Food Research International 175 (2024) 113666

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Anonna crassiflora suppresses colonic carcinogenesis through its antioxidant effects, bioactive amines, and phenol content in rats

Patrícia Felix Ávila [a], Angélica Pereira Todescato [a], Mylena de Melo Carolo dos Santos [b], Luiz Fernando Ramos [b], Isabella Caroline Menon [b], Michele Oliveira Carvalho [a,b], Maysa do Vale-Oliveira [c,d], Flávia Beatriz Custódio [d], Maria Beatriz Abreu Gloria [e], Bruno Martins Dala-Paula [a], Pollyanna Francielli de Oliveira [a,b,*]

[a] *Programa de Pós-graduação em Nutrição e Longevidade, Universidade Federal de Alfenas, Alfenas, UNIFAL, Rua Gabriel Monteiro da Silva, 700, Centro, Alfenas, MG 37130-001, Brazil*

[b] *Laboratório de Genética Humana, Instituto de Ciências da Natureza, Universidade Federal de Alfenas - UNIFAL, Rua Gabriel Monteiro da Silva, 700, Centro, Alfenas, MG 37130-001, Brazil*

[c] *Universidade Federal do Espírito Santo (UFES) campus São Mateus, BR-101, km 60 - Litorâneo, São Mateus, ES 29932-540, Brazil*

[d] *BioTox - Laboratório de Bioquímica e Toxicologia de Alimentos, Departamento de Alimentos, Faculdade de Farmácia, Universidade Federal de Minas Gerais - UFMG, Av. Presidente Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil*

[e] *Laboratório de Controle de Qualidade (LCQ), Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais - UFMG, Av. Presidente Antônio Carlos 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil*

ARTICLE INFO

Keywords:
Marolo
Antioxidant activity
Spermidine
Aberrant Crypt Foci
Chemoprevention

ABSTRACT

Marolo (*Annona crassiflora*) is an underutilized Brazilian Cerrado fruit with few reports in the literature about its bioactive compounds and functional properties. In this context, the chemoprevention against the carcinogen 1,2-dimethylhydrazine (DMH)-induced pre-neoplastic lesions in Wistar rat colon was investigated and correlated with marolo's antioxidant activity and the contents of phenolic compounds and bioactive amines. Total phenolic compounds (TPC) and total flavonoids compounds (TFC) were determined in the marolo pulp extract by spectrophotometric and Ultra-Performance Liquid Chromatography and diode array detection (UPLC-DAD) analysis. Free bioactive amines were determined by High Performance Liquid Chromatography and fluorescence detection (HPLC-FLD) after post column derivatization with o-phthalaldehyde. In addition, the *in vitro* antioxidant activity was determined by DPPH, and ABTS. Wistar rats were treated orally with marolo pulp at 0.7, 1.4 and 2.8 g/kg body weight (bw)/day added to a standard ration. Four subcutaneous injections of DMH (40 mg/kg bw) were used to induce a pre-neoplastic lesion that was assessed by the aberrant crypt foci (ACF) assay. The marolo pulp (fresh weigh) showed high content of total phenolic compounds (9.16 mg GAE/g), with predominance of chlorogenic acid (1.86 μg/g) and epicatechin (0.99 μg/g), and total flavonoids (7.26 mg CE/g), ~85 % of the TPC. The marolo pulp had significant contents of tyramine (31.97 mg/kg), putrescine (20.65 mg/kg), and spermidine (6.32 mg/kg). The marolo pulp inhibited ($p < 0.05$) pre-neoplastic lesions induced by DMH administration at the all concentrations tested. These findings indicate that marolo pulp has a colon carcinogenesis chemopreventive effect, which could be due to, at least in parts, its antioxidant action associated with its phenolics and flavonoids content as well of spermidine.

1. Introduction

Cerrado or Brazilian savanna is the second largest biome present in Brazil, encompassing more than twelve states and occupying approximately 25 % of the total land area of Brazil. Its native flora is characterized by twisted and small trees and it is composed by more than 4000 unexplored species. Due to the large number of endemic species and the continuous threat of extinction, Cerrado was considered a biodiversity hotspot, and, therefore, a priority conservation area (Arruda et al., 2023). Studies involving plants from Cerrado are essential, as they increase the appreciation for this rich biome and, consequently, stimulate its preservation (Prado et al., 2020; Arruda et al., 2019; Arruda,Pereira

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* Corresponding author at: R Gabriel Monteiro da Silva, 700, Centro, Alfenas, MG 37130-001, Brazil.
E-mail address: pollyanna.oliveira@unifal-mg.edu.br (P. Francielli de Oliveira).

https://doi.org/10.1016/j.foodres.2023.113666
Received 26 July 2023; Received in revised form 31 October 2023; Accepted 3 November 2023

& Pastore, 2018; Roesler et al., 2007).

Marolo (*Annona crassiflora*), member of the Annonaceae family, is a native plant of the Brazilian Cerrado and is amongst the most consumed fruits in the region. The pulp can be consumed *in natura* or it can be used in the manufacture of food products, such as ice cream and jellies (Arruda et al., 2019). The fruits are known to have high nutritional value and to be rich in phenolic compounds (Arruda, Pereira & Pastore, 2018), carotenoids, vitamins (Cardoso et al., 2013) and bioactive amines (Dala-Paula, Tavano, & Glória, 2019). Among the food components with health promoting properties, marolo is rich in phenolic compounds, well known for their antioxidant activity (Almeida et al., 2011; Arruda & Pastore, 2019). The presence of other bioactive compounds and their interactions can significantly increase the antioxidant activity, but these properties are poorly investigated in marolo (Arruda et al., 2023; Prado et al., 2020).

