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Chemical Characteristics and Bioactive Compounds in *Juazeiro* Fruits Collected in Different Maturation Stages

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Authors' contributions

This work was carried out in collaboration between all authors. Author JLS elaborated the study, participated in all the steps of conducting and writing the manuscript. Author FBC is the research supervisor, showed the alternatives of conducting and evaluating the data and assisted in the statistical part of the work. Authors AMN and TMG participated in the process of planning and conducting the experiment, besides participating in the writing of results and discussion. Authors MSS and LSS were responsible for the part of the methodology of the manuscript and reviewed the bibliography. Authors MAFN and KPS were of paramount importance in the conduction and evaluation of the experiment, data typing process for later statistical analysis. Authors CRA and KGS gave decision in the correction phase, showing alternatives to enrich the information work, introductory part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The present study aims to assess the bioactive compounds found in *juazeiro* fruits collected during different maturation stages and kept under controlled temperature.

Experimental Design: The experiment was conducted in a completely randomized factorial design (5×4) , in which the first factor consisted of the maturation stages and the second consisted of days of storage.

Location and Duration of Study: The experiment was conducted at the Chemistry, Biochemistry and Food Analysis Laboratory of the Agrifood Science and Technology Center of the Federal University of Campina Grande, Pombal Campus, Paraíba, Brazil, between April and May of 2017.

Methodology: Fruits were collected from *juazeiro* trees located in the Pombal Campus of the Federal University of Campina Grande, and were classified in five maturation stages, according to their skin color. After being classified and cleaned, the fruits were packaged in expanded polystyrene trays, wrapped with a layer of PVC film, and stored under controlled temperature (28±2°C and 55±5% RH) for 6 days. Chemical and bioactive compound analysis was conducted every other day.

Results: In the study conditions, the fruits of juazeiro of all stages of maturation remained apt for analysis and consumption only on the first day of analysis. The results of the analyzes indicate that the advancement of the maturation promotes the reduction of acidity and increase of the soluble solids content, thus providing fruits with a more pronounced sweet flavor.

Conclusion: The *juazeiro* fruits are potential suppliers of phenolic compounds, especially in the first stage of maturation.

Keywords: Ziziphus joazeiro Mart.; potential; soluble solids; phenolic compounds.

1. INTRODUCTION

Located in the Brazilian semiarid region, the Caatinga biome occupies an area of approximately 1.037.517,80 square kilometers, where there are various plants, mainly the *aroeira*, the *mandacaru*, the *amburana* and the *juazeiro*. Despite the great amount of plants, only a few species of the caatinga are valued, and among them is the *juazeiro* (*Ziziphus joazeiro* Mart.). Even though it is present all over the Northeast region of Brazil, only a few studies are directed aim to assess its quality and possible introduction in human nutrition [1,2].

The *Ziziphus joazeiro* has great relevancy, since it is used for several purposes, such as firewood and charcoal; utilization in traditional medicine as an expectorant, in treatment of gastric ulcers and bronchitis; street and garden afforestation; soap, cosmetics and toothpaste manufacture; and serving as animal forage, especially during droughts [3,4].

Juazeiro fruits are edible. They have a big kernel surrounded by a white, sweet mucilaginous pulp, with a pit that splits in half [5,6].

The exploration of the *juazeiro* is limited to predatory extractivism only, despite all its importance and widespread utilization by the

population of the northeastern semiarid, and it is necessary to further research and study it, to enhance its cultivation and domestication [7].

Therefore, the study aimed to assess the chemical and bioactive compounds of juazeiro fruits harvested at different stages of ripeness and stored under controlled temperature, thereby enabling the scope of knowledge about this culture.

2. MATERIALS AND METHODS

The fruits used were collected from plants located in the Agrifood Sciences and Technology Center (CCTA) of the Federal University of Campina Grande (UFCG), located in the Pombal/PB. municipality of Brazil. of geographical coordinates 6°48'16''S and 37°49'15"W, 175 m above sea level. According to Köppen classification, the predominant climate in the region is of the BSh type, hot semiarid, with annual rainfall of 750 mm [8].

