

Chemical characteristics and bioactive compounds in juazeiro fruits picked in different stages of ripening

ABSTRACT

Objective: the present study aimed to measure the bioactive compounds in juazeiro fruits picked in different stages of ripening and maintained under controlled temperature.

Experimental design: the experiment was conducted in a completely random factorial design (5 x 4), in which the first factor featured the ripening stages and the second featured days of storage.

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

Methodology: fruits were picked from juazeiro trees located in the Pombal Campus of the Federal University of Campina Grande, and were classified in five stages of ripening, according to their skin color. After classifying and cleaning, the fruits were packaged in expanded polystyrene trays, wrapped with a layer of PVC film, kept in controlled temperature (28±2 °C e 55±5% UR) for 6 days, and chemical and bioactive compound analysis were conducted every 2 days.

Results: in the study's conditions the juazeiro fruits in every ripening stage were apt for analysis and consumption only in the first day. The results of the analysis indicate that as ripening happens the acidity decreases and there is an increase in the concentration of soluble solids, giving the fruits a sweeter taste.

Conclusion: The juazeiro fruits are potential suppliers of phenolic compounds, especially at first stage of ripening.

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

1. INTRODUCTION

Located in the Brazilian semiarid region, Caatinga occupies an area of approximately 1.037.517,80 km², 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 on 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, few studies are directed towards evaluating its quality and possible introduction in human nutrition. [1, 2].

The *Ziziphus joazeiro* is of great relevancy, since it is put to many 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, in addition to serving as 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 comes down to predatory extrativism only, despite all its importance and widespread utilization by the population of the northeastern semiarid, and incentive to further research and studies about it, to enhance its cultivation and domestication. [7].

Therefore, the main goal is to assess the chemical characteristics and measure bioactive compounds in juazeiro's fruits picked up in different stages of maturation and packed under controlled temperature, thus enabling the acquisition of knowledge of this culture.

2. MATERIAL AND METHODS

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

2.1 Obtention of Vegetable Matter and Experimental Procedures

Fruits were manually picked early in the morning, from 6am to 9am, packed in polystyrene bags and transported to the Chemistry, Biochemistry and Food Analysis of the CCTA.

After being picked, the fruits were selected visually for the absence of injuries in order to obtain uniform, quality samples. After selection, the fruits were classified in stages of ripening according to the color of their skin, following a ripening chart developed by Silva et al. [9], as shown on Figure 1.



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

After classification, fruits were cleaned in current water, to remove superficial dirt, and after that they were packaged in expanded polystyrene involved by a 12 µm PVC film. Each tray contained approximately 150g of juazeiro fruit. The trays were displayed on a stainless steel table and kept under controlled temperature (28 ± 2 °C e $55 \pm 5\%$ UR), and chemical and bioactive compounds assessments were conducted every other day. (days 0, 2, 4 and 6).

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

2.2 Experimental Design

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

2.3 Chemical and Biochemical Compounds Assessment

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

Chemical analysis were realized accordingly to the Instituto Adolfo Lutz recommendations, being the obtained extract evaluated under the following parameters:

- Hydrogenionic Potential: The hydrogenionic potential (pH) was determined with direct reading of the juazeiro fruit extract in a bench digital potentiometer (Digimed brand).

- H^+ Ion Concentration (μM): estimated on the conversion of the values obtained for the pH through the equation $[H^+] = 10^{-pH}$.

68 - Soluble Solids (%): the pulp of the juazeiro fruit was filtered through a layer of cotton and the soluble
69 solid concentration was determined in a digital refractometer (Digital refractometer brand) with
70 automatic temperature compensation. The analysis was made twice for each repetition and whenever
71 necessary, the refractometer was calibrated with distilled water.

72 - Titratable Acidity (% of citric acid): acidity was measured in 5g of pulp, homogenized in a acidized
73 media em 5 g de polpa, made homogenous in 45mL of distilled water. The solution containing the
74 sample was titrated with NaOH 0,1 N until the turning point of the phenolphthalein indicator, being
75 expressed in citric acid percentage.

76 - Soluble Solids and Titratable Acidity Ratio (SS/AT): obtained by dividing the soluble solids value by
77 the values of titratable acidity.

78 The bioactive compounds were assessed by quantifying ascorbic acid, total chlorophyll total
79 Carotenoids, total phenolic compounds, flavonoids and antocyanins, as described:

80 - Ascorbic Acid (mg/100 g): The value of ascorbic acid was estimated through titration, being utilized
81 3g of juazeiro fruit pulp, added of 47ml of oxalic acid 0,5% and titrated against Tillmans Solution until
82 the pink coloring, according to the Instituto Adolfo Lutz Method[10].

