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Piruvato descarboxilase em cladódio jovem de palma minimamente processado

Pyruvate decarboxylase in minimally processed young palm cladode

Descarboxilasa piruvada en cladodia joven procesada minimamente

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Larissa Félix Macêdo
ORCID: https://orcid.org/0000-0002-3854-6397
Universidade Federal de Campina Grande, Brasil
E-mail: larissafelixmcd@gmail.com
Franciscleudo Bezerra da Costa
ORCID: https://orcid.org/0000-0001-6145-4936
Universidade Federal de Campina Grande, Brasil
E-mail: franciscleudo@yahoo.com.br
Ana Marinho do Nascimento
ORCID: https://orcid.org/0000-0002-1808-7445
Universidade Federal de Campina Grande, Brasil
E-mail: anamarinho06@hotmail.com
Jéssica Leite da Silva
ORCID: https://orcid.org/0000-0003-3815-5457
Universidade Federal de Campina Grande, Brasil
E-mail: jessicaleite2010@gmail.com
Osvaldo Soares da Silva
ORCID: https://orcid.org/0000-0002-2029-9279
Universidade Federal de Campina Grande, Brasil
E-mail: osvaldo_so2002@yahoo.com.br
Charlene Maria de Alcântara
ORCID: https://orcid.org/0000-0001-5852-9968
Universidade Federal de Campina Grande, Brasil
E-mail: charlenealcantara7@gmail.com
Tatiana Marinho Gadelha
ORCID: https://orcid.org/0000-0002-4713-1628

Universidade Federal de Campina Grande, Brasil
E-mail: tatianamarinho08@hotmail.com
Álvaro Gustavo Ferreira da Silva
ORCID: http://orcid.org/0000-0001-8977-3808
Universidade Federal de Campina Grande, Brasil
E-mail: gustavosilva012345@gmail.com
Giuliana Naiara Barros Sales
ORCID: https://orcid.org/0000-0002-4909-6774
Universidade Federal de Campina Grande, Brasil
E-mail: giulianasales@outlook.com
Pahlevi Augusto de Souza
ORCID: https://orcid.org/0000-0002-7964-3193
Instituto Federal do Rio Grande do Norte, Brasil
E-mail: pahlevi10@hotmail.com

Resumo

A palma é uma cactácea de grande relevância mundial, sendo os cladódios jovens minimamente processados, uma alternativa viável de consumo para a culinária. Entre as variáveis de palma estudadas, as enzimas, como o piruvato descarboxilase, desempenham ampla influência na qualidade pós-colheita dessas espécies, gerando a oxidação e influenciando nos atributos sensoriais dos cladódios. Logo, objetivou-se estimar a atividade piruvato descarboxilase em cladódios jovens das palmas 'Miúda' - *Nopalea cochenilifera* e 'Orelha de Elefante Mexicana' - *Opuntia tuna,* minimamente processados. O experimento foi realizado no Laboratório de Química, Bioquímica e Análise de Alimentos do Centro de Ciências e Tecnologia Agroalimentar da Universidade Federal de Campina Grande, Campus Pombal, Paraíba. Os cladódios jovens foram minimamente processados e as análises realizadas logo após o processamento, com 24 e 48 horas de incubação sob temperatura controlada (22 ± 2 °C). As análises realizadas foram a atividade da enzima piruvato descarboxilase (PDC), pH, sólidos solúveis, acidez titulável, ratio, açúcares solúveis, clorofila total, carotenóides totais, ácido ascórbico e compostos fenólicos. Houve atividade enzimática da piruvato descarboxilase nos cladódios jovens minimamente processados para as espécies estudadas, com maior atividade nos cladódios da espécie 'Miúda'.

Palavras-chave: *Opuntia* ssp.; Cactácea; Atividade enzimática.

