expressed in enzymatic unit (EU). min^{-1} g^{-1} of tissue.

Polygalacturonase (PG) was extracted according to the methodology proposed by Pressey and Avants (1973). PG was determined by mixing 3.0 mL of this extract with 3.0 mL of 0.25% polygalacturonic acid diluted in sodium acetate buffer at pH 5.0, in a test tube (AR2). In another tube, 3.0 mL of water were placed with another 3.0 mL of the extract, to determine the blank of the sample (AR1).

To obtain the enzymatic activity, the difference between the concentrations of AR1 and AR2, obtained by the Miller method (1959) was made and the result was expressed in US min^{-1} g^{-1} of sample.

The total antioxidant activity (AAT) was determined through the capture of the ABTS+ radical, according to the methodology described by Re et al. (1999) and adapted by Rufino et al. (2007). The spectrophotometric reading was performed six minutes after mixing 30 µL of extract with 3 mL of ABTS•+ radical and the standard curve was prepared using the synthetic antioxidant Trolox at a concentration of 100 – 2000 µM in ethanol. Results were expressed in µmol of Trolox g^{-1} of pulp.

The total antioxidant activity by DPPH was determined according to the methodology described by Brand-Williams et al. (1995) and modified by Sánchez-Moreno et al. (1998). This method is based on the capture of the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) by antioxidants present in the sample, which reduces the absorbance at 515 nm. The antioxidant activity was expressed as the concentration of antioxidant capable of reducing by 50% the initial concentration of the radical (EC50) and expressed in g of pulp g^{-1} of DPPH.

The extracts for the determination of total extractable polyphenols (PET) and total antioxidant activity (AAT) were prepared according to the methodology proposed by Larrauri et al. (1997). Then, this extract was centrifuged in a Hettich centrifuge, model ROTANTA 460R, for 20 minutes at 20 °C at 10,000 rpm. Polyphenols were determined by colorimetric assay using the Folin-Ciocalteu reagent, according to Obanda et al. (1997). Readings were performed in a Pró-Anais® spectrophotometer, model UV-1600 at 700 nm, using the standard curve of 98% gallic acid (dosed at 0, 10, 20, 30, 40 and 50 µg). Results were expressed as gallic acid equivalents (GAE) mg 100 g^{-1} of pulp.

2.3. Statistical analysis

The experiment was carried out in a completely randomized design with five replications of three fruits per stage, the analyzes were carried out separately for the 'Giombo' and 'Rama Forte' cultivars. Data were submitted to analysis of variance by the F test at 5% probability, with the exception of color data, variables a and b; vitamin C; soluble solids; pH; total sugars; total extractable polyphenols; beta-carotene; soluble tannins; astringency index; for 'Rama Forte' cultivar, total antioxidant activity by DPPH and by ABTS in 'Giombo' cultivar and longitudinal diameter; vitamin C; pH; total carotenoids; beta-carotene; soluble tannins; astringency index and total antioxidant activity by DPPH, which were analyzed using the Kruskal-Wallis test at 5% probability (Kruskal and Wallis, 1952). The differences between the means of each stage for each cultivar were compared by Tukey's test at 5% probability with the aid of the R program, version 3.5.1.

3. Results and Discussion

3.1. Physical, chemical and biochemical characteristics

The 'Giombo' and 'Rama Forte' persimmon cultivars have different physical characteristics, as shown in Table 1. Regarding fresh weight, the 'Giombo' cultivar presented an average of 82.31 g and there was no significant difference between the maturation stages, indicating that in the green stage (S1) the fruit had already reached its maximum mass. The 'Rama Forte' cultivar, on the other hand, presented an average of 106.01 g and there was a difference between the maturation stages, in which the semi-ripe stage (S2) stood out with the highest fresh weight, (112.60 g), an increase of 10.6% when compared to the green stage (S1). Moura (2014) found, in experiments carried out in the southeastern region of Brazil, an average

Table 1. Physical characteristics of the green (S1), semi-ripe (S2) and mature (S3) stages of persimmon fruits (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' varieties produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