Bioactive amines are organic bases of low molecular mass and comprised of polyamines (e.g., spermidine – SPD and spermine - SPM) and biogenic amines (tyramine – TYM, putrescine - PUT, histamine – HIM, tryptamine – TRM, phenylethylamine – PHM, cadaverine - CAD). They show high biological activity and, therefore, some are needed for health and survival of plants and humans(Dala-Paula Starling & Gloria, 2021) . SPD exerts antioxidant activity in biological systems through radical scavenging properties, has prominent cardioprotective and neuroprotective effects and stimulates anticancer immunosurveillance in rodent models, thereby contributing to longer life span and healthier aging (Dala-Paula, Starling & Gloria, 2021; Madeo et al., 2018). For instance, epidemiological studies have shown that a polyamine-rich diet is associated with a decreased incidence of breast and colon cancer (Soda, 2022).

Colon carcinogenesis is a multistep process that involves genetic alterations and successive mutations that are translated into a specific morphological sequence, starting with the formation of an adenoma and progression to carcinoma (Lamichhane et al., 2020). The development of chemically induced colon cancer in rodents is also a multistep process that involves a series of pathological alterations ranging from discrete microscopic mucosal lesions such as aberrant crypt foci (ACF) to malignant tumors. These ACF are pre-polyp abnormalities considered preneoplastic lesions because they are present in the colon of rodents treated with chemical carcinogens (Clapper, Chang & Cooper, 2020). In this context polyamines have many biological activities that may inhibit progression of aberrant DNA methylation acting as potential chemopreventive agents on colon carcinogenesis. However, clinical studies with this potential are still missing (Soda et al., 2013; Soda, 2018; Soda, 2022).

The influence of marolo consumption in the modulation of mutagenic/antimutagenic *in vivo* oxidative stress induced by the alkylating agent cyclophosphamide was investigated by Dragano et al. (2010). They did not find either mutagenic or antimutagenic effects on bone marrow cells, however, a potentiation effect was observed with cyclophosphamide. However, scarce information is available for colonic tumorigenesis, in which one important target is inflammation. This process generates proinflammatory cytokines and reactive oxygen and nitrogen species that contribute to oxidative stress, affecting DNA, RNA, lipids, and proteins. Consequently, inflammation provides a favorable microenvironment for malignant transformation and uncontrolled cell proliferation (Lee & Lawrence, 2018). Antioxidant protection against free radical damage is vital to the integrity of cell structures as it interferes with normal cell signaling cascades in tumor formation, affecting activation or expression of upstream transcription factors and kinases (Oliveira et al., 2021). Therefore, both phenolic compounds and amines are considered potent antioxidants and could help prevent the inflammation process in the cells (Prado et al., 2020; Soda, 2022).

Considering the scarcity of studies covering the biological potential of bioactive compounds from marolo pulp, the aim of this study was to investigate the possible protective effect of phenolic compounds and bioactive amines from marolo pulp on colorectal carcinogenesis induced in Wistar rats.

2. Material and methods

2.1. Reagents and chemicals

All reagents and solvents used had a degree of purity compatible with the respective analysis. Standards from each analyte had a degree of purity $\geq$ 95 %.

2.2. Plant material, sample preparation and physico-chemical characterization

The fruits (*Annona crassiflora*) were collected in the rural area of Machado (21°41′58.59″ south latitude, 45°49′14.32″ west longitude), Minas Gerais, Brazil. The fruits were transported to the laboratory up to 24 h after harvesting, washed with distilled water, and manually separated into pulp, seeds, and peel (Fig. 1). The pulp was homogenized in a food processor (Model Ri7630, Philips Walita, São Paulo, SP, Brazil) and was conditioned in plastic bags for subsequent analysis. In a previous study the pulp was characterized regarding some physico-chemical parameters to establish the most suitable ripening stage of the fruit.

2.3. Extraction procedure for the analysis of phenolic compounds and antioxidant activity

The fruit pulp was weighed (2 g) and mixed with 10 mL of methanol/acetone/water (7:7:6 v/v/v) solution and shaked in a vortex for 1 min (Global Trade Technology XH-DU model, Jaboticabal, São Paulo, Brazil). The mixture was kept in an ultrasound bath at 25 °C for 30 min and centrifuged at 4000 x *g* for 5 min at 4 °C (Fanem Excelsa Baby Centrifuge Model 206-BL, Guarulhos, São Paulo, Brazil) and the supernatant was retrieved. Afterwards, 10 mL of the methanol/acetone/water solution was added to the residue, incubation and centrifugation were repeated twice more under the same conditions described above. The collected supernatants were mixed resulting in a final volume adjusted to 30 mL using methanol/acetone/water solution.

2.4. Determination of total phenolics content (TPC)

The TPC of the marolo pulp extract (MP-E) was determined according to the Folin-Ciocalteu assay described by Wang et al. (2020) with minor adaptations. A calibration curve with gallic acid at 1 to 10 μg/mL was prepared ($R^2 \geq 0.9910$). The gallic acid solutions or the diluted samples (160 μL) were mixed with the Folin-Ciocalteu reagent (80 μL) and sodium carbonate (640 μL, 75.0 g/L). After 30 min of reaction protected from light, the absorbance was read at 756 nm using a BelPhotonics Ultraviolet/Visible-M51 spectrophotometer (Monza, Milan, Italy) at 25 °C. The TPC was expressed as mg of gallic acid equivalents per g of marolo fresh pulp (mg GAE/g of marolo fresh pulp).

