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# Antioxidant potencial in subpopulations of *Tacinga inamoena*

**Abstract** – The objective of this work was to determine the contents of bioactive compounds in *Tacinga inamoena* fruits from different subpopulations, as well as to evaluate the genetic diversity among and within these subpopulations based on these contents. The fruits were collected from ten plants from each of the following municipalities: Assú, Apodi, and Parelhas, in the state of Rio Grande do Norte, Brazil. The fruits were analyzed for the contents of vitamin C, carotenoids, betacyanin, betaxanthin, flavonoids, total extractable polyphenols, and antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) methods. The fruits from Parelhas showed the highest content averages, which did not differ significantly from those obtained for total extractable polyphenols in Assú and for vitamin C and antioxidant activity by DPPH in Apodi. The fruits from Apodi and Assú did not differ for carotenoids, betacyanin, betaxanthin, and antioxidant activity by ABTS. Using the unweighted pair-group method with arithmetic mean, the three subpopulations were arranged into the following two clusters: Parelhas; and Assú and Apodi. Among subpopulations, total variance was higher for carotenoids, betacyanin, betaxanthin, and DPPH, whereas, among individuals, carotenoids, betacyanin, betaxanthin, and vitamin C were the contents that most contributed to variation. Fruits from *T. inamoena* are rich in bioactive compounds, and these contents can vary among and within subpopulations from different locations.


**Index terms:** Cactaceae, antioxidant properties, native species, genetic diversity.


## Potencial antioxidante em subpopulações de *Tacinga inamoena*


**Resumo** – O objetivo deste trabalho foi determinar os teores de compostos bioativos de frutos de *Tacinga inamoena* de diferentes subpopulações, bem como avaliar a diversidade genética entre e dentro dessas subpopulações com base nesses teores. Os frutos foram coletados de dez plantas escolhidas em cada um dos seguintes municípios: Assú, Apodi e Parelhas, no estado do Rio Grande do Norte, Brasil. Os frutos foram analisados quanto aos teores de vitamina C, carotenoides, betacianina, betaxantina, flavonoides, polifenóis extraíveis totais, e atividade antioxidante pelos métodos 2,2-difenil-1-picril-hidrazila (DPPH) e 2,2-azino-bis(3-etilbenzotiazolina-6-sulfônico) (ABTS). Os frutos de Parelhas apresentaram as maiores médias de teores, que não diferiram significativamente das obtidas para polifenóis extraíveis totais em Assú e para vitamina C e atividade antioxidante por DPPH em Apodi. Os frutos de Apodi e Assú não diferiram para carotenoides, betacianina, betaxantina e atividade antioxidante por ABTS. Com uso do método de pares e grupos não-ponderados com média aritmética, as três subpopulações foram organizadas nos dois seguintes grupos: Parelhas; e Assú e Apodi.


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Entre as subpopulações, a variância total foi maior para carotenoides, betacianina, betaxantina e DPPH, enquanto, entre os indivíduos, carotenoides, betacianina, betaxantina e vitamina C foram os conteúdos que mais contribuíram para a variação. Os frutos de *T. inamoena* são ricos em compostos bioativos, e esses conteúdos podem variar entre e dentro das subpopulações de diferentes locais.

**Termos para indexação:** Cactaceae, propriedades antioxidantes, espécie nativa, diversidade genética.

## Introduction

In Brazil, a vast plant biodiversity is distributed throughout the country, composing distinct and well-defined biomes (Weichert et al., 2024). However, in these ecosystems, the nutritional and economic values of native and exotic plant species are still little known (Schiassi et al., 2018).

The Caatinga, an exclusive Brazilian biome, harbors numerous endemic plant species, especially from the Cactaceae family (Barbosa et al., 2020), such as *Tacinga inamoena* (K.Schum.) N.P.Taylor & Stuppy, a native species widely distributed along the northeastern and northern regions of the state of Minas Gerais (Barbosa et al., 2020). Popularly known as *cumbeba*, *gogóia*, *pêlo*, or *quipá*, this perennial shrub can reach up to 1.0 m in height, presents a succulent and prickly stem typical of cacti, and produces yellowish fruits weighing around 13 g, rich in bioactive compounds with a high antioxidant activity (Souza et al., 2007; Dantas et al., 2015, 2016; Formiga et al., 2016).