Cultivar	Stage	Fresh Weight (g)	Fruit Shape	Longitudinal Diameter (mm)	Transverse Diameter (mm)	Firmness (N)
Giombo	S1	79.83 a	1.05 a	53.05 a	50.74 a	51.90 a
	S2	83.66 a	1.02 a	54.18 a	53.05 a	52.15 a
	S3	83.26 a	0.95 b	50.66 b	53.18 a	45.24 a
	General Average	83.46	1.01	52.63	52.32	49.76
	CV (%)	6.67	3.92	2.36	3.64	8.40
Rama Forte	S1	101.78 b	0.76 a	46.06 a	60.37 b	49.37 a
	S2	112.60 a	0.73 b	47.17 a	64.93 a	53.43 a
	S3	103.66 ab	0.71 b	44.82 b	63.01 ab	51.11 a
	General Average	106.01	0.73	46.02	62.77	51.30
	CV (%)	5.82	1.88	1.98	2.63	7.97

Means followed by the same lowercase letter in the column do not differ from each other, according to Tukey's test at 5% probability. CV= coefficient of variation. S1 = green: around 90% of the color of the green shell; S2 = semi-ripe: 50% of the green skin color; and S3 = ripe: around 80% of the yellow skin color.

weight of 140 and 150 g for the 'Giombo' and 'Rama forte' cultivars, respectively. In the present study, lower values were observed, possibly because in semi-arid conditions (Figure 1) the persimmon tree undergoes a lot of stress, resulting in a greater amount of fruits per plant and smaller size of these fruits.

Regarding the shape, the 'Giombo' and 'Rama Forte' persimmons showed a significant difference for the longitudinal diameter only in the mature maturation stage (S3). The transversal diameter of the fruits of the two cultivars studied did not differ according to the stage of maturation, as shown in Table 1. According to the data of the longitudinal and transversal diameters presented, it is possible to verify that the persimmons of the 'Giombo' cultivar have an oval shape, with an average of 1.01, whereas the 'Rama Forte' cultivar persimmons have a more rounded/flattened shape, with an average of 0.73. These results corroborate the results found by Cavalcante et al. (2007) and Sá et al. (2018) when working with these persimmon cultivars.

For the fruit firmness variable (Table 1), no difference was observed between the maturation stages in any of the cultivars, with a general average of 49.76 N for the 'Giombo' cultivar and 51.30 N for the 'Rama Forte' cultivar. These firmness results suggest that firmness is not a good indicator of the persimmon ripening stage (Del Bubba et al., 2009), as there was no difference between the stages evaluated. Yet that even during maturation, the persimmon fruits remain firm, thus contributing to a greater acceptability and extension of the post-harvest shelf life of the 'Giombo' and 'Rama Forte' persimmon cultivars.

The skin color of the 'Giombo' and 'Rama Forte' cultivars of Caqui showed similar characteristics (Table 2). In terms of luminosity, the green stage (S1) differed from the others, with lower values of 55.60 and 58.38 for the 'Giombo' and 'Rama Forte' cultivars, respectively. The mature stage (S3) presented the highest chromaticity results for the two cultivars. On the other hand, the hue angle (H°) presented a decreasing behavior in relation to the maturation stages, in which the mature stage presented lower results for the two cultivars. The value of H° in the green stage (S1) was located in the second quadrant (from 90° to 180°), that is, from green to yellow. In the S2 and S3 stages, the values were in the first quadrant (0 to 90°), indicating a variation from yellow to red. The skin color index of the two cultivars showed an ascending value, from green to mature, ranging from -1.90 to 6.02 for 'Giombo' and -1.24 to 4.48 for 'Rama Forte' (Table 2).

The color changes of persimmons during ripening may be related to the degradation of chlorophyll and synthesis of carotenoid pigments, such as cryptoxanthin, zeaxanthin (Daood et al., 1992) and lycopene (Ito, 1971). The data obtained in the color of the skin are in agreement with the evolution in the ripening of the fruits, considering that the lower the color index, the closer it will be to green; the higher, the closer to the red color. Santos et al. (2010) observed in persimmons, harvested physiologically mature, present values of L, C and H° of 34.03; 66.59 and 69.05 for 'Giombo' and 32.28; 63.35 and 72.72 for 'Rama Forte'. In turn, Albuquerque (2018), working with 'Rama Forte' persimmons at three stages of maturation, obtained results for L and C of 52.1 and 41.5 in green persimmons, 51.4 and 43.1 in orange and 48.9 and 42.6 in intense orange, respectively.