2.5. Determination of total flavonoids content (TFC)

The TFC of the MP-E was determined according to Boateng et al. (2008). The aliquots of adequately diluted samples (650 μL) were mixed in a test tube with sodium nitrite (37.5 μL, 5 % w/v) and incubated for 5 min at 25 °C in the dark. After the incubation period aluminum chloride solution (37.5 μL, 10 % w/v) was added, the solution was homogenized and incubated again for 5 min. Sodium hydroxide (250 μL, 1 mol/L) and 350 μL ultrapure water were added to the test tube, the mixture was homogenized and incubated for 5 min. Next, absorbance was read in a BelPhotonics Ultraviolet/Visible-M51 spectrophotometer, at 510 nm. A catechin standard curve at 4.5 to 46 μg/mL was constructed ($R^2 \geq 0.9961$) and the results were expressed as mg of catechin equivalent per g of marolo fresh pulp (mg CE/g of marolo fresh pulp).

Fig. 1. *Anonna crasiflora* Mart. fruit appearance (A), yellowish pulp after pell removing (B), seeds (C) and median sagittal cut highlighting whitish pulp surrounding the seeds (D).

2.6. *UPLC-DAD analysis of the phenolic compounds*

Ultra-Performance Liquid Chromatography coupled with diode array detection (UPLC-DAD) analyses of the phenolic compounds on the MP-E was performed on a Waters ACQUITY UPLC (Waters Corporation, Milford, MA, USA) consisting of a quaternary pump, on-line degasser, autosampler, column temperature controller (35 °C) and a diode array detector set at 271 and 320 nm. Separation was achieved on an Acquity BEH, C18 column (2.1 × 50 mm, 1.7 μm).

Two different methods were used in the separation of phenolic compounds. The first one was used to identify catechin, chlorogenic acid, and gallic acid. The chromatographic conditions were injection volume of 1 μL; mobile phase components were water/formic acid (99.75:0.25, v/v) as eluent A and acetonitrile/formic acid (99.75:0.25, v/v) as eluent B using an isocratic gradient of 95 % of A and 5 % of B, for 8 min, at a flow rate of 0.20 mL/min.

The second method was used to analyze six phenolic compounds (caffeic acid, epicatechin, ferulic acid, *p*-coumaric acid, rutin, and quercetin). The same eluents, flow rate and injection volume of the first method were used. However, the mobile phases gradient conditions were as follows: 0–14 min, 10–30 % B; 14–16 min, 30–70 % B; 16–18 min, 70–100 %; 18–19 min, 100–10 % B.

For both analytical conditions, phenolic compounds were quantified by interpolation in external analytical curves ($R^2 \geq 0.995$) using seven points of standards (1–7 μg/mL), in triplicate. Data were processed using Empower® software and the results were expressed in μg/g of fresh fruit.

2.7. *Bioactive amines determination*

Free bioactive amines were extracted from fruit pulp (5 g) by three successive extractions with 7 mL 5 % trichloroacetic acid (TCA) followed by stirring for 5 min, and centrifugation at 11,180 × *g* at 4 °C/10 min. The supernatants were collected into a 25 mL volumetric flask and filtered through a 0.45 μm filtering membrane. The separation and quantification of nine free bioactive amines in the filtered extract were performed by High Performance Liquid Chromatography and fluorescence detection (HPLC-FLD). The system consisted of a CBM-10 AD controller (Shimadzu Corp., Kyoto, Japan). Luna C18 Phenomenex column (4.6 x 250 mm, 5 μm) and a C18 (4x3 mm) pre-column were used in an oven (CTO-10 ASvp, Shimadzu, Kyoto, Japan) at 30 °C. An auto-injector (SIL-20AHT, with the capacity of 105 1.5-mL vials and a HPLC interface control unit (CBM-20A) were also used.

The mobile phases used were A solution of 0.2 M sodium acetate and 15 mM sodium octanesulfonic acid, pH adjusted to 4.9 with acetic acid; and B, acetonitrile. The gradient was: 0.01–17.99 min/2% B; 18.00–18.99 min/20 % B; 19.00–39.99 min/5% B; 40.00–49.99 min/23 % B; 50.00–50.49 min/35 % B; 50.50–60.00 min./2% B. A post-column derivatization system with *o*-phthaladehyde, and fluorescence detection (RF-551 spectrofluorimetric detector), at 340 and 445 nm of excitation and emission, respectively, were used as described by Dala-Paula et al. (2021). Quantification was undertaken by interpolation in external standard curves ($R^2 \geq 0.9931$) constructed for each of the nine amines (putrescine, cadaverine, agmatine, spermidine, serotonin, phenylethylamine, tryptamine, tyramine, histamine) at nine different concentrations.

2.8. *Antioxidant activity*

2.8.1. *DPPH radical scavenging assay*

The DPPH radical-scavenging assay was used as described by Margraf et al. (2016) with minor adaptations. MP-E aliquots at adequate dilutions (100 μL) were added to a test tube with 650 μL of 2,2-diphenyl-

1-picrylhydrazyl (DPPH; 0.1 mM) prepared in methanol. The samples were incubated protected in the dark for 30 min and the absorbance was measured in a BelPhotonics Ultraviolet/Visible-M51 spectrophotometer at 510 nm, using the same extractor solution as a negative control. The standard curves were prepared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at (1–14.7 nmol/mL). The results were expressed as micromols of Trolox equivalents per gram of marolo pulp (μmol TE/g of marolo pulp).

2.8.2. *ABTS radical cation assay*

The MP-E extracts were submitted to the ABTS radical cation assay . The ABTS radical cation was obtained by mixing 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) solution (7 mM, 5000 μL) with potassium persulfate solution (2.45 mM, 88 μL), followed by incubation for 12 h in the dark at room temperature. Afterwards, the ABTS radical cation solution was diluted in ultrapure water until reaching the absorbance of 0.700 ± 0.005 at 734 nm (ABTS work solution). Aliquots of suitably diluted samples of MP-E (250 μL) were added to the ABTS work solution (750 μL) in a tube test and the absorbance was measured in a BelPhotonics Ultraviolet/Visible-M51 spectrophotometer. The standard curves were prepared with Trolox standard (1–17 nmol/mL). The results were expressed as μmol TE/g of marolo pulp.

