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Biochemical Evaluation of Custard Apple (*Annona reticulata* L.) Fruits in Tepic, Nayarit

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ABSTRACT: The custard apple fruit (*Annona reticulata* L.) is distinguished not only by its pleasant flavor and high nutritional value, but also by the presence of bioactive compounds in its pulp, particularly antioxidants, which may provide health benefits when consumed. However, in Nayarit this plant material is found in the wild, without agronomic management or conservation programs. Therefore, the purpose of this research was to evaluate several antioxidants in the pulp of custard apple fruits. Fruits were collected from four localities in Tepic, Nayarit (Jicote, Trapichillo, 14 de Marzo, and Tepic). Total phenolics, flavonoids, vitamin C, carotenoids, and antioxidant capacity by ABTS, DPPH, and FRAP were quantified. The pulp of the fruits from Tepic and Trapichillo showed the highest phenolic content (254–321 mg AGE/100 g FW), and fruits from Trapichillo also exhibited higher flavonoid levels (1267 mg QE/100 g FW). The pulp of Trapichillo fruits presented the highest antioxidant capacity by the ABTS method, as well as the pulp of Jicote and Trapichillo fruits by the FRAP method. Vitamin C and carotenoids were found in all pulps analyzed; however, no significant differences were observed among fruits from the different localities. In conclusion, antioxidants were detected in the pulp of custard apple fruits collected in Tepic, Nayarit, suggesting that their consumption could be beneficial and that the fruit has significant potential for industrial applications in the medical and food sectors, from the extraction of bioactive compounds, in the manufacture of medicines and in the production of functional foods.

KEYWORDS: Antioxidant capacity; *Annona reticulata*; vitamin C; carotenoids

1 Introduction

The Annonaceae family includes 108 genera and 2400 recognized species worldwide [1], with the genus *Annona* being one of the most representative [2]. In Mexico, the National Biodiversity Information System (SNIB) reports the presence of 19 species of this genus, among which soursop (*Annona muricata* L.), cherimoya (*Annona cherimola* M.), sugar apple (*Annona squamosa* L.), and custard apple (*Annona*

reticulata L.) are included. However, only the first three species are commercially cultivated in states such as Michoacán, Morelos, Yucatán, Veracruz, Tabasco, Nayarit, and Chiapas [3,4]. Different parts of the custard apple tree (commonly known as bullock's heart, ramphal, or custard apple) contain phytochemicals such as tannins, saponins, alkaloids, phenols, glycosides, flavonoids, steroids, terpenoids, and acetogenins. These secondary metabolites have demonstrated pharmaceutical and nutraceutical relevance due to their antioxidant, antimicrobial, anticancer, anthelmintic, larvicidal, anti-inflammatory, analgesic, central nervous system depressant, and antihyperglycemic activities [5–9]. Custard apple pulp has a pleasant taste and is granular, creamy, and aromatic. It also has high nutritional value, as it is rich in crude protein, crude fiber, monosaccharides, calcium, magnesium, iron, and vitamin C [8,10,11]. Furthermore, phenolic compounds, flavonoids, anthocyanins, carotenoids, and antioxidant capacity have been reported in the pulp [12], indicating potential health benefits associated with consumption, including immune modulation and enhanced barrier function [13]. However, in Nayarit this plant material grows wild, without agronomic management or conservation programs to prevent its extinction. Therefore, the objective of this research was to evaluate the presence of selected bioactive compounds in the pulp of custard apple fruits in Tepic, Nayarit. This will help highlight the importance of its consumption and its potential use in pharmaceutical, food, and other industries.