2.1 Acquisition of Vegetable Matter and Experimental Procedures

Fruits were manually collected early in the morning, from 6 to 9am, packed in polystyrene bags and later transported to the Chemistry, Biochemistry and Food Analysis Laboratory of the CCTA. After being collected, the fruits were selected visually for the absence of injuries in order to obtain uniform, quality samples. After selection, the fruits were sorted in five maturation stages according to the color of their skin, following a maturation chart developed by Silva et al. [9], as shown on Fig. 1.

After classification, fruits were cleaned in running water, to remove superficial dirt, and after that, they were packaged in expanded polystyrene trays wrapped in a 12 μ m PVC film. Each tray contained approximately 150 g of *juazeiro* fruit. The trays were placed on a stainless steel table and stored under controlled temperature (28±2°C and 55±5% RH), and chemical and bioactive compounds assessments were conducted every other day (on day 0, 2, 4 and 6).

At the more advanced maturation stages, the analysis was interrupted due to the advanced senescence of the fruits, marked by the development of fungi, elevated softness of the pulp, strange odors and the product's atypical color.

2.2 Experimental Design

The experimental design used was a completely randomized factorial design (5 x 4), in which the first factor consisted of the maturation stages (I, II,III, IV and V) and the second consisted of days of storage (0, 2, 4, and 6), with 4 repetitions, being each repetition constituted by a single tray containing approximately 150 g of fruit.

2.3 Chemical and Biochemical Compounds Assessment

The fruits were manually pulped with the aid of knifes and the pulp was then processed in a domestic blender (Mondial brand), with addition of water in the 1:1 proportion (mass:volume). The results were corrected afterwards.

Chemical analyses were realized according to the Instituto Adolfo Lutz recommendations, and the obtained extract was examined for following parameters:

- pH: The pH was determined with direct reading of the *juazeiro* fruit extract in a bench digital potentiometer (Digimed brand).
- H^+ lon Concentration (μ M): Estimated on the conversion of the values obtained for the pH through the equation $[H^+] = 10^{-pH}$.
- Soluble Solids (%): The pulp of the juazeiro fruit was filtered through a layer of cotton and the soluble solid concentration was determined in a digital refractometer (Digital refractometer brand) with automatic temperature compensation. The analysis was made twice for each repetition and whenever necessary, the refractometer was calibrated with distilled water.
- Titratable Acidity (% of citric acid): Acidity was measured in 5 g of pulp, homogenized in 45 mL of distilled water. The solution containing the sample was titrated against NaOH 0,1N until the turning point of the phenolphthalein indicator, being expressed in citric acid percentage.
- Soluble Solids and Titratable Acidity Ratio (SS/TA): Obtained by dividing the soluble solids value by the titratable acidity value.

The bioactive compounds were assessed by quantifying ascorbic acid, total chlorophyll total carotenoids, total phenolic compounds, flavonoids and anthocyanins, as follows:

 Ascorbic Acid (mg/100 g): The value of ascorbic acid was estimated through titration, by using 3 g of *juazeiro* fruit pulp, added 47 ml of oxalic acid at 0.5% and then titrated against Tillmans Solution until the pink coloring, according to the method of Instituto Adolfo Lutz [10].



Fig. 1. *Juazeiro* fruits classified in five maturation stages, according to the maturation chart of Silva et al. [9]

- Total Chlorophyll and Total Carotenoids (mg/100 g): Determined accordingly to Lichtenthaler [11] with adaptations. Around 0.2 g of the sample was macerated in a mortar with 0.2 g of calcium carbonate (CaCO₃) and 5 mL of cold acetone (80%), which was kept in a dark environment. After that the samples were centrifuged at 10°C and 3.000 rpm for 10 minutes in a refrigerated centrifugal machine (Cientec brand). The supernatant were read in a spectrophotometer (Spectrum brand) in the 470, 646 e 663 nm wavelengths.
- Total Phenolic Compounds (mg/100 g): The phenolic compounds were estimated using the Folin & Ciocalteau method, as described by Waterhouse [12], by mixing 50 µL of filtered juice of the juazeiro fruits with 2075 µL of distilled water and 125 µL of the Folin-Ciocalteau reagent, followed by agitation in a tube agitator (Nova brand), and resting for 5 minutes. After the reaction time, 250 µL of 20% sodium carbonate were added, and subsequently there was a second agitation and resting in water bath (Hemoquímica brand) at 40°C, for 30 minutes. The samples were read in a spectrophotometer (Spectrum brand) at 765 nm.
- Flavonoids and Anthocyanins (mg/100 g): Flavonoids anthocyanins and were determined according to the Francis methodology [13]. Around 0,5 g of the sample were macerated in a mortar with 5 ml of ethanol - HCl (1,5N) in a dark environment and were left to rest for 24 hours in a refrigerator (Consul brand). The samples were filtered with paper filters and readings were done in а spectrophotometer (Spectrum brand) at

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374 nm for flavonoids and at 535 nm for anthocyanins.