The pulp color showed a different behavior from the peel color (Table 2). The luminosity of the green stage differed from the other maturation stages, with higher values for the two cultivars, 63.88 and 76.62 for 'Giombo' and 'Rama Forte', respectively. The chromaticity in the 'Giombo' cultivar differed in all stages, with the highest value for the mature stage, while in the 'Rama Forte' cultivar, the chromaticity of the green and semi-mature

Table 2. Variables of luminosity (L), chromaticity (C), hue angle (H°) and color index (IC) of the skin and palp colors of the green (S1), semi-ripe (S2) and ripe (S3) stages of fruits persimmons (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' cultivars produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

Cultivar	Stage	Shell Color				Pulp Color			
		L	C	H°	IC	L	C	H°	IC
Giombo	S1	55.60 b	42.04 c	96.00 a	-1.90 c	63.88a	39.64 c	71.91 a	5.21 b
	S2	57.88 a	48.22 b	84.85 b	1.56 b	55.13b	44.62 b	67.93 ab	7.51 ab
	S3	58.95 a	56.69 a	70.47 c	6.02 a	55.95b	47.76 a	65.78 b	8.06 a
	General Average	57.48	48.98	83.77	1.89	58.32	44.01	68.54	6.93
	CV (%)	1.47	3.98	2.95	40.4	7.23	4.18	4.32	20.12
Rama Forte	S1	58.38 b	45.30 b	94.14 a	-1.24 c	76.62a	40.55b	80.60 a	2.17 b
	S2	62.56 a	47.6 b	83.54 b	1.80 b	59.71c	46.74a	67.76 c	7.01a
	S3	63.23 a	57.0 a	74.20 c	4.48 a	70.06b	47.46a	74.40 b	4.00 b
	General Average	61.39	50.02	83.96	1.68	68.80	44.92	74.25	4.39
	CV (%)	1.93	5.75	2.74	38.2	5.35	6.82	3.72	28.95

Means followed by the same lowercase letter in the column do not differ from each other, according to Tukey's test at 5% probability. CV= coefficient of variation. S1 = green: around 90% of the color of the green shell; S2 = semi-ripe: 50% of the green skin color; and S3 = ripe: around 80% of the yellow skin color.

stages did not differ statistically, both presented the highest values, 46.74 and 47.46, respectively. The hue angle values of the semi-mature stage of the 'Giombo' cultivar did not differ from the green and mature stages. In the 'Rama Forte' cultivar, they were different for all stadiums. The hue angle values of the pulps of the two cultivars, at all stages, were in the first quadrant (0 to 90°), indicating color variation from yellow to red. The color index of the 'Giombo' cultivar showed the highest value for the mature stage, with a value of 8.06, but there was no significant difference in relation to the semi-mature stage. In the 'Rama Forte' cultivar, the semi-mature stage had the highest color index, with a value of 7.01, differing from the other stages (Table 2). The results obtained for the Giombo cultivar show a small variation in the color of the pulp according to ripening, with the color index varying from yellow to intense yellow. In the Rama Forte cultivar, these results differ a little from what was expected, as the semi-mature stage has a higher value, that is, a more intense yellow color compared to the mature stage. This result can be explained by the presence of light pulps (non-fertilized fruits) and dark chocolate-colored pulps (when they present seeds) in all stages, which influenced the result. Santos et al. (2010) observed more intense flesh coloring in 'Giombo' persimmons (L = 26.51; C = 31.04 and H° = 77.71) and 'Rama Forte' (L = 35.35; C = 43, 23 and H° = 79.66) also produced in the Brazilian semi-arid region, and harvested physiologically mature. In turn, Mendonça et al. (2015), analyzing 'Rama Forte' persimmons harvested with yellow-orange skin color, observed similar chromaticity values of 52.51 and hue angle of 72.07.