2 Materials and Methods

2.1 Plant Material

In April 2021, custard apple trees were georeferenced: six in the locality of Jicote (21°43'30.04" N, 105°03'31.45" W, 198 masl), five in Trapichillo (21°34'24" N, 104°59'2" W, 674 masl), five in the locality 14 de Marzo (21°44'9.12" N, 105°4'26.96" W, 125 masl), and three in Tepic (21°30'34.2" N, 104°53.741" W, 915 masl), all located within the municipality of Tepic, Nayarit. The localities of Tepic and Trapichillo exhibit a semi-warm sub-humid climate with temperatures between 20–22°C, and Jicote and 14 de Marzo exhibit a warm sub-humid climate with temperatures between 24–26°C [14]. All trees were growing either wild or in backyard conditions. From each tree, between three and twenty-one fruits were manually harvested at physiological maturity, for a total of 18 to 38 fruits per locality. Subsequently, fruits were transported in plastic boxes to the Food Technology Unit at the Universidad Autónoma de Nayarit. Plant material was stored in a climate-controlled chamber (Climacell®) at 28 ± 2°C and 95% RH until each fruit reached edible maturity (4 to 7 days). Fruits were peeled and pulped. The pulp was then stored at –20°C until further analysis.

2.2 Preparation of Methanolic Extracts

One gram of pulp was weighed and mixed with 10 mL of 80% methanol. Samples were homogenized using an Ultraturrax homogenizer (T8 IKA® Staufen, Germany) at 18,000 rpm for 15 s. The homogenized samples were centrifuged in a Hermle Z326K centrifuge (Wehingen, Germany) at 10,410× g at 4°C for 20 min. Supernatants were stored at 4°C until subsequent analyses of total soluble phenolics, total flavonoids, antioxidant capacity by ABTS, DPPH, and FRAP, and total carotenoids. All Analyses were made in triplicate.

2.3 Preparation of Oxalic Acid Extracts

One gram of pulp was weighed and mixed with 10 mL of 0.4% oxalic acid, followed by homogenization using an Ultraturrax at 18,000 rpm for 15 s. Samples were centrifuged in a Hermle Z326K centrifuge at 10,410× g at 4°C for 10 min. Supernatants were stored at 4°C for subsequent analysis of vitamin C. Analyses were made in triplicate.

2.4 Total Soluble Phenolics

Total soluble phenolics were quantified using the method described by Singleton et al. [15], based on the Folin–Ciocalteu assay. Polyphenols react with sodium molybdate and sodium tungstate in a basic medium, forming yellow oxides that, upon reduction by phenolic groups, yield an intense blue complex [16]. A total of 50 μL of methanolic extract was mixed with 250 μL of Folin–Ciocalteu reagent and allowed to stand in the dark for 5 min, followed by the addition of 200 μL of 7.5% Na_2CO_3 . Samples were kept in the dark for 30 min. Absorbance was measured at 765 nm using a Multiskan GO spectrophotometer (Thermo Scientific). A standard curve (0–400 mg/L) was prepared using gallic acid, and results were expressed as mg gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW).

2.5 Total Flavonoids

Total flavonoid content was determined following the method of Zhishen et al. [17]. In an alkaline medium, aluminum chloride reacts with flavonoids in the presence of sodium nitrite. A mixture of 50 μL methanolic extract, 100 μL water, and 10 μL 15% NaNO_2 was vortexed and incubated in the dark for 6 min. Then, 15 μL of 10% AlCl_3 was added, followed by 200 μL of 4% NaOH . Absorbance was measured at 510 nm, using a quercetin standard curve (0–400 mg/L). Results were expressed as mg quercetin equivalents per 100 g fresh weight (mg QE/100 g FW).

2.6 Antioxidant Capacity by ABTS (2,2'-Azino-Bis(3-Ethylbenzothiazoline-6 Sulfonic Acid))

Antioxidant capacity was determined using the method of Re et al. [18]. Thirty μL of methanolic extract was mixed with 250 μL of ABTS radical solution adjusted with phosphate buffer to an absorbance of 0.70 ± 0.02 at 734 nm. Samples were incubated in the dark for 7 min. Absorbance was measured at 734 nm. A standard curve (0–150 mg/L) using ascorbic acid was used, and results were expressed as mg ascorbic acid equivalents per 100 g fresh weight (mg AAE/100 g FW).

2.7 Antioxidant Capacity by DPPH (2,2-Diphenyl-1-Picrylhydrazyl Radical)

The DPPH radical scavenging activity was determined using the method of Brand-Williams et al. [19]. Fifty μL of methanolic extract were mixed with 250 μL of DPPH solution adjusted with methanol to an absorbance of 0.70 ± 0.02 at 520 nm. Samples were incubated in the dark for 30 min. Absorbance was measured at 520 nm. A standard curve (0–100 mg/L) was prepared using ascorbic acid, and results were expressed as mg AAE/100 g FW.