2.4 Statistical Analysis

Data obtained was subject to analysis of variance, when a significant effect was detected for the F test. A Tukey test at the 5% probability level was applied. To evaluate the influence of one parameter over the other, Pearson correlation coefficients were determined. Data was analyzed with the aid of the AgroEstat® statistical package [14].

3. RESULTS AND DISCUSSION

In storage, in maturation stages III, IV and V there were fungi, rotting of the fruit, unpleasant odor and elevated softness of the pulp, making analysis impossible. This behavior might be associated to the quickened senescence process.

It can be said that the *juazeiro* fruits have elevated senescence, seen that within 2 days in storage one maturation stage was eliminated, with only stages I and II being suitable to analysis on the sixth day of storage.

3.1 Chemical Analysis

Fig. 2 shows the average values of pH and H^+ ions determined to the *juazeiro* fruits. pH values (Fig. 2A) ranged from 5.73 to 6.33 at the beginning of storage, results close to those encountered by Silva et al. [1], that ranged from 5.65 to 6.89, being superior to the values determined by Cavalcanti et al. [15] who determined a pH of 4.78 to the flour obtained from *juazeiro* fruit.



Fig. 2. pH (A) and H⁺ ions (B) of *juazeiro* **fruits collected in different maturation stages** Vertical bar represents the standard deviation from the mean. CV = variation coefficient

Normally, the pH value tends to increase during storage, because the acids present in the fruit tend to decrease during storage, as they are consumed in respiration or converted to sugars. However, it can decrease as a result of an increase in the acids content, as it occurs with bananas and pineapples, where the acids peak at full maturation [16], resembling the pH of *juazeiro* fruits during storage. Only in stage I there was an increase followed by a decrease in the pH value, probably associated to the maturation stage of the fruit, which were not very ripe.

behavior observed in the H^+ The ion concentration (Fig. 2B) is a reflex of the pH behavior (Fig. 1A), since pH and H^{+} ion concentration are proportional. Among the maturation stages at the beginning of storage, a reduction was observed in the H⁺ ion concentration until maturation stage IV, followed by an expressive increase in stage V. That indicates that the maturation process promotes the increase in concentration of acids, and during storage there was an increase in the H^{+} ion concentration, that once again could be attributed to the increase in the acid levels.

Fig. 3 shows the values obtained for soluble solids, titratable acidity and SS/TA ratio obtained in the *juazeiro* fruit samples. At the beginning of storage there was an increase in the value of soluble solids (Fig. 3A) until maturation stage IV, with a subsequent decrease in maturation stage

V, which can result from the build-up of substrates during development (stages I, II, III and IV) and consumption at the senescence stage (stage V). *Juazeiro* fruits showed high rates of soluble solids, which enhances their utilization in industrial processes. The soluble solid rate is directly related to industrial performance. In practical terms, for each degree ^oBrix of increment of raw material there is an increment of 20% in industrial yield [17].

During storage, two behaviors were observed (Fig. 3A). In stages I, II and III there was an increase in the soluble solids rate, possibly from the advance of the maturation stages during the period of storage, since storage promotes the increase in the solid soluble content. To stages IV and V a decrease in the soluble solids content was observed, possibly due to senescence in which there is consumption of accumulated substrates, reducing the amount of soluble solids in the fruit.

In the literature are found several statements relating the increase of the content of soluble solids in fruits during maturation. Some authors attribute this effect to the hydrolysis of the starch [18], to the respiratory peak of the fruit [19] to processes of biosynthesis [20], among others. Deeper analyzes may clearly indicate what promotes an increase in soluble solids content in *juazeiro* fruits, but in principle the fruit peak can be indicated [9] and probably the breakdown of polysaccharides (hydrolysis).