Abstract

The palm is a cactaceous of great global importance, being the young cladodes minimally processed a viable alternative consumption for cooking. Among the studied palm variables, enzymes play a major role in the post-harvest quality of these species, generating oxidation and influencing the sensory attributes of cladodes. Therefore, the objective was to estimate the pyruvate decarboxylase activity in young cladodes of 'Tiny' palms - *Nopalea cochenilifera* and 'Ear Mexican Elephant' - *Opuntia tuna* minimally processed. The experiment was conducted in the Laboratory of Chemistry, Biochemistry and Food Analysis Center of Science and Technology Agrifood the Federal University of Campina Grande, Campus Pombal, Paraíba. The young cladodes were minimally processed and the analyzes were performed immediately after processing, with 24 and 48 hours of incubation under controlled temperature (22 ± 2°C). The analyzes performed were activity of the enzyme pyruvate decarboxylase (PDC), pH, soluble solids, titratable acidity, ratio, soluble sugars, total chlorophyll, total carotenoids, ascorbic acid and phenolic compounds. There was enzymatic activity of pyruvate decarboxylase from young minimally processed cladodes for the species studied, with greater activity in the cladodes of the species 'Tiny'.

Keywords: *Opuntia ssp*.; Cactaceae; Enzymatic activity.

Resumen

La palma es un cactus de gran relevancia en todo el mundo, con cladodios jóvenes mínimamente procesados como una alternativa viable para el consumo para cocinar. Entre las variables de palma estudiadas, las enzimas juegan un papel importante en la calidad postcosecha de estas especies, generando oxidación e influyendo en los atributos sensoriales de los cladodios. Por lo tanto, el objetivo fue estimar la actividad de la descarboxilasa de piruvato en cladodios jóvenes de la 'Chica' - *Nopalea cochenilifera* y 'Oreja de elefante mexicano' - *Atún Opuntia* palmas mínimamente procesadas. El experimento se realizó en el Laboratorio de Química, Bioquímica y Análisis de Alimentos del Centro de Ciencia y Tecnología Agroalimentaria de la Universidad Federal de Campina Grande, Campus Pombal, Paraíba. Los cladodios jóvenes se procesaron mínimamente y los análisis se realizaron inmediatamente después del procesamiento, con 24 y 48 horas de incubación a temperatura controlada (22± 2 °C). Los análisis realizados fueron la actividad de la enzima piruvato descarboxilasa (PDC), pH, sólidos solubles, acidez titulable, proporción, azúcares solubles, clorofila total, carotenoides totales, ácido ascórbico y compuestos fenólicos. Hubo actividad

enzimática de la piruvato descarboxilasa en los cladodios jóvenes mínimamente procesados para las especies estudiadas, con mayor actividad en los cladodios de la especie 'Chica'.

Palabras clave: *Opuntia ssp*.; Cactacea; Actividad enzimática.

1. Introduction

Opuntia sp. is a cactus native to Mexico, exploited for the production of fruit, forage for animal feed and also production of biofuels, cosmetics, adhesives, glues, dyes, antiperspirants and others (Bezerra *et al*., 2014). As a result, its planting is present in several of the semiarid region, emerging as a food alternative and as a source of income for having easy adaptation to the dry climate due to its physiological characteristics and different purposes (Almeida, Peixoto & Ledo, 2012).

It is a plant that can be used in the elaboration of a range of items for human consumption, although its use is still quite restricted (Pinto *et al*., 2011). There is a large amount of products being generated with cactaceous, including the 'Ear' palm, since they present adequate characteristics for the preparation of food products (Galvão *et al*., 2018).

Despite the great importance of palm cultivation, especially for the northeastern region, research on the use of minimally processed cladodes intended for human consumption is still insufficient, requiring further studies. The minimal processing of palm cladodes for human consumption is considered a viable option for trade in the semiarid regions of northeastern Brazil, presenting itself as an innovation that facilitates consumers' daily lives (Pereira *et al*., 2013).

The importance of the enzymatic study of these plants is evidenced since the condition common to plants in the absence of oxygen is the deviation of aerobic metabolism to the anaerobic pathway. During the absence of O_2, pyruvate undergoes decarboxylation, producing acetaldehyde, in a reaction catalyzed by pyruvate decarboxylase (PDC), which is metabolized by alcohol dehydrogenase (ADH) to ethanol, with the regeneration of NAD+ to aid glycolysis (Kolb & Joly, 2009).