2.9. *Chemopreventive activity evaluation*

Male Wistar rats (*Ratus norvegicus*) with an average of 200 g body weight (bw) were fed with a standard pellet diet and kept in plastic boxes under controlled conditions of temperature (23 ± 2 °C) and humidity (50 ± 10 %), 12 h light–dark cycle, and *ad libitum* access to food and water. The treatment protocol was reviewed and approved by the Ethics Committee on Animal Use of the Federal University of Alfenas, Brazil (0039/2021). The application of 1,2-dimethylhydrazine hydrochloride (DMH) was performed by subcutaneous (sc) injection, on the back of the animals, at four doses of 40 mg/kg bw, administered on the second and fifth days of the first two weeks Oliveira et al. (2023) to induce the preneoplastic lesions in the colorectal tissue. The period for evaluation of the effect on colonic carcinogenesis lasted a total of 4 weeks, which is a sufficient period for the observation of aberrant crypt foci (ACF) formation(Oliveira et al., 2023; Rodrigues et al., 2002) .

Among the studied groups of rats used (n = 6 per group), a positive control [PC, DMH 40 mg/kg body weight (bw)] and a negative control group (NC) received only commercial standard ration. The remaining groups received standard ration containing marolo pulp at doses of 0.7 g/kg/bw/day, 1.4 g/kg/bw/day and 2.8 g/kg/bw/day for 4 weeks. These doses were established based on the daily fruit consumption recommended for men (FAO, 2005). A 1/2 portion of fruit was equivalent to 50 g, 1 portion to 100 g and two portions to 200 g for an individual with 70 kg, equivalent to doses of 0.7, 1.4 and 2.8 g/Kg bw/day, respectively. At the end of the experiment, the animals were euthanized with ketamine (300 mg/kg bw) and xylazine (30 mg/kg bw), the colons were removed, opened longitudinally, and washed with 0.9 % NaCl. Each colon was pinned flat, fixed with 10 % phosphate buffered formalin for 24 h. Each colon was cut into three parts (proximal, middle, and distal) and then stained with 0.2 % methylene blue dissolved in Phosphate saline buffer (PBS) and stored for the ACF assay. The total number of ACF and aberrant crypts (AC) were determined under light microscopy at 10x magnification. The number of ACF and aberrant crypts (AC) in 50 sequential fields were counted on the distal colon, with ACFs characterized by a thick epithelial layer that stains more intensely with methylene blue, and elongated, tortuous, and larger luminal opening relative to surrounding normal crypts (Oliveira et al., 2023).

2.10. *Statistical analysis*

The analytical determinations were performed in triplicate and the results were expressed as mean ± standard deviation. The ACF and AC data were statistically analyzed by analysis of variance (One-way ANOVA), with the calculation of the F statistic and its respective *p*-value. In cases where $p < 0.05$, treatment means were compared by the Tukey's test, with the minimum significant difference defined for $\alpha = 0.05$, using GraphPad Prism® version 8.2.2.

3. Results and discussion

The marolo pulp had high moisture content 74.36 ± 0.37 g/100 g, considerable soluble solids/titratable acidity ratio (36.44) and pH value of 4.41, therefore it was considered fully mature (Della Lucia et al., 2023).

3.1. *Total phenolics and flavonoid compounds in marolo pulp*

The Folin–Ciocalteau assay allowed the estimation of the sum of flavonoids, anthocyanins, and other phenolic compounds present in the sample. The mean of total phenolic compounds (TPC) for the marolo pulp was 9.16 ± 0.36 mg GAE/g fresh weight (fw) (Table 1). According to Vasco et al. (2008), fruits are classified into three categories based on their TPC: low (<1 mg GAE/g fw), medium (1–5 mg GAE/g fw), and high (>5 mg GAE/g fw). Therefore, marolo pulp can be considered as an excellent source of phenolic compounds. The samples had higher TPC levels compared to the marolo fruits from Brasília, Brazil (5.80 ± 1.43 mg GAE/g fw) Siqueira et al. (2013) and from Minas Gerais, Brazil (2.11 ± 0.60 mg GAE/g fw) (Damiani et al., 2011). Compared to other native fruits from Cerrado such as buriti, cagaita, cajá and coquinho (0.58 to 1.73 mg GAE/g fw) Nascimento et al. (2020), marolo pulp was the one with higher TPC levels. The difference in the marolo TPC levels compared to literature values may be due to the edaphoclimatic conditions during cultivation, physiological and genetic factors of the plant, preparation and storage conditions, and extraction method (Carvalho et al., 2022).

Among the several groups of phenolic compounds present in nature, flavonoids stand out for being the most common and widely distributed in the plant kingdom. They are found in practically all plant parts, especially in photosynthetic cells (Arruda, Pereira & Pastore, 2018; Kumar & Pandey, 2013). TFC levels in the marolo pulp (7.26 ± 0.37 CE mg/g fw), accounted for approximately 85 % ($R_{TFC\text{-}TPC} = 0.85$) of the TPC (Table 1). The levels found are like those reported by Siqueira et al. (2013) (5.49 mg CE/g fw). Compared to other fruits from the Brazilian Cerrado, TFC was like tucum (7.16 mg CE/g) and considerably higher than cagaita, mangaba and guariroba (0.02–0.03 mg CE/g) (Siqueira et al., 2013). Flavonoids are often associated with the reduction of the incidence of mortality from cardiovascular diseases (Prado et al., 2020). In addition, there is a strong evidence for the carcinogenesis-preventive effect of quercetin, a flavonoid with strong antioxidative property (Chondrogianni et al., 2010). According to Kurzawa-Zegota et al.