The fruit of *T. inamoena* show physicochemical traits similar to those of *Opuntia ficus-indica* (L.) Mill. fruit (Souza et al., 2007), already being used for fresh consumption and processing, but still in restricted areas in Brazil (Silva et al., 2009). To date, since cactus species are not cultivated, their exploitation has been extractive and illegal (Almeida & Fernandez, 2024).

For the conservation of the species, the basis is research on the genetic relationship among subpopulations that occupy different habitats and may have different genetic characteristics (Barbosa et al., 2020). This genetic variation in morphological and anatomical characteristics is important since it allows plants to adapt and survive to different environmental conditions, such as temperature, light, soil, rainfall, altitude, water stress, nutrient availability, and

geographical position (Pimentel et al., 2023). In this scenario, fruit physicochemical characteristics are also affected by genetic variation, as reported for the morphological and nutritional characteristics of *T. inamoena* fruits from plants occurring in different locations (Silva et al., 2009; Dantas et al., 2015; Formiga et al., 2016).

The objective of this work was to determine the contents of bioactive compounds in *T. inamoena* fruits from different subpopulations, as well as to evaluate the genetic diversity among and within these subpopulations based on these contents.

## Materials and Methods

The experiment was carried out in the state of Rio Grande do Norte, Brazil. The climate of the region, according to Köppen-Geiger' classification, is BSw'h, very hot tropical semiarid, with rainfall concentrated from February to April. Climatic data from July 2016 to April 2017 (Table 1) were obtained from automatic meteorological weather stations in the region and available at Instituto Nacional de Meteorologia (Inmet, 2017) and Sistema de Monitoramento Agrometeorológico (Agritempo, 2017).

For the study, the following three municipalities with subpopulations of *T. inamoena* were chosen: Apodi (5°36.55'S, 37°55.80'W, at 124 m above sea level), Assú (5°34.95'S, 36°56.68'W, at 87 m above sea level), and Parelhas (6°40.36'S, 36°38.83'W, at 290 m above sea level). In each location, ten plants were selected and 50 fruits were collected from each from January to April 2017. Fruit peel showed a predominantly green color with yellowish nuances.

For the evaluation of the content of bioactive compounds, the fruit were packed in plastic bags and transported to the Physiology and Postharvest Laboratory at Universidade Federal Rural do Semi-Árido, in the municipality of Mossoró, in the state of Rio Grande do Norte. The pulp was separated from the peel (epicarp), and the edible fraction (mesocarp + seeds) was homogenized in the T 25 digital Ultra-Turrax equipment (IKA-Werke GmbH & Co. KG, Staufen, Germany). The edible fraction was then packed in plastic pots and stored in a freezer for further evaluation of vitamin C, flavonoids, carotenoids, betacyanins, betaxanthin, total extractable polyphenols, and antioxidant activity.

Vitamin C content was estimated by titration using Tilman's solution (2.6 dichlorophenol at 0.02% indophenol) as in the methodology proposed by Suvarna et al. (2022), and results were expressed as milligram ascorbic acid 100 g<sup>-1</sup> fresh weight. The contents of yellow flavonoids and carotenoids were determined by spectrophotometry according to Kourouma et al. (2020), and results were expressed as milligram 100 g<sup>-1</sup> fresh weight.

Betacyanins and betaxanthins were quantified directly on the homogenized edible fraction. The citrate-phosphate buffer solution (0.05 mol L<sup>-1</sup>, 6.5 pH) was used when necessary to dilute samples so that the readings at 538 nm for betacyanin and 480 nm for betaxanthin remained between 0.8 < absorbance < 1.0. Quantification was performed as described by Stintzing et al. (2005), and results were expressed as milligram 100 g<sup>-1</sup> fresh weight.

To determine total extractable polyphenols and antioxidant activity, the pulp extract was obtained using methanol and acetone (Larrauri et al., 1997). Total extractable polyphenols were determined by spectrophotometry using the Folin-Ciocalteu reagent as described by Kourouma et al. (2020), and results were expressed as milligram gallic acid equivalent (GAE) 100 g<sup>-1</sup> fresh weight. Antioxidant activity was obtained by sequestration of the 2,2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods as proposed by Rufino et al. (2007a, 2007b). The results of ABTS were expressed as µmol Trolox g<sup>-1</sup> fresh

weight, and those of DPPH as gram of pulp per gram of DPPH to be reduced in 50% (effective concentration<sup>50</sup>) by the radical.