2.8 Antioxidant Capacity by FRAP (Ferric Reducing Antioxidant Power)

FRAP antioxidant capacity was determined according to Benzie & Strain [20]. Thirty μL of methanolic extract were mixed with 250 μL of FRAP reagent containing TPTZ (2,4,6-tripyridyl-s-triazine), FeCl_3 , and 0.3 M acetate buffer. Samples were incubated in the dark for 30 min. Absorbance was measured at 595 nm. A standard curve (0–30 mg/L) using ascorbic acid was used, and results were expressed as mg AAE/100 g FW.

2.9 Vitamin C

Vitamin C content was determined following the method of Dürüst et al. [21]. Fifty μL of oxalic acid extract were mixed with 50 μL acetate buffer and 400 μL of DCPI reagent (2,6-dichlorophenolindophenol). All procedures were conducted at a cold temperature. Absorbance was measured at 520 nm using a standard curve (0–100 mg/L) of ascorbic acid. Results were expressed as mg AAE/100 g FW.

2.10 Total Carotenoids

Total carotenoid content was determined according to Braniša et al. [22], which quantifies chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids. Two hundred μL of methanolic extract were measured at 663, 647, and 470 nm. Concentrations were calculated using the following equations:

- $\text{Chl a} = 16.72(A_{663}) - 9.16(A_{647})$
- $\text{Chl b} = 34.09(A_{647}) - 15.28(A_{663})$
- $\text{Total carotenoids } (\mu\text{g/mL}) = [1000(A_{470}) - 1.63(\text{Chl a}) - 104.96(\text{Chl b})]/221$

Results were expressed as mg β -carotene per 100 g fresh weight (mg β -carotene/100 g FW).

2.11 Statistical Analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA) and Tukey's mean comparison test at a significance level of $p \leq 0.05$. Pearson correlation analysis was also performed among variables. Statistical analyses were conducted using SAS[®] software version 9.0 for Windows.

3 Results and Discussion

3.1 Total Soluble Phenolics

The highest concentration of total soluble phenolics were recorded in fruits from the localities of Tepic and Trapichillo, with values of 321.50 ± 50.66 mg GAE/100 g FW and 254.24 ± 16.74 mg GAE/100 g FW, respectively ($p \leq 0.05$), whereas the lowest concentration was recorded in fruits from 14 de Marzo (144.81 ± 6.23 mg GAE/100 g FW) (Table 1). These differences may be attributed to abiotic stress caused by the concrete surfaces surrounding the habitat of trees in Tepic and Trapichillo, which leads to increased soil temperatures and induces water stress, thereby enhancing the synthesis of secondary metabolites, such as phenolic compounds, as a physiological response to these stress conditions [23]. Phenolics have diverse physiological roles in plants, frequently involving growth, resistance to pathogens or diseases, and pigmentation [24]. Additionally, factors such as temperature, radiation, nutrition, and irrigation can affect phenolic content [25]. A higher phenolic content could increase fruit marketability due to their antioxidant properties [26,27], as consumers increasingly seek foods with health-promoting qualities [28]. Recent studies on phenolic dynamics during fermentation also highlight their stability and bioactivity, which may support the valorization of custard apple products. In custard apple fruits from Yucatán, Mexico, phenolic concentrations of 358.25 mg GAE/100 g FW have been reported [12], which are higher than those found in this study. Differences may be due to environmental conditions (temperature and relative humidity) and soil type variations between the states of Yucatán and Nayarit. Phenolics have also been reported in other Annonaceae species such as soursop, chincuya, and cherimoya, with values of 154, 181, and 366 mg GAE/100 g FW, respectively; the values for soursop and chincuya are within the range found in our study [29].