83 - Total Chlorophyll and Total Carotenoids (mg/100 g): determined accordingly to Lichtenthaler [11]
84 with adaptations. Around 0,2 g of the sample was macerated in a mortar with 0,2 g of calcium
85 carbonate (CaCO_3) and 5 mL of cold acetone (80%), kept in a dark environment. After that the
86 samples were centrifugated at 10 °C and 3.000 rpm for 10 minutes in a refrigerated centrifugal
87 machine (Cientec brand), the supernatant were read in a spectrophotometer (Spectrum brand) in the
88 470, 646 e 663 nm wavelengths.

89 - Total Phenolic Compounds (mg/100 g): the phenolic compounds were estimated using the Folin &
90 Ciocalteu method, as described by Waterhouse [12], by mixing 50 μL of filtered juice of the juazeiro
91 fruits with 2075 μL of distilled water and 125 μL of the Folin-Ciocalteu reagent, followed by agitation
92 in a tube agitator (Nova brand), and resting for 5 minutes. After the reaction time, 250 μL of 20%
93 sodium carbonate were added, and finally there was a second agitation and resting in water bath
94 (Hemoquímica brand) at 40 °C, for 30 minutos. The samples were read in a spectrophotometer
95 (Spectrum brand) at 765nm.

96 - Flavonoids and Antocyanins (mg/100 g): flavonoids and antocyanins were determined according to
97 the Francis methodology [13]. Around 0,5 g of the sample were macerated in a mortar with 5ml of
98 ethanol - HCl (1,5 N) in a dark environment and were left to rest for 24 hours in a refrigerator (Consul
99 brand). The samples were filtered with paper filters and readings were done in a spectrophotometer
100 (Spectrum brand) at 374 nm for flavonoids and at 535 nm for antocyanins.

101 2.4 Statical Analysis

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

106 3. RESULTS AND DISCUSSION

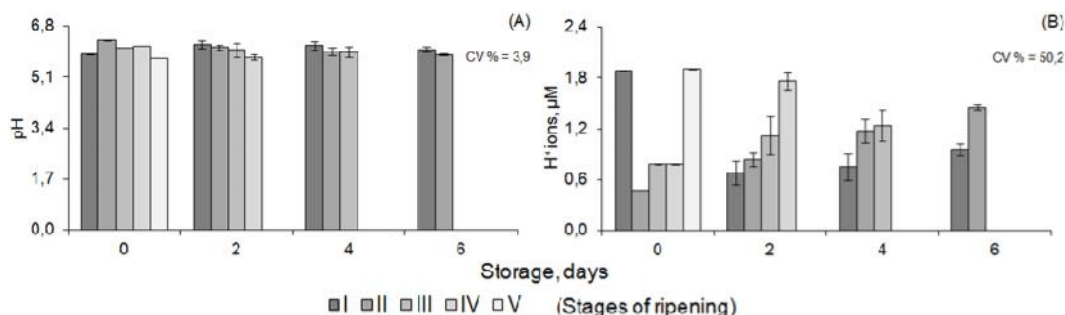
107 In storage, in the ripening stages III, IV and V there were fungi, rotting of the fruit, unpleasant odor
108 and elevated softness of the pulp, making analysis impossible. This behaviour might be associated to
109 the quickened senescence process.

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

112 3.1 Chemical Analysis

113 Figure 2 shows the average values of pH and H^+ ions determined to the juazeiro fruits. pH values
114 (Fig. 2A) ranged from 5,73 to 6,33 at the beginning of storage, results close to those encountered by

115 Silva et al. [1], that ranged from 5,65 to 6,89, being superior to the values determined by Cavalcanti et
116 al. [15] that determined pH of 4,78 to the flour obtained from juazeiro fruit.

