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Digestion and Colonic Fermentation of Cactus Fruits from the Brazilian Semiarid Region (*Pilosocereus gounellei* and *Cereus jamacaru*) Impacts Bioaccessibility and Functional Potential

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ABSTRACT: This study evaluated the bioactivity and metabolite production of cactus fruits after gastrointestinal digestion and in vitro colonic fermentation. After gastrointestinal digestion, there were a 2.27- and 3.34-fold increase in total phenolic compounds and antioxidant capacity in *xique−xique* and a 1.46- and 1.98-fold increase for *mandacaru*, respectively. Compounds such as quercetin, procyanidin B2, and vanillin increased for both fruits in the intestinal phase, while gallic and ferulic acids decreased. The final pH after 72 h of fermentation was 6.11 and 5.89 for *xique−xique* and *mandacaru* fruits, respectively. Flavonoids in both fruits are consumed during fermentation, leading to a reduction of total phenolic content and antioxidant capacity but to an increase in phenolic acid content. The highest production of acetic, propionic, and butyric acids (228.45; 128.32; and 72.20 mg/L, respectively) was observed after *mandacaru* fermentation. Thus, *xique−xique* and *mandacaru* fruits show promising potential for the preparation of foods with prebiotic claims.

KEYWORDS: *cactaceae, xique−xique, mandacaru, functional food, bioactive compounds, prebiotic effect*

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INTRODUCTION

Species from the Cactaceae family are unconventional food plants that, in addition to offering excellent nutritional value and great technological potential, play a significant socio-economic role in the subsistence of populations in regions like the Brazilian semiarid region.[1] *Pilosocereus gounellei* and *Cereus jamacaru*, popularly known as *xique−xique* and *mandacaru*, are native species that occur exclusively in the Brazilian Caatinga.[13] Currently, these species are commercially underused, but recent studies suggest they hold great potential across various industrial sectors. Furthermore, they can be incorporated into foods such as bread, cakes, ice cream, yogurt, jam, and other products.[2]

In terms of chemical composition, the fruits of these species stand out for their abundance of nutrients, including carbohydrates such as glucose, fructose, sucrose, and maltose and oligosaccharides, such as nystose. In addition, they are excellent sources of dietary fiber (approximately 40.4% in *xique−xique* fruits and 34.4% in *mandacaru* fruits) and phenolic compounds, such as catechin, epicatechin, quercetin, *p*-coumaric acid, and rutin.[3−5]

As they are good sources of nutrients and bioactive compounds, these species are associated with several beneficial effects on health, such as antioxidant, prebiotic, antinociceptive, and gastroprotective properties. They also demonstrate potential to inhibit digestive enzymes and protein glycation, suggesting benefits in controlling diabetes.[2,6,7,13]

Although the functional value of these plants is often related to the presence of secondary metabolites, such as phenolic compounds, the bioactivity of these molecules is influenced by a series of factors, such as concentration in food, release in the food matrix, chemical structure, and conjugation with other compounds.[8,9] In reality, phenolic compounds are often associated with various components of the plant matrix, including dietary fiber, which the body cannot digest or absorb, forming what are known as antioxidant dietary fibers (ADF).[10] In this context, only about 5−10% of phenolic compounds are bioaccessible in the small intestine, while most of these compounds, because they are bound to other molecules, including dietary fiber, reach the large intestine. There, they are fermented by the microbiota present in the colon, generating the production of phenolic metabolites and short-chain fatty acids, substances that help modulate the microbiota and provide a series of health benefits.[10−14]

Although recent studies have highlighted the potential of *xique−xique* and *mandacaru* cladodes in modulating human gut microbiota,[12,15,16] there is currently limited information in the literature regarding the effects of the digestive process and the prebiotic potential of their fruit. Therefore, the objective of this study was to evaluate the impact of gastrointestinal digestion and in vitro colonic fermentation on *xique−xique* and

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mandacaru fruits, aiming to demonstrate their potential as ingredients for developing new products with functional claims to enhance consumer health.

MATERIAL AND METHODS

Sample Acquisition and Preparation. *Xique−xique* and *mandacaru* fruits were collected during the months of October and November 2022 in the city of Solânea in the interior of Paraíba state. The previously identified samples (accession nos 208.839 and 208.840) were deposited in the Herbarium-UEC of the Institute of Biology of the State University of Campinas UEC-UNICAMP. The collected whole fruits were washed with distilled water, processed, frozen (−20 °C), and immediately lyophilized. After the lyophilization process, the samples were ground in a knife mill to obtain a powder. The samples thus obtained were stored at −20 °C in dark packaging until further use.

Gastrointestinal Digestion. The in vitro gastrointestinal digestion process was carried out according to the INFOGEST 2.0 protocol outlined by Brodkorb et al. (2019).[17] Briefly, 1 g freeze-dried samples of *xique−xique* and *mandacaru* were mixed with 1 mL of oral fluid containing 1.5 mmol $CaCl_2$ and incubated with continuous mixing for 2 min at 37 °C and pH 7, representing the oral phase. From this phase, 0.5 mL was collected and centrifuged at 4000*g* for 5 min, and the supernatant was filtered using 0.20 μm PTFE filters for further analysis. The remaining oral content (≈1.5 mL) was then combined with 2 mL of gastric fluid containing a mixture of 0.15 mmol $CaCl_2$, 2000 U/mL pepsin, and 60 U/mL gastric lipase. The pH of the gastric phase was adjusted to 3 using 1 M HCl, and the samples were incubated with continuous mixing for about 2 h at 37 °C. The reaction was halted by adjusting the pH to 7.0 by adding 1 M NaOH solution. Afterward, an aliquot of 1 mL was collected and centrifuged at 4000*g* for 5 min, and the supernatant was filtered using 0.20 μm PTFE filters. For the intestinal phase, the remaining gastric phase volume was combined with 4 mL of intestinal fluid containing 10 mmol bile salts, 0.6 mmol $CaCl_2$, and pancreatin with a trypsin activity of 100 U/mL. This mixture was incubated with continuous mixing for 2 h at 37 °C and pH 7. An aliquot of 1 mL from the intestinal phase was collected, centrifuged at 4000*g* for 5 min, and filtered through 0.20 μm PTFE filters. This process was carried out in triplicate, and a blank was prepared by replacing the fruit samples with ultrapure water, following the same protocol steps. All supernatants collected from the different phases were used for gastrointestinal digestion analysis, while the pellet and approximately 10% of the supernatant from the intestinal phase were used for in vitro colonic fermentation.