A descriptive statistics analysis was carried out. The variables flavonoids and total extractable polyphenols did not present a normal distribution. The subpopulations were compared using a non-parametric analysis. In addition, the effect of subpopulation was tested using the analysis of variance-type statistics, as presented by Brunner et al. (2017) and implemented in the rankFD R package (Konietschke et al., 2022). The relative treatment effect was used to estimate the difference between groups, indicating the probability that a randomly selected case from one group will exhibit a higher value than a case randomly selected from the entire sample (Konietschke et al., 2010). Pairwise multiple comparisons by Tukey's criterion were conducted according to the procedures proposed by Noguchi et al. (2020) using the nparcomp R package (Konietschke et al., 2015). These procedures offer control of the Type I error rate and effect size in the same metric as Cohen's d.

To study the genetic divergence among the accessions, the distance matrix between individuals was estimated by the standardized Euclidean distance ( $\sim Z$ ,  $\mu = 0$ ,  $\sigma = 1$ ). A dendrogram was constructed using the hierarchical method: unweighted pair-group method with arithmetic mean (UPGMA). The choice of this method over others was determined by the estimation of the cophenetic correlation coefficient between the original and final distance matrices.

**Table 1.** Temperature, rainfall, and humidity data from July 2016 to April 2017 in the municipalities of Parelhas, Apodi, and Assú in the state of Rio Grande do Norte, Brazil.

Month/Year	Temperature (°C)			Rainfall (mm)			Air relative humidity (%)		
	Parelhas	Apodi	Assú	Parelhas	Apodi	Assú	Parelhas	Apodi	Assú
July 2016	28.07	28.20	27.94	0.0	0.0	0.0	54.93	57.26	57.18
August 2016	28.54	28.12	28.45	0.0	0.0	0.0	52.95	58.72	57.46
September 2016	29.20	28.56	20.08	3.4	0.0	0.0	55.96	65.07	54.75
October 2016	29.75	29.23	20.39	0.0	0.0	0.0	56.18	64.57	56.15
November 2016	30.10	29.35	29.33	0.0	0.0	0.0	50.40	68.33	57.72
December 2016	30.18	28.91	29.68	7.10	54.40	0.0	50.55	67.69	59.17
January 2017	30.40	29.14	29.69	17.10	15.30	16.00	55.25	60.30	63.75
February 2017	28.80	28.21	29.00	216.70	217.70	123.10	63.63	67.91	65.60
March 2017	27.60	27.25	28.34	172.80	117.60	307.70	76.88	77.68	73.92
April 2017	28.00	27.41	28.38	131.00	144.70	118.30	68.92	75.80	72.15
Mean	26.14	28.44	27.12	91.3	109.9	141.3	58.6	66.3	61.8

Source: Agritempo (2017) and Inmet (2017).

The non-hierarchical data clustering algorithm K-means was also used to group the individuals. This algorithm aims to iteratively minimize the distance of data points from  $k$  centers (Azevedo, 2024). The genetic divergence analyses were performed using the multivariate analysis (Azevedo, 2024), as well as the heatmap (Kolde, 2019) and factoextra (Kassambara & Mundt, 2020) packages from the R, version 4.3.1, software (R Core Team, 2025).

## Results and Discussion

The analysis of variance-type statistics indicated that the effect of subpopulation was significant ( $p < 0.05$ ) for almost all evaluated characteristics, except for yellow flavonoids and total extractable polyphenols (Table 2).

Post-hoc tests showed significant differences at  $\alpha = 0.05$  between the subpopulations in terms of vitamin C, carotenoids, betacyanins, betaxanthin, ABTS, and DPPH. The subpopulation of Parelhas, the municipality with the highest altitude, showed the highest relative treatment effect, which did not differ from that of Apodi for vitamin C and DPPH (Table 2). The subpopulations of Apodi and Assú did not differ regarding flavonoids, carotenoids, betacyanins, betaxanthin, total extractable polyphenols, and ABTS.

The cluster analysis using the UPGMA method classified the three subpopulations into two groups (Figure 1): one formed by ten individuals from Parelhas, most of them showing higher values of betaxanthin, betacyanins, carotenoids, and, at a lower extent, vitamin C (green and red areas); and the second

by individuals from Apodi and Assú that presented lower estimates for these characteristics (blue area).