Thus, the present study aimed to detect the enzymatic activity of pyruvate decarboxylase (PDC) and the physical-chemical characteristics in the minimally processed young palm cladode, since this enzyme can influence the post-harvest quality of the young cladode and, thus, identify the incubation time that highlights the activation of the fermentative metabolism in the studied palms.

2. Material and Methods

A research is done to bring new knowledge for society according to Pereira et al. (2018). In that research it were used palm cladodes of the species 'Tiny' *Nopalea cochenilifera* (L.) Salm Dyck and 'Ear Mexican Elephant' *Opuntia tuna* (L.) Mill. between 8 and 30 days and 12-16 cm long, grown at

the Experimental Farm Rolando Enrique Rivas Castellón of the Federal University of Campina Grande, Campus Pombal, PB, located in the city of São Domingos, PB and located at 6° 48 ' 41.1 S, 37° 56'11.3 W. According to the Köppen classification, adapted to Brazil, the characteristic climate of the city of São Domingo is Aw, that is, tropical climate with dry winter season, average temperature of 26.7 °C , annual rainfall around 843 mm year^{-1} (Climate-Data, 2020).

They were harvested manually in the early morning from 6:50 am to 7:30 am, with a fresh appearance and characteristic color, avoiding damaged cladodes. Then, they were taken to the Chemistry, Biochemistry and Food Analysis Laboratory of the Center for Science and Agri-food Technology at the Federal University of Campina Grande, Campus Pombal.

The cladodes were stored in plastic trays previously sanitized and identified, being visually selected again for the absence of injuries in order to obtain uniform quality samples. When cleaning, the cladodes were washed in running water to remove residues adhered to the surface, after which they were subjected to sanitization in a solution of (Neoclor®: 100% sodium dichloroisocyanurate) at a concentration of 200 ppm for 10 minutes, followed by rinsing in a solution of (Neoclor®: 100% sodium dichloroisocyanurate) at 5 ppm for 10 minutes.

The cut was performed manually with the aid of stainless steel blades, removing the spines and the edges of the cladodes. The cladodes were sliced in a mechanical vegetable processor (Robot Coupe CL 50 Ultra) in a 2 mm thick blade, being placed longitudinally to obtain cross-sections, followed by selection to remove uneven slices.

The samples were stored in plastic containers of 1.6 liters at controlled temperature (22±2 °C), the containers were closed with their respective lids in order to promote the condition of anaerobiosis, with determination of enzymatic activity and other analyzes at intervals of 0, 24 and 48 hours.

Pyruvate decarboxylase – PDC E.C. 4.1.1.1 (EU min^{-1} g^{-1} MF): The extraction was adapted from Ke *et al*. (1994) and Mitchell and Jelenkovic (1995). PDC activity was estimated from NADH oxidation as described by Ke *et al*. (1994), spectrophotometrically expressing the results as protein or per gram of fresh mass.

Hydrogenionic potential: It was determined directly in a digital bench pot (Digimed-DM-22). Soluble solids (%): The cellular juice was extracted from minimally processed palm cladode, crushed with the aid of a microprocessor (RI7632 650W - Arno) and the juice filtered in two layers of cotton, using a digital refractometer (Megabrix BZW45) with automatic temperature compensation.

Titratable acidity (%): 3.0 g of the sample was weighed, homogenized in 47 ml of distilled water. The solution was titrated with 0.1 N NaOH until it reached the turning point of the phenophthalein indicator, confirmed by the pH range of the indicator of 8.2. The titratable acidity was expressed as a percentage of abundant acid in the palm equivalent to the amount of 0.1 N NaOH spent in the titration. The procedure was performed according to the Analytical standards of the Adolfo Lutz Institute (2008).

Ratio: It was determined by the division between the levels of soluble solids and the values of the titratable acidity.

Soluble sugars (g 100 g^{-1}): They were estimated as described by Yemm and Willis (1954) with adaptations. About 2.0 g of the sample was macerated in 3 ml of distilled water and made up to 75 ml, the extract filtered through filter paper and an aliquot of 15 µL of the diluted extract plus 985 µL of distilled water and 2000 µL of Antrona were used for reaction in boiling water, for 10 minutes, followed by cooling, in water with ice, until room temperature. The readings were taken at 620 nm, in a spectrophotometer (Spectrum SP-1105).