The soluble solids contents of the two cultivars averaged 20.46%, and there was no difference between the maturation stages. It was observed that the total sugars and reducing sugars of the two cultivars of persimmons present higher values in the mature stage. The pH values of the 'Giombo' cultivar did not differ in relation to the maturation stage, with an overall average of 5.83. In the 'Rama Forte' cultivar, the fruits of the green and semi-ripe stages had the highest pH values, 5.90 and 5.88, respectively. The titratable acidity values of the 'Giombo' cultivar were higher for the green stage. In the 'Rama Forte' cultivar, there was no significant difference between the maturation stages, with an average of 0.20 g of malic acid 100g^{-1} (Table 3).

The soluble solids of the two cultivars of persimmons did not vary with the stage of maturation. However, the total and reducing sugars of the two cultivars increased in the mature stage, indicating that there was an accumulation of sugar with the advancement of maturation. The titratable acidity did not vary between the maturation stages of the "Rama Forte" cultivar, it presented a significant difference only for the mature stage of the 'Giombo' cultivar, in which there was a reduction, probably, during the ripening of the fruits of the "Giombo' cultivar, acids were used in the respiratory process or converted into sugars (Chitarra and Chitarra, 2005). In relation to pH, there was a reduction in the mature stage of both cultivars. Similar results were observed in 'Rama Forte' persimmons (5.59) harvested with the color of the yellow-orange epidermis (Mendonça et al., 2015).

The relationship between sugars and organic acids is a determining factor for the flavor of the fruit, in which a reduction in acidity is expected to occur during fruit maturation (Martineli et al., 2013), to obtain a fruit with flavor. more palatable for fresh consumption. However, the titratable acidity and soluble solids ratio only showed a significant difference for the mature stage of the 'Giombo' cultivar.

Table 4 presents the values of Vitamin C (Vit. C), total carotenoids (TC), beta-carotene (BC) and astringency index (AI). The persimmon cultivars behaved differently in terms of vitamin C content. In the 'Giombo' cultivar, the green and semi-ripe stages presented values lower than S3, 22.40 and 23.51 mg 100 g^{-1} of vitamin C content, respectively.

Table 3. Soluble solids (SS), hydrogenic potential (pH), titratable acidity (ATT), soluble solids/titratable acidity ratio (SS/ATT), total sugars (AT) and reducing sugars (AR) of the green (S1), semi-mature (S2) and ripe (S3) of persimmon fruits (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' cultivars produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

Cultivar	Stage	SS (°Brix)	AT (g/100g)	AR (g/100g)	pH	ATT (g malic acid 100g^{-1})	SS/ATT
Giombo	S1	20.60 a	11.54 b	14.32 b	5.78 a	0.20 a	102.83 b
	S2	19.92 a	11.93 b	13.90 b	5.94 a	0.16 b	129.67 b
	S3	20.10 a	16.66 a	19.05 a	5.77a	0.12 b	180.19 a
	General Average	20.21	13.38	15.75	5.83	0.16	137.56
	CV (%)	7.40	11.30	11.54	5.67	13.54	13.81
Rama Forte	S1	19.75 a	18.67 b	3.69 b	5.90 a	0.21 a	96.35 a
	S2	21.63 a	17.65 b	3.21 b	5.88 a	0.20 a	102.64 a
	S3	20.79 a	23.44 a	14.12 a	5.63 b	0.19 a	108.49 a
	General Average	20.72	19.92	7.01	5.81	0.20	102.49
	CV (%)	5.62	12.16	8.16	1.60	13.09	9.68

Means followed by the same lowercase letter in the column do not differ from each other, according to Tukey's test at 5% probability. CV= coefficient of variation. S1 = green: around 90% of the color of the green shell; S2 = semi-ripe: 50% of the green skin color; and S3 = ripe: around 80% of the yellow skin color.