The vertical bar represents the standard deviation from the mean. CV = variation coefficient

Analyzing Fig. 3B, it has seen that the acidity of the fruits in the beginning of the storage varied from 0.26 to 0.44%, values higher than the ones observed by Silva et al. [1] that ranged from 0.12 to 0.14%. During maturation, a regular behavior of increase or decrease of acidity was not observed, and this fact could be related to the maturation of the fruit. According to Chitarra [21], during maturation, fruits quickly lose acidity, but in some cases, there is a small increase in the concentration as maturation happens, behavior observed in the *juazeiro* fruit, since there was not a regular decrease of acidity related to the maturation stage.

In relation to the SS/TA ratio (Fig. 3C), throughout the storage there was an increase in relation to the maturation stages, indicating that the fruits tasted sweeter, since there was an increment in the values of this ratio. The SS/TA ratio has been used as an index for assessing palatability, giving an idea of the balance between sugars and acidity [21]. To Mattedi et al. [22] the elevated value for the SS/TA ratio brings a smoother taste (sweeter and less acid), as low values promote acidic taste, making the latest maturation stages (IV and V) more interesting to commercialization. During storage, the SS/TA ratio generally increases what is probably a reflection of the conversion of acids in sugars, a behavior similar to the one found in determining soluble solids (Fig. 3A).

The obtained results of the Pearson correlation coefficients among the chemical characteristics of *juazeiro* fruits are shown in Chart 1.

Every chemical characteristics showed a positive correlation among themselves, but it was observed that there is a high positive correlation between pH and Titratable Acidity, seen that they showed a similar behavior with small variations between maturation stages and during storage.

A high positive correlation between the SS/TA ratio and soluble solids was verified, as expected, since the soluble solids have direct influence in the SS/TA ratio, for the higher the

soluble solid concentration is, the higher the SS/TA ratio will be. As for the titratable acidity, a low correlation with the SS/TA ration was seen, as they are inverse parameters and the higher the acidity, the lower the SS/TA ratio.

3.2 Bioactive Compounds Assessment

Fig. 4 illustrates the results obtained for the variables ascorbic acid, total chlorophyll and carotenoids in the *juazeiro* fruits. They had considerable quantities of ascorbic acid (Fig. 4A), ranging from 6.4 to 8.9 mg/100 g at the beginning of storage, a fact of high relevancy, because the amount of ascorbic acid is used and a food quality index [21].

In storage, for stages I, II and III there was a decrease in ascorbic acid, and for stage IV there was a considerable increase in this parameter. According to Chitarra [21] the decrease in the amount of ascorbic acid during maturation is subsequent to direct action of the ascorbic acid oxidase enzyme, or by the action of oxidant enzymes such as the peroxidase.

For each day in storage, there was a decrease in the total chlorophyll levels. (Fig. 4B), which is subsequent to maturation, a chlorophylldegrading fact. To Chitarra [23] the green color in vegetables is subsequent to the presence of chlorophyll and the loss of green color is associated with their degradation. The rapid loss of chlorophyll and subsequently, of the green color of tissues during maturation is associated to the fact that the chloroplasts and their thylakoid membranes disintegrate. Many factors can influence alone or together, such as the pH, influenced by the increase of organic acids in the vacuoles, oxidative systems and chlorophyllase.

As for stage V, in the beginning of storage a small increase in the chlorophyll concentration was perceived, that could be subsequent to the non-uniformity in classification of the maturation stages, since the only factor considered during classification was the fruit's color.