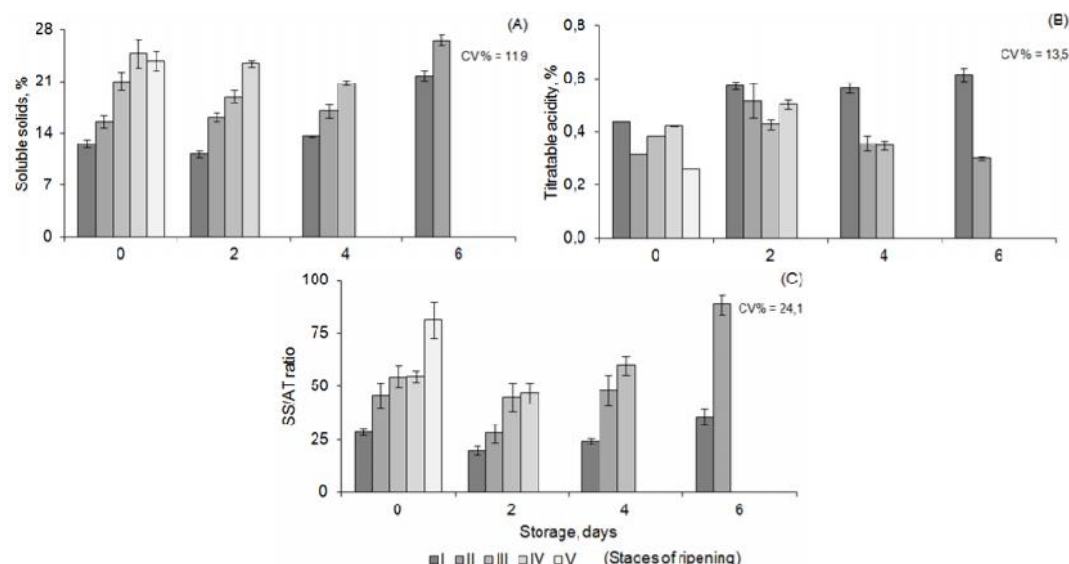


117 **Fig. 2. pH (A) and H⁺ ions (B) of juazeiro fruits picked in different stages of ripening. Vertical**
118 **var represents the standard deviation from the mean. CV = variation coefficient.**
119

120 Normally, the pH value tends to increase during storage, because the acids present in the fruit tend to
121 decrease during storage, as they are consumed in respiration or converted to sugars. However, they
122 can decrease resulting of acids increase, as it occurs with bananas and pinapples, where the acids
123 peak at full maturation. [16]. Resembling the behavior of pH of juazeiro fruits during storage. Only in
124 stage I there was an increase followed by a decrease in the pH value, probably associated to the
125 ripening stage of the fruit, that weren't totally ripe.

126 The behavior observed in the H⁺ ion concentration (Fig. 2B) is a reflex of the pH behavior (Fig. 1A),
127 being the answers inverse, once the higher the pH, the higher the H⁺ ion concentration. Among the
128 ripening stages at the beginning of storage, a reduction was observed in the H⁺ ion concentration until
129 ripening stage IV, followed by an expressive increase in stage V, indicating that the ripening process
130 promotes the increase in concentration of acids, and during strage there was an increase in the H⁺ ion
131 concentration, that once again could be attributed to the increase in the acid levels..

132 Figure 3 shows the values obtained for soluble solids, titratable acidity and SS/AT ratio obtained in the
133 juazeiro fruit samples. At the beginning of storage there was an increase in the value of soluble sollids
134 (Fig. 3A) until ripening stage IV, with subsequent decrease in ripening stage V, which can result from
135 the build-up of substrates during development (stages I, II, III and IV) and consumption at the
136 senescence stage (stage V). Juazeiro fruits showed high rates of soluble solids, what enhances their
137 utilization in industrial processes. The soluble solid rate is directly related to industrial performance,
138 the higher the soluble solid rate, the higher the performance in the process of pulp concentration In
139 practical terms, for each degree °Brix of increment of raw material there is an increment of 20% in
140 industrial yield [17].



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Fig. 3. Soluble solids (A), titratable acidity (B) and SS/AT ratio (C) of juazeiro fruits picked in diferente ripening stages. The vertical bar represents the standard deviation from the mean. CV = variation coefficient.

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

Analysing Fig. 3B, it's 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] from 0,12 to 0,14%. During ripening, a regular behavior of increase or decrease of acidity was not observed, and this fact could be related to the ripening of the fruit. According to Chitarra; Chitarra [18], fruits during ripening quickly lose acidity, but in some cases there is a small increase in the concentration as ripening goes, behavior observed in the juazeiro fruit, since there wasn't a regular decrease of acidity related to the ripening stage.