Table 4. Vitamin C (Vit. C), soluble tannins (TS), total carotenoids (CT), beta-carotene (BC), astringency index (AI) of green (S1), semi-ripe (S2) and ripe (S3) stages of persimmon fruits (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' cultivars produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

Cultivar	Stage	Vit. C (mg 100g^{-1})	TS (mg 100g-^{1})	CT (mg 100g^{-1})	BC (mg 100g^{-1})	AI Grades
Giombo	S1	22.40 b	633.21 a	0.31 b	0.033 b	3.53 a
	S2	23.51 b	356.56 b	0.49 b	0.024 b	2.13 a
	S3	36.76 a	63.30 c	1.54 a	0.142 a	1.00 b
	General Average	27.56	351.03	0.78	0.066	2.22
	CV (%)	17.39	21.28	24.62	108.26	39.81
Rama Forte	S1	32.60 a	1166.24 a	0.30 a	0.031 b	3.60 a
	S2	37.12 a	1122.24 a	0.17 b	0.050 a	4.20 a
	S3	16.98 b	517.99 b	0.41 a	0.100 a	2.33 b
	General Average	28.90	935.49	0.29	0.060	3.38
	CV (%)	42.85	20.14	24.33	36.10	19.75

Means followed by the same lowercase letter in the column do not differ from each other, according to Tukey's test at 5% probability. CV= coefficient of variation. S1 = green: around 90% of the color of the green shell; S2 = semi-ripe: 50% of the green skin color; and S3 = ripe: around 80% of the yellow skin color.

The mature stage, on the other hand, presented a content of 36.76 mg 100 g^{-1} of vitamin C. The 'Rama Forte' cultivar also showed a difference between the mature stage and the other stages, but in this cultivar the ripe fruits presented lower content (16, 98 mg 100 g^{-1}), when compared with the fruits of the green and semi-ripe stages, respectively. Del Bubba et al. (2009), analyzing 'Kaki Tipo' and 'Rojo Brilhante' persimmons, found a similar behavior to that of the 'Rama Forte' cultivar, with a decrease in vitamin C content with advancing maturation.

The content of soluble tannins (TS) in the 'Giombo' cultivar decreased as the fruit maturation level increased, with a lower content in the mature stage, 63.30 mg 100 g^{-1}. In the 'Rama Forte' cultivar, the behavior was similar to that of the 'Giombo' cultivar, however, they presented higher contents of soluble tannins than the 'Giombo' cultivar. The mature stage of the 'Giombo' cultivar presented 517.99 mg 100 g^{-1} of soluble tannins, while the green and semi-ripe stages presented higher tannin contents, but did not differ from each other. The decrease in the content of soluble tannins during maturation occurs because the tannin molecules are polymerized during the maturation process, becoming insoluble and, consequently, impossible to react with the enzymes present in the saliva (Brecht et al., 2010; Chitarra and Chitarra, 2005). According to Vidrhi et al. (1994), the astringency of persimmon is no longer perceived and the fruits become edible when the concentration of soluble tannins is less than 0.1%. The 'Giombo' and 'Rama Forte' cultivars can be considered edible in the mature stage, since they presented 0.06 and 0.5% of soluble tannins, respectively.

The content of total carotenoids was similar in the green and semi-mature stages of the 'Giombo' cultivar, and higher in the mature stage, which presented 1.54 mg 100 g^{-1}. In the 'Rama Forte' cultivar, the content of total carotenoids in the green and mature stages were similar, 0.30 and 0.41 mg 100 g^{-1}, respectively, whereas the semi-ripe presented only 0.17 mg 100 g^{-1} of the of carotenoids. In persimmon fruits, carotenoids accumulate during fruit maturation and post-harvest ripening (Qi et al., 2021), as seen in the 'Giombo' cultivar. This behavior was not observed for the 'Rama' Forte' cultivar, this result may be related to the level of gene expression of *DkPSY*, *DkZDS*, *DkLBYb* and *DkBCH*, since these genes are directly related to carotenoid biosynthesis in persimmon fruits, and may be expressed differently depending on the cultivar (Qi et al., 2021), however the present study did not perform any gene expression analysis to prove this result. This result corroborates the results obtained in the pulp color of the two cultivars. Regarding the determination of beta-carotene, it was found that in the 'Giombo' cultivar, the green and semi-mature stages were similar, 0.033 and 0.024 mg 100 g^{-1}, respectively, while the mature stage had the highest value of beta-carotene, 0.142 mg 100 g^{-1}. In 'Rama Forte' persimmons, the green stage had the lowest beta-carotene content (0.031 mg 100 g^{-1}), the semi-ripe and mature stages were similar with 0.050 and 0.100 mg 100 g^{-1}, respectively. In the determination of the astringency index, the behavior of the two cultivars was similar, the green and semi-mature stages did not differ and presented lower value than the mature stage. The values for 'Giombo' were 3.53; 2.13 and 1.00 and for 'Rama Forte' they were 3.60; 4.20 and 2.33 for stages S1, S2 and S3, respectively. The astringency index represents, through a qualitative scale, the content of soluble tannins and is directly related to the astringency of the fruit. The mature stage for both cultivars showed a lower astringency index value, however, 'Rama Forte' persimmons remained more astringent even in the mature stage, this astringency reduction result corroborates the behavior reported by Campo-Dall'Orto et al. (1996) for 'Giombo' and 'Rama Forte' persimmons.