Table 1
Total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant activity by DPPH and ABTS•+ assays found in marolo pulp.

Analytical parameters	Content (fw)
TPC (mg GAE/g)	9.16 ± 0.36
TFC (CE mg/g)	7.26 ± 0.37
$R_{TFC\text{-}TPC^{*}}$	0.85 ± 0.73
$ABTS^{\bullet +}$ (μmol TE/g)	66.15 ± 5.96
DPPH (μmol TE/g)	65.94 ± 4.23

RTFC-TPC: relationship between total flavonoids and total phenolic compounds; $ABTS^{\bullet +}$- 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH- 2,2-diphenyl-1-picrylhydrazyl; TE- Trolox equivalent (μmol/g); GAE - Gallic acid equivalent (mg/g); CE - Catechin equivalent (mg/g). Data represent mean values ± standard deviations – SD (n = 3) expressed in fresh weight (fw) of marolo pulp.

(2012), the daily intake of 23 mg quercetin leads to a carcinogenesis-preventive effect of 70 %.

3.2. Phenolic profile by UPLC-DAD

Seven different polyphenols (out of 9) were identified and quantitated by UPLC-DAD in the marolo pulp (Table 2). The one found at the highest concentration was chlorogenic acid (1.86 ± 0.03 µg/g) representing around 40 % of phenolics compounds identified in the pulp, followed by epicatechin (0.99 ± 0.11 µg/g, 21 %). Caffeic, *p*-coumaric and ferulic acid were the phenolic acids identified in smaller amounts while quercetin and rutin were the less abundant flavonoids (Table 2). Illustrative chromatograms are available in the supplementary material (**Figure S1**).

Ramos et al. (2023) detected a different phenolic profile in marolo pulp from four different cities in Minas Gerais (Brazil), by mass spectrometry and paper spray ionization. These results corroborate that several factors can affect the profile of phenolic compounds in fruits, including environmental factors, such as temperature, light intensity, soil, harvest time and plant part (Demir et al., 2022; Nkhata Malunga et al. (2022). In addition, the method, and the extraction, including solvent, and others variables, can affect the results (Chen, Zhang, Chen, Han, & Gao, 2017).

Chlorogenic acid (3-*o*-caffeoyl-quinic acid) was the major phenolic compound identified in the marolo pulp. It is a naturally occurring non-flavonoid polyphenol in green coffee beans, teas, certain fruits, and vegetables (Rashidi et al., 2022). According to Sato et al. (2011) it showed a stronger superoxide anion (O_2–)-scavenging activity compared to ascorbic acid and α-tocopherol, in ischemia–reperfusion injury.

Another compound found at significant amounts was epicatechin. This flavonoid is also present in apples, blackberries, broad beans, cherries, black grapes, pears, raspberries and chocolate (2–48 mg/100 g) (Gadkaria & Balaraman, 2015).

3.3. Antioxidant activity

DPPH and ABTS radicals are the two most widely used chromogenic compounds to determine the antioxidant activity of biological materials, including studies correlations with antioxidant of natural sources with human colon cancer cell line and antitumoral effect (De Sousa et al., 2004; Encalada et al., 2011). Both were characterized by considerable reproducibility and stability under certain assay conditions. (Encalada et al., 2011). The protective effect against colon carcinogenesis of phenolic compounds and amine could be explained in part by their antioxidant activity, because they may alter the levels of reactive oxygen species generated by cancer cells. Therefore, natural antioxidants are emerging as an option to selectively target carcinogenic cells.

A DPPH radical scavenging activity of 65.94 ± 4.23 µmol TE/g fresh weight was found for the marolo pulp, and it was 65.94 ± 5.96 µmol TE/g for the cation radical ABTS (Table 1). A previous study with marolo pulp from Brasilia reported similar values, e.g., 54.3 and 67.3 µmol TE/g for the ethyl acetate and aqueous extracts, respectively (Siqueira et al., 2013).

Regarding the ABTS assay, the marolo pulp showed lower antioxidant activities than other marolo extracts, also reported in fresh weight, equivalent to 131.58 ± 19.61 µmol TE/g Souza et al. (2012). Compared to other non-traditional Brazilian fruits rich in antioxidants, the results for marolo are similar to açaí mangaba, umbu, cashew (64.5 to 79.4 µmol TE/g); lower than jambolão gurguri, puçá-coroa-de-frade, murta, uvaia, jabuticaba, puçá-preto, murici, juçara and acerola (125 to 953 µmol TE/g); and higher than camu-camu, carnauba, bacuri, cajá (1.23 to 18.1 µmol TE/g) Rufino et al. (2010). The different antioxidant activity reported for the Brazilian fruits result from several factors, including botanic origin, environmental aspects, ripening, variety, synergistic and antagonistic effects and extraction method (Silva, Vilas Boas & Xisto, 2013).

The marolo pulp had compounds with high antioxidant potential, which justifies the considerable antioxidant activity found. In addition, several studies have shown a high correlation between the amount of TPC and the antioxidant activity (Almeida et al., 2011; Arruda, Pereira & Pastore, 2017; Rufino et al., 2010; Roesler et al., 2007). However, other compounds such as carotenoids, tocopherols, organic acids, which are present in marolo but not included in this study, could be present in the extracts studied and, thus, they could contribute to the antioxidant potential of the marolo pulp (Prado et al., 2020).

The antioxidant activity of food products must be evaluated by more than one method so that the different characteristics based on the stable free radical scavenge (for example, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$) by antioxidants and it was possible to obtain useful information about the antioxidant activity of all compounds present in the food matrix. Both methods are based on sample antioxidants' ability to reduce the radicals by electron transferences and/or hydrogen atoms measured by absorption, which decrease at 517 nm (DPPH) and 734 nm (ABTS) (Moo-Huchin et al., 2014).