3.2 Total Flavonoids

Total flavonoid content was significantly higher ($p \leq 0.05$) in fruits from Trapichillo (1267.14 ± 141.79 mg QE/100 g FW) compared to those from 14 de Marzo (279.08 ± 64.97 mg QE/100 g FW) (Table 1). Both abiotic (water deficit) and biotic (pathogens) stress can positively influence flavonoid production [25]. In fruit, flavonoid levels typically increase during ripening due to starch hydrolysis into sugars, which favors the synthesis of shikimic acid and mevalonic acid, precursors of phenylpropanoid compounds [30]. On the other hand, flavonoids possess antioxidant capacity and have been associated with preventing

diseases related to free radicals [31]. Factors such as maturation stage, geographic origin, environmental conditions during growth, and genetic variability may influence flavonoid content [29]. In this regard, higher flavonoid levels in Trapichillo fruits may be associated with insect borer damage (visible perforations and empty seeds), which was not observed in fruits from 14 de Marzo. Other factors that may increase flavonoid production include solar radiation, nitrogen stress, defoliation, and drought [32].

Moo-Huchin et al. [12] reported 418.24 mg QE/100 g FW in custard apple fruits from Yucatán, values within the range of this study. Flavonoids have also been reported in soursop and cherimoya, with concentrations of 10.13 and 1.84 mg QE/100 g FW, respectively [29], which are considerably lower than those observed in this study, likely because they correspond to different species despite belonging to the same family.

Table 1: Total soluble phenolic compounds and flavonoids in custard apple pulp from four localities of Tepic, Nayarit, México.

Localities	Phenols (mg AGE/100 g FW)	Flavonoids (mg QE/100 g FW)
Jicote	233.51 ± 16.69 ^{ab}	973.75 ± 136.15 ^{ab}
Trapichillo	254.24 ± 16.74 ^a	1267.14 ± 141.79 ^a
14 de Marzo	144.81 ± 6.23 ^b	279.08 ± 64.97 ^c
Tepic	321.50 ± 50.66 ^a	719.63 ± 73.17 ^{bc}
LSD	90.915	497.75
CV	50.41	76.27

Mean ± standard error; means with similar letters in the same column are statistically equal (Tukey, $p \leq 0.05$). AGE: Acid Galic Equivalents. QE: Quercetin Equivalents. LSD: Least Significant Difference. CV: Coefficient of Variation.

3.3 Antioxidant Capacity by ABTS

Fruits from Trapichillo exhibited the highest antioxidant capacity ($p \leq 0.05$) by the ABTS method (134.64 ± 0.54 mg AAE/100 g FW), compared to fruits from Tepic and 14 de Marzo (Table 2). Antioxidants can prevent the development of degenerative diseases such as cancer, heart disease, obesity, type 2 diabetes, hypertension, premature aging, and inflammatory disorders [33]. The ABTS method evaluates the ability of the sample to neutralize free radicals mainly through electron transfer [34]. In custard apple fruits from Yucatán, antioxidant capacity by ABTS has been reported at 189.66 mg AAE/100 g FW [12], higher than the values found here. In other Annonaceae species, values of 79.6 and 93.16 mg AAE/100 g FW have been reported in soursop and sugar apple, respectively [35,36], lower than those in our study. De los Santos-Santos et al. [37] reported 105.6 mg AAE/100 g FW in soursop. Singh et al. [38] reported that differences in antioxidant capacity may be influenced by genotype, fruit ripening stage, and edaphic factors. In this study, soil type may have played a role since Tepic has umbrisol soil, compared to lluvisol and nitisol soils in other localities. The soil of Tepic is less fertile and prone to erosion due to surface acidity [14].

3.4 Antioxidant Capacity by DPPH

Antioxidant capacity by DPPH showed no significant differences ($p \leq 0.05$) among fruits from the different localities (Table 2), with values ranging from 85.60 to 89.66 mg AAE/100 g FW. DPPH radical inhibition is often associated with phenolic compounds capable of donating electrons to neutralize free radicals [39]. Previous studies reported 3.43 mg and 81.10 mg AAE/100 g FW in custard apple from Sri Lanka and Yucatán, respectively [12,40], both lower than values found here. In contrast, data reported by De los Santos-Santos et al. [37] in soursop fruits showed values of 243.59 mg AAE/100 g FW. Differences likely relate to edaphoclimatic conditions and species differences within the Annonaceae family. Chavan

et al. [41] noted that climatic and soil conditions can influence antioxidant activity. Additionally, Rayar & Manivannan [42] reported compounds with antioxidant activity against breast cells, analyzed by DPPH. Differences between ABTS and DPPH results may be attributed to differences in experimental conditions, radical structure, and antioxidant polarity [43]. ABTS detects both hydrophilic and lipophilic antioxidants, while DPPH is more sensitive to less polar compounds [44].