The cophenetic correlation estimate ( $r = 0.81$ ,  $p < 0.01$ ) showed that matrices of original and final distances were similar and that there was little loss of information when the individuals were hierarchized in groups, indicating that the UPGMA is reliable. The K-mean analyses also formed two clusters (Figure 2), confirming the hierarchical clustering through the UPGMA (Figure 1).

The obtained results revealed genotypic diversity in fruits traits, with differences in betalains, carotenoids, flavonoids, polyphenols, and vitamin C contents (Table 2 and Figure 1). The fruits from Parelhas presented higher contents of betaxanthin, betacyanins, carotenoids, and vitamin C than those from Assú and Apodi.

Most variation in vitamin C content occurred within the subpopulation, which may be due to differences in environmental temperature, light intensity, and air relative humidity even in the same geographic location (Rufino et al., 2009). Among individuals, the obtained values ranged from 37.11 to 60.43 mg 100 g<sup>-1</sup> fresh weight, being higher than those of 42.01 and 26.85 mg 100 g<sup>-1</sup> fresh weight found by Dantas et al. (2015) and Formiga et al. (2016), respectively. The vitamin C content of some *T. inamoena* fruits also exceeded those of 16 to 58 mg 100 g<sup>-1</sup> and of 42.45 mg 100 g<sup>-1</sup> obtained by Najwa & Azrina (2017) and Cardoso et al. (2011) for widely consumed fruits such as citrus (*Citrus* spp.) and strawberry (*Fragaria vesca* L.), respectively. Therefore, *T. inamoena* fruits can be a source of dietary vitamin C for humans. According to Najwa & Azrina (2017), the daily consumption of

**Table 2.** Estimates of the analysis of variance-type statistics (ATS) and relative treatment effect for eight fruit traits evaluated for three *Tacinga inamoena* subpopulations in the municipalities of Apodi, Assú, and Parelhas in the state of Rio Grande do Norte, Brazil<sup>(1)</sup>.

Subpopulation	Relative treatment effect <sup>(2)</sup>							
	Vitamin C	FLAV	CARO	BETC	BETX	PET	ABTS	DPPH
Apodi	0.59a	0.49a	0.31b	0.34b	0.33b	0.37a	0.36b	0.69a
Assú	0.24b	0.49a	0.35b	0.34b	0.34b	0.65a	0.46b	0.22b
Parelhas	0.66a	0.52a	0.85a	0.79a	0.82a	0.48a	0.67a	0.59a
ATS	9.57**	0.05 <sup>ns</sup>	27.8**	14.58**	22.83**	2.51 <sup>ns</sup>	3.20*	13.02**