3.4. Bioactive amines in marolo pulp

Among the nine investigated amines, three were detected in the marolo pulp (Table 3). The monoamine TYM was predominant (31.97 ± 0.52 mg/kg), representing 54.2 % of the total amines, followed by the diamine putrescine (PUT) (20.65 ± 1.06 mg/kg) 35.1 %, and the polyamine spermidine (SPD) (6.32 ± 0.59 mg/kg) 10.7. To the best of our knowledge, this is the first study of bioactive amines in marolo. Representative chromatograms are available in the supplementary material (**Figure S2**).

With respect to the levels of these amines in the marolo pulp compared to other fruits and vegetables, marolo had higher PUT content compare to mango, pineapple, papaya, passion fruit, broccoli, cauliflower, tomato, *jiló*, parsley, spinach, capers, cassava and heart of palm

Table 2
Levels of some phenolic acids and flavonoids in marolo pulp, chromatographic conditions, linearity and limits of quantification and detection.

Class	Phenolic compound	RT (min)	λ (nm)	R^2	LOD (µg/g)	LOQ (µg/g)	Concentration (µg/g - fw)
Phenolic acids	Chlorogenic acid	6.05	320	0.99	0.06	0.19	1.86 ± 0.03
	Caffeic acid	2.46	320	0.99	0.02	0.06	0.52 ± 0.03
	p-Coumaric acid	4.11	320	0.99	0.02	0.06	0.23 ± 0.06
	Ferulic acid	5.10	320	0.99	0.05	0.16	nq
	Gallic acid	1.08	271	0.99	0.02	0.05	nd
Flavonoids	Epicatechin	2.66	271	0.99	0.38	1.28	nq
	Quercetin	11.01	271	0.99	0.05	0.17	0.53 ± 0.01
	Rutin	5.64	271	0.99	0.02	0.08	0.46 ± 0.01
	Catechin	5.45	271	0.99	0.04	0.12	nd

RT- retention time; λ- detection wavelength; R^2- adjusted coefficient; LOD- limit of detection; LOQ- limit of quantification; nq: not quantified; nd: not detected. Data represent mean values ± standard deviations expressed in fresh weight (fw) of marolo pulp.

Table 3
Levels of some bioactive amines in marolo pulp, chromatographic conditions, linearity and limits of quantification and detection.

Bioactive amines	RT (min)	R^2	LOD (mg/ 100 g)	LOQ (mg/ 100 g)	Content (mg/ kg - fw)
PUT	35.59	0.99	0.03	0.08	20.65 ± 1.06
CAD	36.69	0.99	0.03	0.08	nd
AGM	49.15	0.99	0.06	0.16	nd
SPD	52.11	0.99	0.03	0.08	6.32 ± 0.59
PHM	53.89	0.99	0.03	0.08	nd
SRT	41.98	0.99	0.05	0.16	nd
TRM	55.04	0.99	0.05	0.14	nd
HIM	40.16	0.99	0.03	0.08	nd
TYM	38.15	0.99	0.05	0.14	31.97 ± 0.52

PUT-putrescine; CAD-cadaverine; AGM-agmatine SPD-spermidine; PHE-phenylethylamin; SRT-serotonin; TRM-tryptamine; HIM-histamine; TYM-tyramine; nd-not detected. Values were reported as the mean ± standard deviation and were expressed in fresh weight (fw) of pulp.

(0.9 to 17. 9 mg/kg); and lower than eggplant and bean sprouts (21.3 to 31.9 mg/kg) (Dala-Paula, Starling, & Gloria. 2021; Santiago-Silva, Labanca, & Gloria, 2011). The increase in PUT content in some vegetables can result from the response to adverse conditions during developments, including, drought, heat, salt and oxidative stresses (Dala-Paula, Starling & Gloria, 2021). PUT is an obligate intermediate in the synthesis of SPD from ornithine, arginine or citrulline (Madeo et al., 2020). Significant levels of PUT in foods may be undesirable from a sensory point of view, considering that this is a volatile amine with the potential to impart a putrid taste and odor (Reis et al., 2020a).

In the case of SPD, similar levels were reported for pineapple (7.0 mg/kg), lower levels for mango, papaya and guava (3.8 to 5.4 mg/kg) and high content for passion fruit (30.5 mg/kg) (Santiago-Silva, Labanca & Gloria, 2011), and mushrooms (32.3 to 172.0 mg/kg) (Reis et al., 2020b). Based on the SPD in marolo pulp, it could be considered as sources of polyamines, which is valued due to its association with growth, health promotion and antioxidant properties. A high dietary intake of SPD is correlate with low mortality risk of vascular diseases, cancer, or heart failure, thereby contributing to longer life span and healthier aging (Madeo et al., 2020). Therefore, based on the SPD levels marolo pulp can be considered a source of this polyamine and the consumption should be encouraged. SPD is a natural polyamine found in many foods, as well as PUT, which is an obligate intermediate in the synthesis of SPD from ornithine, arginine or citrulline (Madeo et al., 2020). According to Dala-Paula, Starling & Gloria (2021), SPD is generally the predominant polyamine in plant-derived food, with higher contents in legumes and cereals.