Figure 2 shows the results of activity of the pectin methyl esterase (PME) and polygalacturonase (PG) enzymes, total antioxidant activity (AAT) and total extractable polyphenols (PET).

The activity of the pectin methyl-esterase (PME) enzyme of the 'Giombo' persimmon cultivar showed significant differences between the three maturation stages, with the highest activity in the mature stage, 1739.95 EU $min^{-1}g^{-1}$, while the polygaturonase (PG) showed no difference between maturation stages, with a mean of 1516.6 EU min^{-1} g^{-1} (Figure 2A and B). In the 'Rama Forte' cultivar, there was no difference between the maturity stages of PME and the general average was 1594.08 E.U. $min^{-1}g^{-1}$. On the other hand, PG activity showed a significant difference for the mature stage, with a value of 761.75 E.U. $min^{-1}g^{-1}$. The activity of the PME enzyme precedes and facilitates the action of PG, demethylating the C6 of each protopectin unit, enabling recognition by PG, which, in turn, catalyzes the hydrolysis of β-1,4 bonds between galacturonic acid residues within the pectin chain, culminating in an increase in fruit softness (Cheftel and Cheftel, 1992; Bicalho et al., 2000; Manrique and Lajolo, 2004). Moraes et al. (2011) observed that PME activity in 'Giombo' persimmons increased days before the increase in PG activity during storage. Although a statistical difference was observed in the activity of the hydrolytic enzymes of the cell wall, it was not sufficient to trigger a significant effect on the firmness values for both cultivars, since there was no significant difference in firmness between the maturation stages (Table 1).

For the total antioxidant activity (AAT) (Figure 2C) by the ABTS method, in the 'Giombo' cultivar, there was no significant difference between the green and semi-ripe stages, the mature stage differed with a lower value (3.09 µM Trolox g pulp- 1). By the DPPH method for the 'Giombo' cultivar, there was no significant difference between the semi-ripe stage and the others, however the green and mature stages differed from each other, presenting an average content of 17921.67 g of pulp g of DPPH-1(37, 59% scavenging of the DPPH radical). Both methods suggest a reduction in the antioxidant capacity in the ripe 'Giombo' cultivar, this result may be related to a greater reduction in the content of total extractable polyphenols and soluble tannins at this stage. These results corroborate the results obtained by Novillo et al. (2016), when studying the maturation stages of 10 persimmon cultivars, including the cultivar 'Giombo'. In the 'Rama Forte' cultivar, there was no significant difference in antioxidant activity by the ABTS method, with an average content of 85.19 µM Trolox g pulp-1 (Figure 1C). By the DPPH method, this same cultivar showed difference in AAT between semi-ripe and mature (Figure 2D). The green stage did