Total chlorophyll (mg 100 g^{-1}) and total carotenoids (µg 100 g^{-1}): They were determined according to the method of Lichtenthaler (1987). Weighed 0.2 g of the sample, placed it in a mortar with 0.2 g of calcium carbonate and 3 ml of 80% acetone, macerated and transferred the extract to a falcon tube completing the volume to 5 ml. Afterwards, it was centrifuged for 10 minutes at 10 °C and 3000 rpm. An aliquot was taken up in a cuvette and made up readings on a spectrophotometer (Spectrum SP-1105) absorbance at 470, 646 and 663 nm.

Ascorbic acid (mg 100 g^{-1}): 3.0 g of the crushed sample was weighed, made up to 47 mL with 0.5% oxalic acid and titrated against Tillmans' solution (2.6 dichlorophenol indophenol 0.2%) until the turning point as described by Adolfo Lutz Institute (2008).

Phenolic compounds (mg 100 g^{-1}): They were estimated using the method described by Waterhouse (2006), by mixing 400 µL of filtered palm juice with 1,725 µL of distilled water and 125 µL of the reagent Folin-Ciocalteu, followed by agitation and rest for 5 minutes, after the reaction time 250 µL 20% sodium carbonate was added. The tubes rested for 30 minutes in a water bath at 37 ºC. The standard curve was prepared with gallic acid and the readings were performed on a spectrophotometer (Spectrum SP-1105) at 765 nm.

The comparison between treatments was carried out by the analysis of anova variance in a factorial scheme (2x3), which corresponds to two palm species and three incubation times, using the Tukey test with a 5% probability level. The data were analyzed using the software AgroEstat® (Barbosa & Maldonato Júnior, 2015).