In relation to the SS/AT ratio (Fig. 3C), evenly throughout the storage there was an increase in relation to the ripening stages, indicating that the fruits were tasting sweeter, since there was an increment in the values of this ratio. The SS/AT ratio has been used as an index for assessing palatability, giving an idea of the balance between sugars and acidity [18]. To Mattedi et al. [19] the elevated value for the SS/AT ratio gives an easier taste (sweeter and less acid), as low values promote acidic taste, making the latest ripening stages (IV and V) more interesting to commercialization. During storage, the SS/AT ratio generally increases what is probably a reflexo f the conversion of acids in sugars, a behavior similar to the one found in determining soluble solids. (Fig. 3A).

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

Chart 1. Pearson correlation coefficients(r) among chemical characteristics of juazeiro fruits picked in diferente stages of ripening.

Chemical Characteristics	pH	H ⁺ Ions	Soluble Solids	Titratable Acidity
H ⁺ ions	0,51763**	—	—	—
Soluble Solids	0,89337**	0,42852**	—	—
Titratable Acidity	0,90115**	0,54344**	0,73999**	—
SS/AT Ratio	0,74538**	0,31317**	0,90418**	0,45545**

**** considerable at 5% probability level.**

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 ripening stages and during storage.

It was also verified a high positive correlation between SS/AT ratio and soluble solids, this fact was expected, because the soluble solids have direct influence in the SS/AT ratio, as higher the soluble solid concentration is, the higher the SS/AT ratio will be. As for the titratable acidity a low correlation with the SS/AT ration was seen, as they are inverse parameters and the higher the acidity, the lower the SS/AT ratio.

3.2 Bioactive Compounds Assessment

Figure 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. [18].

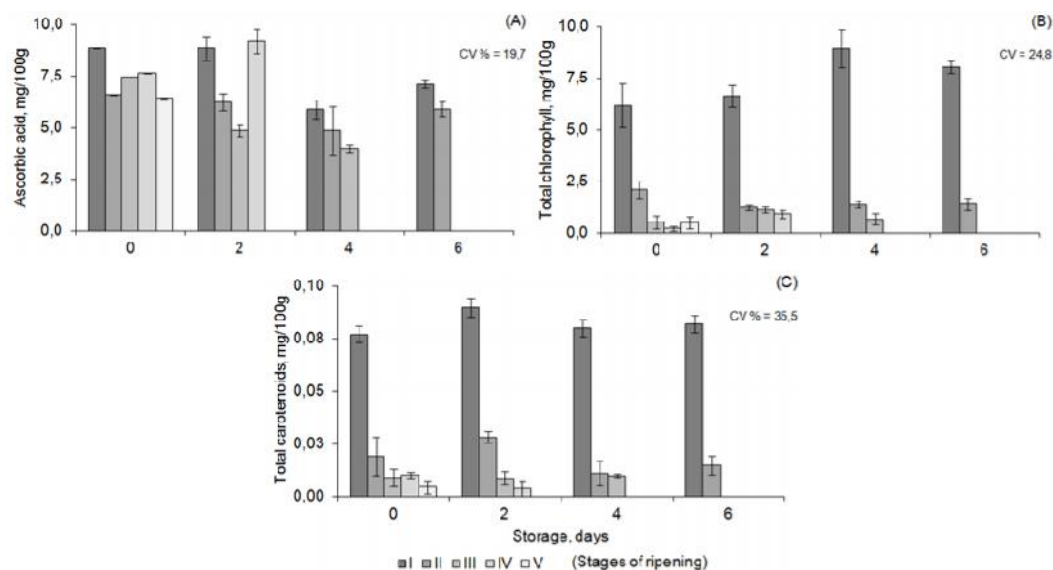


Fig. 4. Ascorbic acid (A), total chlorophyll (B) and total carotenoids (C) of juazeiro fruits picked up during diferente stages of ripening. The vertical bar represents the standard deviation from the mean. CV = variation coefficient.

During 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; Chitarra [18] the decrease in the amount of ascorbic acid during ripening is subsequeute to direct action of the ascorbic acid oxidase enzyme, ir 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 the advance of ripening, a chlorophyll-degrating fact. To Chitarra; Chitarra [20] 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 ripening is associate to the fact that the chloroplasts and their thylakoid membranes disintegrate. Many factors can influence alone or togheter, among them the pH influenced by the increase of organic acids in the vacuoles, oxidative systems and chlorophylasis.

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

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 ripening stage promotes degradation of the analysed pigments. A similar behavior was observed by Silva et al. [21] during the assessment of quantitative composition of camu-camu fruits, when a decrease in the total carotenoid content as ripening went on.

Lima et al. [22] affirmed that during ripening there can 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 also be attributed possibly to enzymatic action.