In Vitro Colonic Fermentation. In vitro colonic fermentation was conducted as described by Guo et al. (2020).[18] Buffered colon medium (1 L) was prepared containing a mixture of K_2HPO_4 (5.22 g), KH_2PO_4 (16.32 g), $NaHCO_3$ (2 g), yeast extract (2 g), peptone (2 g), mucin (1 g), L-cysteine HCl (0.5 g), and Tween-80 (2 mL) in water. A phosphate buffer (1 L) for the fecal inoculum was prepared using K_2HPO_4 (8.8 g), KH_2PO_4 (6.8 g), and sodium thioglucolate (0.1 g). Anaerobic bags were used to collect the fresh fecal sample from four nonsmoking and healthy adults who reported not having had any gastrointestinal disorders or taken antibiotics for at least 3 months prior to the experiment. In accordance with the guidelines of the Medical Ethics Advisory Committee of Wageningen University (METC-WU), this research did not require ethical approval.

The fecal inoculum was prepared by adding 40 g of fresh feces (approximately 10 g from each donor) to 200 mL of phosphate buffer in a Stomacher Bag Mixer, and the mixture was homogenized for 10 min at 300 rpm. The suspension was then transferred to 50 mL centrifuge tubes and centrifuged for 2 min at 500*g*. The supernatant was collected and used as the final fecal inoculum. For inoculation, 0.5 g of the gastrointestinal digestion pellet plus 10% of the supernatant from the intestinal phase was mixed with 7.5 mL of buffered colon medium in penicillin vials, which were sealed with rubber and aluminum caps. The vials were then flushed with nitrogen gas for 15 min. Then, 2 mL of the fecal inoculum was added to each penicillin vial, and the vials were incubated at 37 °C while being shaken at 300 rpm. Samples were collected from each penicillin vial using a syringe at times 0, 3, 7, 18, 24, and 48 h. At each sample collection, the pH value was measured before the samples were centrifuged and frozen at −20 °C for later analysis. The negative control consisted of the addition of basal medium plus deionized water in place of the samples and freshly prepared fecal inoculum.

pH Assessment. The change in the pH of the samples at different times during the in vitro colonic fermentation process was carried out using a digital pH meter (model 320, Metler-Toledo, USA) according to the procedures described by AOAC (2006).[19]

Analysis of Metabolites and Determination of Antioxidant Capacity. *Determination of Total Phenolic Content.* The total phenolic compound content in the digested samples and after in vitro colonic fermentation was measured using the Folin−Ciocalteau method, following the procedure outlined by Singleton et al. (1999).[20] Gallic acid was employed to create an analytical curve with absorbance measurements taken at 760 nm. The results were reported as milligrams of gallic acid equivalent (GAE)/g of freeze-dried sample (fd).

Determination of Total Flavonoid Content. This assay was conducted using catechin to establish an analytical curve, following the procedures previously outlined by Zhishen et al. (1999).[21] The absorbance of each sample was recorded at 510 nm by using a microplate reader. The total flavonoid content of the samples, obtained after gastrointestinal digestion and in vitro colonic fermentation, was expressed as milligrams of catechin equivalent (CE)/g fd.

Antioxidant Capacity. The antioxidant potential of the samples was assessed using the ABTS assay, following the methodology outlined by Re et al. (1999).[22] After the formation of the $ABTS^+$ radical, the absorbance of the reaction mixture was measured at 734 nm. Trolox was used to prepare the analytical curve, and the results were expressed as μM Trolox equivalent TE/g of fd.

Determination of Phenolic Metabolites. The phenolic metabolites were identified according to the method described by Peña-Correa et al. (2023),[23] with some modifications. Initially, a 500 μL aliquot of the filtered sample (using 0.20 μm PTFE filters) was mixed with 500 μL of 50% methanol (MeOH) and vortexed for 30 s. The samples were analyzed using a Nexera UPLC system (Shimadzu Corp., Kyoto, Japan) coupled to a triple quadrupole mass spectrometer LCMS-8050 (Shimadzu Corp., Kyoto, Japan). A 5 μL injection was made using an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm), connected to an Acquity UPLC BEH C18 VanGuard precolumn (1.7 μm, 2.1 mm × 5 mm, Waters Chromatography, Netherlands). The flow rate was set to 0.3 mL/min, and the column temperature was maintained at 40 °C. The mobile phases consisted of 0.1% formic acid (solvent A) and 0.1% acetonitrile containing formic acid (solvent B). The elution gradient was as follows: (0.0 min, 5% B), (0.5 min, 5% B), (2.0 min, 35% B), (7.5 min, 95% B), (9.5 min, 95% B), (9.6 min, 5% B), and (13.5 min, 5% B).

Ionization was performed by electrospray with an energy of 4.0 kV, and the probe temperature was set to 300 °C. The desolvation line and heat block temperatures were set to 250 and 400 °C, respectively, while the collision-induced dissociation (CID) gas pressure was set to 4 kPa. The flow rates for the drying gas, nebulizer gas, and heating gas were set to 10, 3, and 10 mL/min, respectively. Optimization of ionization parameters, including the prebias electrode voltage for Q1 (entrance energy potential of the collision cell), the collision energy for Q2, the prebias for Q3 (exit energy potential of the collision cell), the parent ion, and the m/z fragments for the multiple reaction monitoring transitions, was performed using standard solutions of the target analytes (10−20 ppm concentration) and supporting software (Shimadzu Corporation, Kyoto, Japan). The retention time and time window for MS data acquisition were also optimized for single reaction monitoring (SRM).

To construct the heatmap, which uses a color-coded thermometer to represent the concentration of metabolites in each analyzed sample, the area of each compound was normalized using the formula Log_2

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(peak area/median of peak areas from all samples, including the four fermentation times).

Short-Chain Fatty Acid Analysis. Short-chain fatty acid analysis was performed using a Shimadzu GC-2014 gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector (FID), a Stabilwax-DA fatty acid-free capillary column (1 μm × 0 0.32 mm × 30 m) (Restek, Bellefonte, PA, USA), and a split injector, according to adjustment conditions described by Guo et al. (2020).[18] Nitrogen was used as a carrier gas, and standard solutions of acetic, propionic, butyric, isovaleric, valeric, and isobutyric acids in different concentrations were prepared and used for identification and quantification, with the results expressed as mg/L.