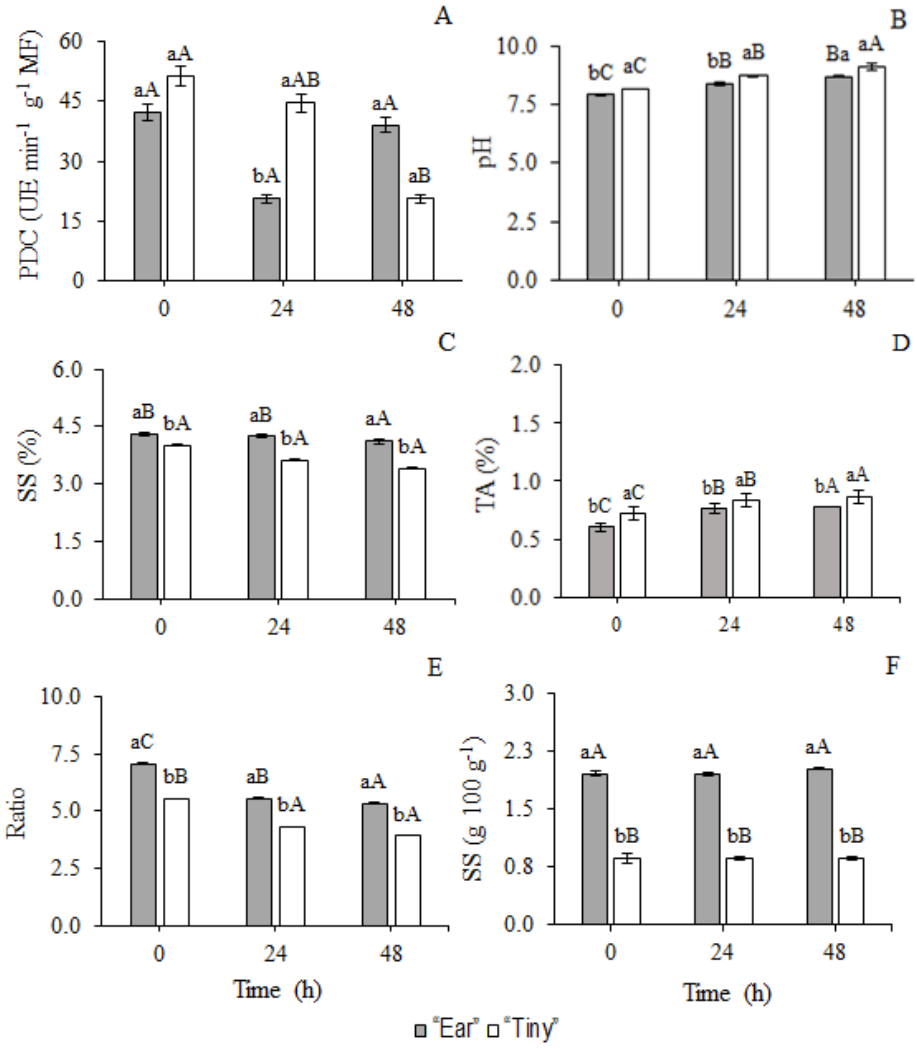
3. Results and Discussion

Minimally processed cladodes stood out with different levels of production of pyruvate decarboxylase (PDC) activity, showing significant interaction (Figure 1A). It was found that the palm cladodes 'Tiny' enzyme activity was greater at 0 and 24 hours of incubation, whereas the palm 'ear' its activity was less. It was observed that there was a drop in activity in the cladodes in the palm 'Tiny' minimally processed for as long incubation. However, the Palm 'ear' was a decrease in 24 hour period followed by an increase to complete at 48 hours of incubation.

According to Fante *et al*. (2010), when the plant is subjected to anaerobic conditions, pyruvate decarboxylase acts by breaking down the pyruvate present in the cytosol of cells producing acetaldehyde, which is metabolized by alcohol dehydrogenase to ethanol, with the regeneration of NAD + to support glycolysis. It is worth noting that this enzyme is a precursor to the enzyme alcohol dehydrogenase, as it is a direct relationship between the expressions of these two enzymes.

It was observed that the pH value did not show significant interaction (Figure 1B). The differences being observed in the isolated factors. Note that the pH of the minimally processed palm Nopalea cochenilifera (L.) Salm Dyck - 'Tiny' increased from 8.2 to 9.1 after 48 hours of incubation. This behavior was also observed in the palm Opuntia tuna (L.) Mill - 'Ear', with an increase from 7.9 to 8.7. According to Alves, Boas and Boas (2010), the increase in pH in minimally processed products may be associated with the consumption of organic acids by the respiratory process. Therefore, as the samples were incubated for 48 hours, there was probably an increase in breathing causing a significant increase in pH.

Figure 1. Enzymatic activity of pyruvate decarboxylase, PDT (A) hydrogen potential, pH (B), soluble solids, SS (C), titratable acidity, TA (D), ratio (E) and soluble sugars, SS (F) in young minimally processed palm cladode. Species followed by the same lowercase letter and conservation times followed by the same capitalized letter do not differ by the Tukey test at the 5% probability level.



Source: Autors.

Soluble solids showed a significant difference with isolated variations between factors (Figure 1C). The results of soluble solids decreased from 4.0 to 3.4% in the minimally processed 'Tiny' palm cladodes, while in the 'Ear' palm the values decreased from 4.3 to 4.1%. This implies that there was a significant degradation of the soluble solids during the incubation period of the samples. According to Chitarra and Chitarra (2005), this decrease in the levels of soluble solids may be associated with the degradation of secondary compounds present in the samples in response to the minimum processing steps.

In the titratable acidity there was a significant interaction between the evaluated treatments (Figure 1D). There was an increase in acidity values after 48 hours of incubation, equivalent to 0.78% in the minimally processed palm cladodes of the species 'Ear' and 0.87% in the 'Tiny' palm, being possible to observe the relationship between the acidity and pH, as the acidity tries to decrease when the pH increases (Figure 1B). Galvão *et al*. (2018) when studying quality of minimally processed palm, he observed that the acidity in palm shoots varied from 0.6 to 0.8%, being close to those found in this work. According to Alves, Boas and Boas (2010) organic acids tend to reduce during the oxidation process in the cycle of tricarboxylic acids due to the respiration process.