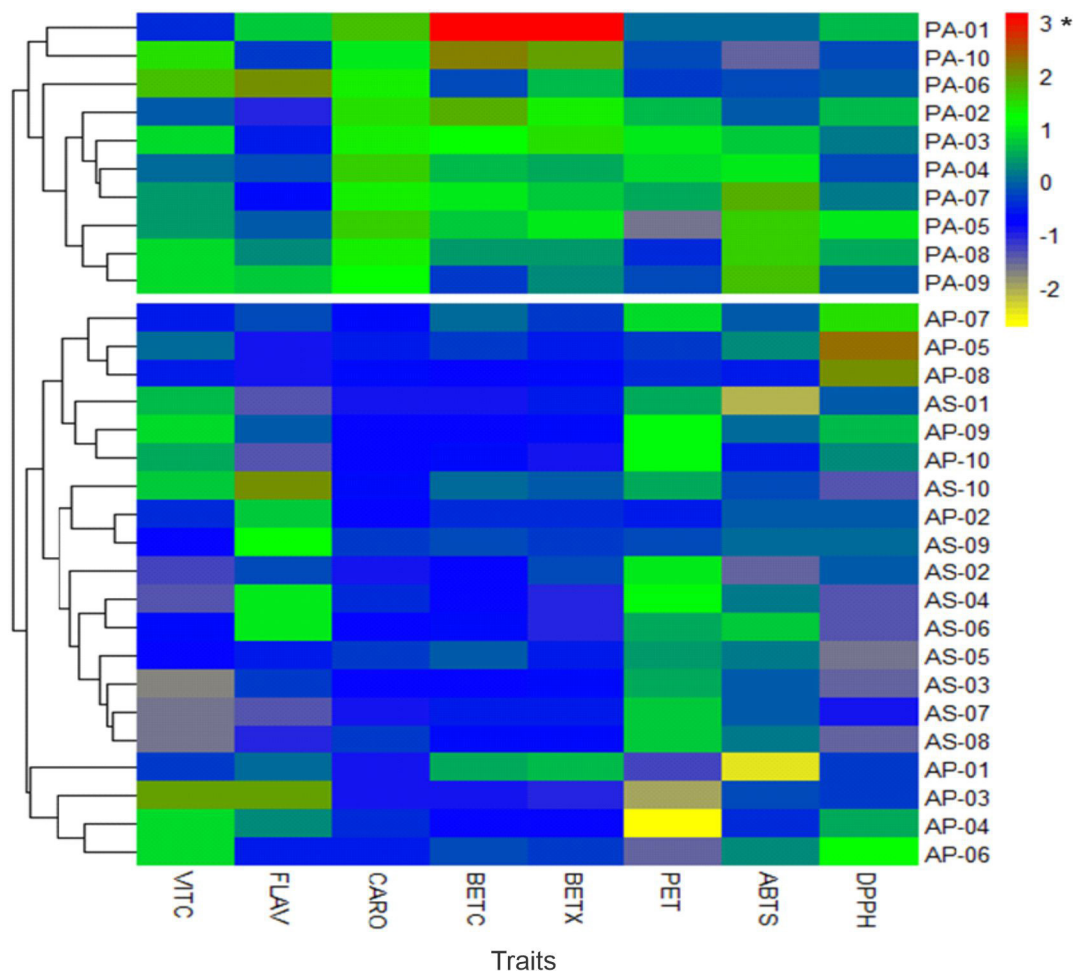
<sup>(1)</sup>Values of the relative treatment effect with equal letters, in the same column, do not differ ( $p > 0.05$ ). <sup>(2)</sup>FLAV, yellow flavonoids; CARO, total carotenoids; BETC, betacyanin; BETX, betaxanthin; PET, total extractable polyphenols; ABTS, total antioxidant activity by the 2,2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) method; DPPH, total antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. \* and \*\*,  $p < 0.05$  and  $p < 0.01$ , respectively. <sup>ns</sup>Nonsignificant at  $\alpha = 0.05$ .

100 g of *T. inamoena* meets the recommendation of 45 mg of vitamin C for adults.

In addition to vitamin C, *T. inamoena* fruits also presented substantial contents of carotenoids, flavonoids, betacyanins, and betaxanthins. However, these contents varied greatly among the studied subpopulations, which can be attributed to genetic variability, but also to the different plant responses to environmental conditions. Irrespectively of site locations, flavonoids ranged from 6.95 to 12.04 mg 100 g<sup>-1</sup>, a content five times greater than that of 1.77 mg 100 g<sup>-1</sup> reported by Formiga et al. (2016). Carotenoid content ranged from 0.12 to 0.83 mg 100 g<sup>-1</sup>,

a value similar to that of 0.47 mg 100 g<sup>-1</sup> obtained by Souza et al. (2007).

The betalain compounds betaxanthin (red-violet) and betacyanins (yellow) represented 2.38 and 2.75 mg 100 g<sup>-1</sup> of the edible fraction in *T. inamoena* fruits, respectively. These values were superior to those of 0.64 and 1.79 mg 100 g<sup>-1</sup> found by Dantas et al. (2015), respectively. The variation in these contents among subpopulations may be attributed to differences in the environmental conditions among the locations where the plants grow since betalains are sensitive to light and pH (Almeida & Fernandez, 2024). In the present work, the municipalities where the fruits were



**Figure 1.** Dendrogram of traits of fruits of *Tacinga inamoena* collected in the municipalities of Parelhas (PA), Apodi (AP), and Assú (AS), in the state of Rio Grande do Norte, Brazil. VITC, Vitamin C; FLAV, yellow flavonoids; CARO, total carotenoids; BETC, betacyanin; BETX, betaxanthin; PET, total extractable polyphenols; ABTS, total antioxidant activity by the 2,2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) method; and DPPH, total antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. \*The color bar corresponds to the range of standardized Euclidean distances.

collected have a high incidence of sunlight and high temperatures due to their semiarid climate. In another study, the higher the altitude, the higher the content of all the phenolic acids (Guevera-Terán et al., 2022). Moreover, the contents of betalain compounds can also be affected by fruit ripening stage (Dantas et al., 2016) and genetic variation (Stintzing et al., 2005).