3.5 Antioxidant Capacity by FRAP

Fruits from Jicote and Trapichillo exhibited the highest antioxidant capacity by the FRAP method (1515.43 ± 295.49 and 1720.83 ± 266.13 mg AAE/100 g FW, respectively) ($p \leq 0.05$) (Table 2). FRAP values may be associated with increased metabolic activity during fruit ripening and the climacteric peak, increased concentrations of ascorbic acid during postharvest, and the accumulation of phenolic compounds [45]. However, FRAP results may be influenced by a variety of antioxidant substances, each with specific mechanisms of action [46]. An increase in antioxidant capacity has been reported in dairy foods due to the addition of anona pulp [47]. In soursop fruits, this capacity has previously been shown reporting FRAP values of 119.28 mg AAE/100 g FW [48] and 62 mg AAE/100 g FW [35], both much lower than those found in this study. Differences may be due to different extraction solvents or species variation within the Annonaceae family. On the other hand, higher FRAP values compared to ABTS and DPPH may be due to the fact that, in the FRAP reaction, all reducing substances—not only phenolic compounds—can participate, and their activity depends on redox potential, pH, and chemical structure [49].

Table 2: Antioxidant capacity by ABTS, DPPH and FRAP in pulp of custard apple from four localities of Tepic, Nayarit, México.

Localities	ABTS (mg AAE/100 g FW)	DPPH (mg AAE/100 g FW)	FRAP (mg AAE/100 g FW)
Jicote	130.56 ± 1.59^{ab}	89.66 ± 0.42^a	1515.43 ± 295.49^a
Trapichillo	134.64 ± 0.54^a	89.56 ± 0.45^a	1720.83 ± 266.13^a
14 de Marzo	124.60 ± 3.47^b	85.60 ± 2.46^a	395.47 ± 79.81^b
Tepic	110.37 ± 2.07^c	89.56 ± 0.33^a	349.69 ± 49.35^b
LSD	7.59	4.10	999.91
CV	7.93	6.12	116.25

Mean \pm standard error; means with similar letters in the same column are statistically equal (Tukey, $p \leq 0.05$). AAE: Ascorbic acid equivalents. LSD: Least Significant Difference. CV: Coefficient of Variation.

3.6 Vitamin C

Vitamin C is a major natural antioxidant [36]. Its concentration in fruit generally peaks at physiological maturity and subsequently declines as fruits reach edible maturity, a process associated with increased levels of galactose and mannose, key precursors involved in vitamin C biosynthesis [37]. As an antioxidant and cofactor in redox reactions, ascorbate plays a key role in activating epigenetic mechanisms involved in cellular differentiation, with deregulation potentially linked to certain cancers [50]. In this study, fruits from all four localities showed statistically similar vitamin C concentrations (34.11 to 41.89 mg AAE/100 g FW) ($p > 0.05$) (Table 3). These values are higher than those reported by Moo-Huchin et al. [12], who found 23.02 ± 1.94 mg AAE/100 g FW. Variations among samples may be related to genotypic diversity, environmental differences among regions, water and soil pH, temperature, and nutrient availability—all of which influence vitamin C biosynthesis [51].

3.7 Total Carotenoids

Carotenoids are not only precursors of vitamin A but also contribute significantly to antioxidant capacity [52]. There are two types of carotenoids: carotenes (oxygen-free) and xanthophylls (oxygen-containing). Carotenoids are hydrocarbons that contain an extensive system of conjugated double bonds; this characteristic is primarily responsible for their interaction with free radicals and singlet oxygen, and therefore they act as effective antioxidants [53]. No significant differences ($p > 0.05$) in total carotenoid content were observed among fruits from the different localities (Table 3), with values ranging from 12.69 to 16.46 mg β -carotene/100 g FW. These values are higher than those reported by Moo-Huchin et al. [12] for the same fruit (2.25 ± 0.4 mg β -carotene/100 g FW). Variations among accessions may be attributed to genotypic variation and pre- and postharvest conditions [54].