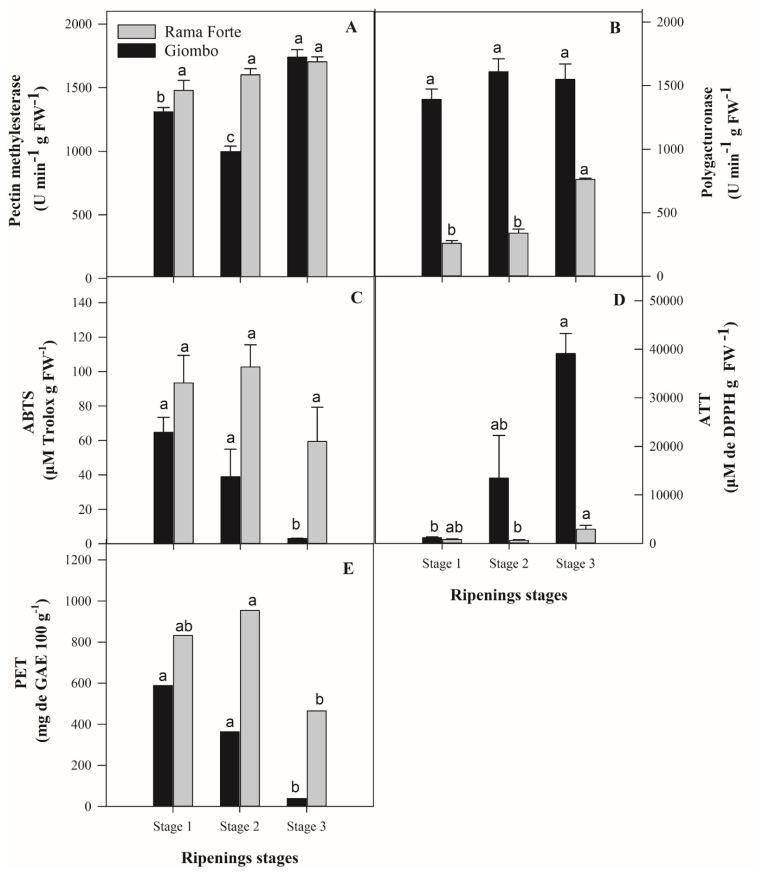

Figure 2. Pectin methyl esterase-PME (A) and polygalacturonase-PG (B); total antioxidant activity by ABTS (C) and DPPH method (D) and total extractable polyphenols-PET (E) of persimmon fruits (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' cultivars, harvested at three stages of maturation: green (Stage 1), semi-ripe (Stage 2) and ripe (Stage 3) produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

not differ from the others, and the general average was 1451.87 g pulp g DPPH (81.85% scavenging of the DPPH radical). The 'Rama Forte' cultivar showed the lowest oscillation in AAT between the stages of maturation, a result similar to that observed in the determinations of PET, soluble tannins and astringency index.

Park et al. (2006) observed a high correlation between the content of compounds polyphenolic compounds found in extracts of persimmon (*Diospyros kaky* L. var. Triumph) and the percentage of oxidation inhibition by the DPPH free radical capture test, which may explain the reduction of AAT in the persimmons of the present work Tessmer et al. (2014), characterizing astringent fruits in seven stages of maturation, mentions the relationship between the antioxidant capacity in persimmons and the natural loss of astringency, as the values decrease over time. Tessmer et al. (2014) found for AAT (% DPPH sequestration) in 'Giombo' persimmons a variation in S1 of 95.86% and 81.19% in E7 of maturation, while for 'Rojo Brilhante' it was 92.46% and 12.60%, concentrations higher than those found in this work for the Giombo cultivar. Rufino et al. (2010), analyzing bioactive compounds and antioxidant activity of 18 non-traditional tropical fruits using the ABTS method, found positive and significant correlations between vitamin C content and total extractable polyphenol content.

In the 'Giombo' 'cultivar,' the reduction in the content of total extractable polyphenols (PET) was quite significant with increasing maturation. The PET content in the mature stage was 38.60 mg of GAE 100 g^{-1}, 12 times lower than in the green and semi-mature stages. While in the 'Rama Forte' cultivar, the semi-mature stage presented 953.39 mg of GAE 100 g^{-1}, thus standing out with a higher PET content, but without significant difference in relation to the green stage, 832.12 mg of GAE 100 g^{-1}, similar to the semi-mature stage, as well as in the 'Giombo' cultivar, the mature stage had the lowest PET content, 465.40 mg of GAE 100 g^{-1}, respectively (Figure 2E). The reduction of phenolic compounds during the ripening of persimmon fruits can be attributed to a series of biochemical and enzymatic changes of certain phenols during the ripening process, including hydrolysis of glycosides by glycosidases, oxidation of phenols by phenoloxidases and polymerization of phenols free (Robards et al., 1999).