TYM levels in marolo pulp was lower than in some species of fresh mushrooms (59.8 mg/kg) (Reis et al., 2020b); but, higher than in eggplant, scarlet eggplant, green onion, parsley, spinach and hearts of palm (1.8 to 23.0 mg/kg) (Dala-Paula, Starling & Gloria, 2021). At low concentrations, TYM can play important roles, such as neuromodulation and mood elevation effects (Reis et al., 2020b). However, if consumed in excessive amounts, TYM can cause adverse effects in human health, such as hypertension, vasoconstriction, pupil dilatation, migraines and cerebral hemorrhages. No observed adverse effect levels (NOAEL) were established as 600, 50 and 6 mg of TYM/person/meal for healthy individuals not taking monoamine oxidase inhibitors (MAOI), for individuals taking third generation MAOI and for individuals taking classical MAOI drugs, respectively. The levels of TYM in marolo pulp did not cause adverse effects to healthy individuals who are not using MAOI, and it would possibly not cause adverse effects in individuals using third-generation MAOI (50 mg/meal). However, consumption of approximately 1 cup (200 g) of pulp in one meal would reach the NOAEL (6.0 mg/meal) for individuals sensitive to this monoamine (e.g., which are using classic MAOI) (EFSA, 2011). The consumption of marolo, a fruit rich in bioactive compounds, can trigger migraine attacks and raise blood pressure in certain population groups with special needs. This is an important finding and more studies are necessary to understand factors that can affect the level of TYM in marolo pulp.

TYM is a neuroactive and vasoactive amine which has also shown anti-inflammatory properties, and inhibition of the production of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) (Ko, Ahn & Oh., 2015). TYM in the urine of metabolic syndrome patients correlated inversely with multiple biomarkers of inflammation and cardiometabolic risk factors, contributing to new insights of its pathogenesis Patel et al. (2019). It is interesting to observe the presence of TYM in marolo, which is not widespread in vegetables, but present in a few, including eggplant, tomato, spinach, chard, and asparagus (Dala-Paula, Starling & Gloria, 2021; Sánchez-Pérez et al. (2018). TYM is synthesized from the decarboxylation of tyrosine and an intermediate in the synthesis of other phenolic amines – octopamine and synephrine (Vieira, Theodoro & Gloria, 2006).

3.5. *Chemoprotective effect on colorectal carcinogenesis*

The presence of ACF and AC in the distal portion of the colon of each animal was used as a parameter to evaluate the chemopreventive effect of the marolo pulp (Fig. 2). The number of ACF and AC observed in each experimental group is illustrated in (Fig. 2-**IA and** Fig. 2-**IB**). Colonic pre-neoplastic lesions were observed in all animal's tissue after the administration of DMH. Representative micrographs from ACF assay are presented with absence of ACF, normal tissue structure, found in negative control (Fig. 2-**IIA**) and ACF containing one AC, pre-neoplastic tissue, found in DMH-treated groups (Fig. 2-**IIB**). The treatment groups with marolo pulp at doses of 0.7, 1.4 and 2.8 g/kg/b.w./day combined with DMH exhibited a statistically significant reduction on frequencies of ACF approximately 54 %, 58 % and 70 %, respectively (Fig. 2-**IA**), as well as reduction on AC of 47 %, 53 %, 64 %, respectively (Fig. 2-**IB**). Our results also shows that there were no significant differences in feed intake between the treated groups (Fig. 2-**IIIB**). However, a small reduction in water intake was identified in all groups that received marolo pulp combined with DMH treatment when compared to the negative control (Fig. 2-**IIIA**). These differences indicate that marolo pulp consumption automatically reduces the water intake.

DMH is a procarcinogen that require metabolic activation to form DNA-reactive products (Oliveira et al., 2021; Venkatachalam et al., 2020). Methylazoxymethanol (MAM), the reactive metabolite of DMH readily yields a methyldiazonium ion that alkylates macromolecules (DNA, RNA, or protein) in the liver and colon by enzymatic and non-enzymatic processes (Venkatachalamet al., 2020). Alkylation of the oxygen atoms present in nitrogenous bases stimulates the mispairing of DNA, which can be critical in mutagenesis and carcinogenesis (Oliveira et al., 2021; Venkatachalam et al., 2020). Thus, DMH induces carcinogenesis by methylation and by increasing the production of reactive oxygen species. Therefore, based on the DNA damage mechanism exerted by DMH, the protective effect of marolo pulp could be related to its antioxidant activity. The antioxidant activity was demonstrated by DPPH and ABTS assays, which can result, at least in part, from the contents of phenolic compounds and amines (specially spermidine) present in the marolo pulp.

Some studies suggested that increased polyamine intake has a suppressive effect on carcinogenesis in healthy individuals (Soda, 2018; Soda, 2022). This evidence possibly corroborates with the reduction on the frequency of AC and ACF observed in the treatments with marolo pulp, suggesting a carcinogenesis protective effect probably induced by the content of polyamines (spermidine) (Table 2). Therefore, according to Soda (2022) it is reasonable to assume that increased polyamine intake may suppress neoplastic diseases. Soda et al. (2013) tested this hypothesis in rats fed with chows containing different polyamine concentrations. The treatments were combined with the administration DMH of (20 mg/kg- bw) once a week for 12 consecutive weeks. The rats which were fed with the high-polyamine chow had a lower incidence of