Chart 1. Pearson correlation coefficients (r) among chemical characteristics of *juazeiro* fruits collected during different maturation stages

Chemical characteristics	рН	H⁺lons	Soluble solids	Titratable acidity			
H [⁺] ions	0.5176**	_	-	_			
Soluble Solids	0.8934**	0.4285**	-	_			
Titratable Acidity	0.9012**	0.5434**	0.7310**	_			
SS/AT Ratio	0.7454**	0.3132**	0.9042**	0.4555**			

**considerable at 5% probability level



Fig. 4. Ascorbic acid (A), total chlorophyll (B) and total carotenoids (C) of *juazeiro* fruits collected up during different maturation stages The vertical bar represents the standard deviation from the mean. CV = variation coefficient



Fig. 5. Total Phenolic compounds (A), flavonoids (B) and anthocyanins (C) of *juazeiro* fruits collected during different maturation stages

The vertical bar represents the standard deviation from the mean. CV = variation coefficient

Simultaneously to the decrease of chlorophyll during the beginning of storage (Fig. 4B), a decrease in the total carotenoids content was observed, (Fig. 4C), leading to the understanding

that the advance of the maturation stage promotes degradation of the analyzed pigments. Silva et al. [24] observed a similar behavior during the assessment of quantitative

	Ascorbic acid	Chlorophyll	Carotenoids	Phenolic compounds	Flavonoids
Chlorophyll	0.5034**				
Carotenoids	0.5264**	0.9269**			
Phenolic	0.7837**	0.5469**	0.5491**		
Compounds					
Flavonoids	0.7038**	0.8986**	0.9049**	0.7799**	
Antocyanins	0.5851**	0.6479**	0.6652**	0.7231**	0.7702**

Chart 2. Pearson correlation coefficients (r) among bioactive compounds pof *juazeiro* fruit collected in diferente maturation stages

**considerable to 5% level of probability

composition of *camu-camu* fruits, when a decrease in the total carotenoid content as maturation advanced.

Lima et al. [25] affirmed that during maturation there could be a decrease in the total carotenoid content, related to oxidative reactions of pigments or phenolic compounds, resulting in the darkening of the pulp, indicating the beginning of senescence. The behavior of total carotenoids during storage was similar to the one observed in chlorophyll results (Fig. 4B), and could be attributed possibly to enzymatic action.

Fig. 5 shows the results regarding to phenolic compounds, flavonoids and anthocyanins in *juazeiro* fruit.

Obtained results indicate that the *juazeiro* fruit are potential sources of phenolic compounds (Fig. 5A), presenting in the beginning of storage 647.9 mg/100 g (stage I) and 389.5 mg/100 g (stage V), aspect of great importance, as it makes *juazeiro* fruits a source of bioactive compounds, which are substances that have antioxidant activity, suppressing the action of free radicals.

During storage there was an accumulation of phenolic compounds, it could be subsequent to the secondary metabolism of the fruits that promotes the synthesis of many substances, phenolic compounds among them [26]. Some authors [27,28] affirm that at the beginning of the maturation the fruits present high levels of phenolic compounds that decrease during the development and reach a minimum during the last phase of maturation, which precedes the final accumulation of the sugars.

Fig. 5B represents the behavior of flavonoids in relation to the stage of maturation. It is noted, that independently to storage, the highest flavonoid content was obtained in stage I. During the storage, there was an increase in the flavonoid content for all maturation stages.

According to Huber; Rodriguez-Amaya [29] the variations observed for the flavonoids could be attributed to genetic factors and influenced by edaphoclimatic factors and even by food processing.

The results for anthocyanins (Fig. 5C) varied from 0.018 to 0.006 mg/100 g in the beginning of storage, and did not show great variation during storage. Answers were similar to the flavonoids behavior already expected, since anthocyanins are part of the flavonoid group. The similarity was observed during both the beginning and the end of the storage.

Chart 2 shows the results obtained for the Pearson correlation coefficients among the bioactive compounds of *juazeiro* fruit assessed in this research.

All bioactive compounds showed positive correlation between them, as expected, because there was a decrease in all bioactive compounds as the maturation stage advanced. During storage there was also observed a similar behavior between different maturation stages.

There was an intense correlation between total carotenoids and chlorophyll and with flavonoids, subsequent to the similar behavior between both maturation stages and storage.

4. CONCLUSION

Storage under controlled temperature was not efficient in extending the shelf life of the *juazeiro* fruit, once after 2 days of storage the maturation stage V was discarded, getting to the 6 days of storage with two stages suitable to analysis.

Chemical analyses demonstrate that as maturation occurs, the fruits taste sweeter, thanks to the increased solid soluble content and decreased acidity, and as such, fruits in later maturation stages are more consumed.

First stage of maturation fruits had higher concentration of phenolic compounds, indicating that they are potential sources of bioactive compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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