Statistical Analysis. The analyses were conducted in triplicate using a completely randomized design, with results expressed as the mean ± standard deviation. All data were subjected to analysis of variance (ANOVA), and when significant differences between means were detected, the Tukey test was applied at a 5% significance level to evaluate the differences. All data were processed using Statistica software version 7.0 (STATSOFT, 1994).[24]

RESULTS AND DISCUSSION

In Vitro Gastrointestinal Digestion. The graphs in Figure 1 present the values for total phenolic compounds, total flavonoids, and antioxidant capacity before and after in vitro gastrointestinal digestion of *xique−xique* and *mandacaru* fruits. The crude (undigested) sample of *mandacaru* fruit presented values approximately 1.5, 3.2, and 1.7 times higher for total phenolics, total flavonoids, and antioxidant capacity, respectively, than that of the undigested sample of *xique−xique* fruits. The content of total phenolic compounds and the antioxidant capacity observed in this study for the undigested sample of *mandacaru* fruits (23.26 mg GAE/g of sample and 18.38 μmol TE/g, respectively) were also higher than the values reported by de Vasconcelos Medeiros et al. (2024), which presented 21.35 mg GAE/g of sample and 4.24 μmol TE/g, respectively.[5] For *xique−xique* fruit, the total phenolic compound values reported here were lower than those observed by Marciel and colleagues (2015) (127.9 mg of GAE/g of sample). However, the total flavonoid content they reported (2.42 mg CE/g sample) was similar to that found in this study (2.76 mg CE/g sample).[25]

As expected, the content of total phenolic (Figure 1A) compounds decreased significantly ($p < 0.05$) during the oral phase for both fruit, showing reductions of 2.5-fold for *mandacaru* and 1.9-fold for *xique−xique*, compared to that for the undigested samples. This behavior can be attributed to the physical−chemical composition of both fruits, combined with the neutral pH and the short contact time with the enzymes present in this phase, which, when associated, can contribute to a decrease in the bioaccessibility of the bioactive compounds, impacting their availability for absorption in the body.[26]

However, during the gastric and intestinal phases, the total phenolic content in both fruits increased significantly. After the gastric phase, the value increased by 26% for *mandacaru* and 9.3% for *xique−xique* compared to that for the undigested samples. Similarly, in the intestinal phase, *xique−xique* showed an increase of 56%, while *mandacaru* showed an increase of 32%. This process is driven by chemical changes during digestion, which can include the breaking of bonds at different pH conditions, as well as the action of digestive enzymes. This leads to the release of molecules bound to various components of the samples, resulting in higher concentrations of bioactive compounds, such as phenolics.[27] Different results were reported by Medina-Pérez et al. when evaluating the effect of the intestinal digestion process on the phenolic compound

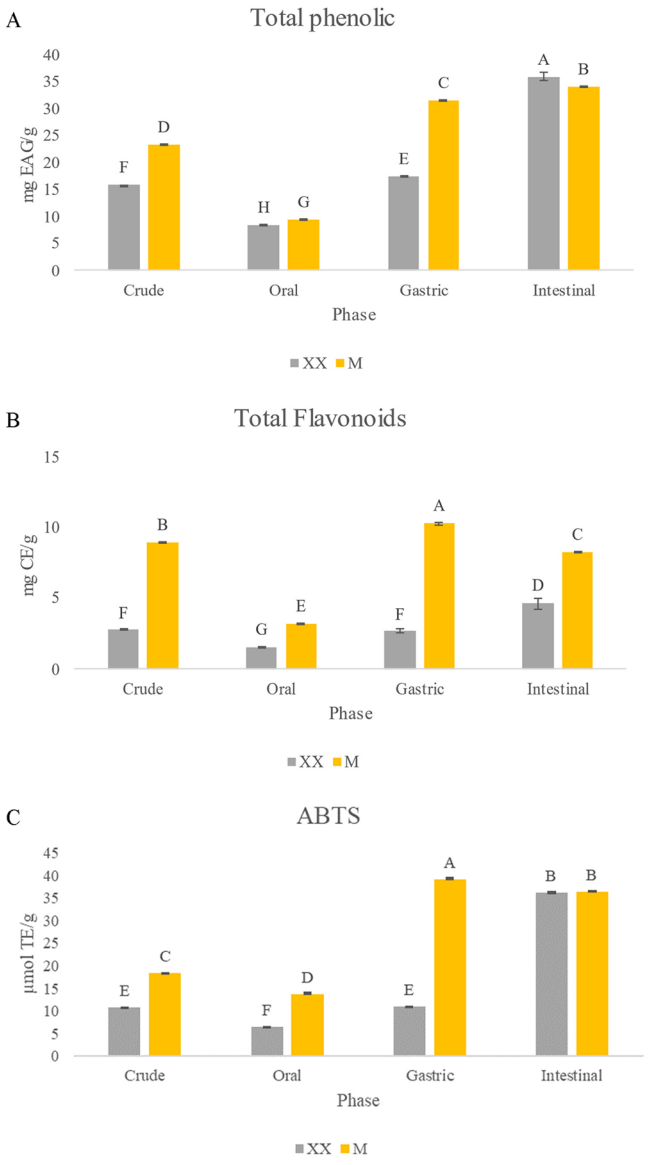


Figure 1. Total phenolics (A), total flavonoids (B), and antioxidant (C) capacity of the *xique−xique* and *mandacaru* fruits during the in vitro gastrointestinal digestion. GAE: Gallic acid equivalents. CE: Catechin equivalents. TE: Trolox equivalents. Equal lowercase letters mean that there was no statistical difference between the means by the Tukey's test at 5% significance ($p \leq 0.05$).

content of *xoconostle* (*Opuntia oligacantha*) fruits. According to the authors, after the intestinal phase, the total phenolic compound content decreased, but a bioaccessibility of 70.4% was observed.[28] A similar trend was observed by Chaalal et al., who studied three varieties of prickly pear (*Opuntia ficus-indica*): yellow, red, and yellow-red. After the three digestion phases, the total phenolic content decreased compared to that in the undigested samples. For example, in the yellow variety, TPC was reduced by 87% in the oral phase and by 71% in the intestinal phase compared to that in the undigested samples.[29]

Regarding the total flavonoid content (Figure 1B), *mandacaru* showed an increase of 13% after the gastric phase compared with the undigested sample, but there was a reduction of approximately 8% in the intestinal phase. As for the *xique−xique* fruit, no significant increase was observed in the gastric phase; however, an increase of approximately 40%