Knowing the content of betalains is important due to the potential use of these compounds as a food colorant, which may allow the development of new food products without using artificial colorants (Stintzing et al., 2005; Fernández-López et al., 2018). In the present study, *T. inamoena* fruits showed equivalent or even higher betalain contents than those of fruits of other cacti, such as *Opuntia* spp., considering the range from 0.50 to 20.95 mg 100 g<sup>-1</sup> for betacyanin and from 1.08 to 11.49 mg 100 g<sup>-1</sup> for betaxanthin (Aparicio-Fernández et al., 2017).

Total extractable polyphenols ranged from 11.83 to 19.0 mg GAE 100 g<sup>-1</sup> fresh weight, which are values lower than those found by Formiga et al. (2016) for plants from another region in Brazil. However, the content of total extractable polyphenols in *T. inamoena* fruits is still higher than those of 15.63, 1.60, and 2.68

reported by Petkovska et al. (2016), Su et al. (2019), and Liu et al. (2019) for other popularly consumed fruits, such as apple [*Malus domestica* (Suckow) Borkh.], litchi [*Litchi chinensis* Sonn.], and peach [*Prunus persica* (L.) Batsch], respectively.

Given the importance of polyphenols for human health, future studies must focus on how to obtain the non-extractable fraction from *T. inamoena* fruits during the aqueous-organic treatments commonly used to determine polyphenol content in foods, especially since the polyphenols found in this fraction present a low molecular weight associated with macromolecules (proteins and dietary fiber) that act as macromolecular antioxidants (Pérez-Jiménez & Saura-Calixto 2015, 2018). According to these same authors, the non-extractable fraction is found in a higher amount than total extractable polyphenols in a range of fruit and vegetable species.

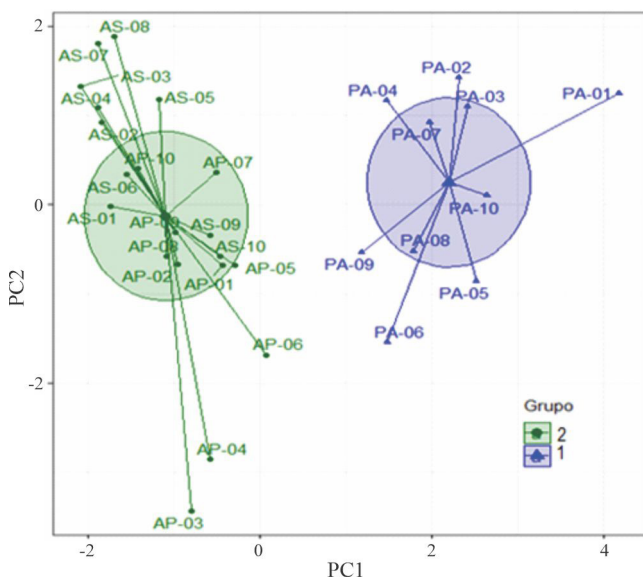
Although *T. inamoena* is a Caatinga species that is still little known, its fruits show potential as alternatives to meet the demands of domestic and foreign markets searching for new flavors, colors, textures, and bioactive compounds. In addition, the fruits collected from the three experimental sites presented extremely high contents of bioactive compounds, meaning that they could be recommended for the diet of the local population, replacing imported fruits consumed traditionally.

## Conclusions

1. Fruits of *Tacinga inamoena* are rich in bioactive compounds such as betalains, flavonoids, polyphenols, and vitamin C.
2. In the Brazilian state of Rio Grande do Norte, the fruits from the municipality of Parelhas show higher contents of carotenoids, betacyanins, and betaxanthins than those from the municipalities of Assú and Apodi.
3. The fruits of *Tacinga inamoena* from the municipalities of Assú and Apodi are genetically similar.

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**Figure 2.** Clustering analysis using the k-means method for fruits of *Tacinga inamoena* collected in the municipalities of Parelhas (PA), Apodi (AP), and Assú (AS) in the state of Rio Grande do Norte, Brazil. PC1, principal component 1; and PC2, principal component 2.

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Data in article: research data are available in the published article.

### Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

### Conflict of interest statement

The authors declare no conflicts of interest.

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