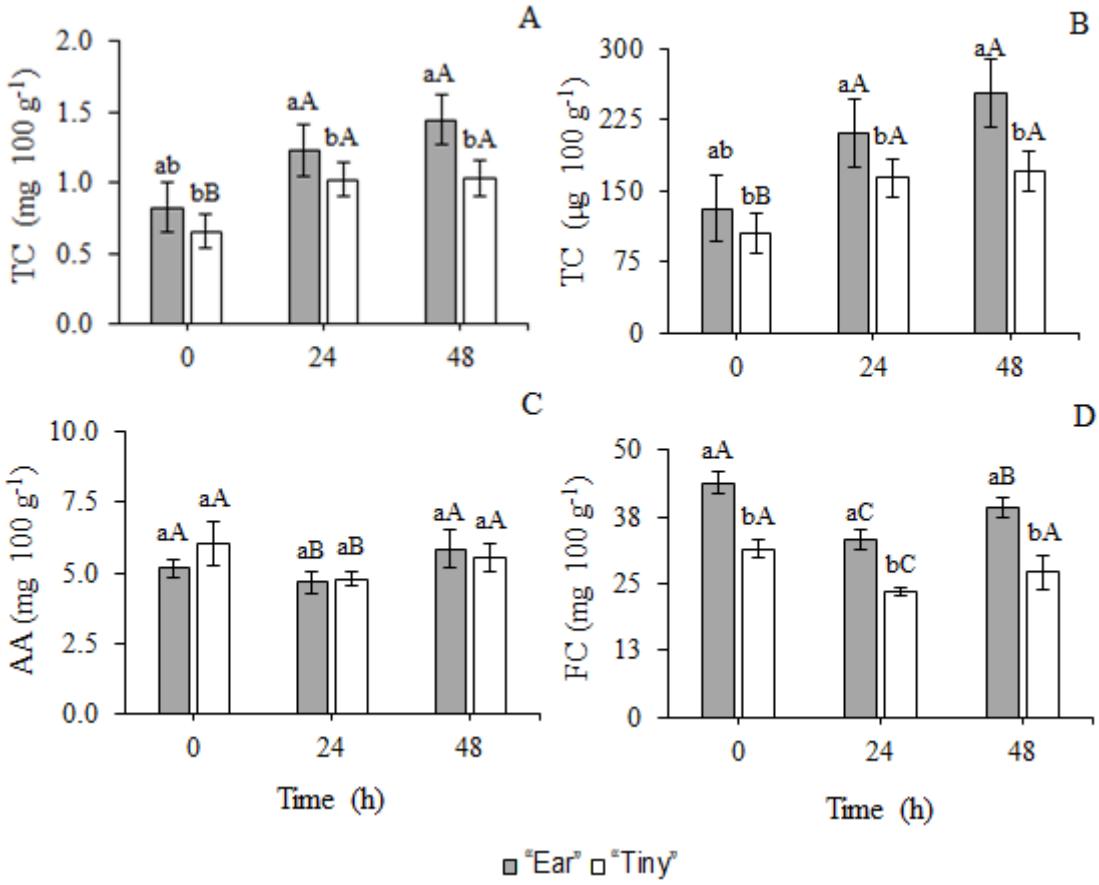
It was observed that the ratio between soluble solids and titratable acidity showed a significant interaction (Figure 1E). There was a decrease in values from 7.1 to 5.3 in the palm 'Ear' and from 5.6 to 3.9 in the palm 'Tiny'. The results obtained for the ratio indicate that the minimally cladodes processed before incubation have a pleasant taste, since according to Mattedi *et al*. (2011) the high value for the ratio allows for mild flavor, while low values, acid flavor.

In the total soluble sugars there was a significant interaction between the evaluated treatments (Figure 1F). It was found that the soluble sugars in the species 'Ear' after storage were 2.0 g 100 g^{-1}. In the 'Tiny' species, the value of 0.9 g 100 g^{-1} was maintained after the incubation time. Santos *et al*. (2005) mentions that soluble sugars are responsible for the sweet taste of fruits and vegetables, being an important feature in consumer acceptance.