Table 3: Vitamin C and total carotenoids in pulp of custard apple from four localities of Tepic, Nayarit, México.

Localities	Vitamin C (mg AAE/100 g FW)	Carotenoids (mg β -caroteno/100 g FW)
Jicote	34.15 ± 3.42^a	14.16 ± 0.89^a
Trapichillo	38.99 ± 3.21^a	16.46 ± 1.15^a
14 de Marzo	34.11 ± 4.17^a	13.14 ± 1.79^a
Tepic	41.89 ± 1.12^a	12.69 ± 0.38^a
LSD	13.38	4.44
CV	47.40	41.28

Mean \pm standard error; means with similar letters in the same column are statistically equal (Tukey, $p \leq 0.05$). AAE: Ascorbic acid equivalents. LSD: Least Significant Difference. CV: Coefficient of Variation.

3.8 Pearson Correlation Analysis

Pearson correlation analysis (Table 4) indicated a strong positive correlation (greater than 0.6) between phenolics and carotenoids, as well as antioxidant capacity by ABTS and DPPH. This suggests that phenolic compounds contribute significantly to antioxidant activity, as reported in other fruits [55]. Differences in correlations between phenolic compounds and antioxidant capacity may be due to the presence of reducing agents such as ascorbic acid, minerals, and carotenoids, as well as genetic, agro-economic, and environmental factors [12]. On the other hand, the strongest positive correlation (0.982) was observed between antioxidant capacity measured by ABTS and DPPH, higher than the value of 0.82 reported by Moo-Huchin et al. [12].

Table 4: Pearson correlation between physicochemical, phytochemical and antioxidant capacity in pulp of custard apple.

	TSS	TA	FRAP	Vit C	TC	DPPH	ABTS	TF	Flav	pH	IM
TSS	1	0.284	0.215	-0.058	-0.192	-0.177	-0.159	-0.362	-0.306	-0.282	0.295
TA		1	0.168	0.128	-0.069	-0.069	-0.037	-0.173	-0.102	-0.396	-0.759
FRAP			1	0.084	0.375	0.456	0.495	0.285	0.243	0.131	0.003
Vit C				1	0.322	0.414	0.458	0.253	0.454	-0.055	-0.105
TC					1	0.786*	0.795*	0.615*	0.467	0.158	-0.021
DPPH						1	0.982*	0.740*	0.582*	0.270	-0.009
ABTS							1	0.693*	0.608*	0.204	-0.035
TF								1	0.646*	0.400	-0.031
Flav									1	0.213	-0.089
pH										1	0.271
IM											1

TSS: Total soluble solids. TA: Titratable acidity. Vit C: Vitamina C. CT: Total Carotenoids. DPPH: Antioxidant Capacity by the 2,2-diphenyl-1-picrylhydrazyl radical. ABTS: Antioxidant capacity by the method 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). TF: Total phenols. Flav: Flavonoids. pH: potential of hydrogen. IM: Maturity index. (* $p \leq 0.05$).

4 Conclusions

The pulp of fruits from Trapichillo and Jicote exhibited the highest concentrations of total soluble phenolics, total flavonoids, and antioxidant capacity as assessed by the ABTS and FRAP assays. Additionally, elevated levels of total soluble phenolics were detected in the pulp of fruits from Tepic. Vitamin C, total carotenoids, and antioxidant capacity determined by the DPPH method were identified in all analyzed pulp samples. Overall, the fruit pulps from the municipality of Tepic demonstrated the presence of a diverse profile of antioxidant compounds, suggesting that their consumption may confer health benefits and highlighting their substantial potential for the pharmaceutical industry, with the extraction of bioactive compounds and the manufacture of medicines and for the food industry, through the addition of pulp to produce nutraceutical products. Therefore, it is necessary to develop an anona crop with agronomic management and to create a germplasm bank for its conservation.

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