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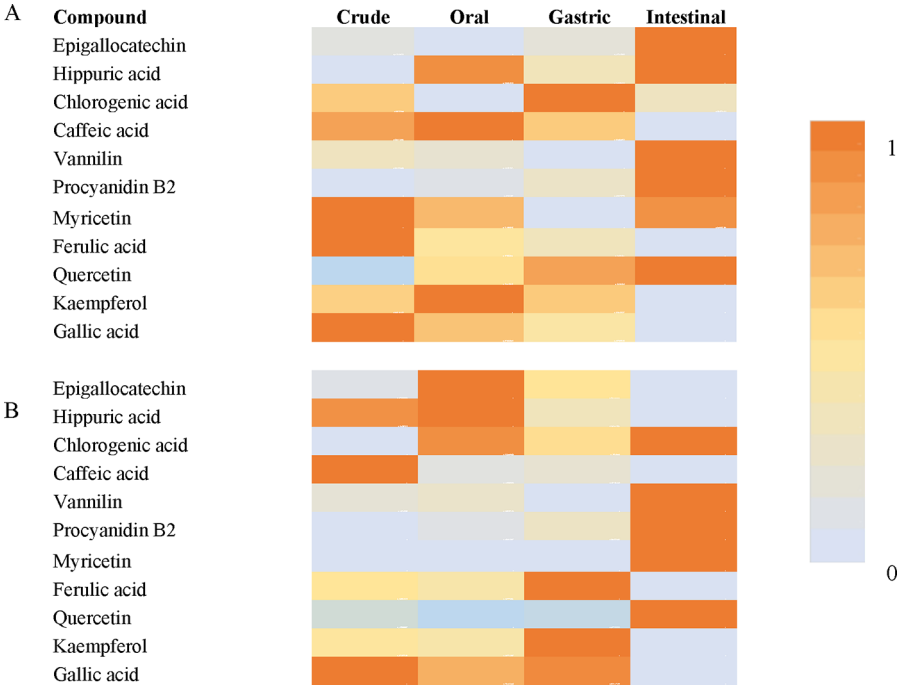


Figure 2. Bioaccessibility of phenolic compounds during in vitro gastrointestinal digestion of *xique−xique* (A) and *mandacaru* (B) fruits.

occurred after intestinal digestion. According to Dobson et al. (2019), differently from what was observed for the content of total phenolic compounds, some flavonoids can also interact with other food components, directly impacting their bioavailability and bioaccessibility during the gastrointestinal digestion process.[30] Some phenolic compounds can be bound to sugars; in this case, they are considered in their glycosylated form. Oteiza et al. reported that this binding increases the resistance of flavonoids to gastrointestinal conditions.[31] Ref 32 studied the effects of in vitro gastrointestinal digestion on the bioaccessibility of phenolic compounds from raw and cooked *O. ficus-indica* cladodes and observed that the total amount of flavonoids decreased by 48% after the gastric phase and by 37% after the intestinal phase. Similarly, ref 29 reported an approximately 3-fold reduction in total flavonoid content in prickly pear variety extracts after the intestinal phase.

The variation in the amount of total phenolic and flavonoid compounds in the different phases of digestion directly affected their antioxidant potentials (Figure 1C). The antioxidant capacity of *mandacaru* increased by 53% and 50% after gastric and intestinal digestion, respectively. Similarly, the antioxidant capacity of *xique−xique* fruits in the intestinal phase (36.20 μmol of TE/g) showed a significant increase compared to that of the undigested sample (10.81 μmol of TE/g) and the gastric phase (10.98 μmol of TE/g), resulting in an increase of approximately 3.34- and 3.29-fold when compared to the respective phases. However, there was no significant difference ($p < 0.05$) between *xique−xique* and *mandacaru* in the intestinal phase. According to Cömert & Gökmen (2022), changes in antioxidant potential during the digestive process, such as those observed for *xique−xique* and *mandacaru* fruits, may be related to the deprotonation of hydroxyl groups in the aromatic rings of phenolic compounds, which may lead to an increase in this capacity under intestinal conditions.[33] Different results from those observed in this study were reported by Balarabe and colleagues, who found a 77% reduction in the antioxidant capacity (1713 ± 10 to 388 ± 55 mg TE/100 g DW) of *baobab* fruits (*Adansonia digitata*) after the intestinal phase.[34] On the other hand, Choo et al. (2019) observed an increase of 11.46% and 8.17% in the antioxidant capacity, measured by the ABTS assay, in the samples of red dragon fruit drink and red dragon fruit juice (*Hylocereus polyrhizus*) after gastrointestinal digestion, respectively.[35]

The heatmap in Figure 2 illustrates the relative abundance of phenolic compounds in *xique−xique* and *mandacaru* fruits along with their release during in vitro gastrointestinal digestion. In undigested *xique−xique* fruit, the predominant phenolic compounds were myricetin, ferulic acid, gallic acid, and hesperetin. In contrast, *mandacaru* fruit primarily contained caffeic and gallic acid. During the oral phase of *xique−xique*, there appears to have been an increase in the concentration of caffeic acid and kaempferol. Epigallocatechin and chlorogenic acid were detected in the lowest concentrations. In *mandacaru* fruit, epigallocatechin, hesperetin, and chlorogenic acid were the most abundant compounds, while myricetin and quercetin were found in the lowest concentrations. In the gastric phase, there was a notable release of compounds, with chlorogenic acid increasing for *xique−xique* and ferulic acid, kaempferol, and gallic acid being more prominent for *mandacaru*. Conversely, the most significant changes in the concentration of certain phenolic compounds occurred during the intestinal phase, with vanillin, procyanidin B2, and quercetin increasing in both fruits. Meanwhile, there was a decrease in the levels of caffeic acid, kaempferol, gallic acid, and hesperetin.