Principal component analysis (APC) shows that the three maturation stages have different characteristics, as well as the two cultivars of persimmons. In addition, it is possible to observe that the green stage shares similar characteristics for the two cultivars (Figure 3).

The 'Giombo' cultivar has higher antioxidant activity by the DPPH and ABTS methods, as well as higher acidity and higher tannin and polyphenol content than the 'Rama Forte' cultivar. This gives it greater astringency (Figure 4). On the other hand, the 'Rama Forte' cultivar has a higher content of carotenoids and total and reducing sugars, and therefore, characteristics that make it more palatable than the 'Giombo' cultivar.. In addition, 'Rama Forte' presents higher values of luminosity and chromaticity, indicating that its bark is more yellowish, due to its higher content of carotenoids. These characteristics were the main responsible for distinguishing the two cultivars. The content of soluble solids and vitamin C, as well as the firmness and pH of the pulp did not differ much between the two cultivars. The two main components explain 6.98% of the total variance, and eigenvalues greater than 1.0 were considered significant.

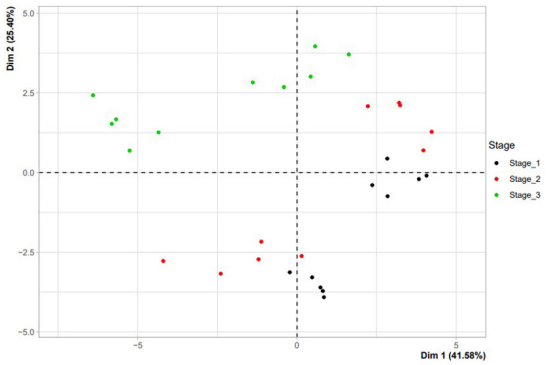

Figure 3. Analysis of the principal components (APC) of the fruits of persimmons (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' varieties, in the green stages (around 90% of the green skin color), semi-ripe (50% of the green bark) and mature (around 80% yellow bark), produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

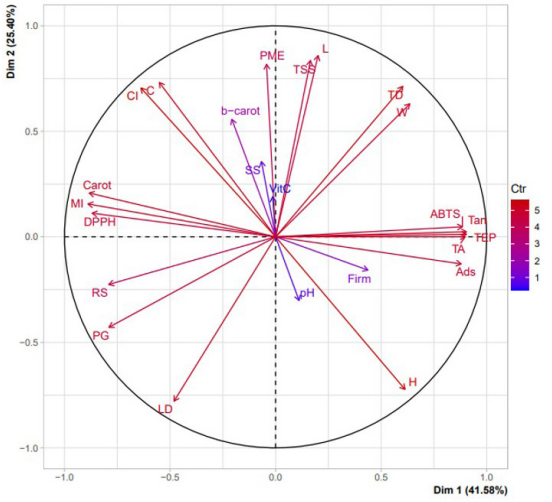

Figure 4. Analysis of the principal components (APC) of the fruits of persimmons (*Diospyros kaki* L.) of the 'Giombo' and 'Rama Forte' varieties, in the green stages (around 90% of the green skin color), semi-ripe (50% of the green bark) and mature (around 80% yellow bark), produced in the Brazilian semiarid, Vale do Jaguaribe-CE, Brazil.

4. Final Considerations

Persimmon fruits of the 'Giombo' and 'Rama Forte' cultivars produced in semi-arid regions have greater antioxidant capacity in the green and semi-ripe stages, but the high astringency index of the fruits at these stages makes them unsuitable for consumption in natura, without

previous treatment for removal astringency and can be used for processing purposes to obtain persimmon by-products. However, the mature stage is the most appropriate for harvesting and marketing the fruits in natura, which presented the best physicochemical quality characteristics and a firmness that allows good conditions for transport and commercialization when ripe, at S3 stadium, with a totally yellow skin.

In addition, the present study shows that persimmon fruits can be cultivated in semi-arid conditions and present characteristics similar to those produced in regions with more appropriate climatic conditions for the development of the crop.

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