The total chlorophyll in the minimally processed palm cladodes differed separately between the types of palm and between the incubation times (Figure 2A). The values were from 0.8 to 1.4 mg 100 g^{-1} in the 'Ear' palm and 0.7 to 1.0 mg 100 g^{-1} in the 'Tiny' palm. The increase in chlorophyll in the two palm cultivars during storage may be related to pheophytins and may be reversed to compounds with the same spectral attributes as chlorophyll by complexing copper and zinc in place of the hydrogens in the porphyrin ring (Fennema, 2010), Thus, it can be said that it is possible for other compounds to be identified in the same absorbance as chlorophyll and thus cause an increase in absorbance values.

The results of the carotenoids differed significantly with the isolated factors, showing an increase from 131 to 253 µg 100 g^{-1} in the 'Ear' palm cladode and 104 to 170 µg 100 g^{-1} in the 'Tiny' palm cladode (Figure 2B). It was identified that after the incubation time of the minimally processed palm cladodes, the carotenoids were concentrated, that is, there was no oxidation of the samples. This increase in the carotenoid content can be attributed to a greater ease of extraction, since there may have been inactivation of oxidative enzymes that denature carotenoid-protein complexes existing in plant cells (Campos *et al*., 2008).

Figure 2. Total chlorophyll, TC (A), total carotenoids, TC (B), ascorbic acid, AA (C) and phenolic compounds, PC (D) in young minimally processed palm cladode. Species followed by the same lowercase letter and conservation times followed by the same capital letter do not differ by the Tukey test at the 5% probability level.



Source: Authors.

The values of ascorbic acid differed within the factors, in the minimally processed cladodes of the 'Ear' palm, an increase from 5.2 to 5.8 mg 100 g^{-1} was observed (Figure 2C). However, in the processed cladodes of the 'Tiny' palm there was a reduction from 6.0 to 5.5 mg 100 g^{-1}. The heterogeneity of the samples or loss of mass between their removal from the storage conditions and the analysis of this variable can cause an increase in ascorbic acid (Rinaldi *et al*., 2009).

This being a possible explanation for the increase in the levels of ascorbic acid in the cladodes of the palm 'Ear'. The reduction in the levels of ascorbic acid for the 'Tiny' palm may have occurred as a result of the physical damage caused during processing, since, according to Moraes *et al*. (2010), the cuts made during processing increase the respiration rate and the production of ethylene by the tissues, promoting chemical and biochemical reactions that modify the sensory quality, affecting several attributes and also the nutritional value, affecting, above all, the vitamin content.

The contents of phenolic compounds showed a significant difference in the isolated factors, with the values obtained by the 'Tiny' palm being smaller (Figure 2D). The phenolic compounds in the minimally processed 'Ear' palm cladodes, reduced from 43.9 to 39.3 mg 100 g^{-1} after 48 hours of incubation. This behavior was also seen in the 'Tiny' palm, with a reduction from 31.6 to 27.1 mg 100 g^{-1}. The decrease in the levels for the two varieties of palms occurred because of injuries to plant tissues, due to minimal processing (Galvão *et al*., 2018).

Ramírez-Moreno *et al*. (2013) working with raw sprouts lyophilized found extractable polyphenols content of between 458-282 mg 100 g^{-1}, with lower values found in the search cited by the authors. Oliveira *et al*. (2012) informs that these compounds are natural constituents that exert antioxidant action due to their chemical structure, and may provide several biological benefits in the plant, in addition to being an important component for plants, since they can influence the oxidative metabolic activity of tissues.

4. Conclusion

There was enzymatic activity of pyruvate decarboxylase in young minimally processed cladodes in the species studied, with greater activity being found in the cladodes of the species 'Tiny' when incubated for 24 h. The cladodes of minimally processed palm can be included in human food, since it has nutritional potential and thus be an alternative for the use of this species.

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Porcentagem de contribuição de cada autor no manuscrito

Larissa Félix Macêdo - 15%

Franciscleudo Bezerra da Costa - 15%

Ana Marinho do Nascimento - 15%

Jéssica Leite da Silva - 10%

Osvaldo Soares da Silva - 7,5%

Tatiana Marinho Gadelha - 7,5%

Charlene Maria Alcântara - 7,5%

Álvaro Gustavo Ferreira da Silva - 7,5%

Giuliana Naiara Barros Sales - 7,5%

Pahlevi Augusto de Souza - 7,5%