The variations in polyphenol content throughout the digestive process can be attributed to factors such as the chemical characteristics of the samples as well as the pH conditions of the medium. In acid conditions, like those observed during the gastric phase, the stability of some polyphenols can be compromised, leading to increased degradation and potentially reduced bioaccessibility in the stomach. Conversely, evidence suggests that the stability and release of certain phenolic compounds may improve under

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alkaline conditions. Compounds such as quercetin and vanillin appear to have greater bioaccessibility in the intestine, possibly due to the break of glycosidic bonds caused by pH changes, which enhances the absorption of aglycones.[36,37]

In a study recently carried out to verify the effect of the digestive process on the bioaccessibility of phenolic compounds from *xique−xique* and *mandacaru* fruits, it was observed that contents of compounds such as quinic acid, rutin, *p*-coumaric acid, and epigallocatechin decreased in both fruits in the intestinal phase, while an increase in the concentration of catechin was observed for *xique−xique*.[38] Similarly, the concentration of rutin, kaempferol, quercetin-3-*O*-neohesperidosyl-rhamnoside, epigallocatechin-glucoside, isorhamnetin glucosyl-rhamnosyl-rhamnoside, and other phenolic compounds also decreased after the digestive process of whole fruit extracts of cactus berry (*Myrtillocactus geometrizans*).[39] In another study, it was found that the total content of isorhamnetin derivates, quercetin derivates, and kaempferol derivates in cladodes of *Opuntia Ficus-indica* reduced by approximately 62%, 65%, and 49%, respectively, after gastrointestinal digestion.[32]

In Vitro Colonic Fermentation. As can be seen in Figure 3, the *xique−xique* and *mandacaru* fruit caused a greater reduction in pH throughout the in vitro colonic fermentation process when compared to the control sample ($p \leq 0.05$). The greatest reduction was observed for *mandacaru* fruit, which resulted in a final pH value of 5.89 after 48 h. *Xique−xique*, in turn, despite causing a greater reduction after 24 h (6.29) when compared to the control (6.60) and *mandacaru* (6.35), presented a final pH of 6.11. The highest pH was observed for the control treatment throughout the entire period evaluated. These results may indicate the prebiotic potential of *xique−xique* and *mandacaru* fruits since according to Martins and colleagues, the reduction in pH may indicate the potential of a substrate to be used as a carbon source, stimulating the growth and metabolism of probiotic microorganisms.[15] Furthermore, pH reduction is an important parameter to determine the functional potential of a substrate. Fermentation of prebiotic foods by the intestinal microbiota leads to the production of metabolites such as short-chain fatty acids (SCFAs), lactate, and gases such as CO_2 and H_2, which can result in a decrease in colonic pH (approximately 5.7). This acidification plays an important role in regulating cellular processes, as well as selectively stimulating the growth and activity of intestinal bacteria potentially associated with improved host health.[40,41]

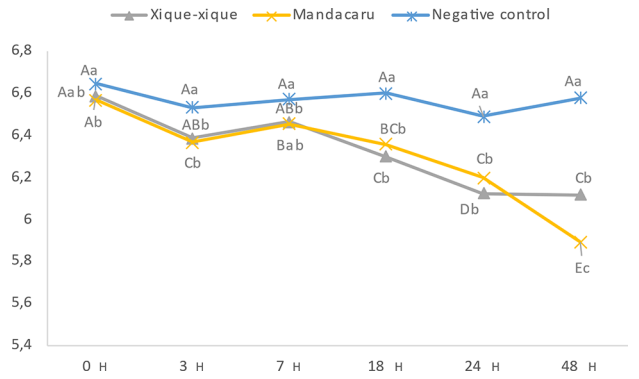


Figure 3. pH of *xique−xique* and *mandacaru* fruits during the in vitro colonic fermentation process. Capital letters indicate statistical difference between the treatment at different times by the Tukey's test at 5% significance ($p \leq 0.05$). Lowercase letters indicate statistical difference between treatments at the same time by the Tukey's test at 5% significance ($p \leq 0.05$).

The pH results found in this study are higher than those observed by Martins et al., who, when studying the prebiotic potential of *mandacaru* cladode flour, observed a final pH of 4.5 after 48 h. According to the authors, the results found for this sample were even higher than the pH values found for samples containing sugars such as fructooligosaccharides and glucose (final pH of 3.5).[15] On the other hand, the results observed for *xique−xique* and *mandacaru* were lower than those observed by Sánchez-Recillas and collaborators, using samples of *Garambullo* (*M. geometrizans*), a species of cactus native to Mexico, did not observe a reduction in pH during the in vitro colonic fermentation process, and the final pH value was 7.28; higher than that observed for the control sample (6.83) after 24 h of fermentation.[42] Therefore, it can be seen that the final pH after colonic fermentation depends on the samples used and, consequently, on their chemical composition.

Figure 4 presents the results regarding the content of total phenolics, total flavonoids, and antioxidant capacity throughout the in vitro colonic fermentation process. As can be seen, there was a statistical difference ($p \leq 0.05$) between the samples and the control. In addition, it was possible to verify a decrease in the content of total phenolic compounds in both fruits during the fermentation process. However, the greatest decrease was observed after 48 h for *xique−xique*, which decreased approximately 2.19-fold (from 5.38 to 2.45 mg EAG/g). At 48 h, the greatest decrease in the content of phenolic compounds was also observed for *mandacaru* (5.28 to 4.14 mg EAG/g); however, this decrease was smaller than that observed for *xique−xique* fruits.

Different behaviors could be observed for the total flavonoid content (Figure 4). In general, there was an increase in the total flavonoid content up to 7 h of fermentation for both samples evaluated, except for the control, which did not present a statistical difference throughout the entire period evaluated. *Mandacaru* presented the highest content of total flavonoids in 7 h (1.06 mg CE/g), this value being approximately 1.26-fold higher than that observed in the same period for *xique−xique* (0.84 mg CE/g). However, after 18 h of fermentation, a reduction in the total flavonoid content was observed for both fruits, with the lowest values being observed after 48 h. *Mandacaru* decreased by approximately 62%, while the decrease for *xique−xique* was even greater, around 83%.