Figure 5 shows the resuts referente to the phenolic compounds, flavonoids and antocyanins in juazeiro fruit.

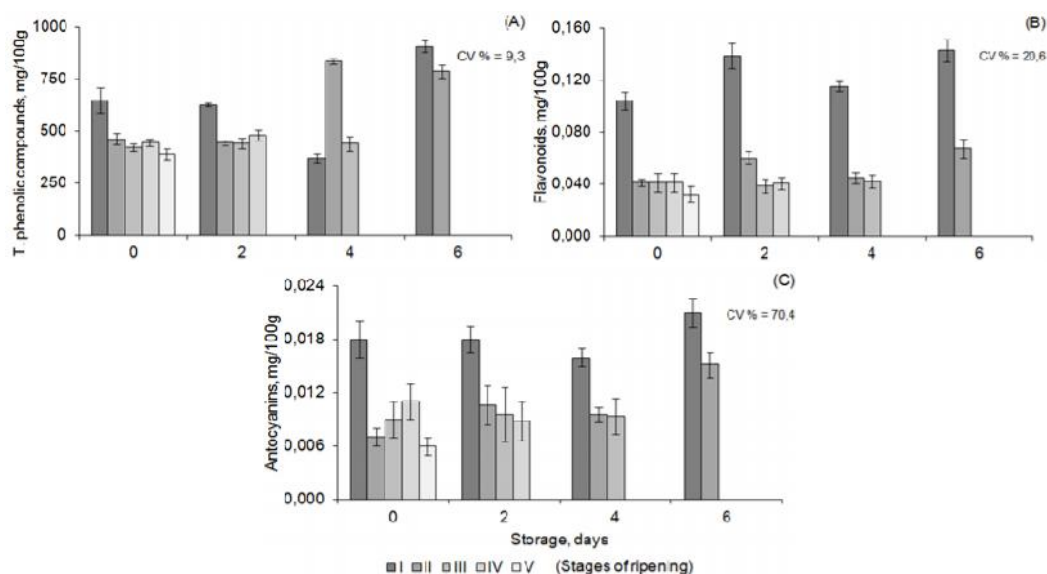


Figure 5. Total Phenolic compounds (A), flavonoids (B) e antocyanins (C) of juazeiro fruits picked during diferente stages of ripening. The vertical bar represents the standard deviation from the mean. CV = variation coefficient.

Obtained results indicate that the juazeiro fruit are potential sources of phenolic compounds (Fig. 5A), obtaining in the beginning of storage 647,9 mg/100g (stage I) and 389,5 mg/100g (stage V), aspect of great importance, as it makes juazeiro fruit a source of bioactive compounds, which are substances that have antioxidant activity, that act 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, the phenolic compounds being among them. [23].

Fig. 5B represents the behavior of flavonoid in relation to the ripening stage. 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 ripening stages. According to Huber; Rodriguez-Amaya [24] 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 antocyanins (Fig. 5C) varied from 0,018 to 0,006 mg/100 g in the beginning of storage, and didn't show great variation through storage. Answers were similar to the flavonoids' behavior already expected, since antocyanins 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.

Chart 2. Pearson correlation coefficients (r) among bioactive compounds pof juazeiro fruit picked in diferente stages of ripening.

	A. Acid	Chlorophyll	Carotenoids	P. Compounds	Flavonoids
Chlorophyll	0,5034**	---	---	---	---
Carotenoids	0,52643**	0,92691**	---	---	---
P. Compounds	0,78366**	0,54690**	0,54914**	---	---
Flavonoids	0,70376**	0,89857**	0,90492**	0,77992**	---
Antocyanins	0,58505**	0,64790**	0,66515**	0,72306**	0,77016**

**considerable to 5% level of probability.

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

244 There was an intense correlation between total carotenoids and chlorophyll and with flavonoids,
245 subsequence to the similar behavior both between ripening stages and storage.

246 **4. CONCLUSION**

247 Storage under controlled temperature was not efficient in extending the shelf life of the juazeiro fruit,
248 once after 2 days of storage the ripening stage V was discarded, getting to the 6 days of storage with
249 2 stages apt to analysis.

250 Chemical analysis demonstrate that as ripening occurs, the fruits taste sweeter, thanks to the
251 increased solid soluble content and decreased acidity, and as such the later stages of ripening are
252 more consumed.

253 First stage of ripening fruits had higher concentration of phenolic compounds, indicating the they are
254 potential sources of bioactive compounds.

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