The antioxidant potential of the samples was also influenced by the in vitro colonic fermentation process. As observed in Figure 4, the antioxidant capacity measured by the ABTS assay decreased over time. The lowest antioxidant capacity was observed after 48 h (17.69 μmol TE/g for *mandacaru* and 14.79 μmol TE/g for *xique−xique*). The reduction in the antioxidant potential of the samples may be related to the decrease in the content of phenolic compounds since these compounds are secondary metabolites of plants, recognized for their antioxidant capacity.[8] In turn, the decrease in these compounds may be related to their use by microorganisms since they have prebiotic potential and can be metabolized by the colon microbiota, thus favoring the development of microorganisms that are beneficial to the host's health, in addition to promoting anti-inflammatory, antioxidant, and antiproliferative actions.[43]

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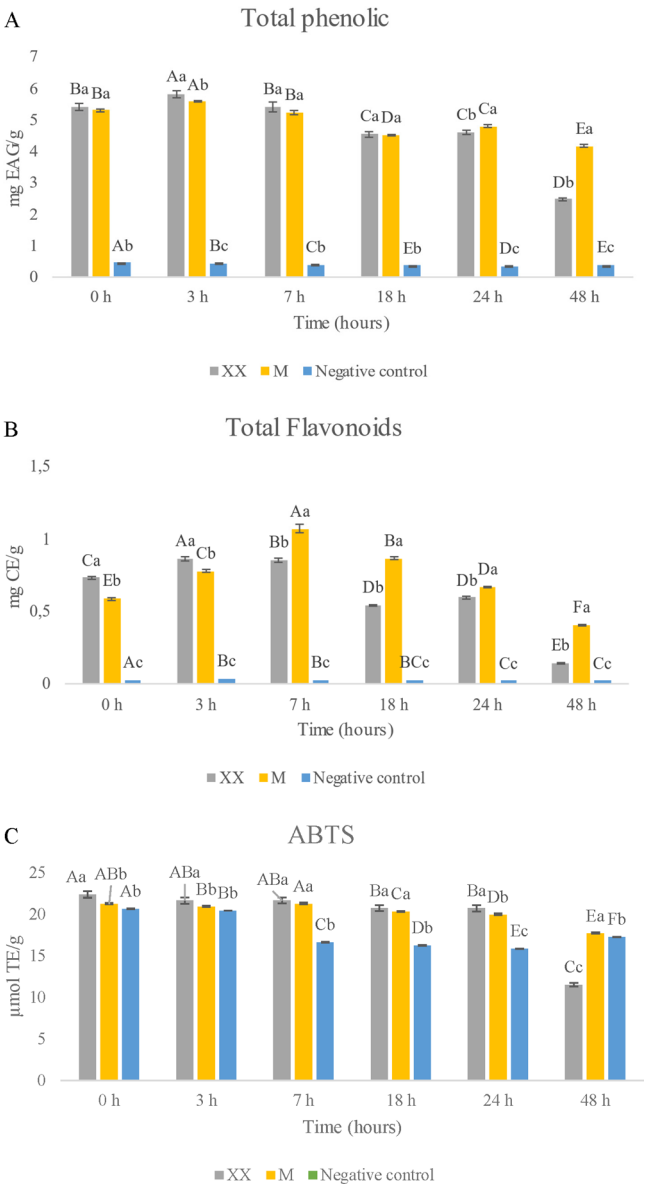


Figure 4. Total phenolics (A), total flavonoids (B), and antioxidant capacity (C) of the *xique–xique* and *mandacaru* fruits during the in vitro colonic fermentation. Capital letters indicate statistical difference between the treatment at different times by the Tukey's test at 5% significance ($p \leq 0.05$). Lowercase letters indicate statistical difference between treatments at the same time by the Tukey's test at 5% significance ($p \leq 0.05$).

Little information can be found in the literature about the prebiotic potential of species from the Cactaceae family; moreover, this is the first study to evaluate the prebiotic potential of *xique–xique* and *mandacaru* fruits. The prebiotic potential of the flour obtained from *mandacaru* cladodes was previously studied by Martins et al. According to the authors, the antioxidant potential decreased from 89.13 to 85.38% and from 62.33 to 54.29% after 48 h of fermentation, measured by the ABTS and DPPH tests, respectively.[15] de Sousa Rocha et al., when studying the potential prebiotic effects of cladodes of *Opuntia dillenii*, Cactaceae, commonly known as prickly pear, found a reduced content of phenolic compounds. However, different from the results observed in this study, they observed an increase in the antioxidant potential of the samples after 24 h of fermentation for the ABTS assay (from 0.22 ± 0.01 to 0.28 ± 0.01 μmol Trolox TEAC/g), and for the FRAP assay, the increase was even greater, approximately 1.45-fold (2.84 ± 0.02 to 4.13 ± 0.02 μmol $FeSO_4$/g).[44] In a study on the effects of in vitro colonic fermentation of phenolic compounds from light roasted coffee, it was found that the values of total phenolic compounds decreased significantly after 4 h of fermentation and then remained at similar levels (with some fluctuations) up to 24 h. As for total flavonoids, the values decreased significantly after 2 h of fermentation and gradually increased and then tended to remain stable after 8 h of fermentation. For the antioxidant activity, it was observed that the potential measured by the DPPH assay increased significantly after 4 h of fermentation and then remained at a similar level up to 24 h of fermentation.[45]

The content of phenolic compounds and their metabolites during in vitro colonic fermentation of *xique–xique* and *mandacaru* fruits can be observed in Figure 5. In general, for

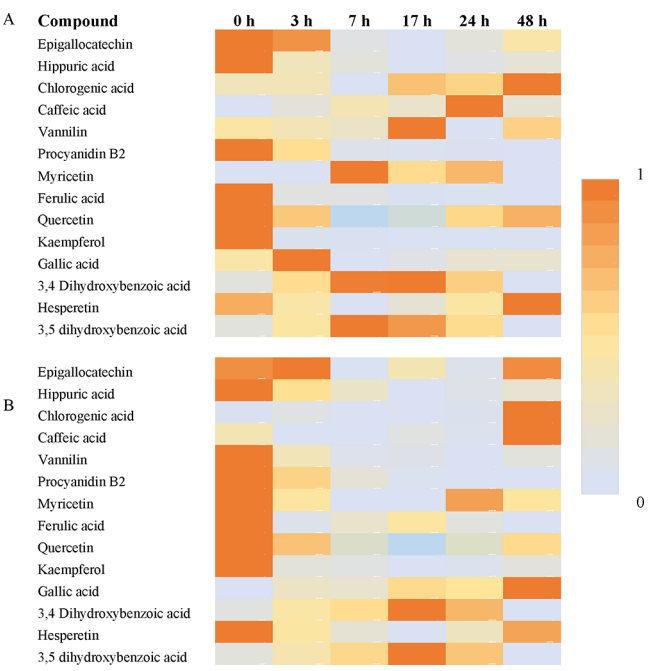


Figure 5. Release of phenolic metabolites during in vitro colonic fermentation of *xique–xique* (A) and *mandacaru* (B) fruits.

both fruits, the highest concentration for most of the phenolic metabolites tested can be seen at time zero, except for caffeic acid and myricetin in *xique–xique* and gallic acid, 3,4-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid in *mandacaru*. With the onset of batch fermentation, the concentrations became lower. In both samples, a moderate reduction in the concentration of these compounds can be observed after 3 h of batch fermentation, except for epigallocatechin in both fruits and gallic acid in *xique–xique*.

After 7 and 17 h of fermentation for *xique–xique* fruit, 3,4-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid showed increased concentration before declining at subsequent time points. Caffeic acid emerged as the dominant polyphenol at 24 h. By 48 h, chlorogenic acid and hesperetin exhibited higher concentrations than that at earlier time points, while procyanidin B2, myricetin, ferulic acid, and both forms of dihydroxybenzoic acid were among the least concentrated. In *mandacaru*, epigallocatechin was the most abundant at 3 h,

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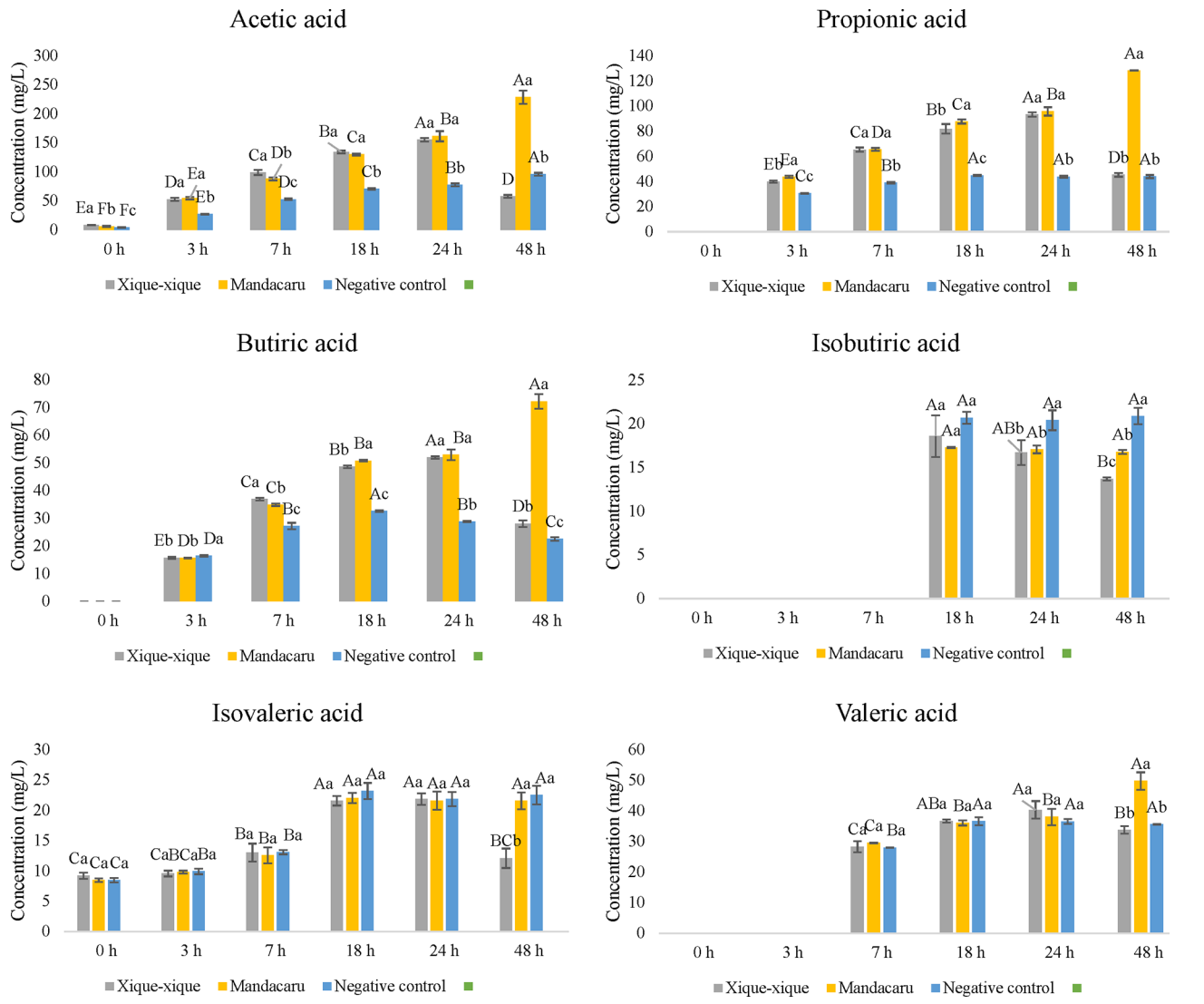


Figure 6. Production of short-chain fatty acids (SCFAs) during in vitro colonic fermentation of *xique–xique* and *mandacaru* fruits. Capital letters indicate statistical difference between the treatment at different times by the Tukey's test at 5% significance ($p \leq 0.05$). Lowercase letters indicate statistical difference between treatments at the same time by the Tukey's test at 5% significance ($p \leq 0.05$).

accompanied by moderate levels of other metabolites, while caffeic and ferulic acid were the least concentrated. At 7 h, all polyphenols reached their lowest concentrations. By 17 h, 3,4-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid were the most concentrated across all time points. After 48 h, chlorogenic acid, caffeic acid, gallic acid, hesperetin, and epigallocatechin dominated, while procyanidin B2, ferulic acid, and both forms of dihydroxybenzoic acid were the least concentrated.

The reduction in some phenolic metabolite levels over time is consistent with findings that polyphenols are degraded by microbial action, being transformed into simpler phenolic acids and metabolites that are readily absorbed. Studies on gut microbiota and polyphenol biotransformation, such as those by Tomás-Barberán et al. (2016), have documented this process extensively, noting that compounds like epicatechin and quercetin degrade into metabolites, enhancing bioaccessibility and potential health benefits.[46] Compounds such as caffeic acid and myricetin in *xique–xique* and 3,4-dihydroxybenzoic acid in *mandacaru*, showing increased concentrations during later time points, align with research that suggests microbial transformation processes or delayed release. This pattern has been observed with caffeic acid in particular, which tends to have a prolonged presence during fermentation The increase in contents of compounds like chlorogenic acid and hesperetin at later fermentation stages is also reported in the literature. Prolonged fermentations of dietary fibers and plant matrices show similar behavior, with chlorogenic acid derivatives releasing gradually, contributing to microbial stability and prolonged metabolic activity.[46−48] The presence of compounds such as myricetin and 3,4-dihydroxybenzoic acid over multiple fermentation stages may be due to microbial rebiosis, where microbial shifts influence polyphenol availability. This phenomenon, noted in studies on gallic acid and catechins, supports the idea of polyphenol−microbiome interactions influencing microbial degradation and synthesis over time.[49]

The results regarding the production of short-chain fatty acids (SCFAs) can be seen in Figure 6. SCFAs such as acetate, propionate, and butyrate are metabolites formed from the bacterial fermentation of prebiotics in the colon, and their concentration varies along the intestine, with the highest concentrations generally being observed in the proximal

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colon.[40] The production of SCFAs by probiotic bacteria plays an important role in maintaining host health since these metabolites can serve as an energy source for colonocytes and enterocytes, in addition to affecting the integrity of gastrointestinal epithelial cells, glucose homeostasis, lipid metabolism, appetite regulation, and immune function.[40,50] In general, carbohydrates and fiber are considered the main sources of energy for the gut microbiota; however, recent evidence has indicated that other compounds such as proteins and polyunsaturated fatty acids and bioactive compounds such as phenolic compounds may have prebiotic potential and stimulate the production of short-chain fatty acids.[51] Additionally, dietary fiber is often linked to antioxidant compounds, such as polyphenols (ADF), offering various health benefits. It is metabolized in the colon, where it serves as a prebiotic for the bacterial microbiota, promoting health benefits for the host.[52]

As shown herein, the fermentation of *xique−xique* and *mandacaru* fruits caused an increase in the production of short-chain fatty acids when compared to the control ($p \leq 0.05$), mainly in the content of acetate, propionate, and butyrate after 48 h of fermentation. The highest content of short-chain fatty acids was found for *mandacaru* fruit after 48 h, which presented an increase of approximately 38-, 2.93-, 4.62-, and 49.78-fold in the values of acetic, propionic, butyric, and valeric acids, respectively. In turn, for the *xique−xique* fruit, the highest contents of acetic, propionic, and butyric acid were found after 24 h of fermentation (155.25, 93.28, and 51.93, respectively). Despite presenting higher values than the control after 48 h, the contents of acetic, propionic, and butyric acids for the *xique−xique* fruit were, respectively, 74.75, 64.69, and 61.19% lower than the values observed for the *mandacaru* fruit. Regarding the levels of isobutyric and isovaleric acid, there was a small production of these acids throughout the in vitro colonic fermentation process, with the greatest production of these metabolites being observed in the control treatment after 24 and 48 h.

Previously, the potential of *xique−xique* cladode juice was tested in probiotic cultures. The results obtained demonstrated that the drink at a concentration of 20 g/L presented prebiotic potential, with the greatest production of lactic acid (increase from 1.55 to 3.40 g/L) and acetic acid (increase from 3.20 to 3.63 g/L) being found when incubated with *L. paracasei* L-10 after 48 h of fermentation.[16] Similarly, the *mandacaru* cladode flour also led to an increase in the production of SCFAs when used as a carbon source for different probiotic cultures. According to the authors, the highest production of these acids was observed when incubated with *L. acidophilus* LA-05, demonstrating an increase from 0.53 to 2.29, 2.03 to 2.47, and 1.21 to 1.66 g/L for lactic, acetic, and propionic acids, respectively.[15] In another study, the prebiotic potential of mucilage (MO) and pectic-derived oligosaccharides (PO) from *O. ficus-indica* cladodes was evaluated, and it was found that the SCFA production increased by 35% (71.05 mmol/L) and 16% (62.57 mmol/L), respectively, after 12 h of fermentation. The production of propionate and butyrate were the most affected, with the propionate content doubling during the MO treatment (21.57 mmol/L) and a 50% increase being observed with the PO treatment (15.52 mmol/L). In addition, the butyrate content increased by about 50% for both treatments (MO 19.06 and PO 12.72 mmol/L).[50] In another study carried out to verify the prebiotic potential of phenolic compounds from the peel of jaboticaba fruits (*Myrciaria trunciflora*), it was also possible to verify an increase in the production of short-chain fatty acids, mainly acetic, propionic, and butyric acids, after 48 h of fermentation.[11] According to the authors, the increase in the production of SCFA content is probably associated with the fermentation of phenolic compounds as well as soluble fiber present in the fruit,[11] which could also justify our results, considering that the fruits analyzed are excellent sources of polyphenols and have significant levels of dietary fiber (approximately 34% to 40%), of which approximately 29.55% and 30.48% are insoluble fibers, while 10.85% and 3.92% are soluble fibers in the *xique−xique* and *mandacaru* fruits, respectively.[3]

In conclusion, *xique−xique* and *mandacaru* fruits have high nutritional and functional value, and their bioactivity was influenced by the process of in vitro colonic digestion and fermentation. In general, an increase in the total phenolic content and antioxidant capacity was observed in the intestinal phase for both fruits. However, the flavonoid content showed distinct behavior after digestion, being higher in the intestinal phase for *mandacaru* and lower in *xique−xique*. A greater release of compounds such as vanillin, procyanidin B2, and quercetin was observed for both fruits in the intestinal phase. In contrast, the concentration of kaempferol, gallic acid, hesperetin, and caffeic acid decreased after the digestive process. During colonic fermentation, the fruits were responsible for a greater reduction in the pH of the medium. Likewise, there was a decrease in the total content of phenolic compounds and flavonoids, which probably contributed to the reduction in the observed antioxidant capacity. Some phenolic compounds, such as myricetin, kaempferol, procyanidin B2, hippuric acid, vanillin, and ferulic acid, appear to have been used by the microbiota, resulting in a reduction in their concentrations throughout the fermentation process. On the other hand, a greater production of phenolic metabolites was observed, including chlorogenic acid, caffeic acid, and gallic acid, in addition to a significant increase in the production of short-chain fatty acids, mainly acetic acid, propionic acid, and butyric acid.

Although the prebiotic potential of *xique−xique* and *mandacaru* fruits was demonstrated in this study, it is essential to carry out further investigations to understand how the ingestion of these fruits influences the gut microbiota and its interaction with human health. Techniques such as the use of the Simulator of the Human Microbial Ecosystem (SHIME) and 16S genetic sequencing analysis may be essential in this process. These studies are crucial to confirm the true potential of these fruits as functional foods, which will undoubtedly positively impact their nutritional value and their viability for commercial exploitation.

■ ASSOCIATED CONTENT

Data Availability Statement

All data supporting the conclusions of this study are available from the corresponding author upon reasonable request.

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Notes

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