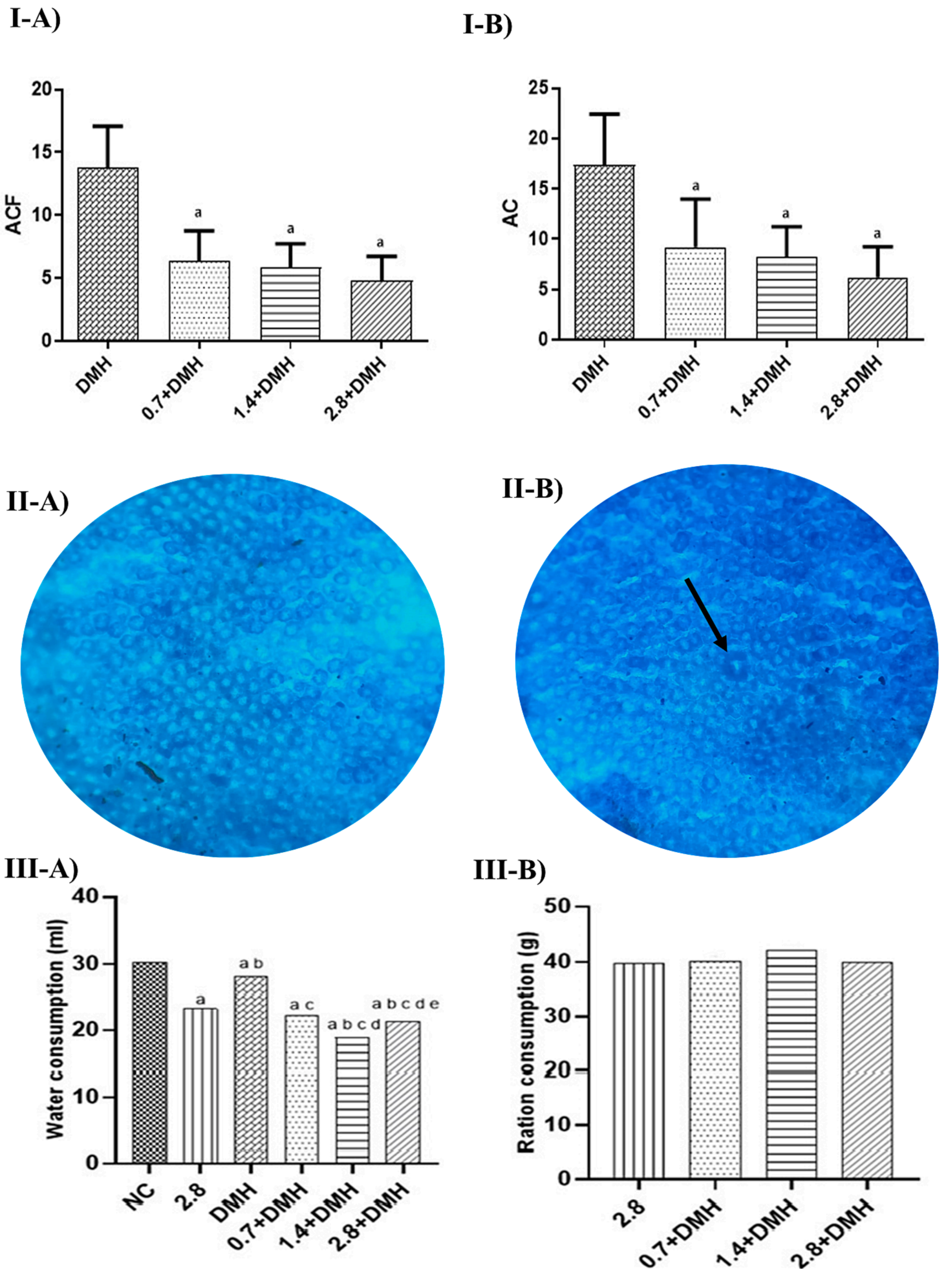

Fig. 2. Chemopreventive effect of the marolo pulp. I-Aberrant Crypt Foci (ACF) (A) and Aberrant Crypt (AC) (B) observed in the distal colon of Wistar rats. II-Representative micrographs from ACF assay (A) normal tissue with absence of ACF (negative control) and (B) ACF containing one AC (black arrow) (DMH-treated groups). III- Water (A) and food (B) consumption observed in the different treatment groups. Data are mean ± standard deviation. DMH: 1,2 Dimethylhydrazine (40 mg/kg bw). One Way ANOVA and Tukey's post-test. ^{a}Significantly different of NC group, ^{b}Significantly different of 2.8 group, ^{c}Significantly different of DMH group, ^{d}Significantly different of 0.7 + DMH group, ^{e}Significantly different of 1.4 + DMH group.

neoplastic growth (mostly colon cancer). Similar results were obtained by Wada et al. (2002) which observed an enhancement of tumorigenesis by increased polyamine (spermidine) intake (Wada et al., 2002). The polyamine content in marolo pulp (Table 3) were in the range of 171—685 μmol for spermidine. The equivalent ingested polyamines doses from marolo pulp are higher than the polyamines intake doses covered in the studies that reported decreased tumorigenesis associated with polyamines intake mentioned above. Therefore, the equivalent dose ingested in the treatments could be considered enough to suggest that the polyamine, spermidine, and the diamine, putrescine, could be supporting compounds in marolo pulp on colorectal carcinogenesis protector effect presented in this work.

Current studies have reported that oxidative damage might be prevented, reduced, or even reversed with antioxidant supplementation (Prasanth et al., 2019; Boots et al., 2020). In this context Cerrado plants can adapt to adverse situations, biosynthesize molecules (secondary metabolites) to protect them from biotic and abiotic factors. Secondary metabolites, such as phenolic compounds, have been reported to act as cytotoxic and cytostatic substances, what can culminate in chemopreventive potential (Formagio et al., 2015). Marolo pulp extracts showed a high content of phenolic compounds (Table 1) and significant antioxidant activity, which suggested that the chemopreventive potential could also be associated with the phenolic compounds content present in the pulp. This is strengthened by the fact that oxidative stress has been related to the progression of preneoplastic lesions (Tauchen et al., 2016).

The antioxidant protection exerted by marolo pulp could prevent free radical damage, which is vital for the integrity of cell structures. This is so, because oxidative damage interferes with the normal cell signaling cascades, affecting activation or expression of transcription factors and kinases. Certainly, a range of compounds, such as alkaloids, annonaceous acetogenins, tocols, carotenoids, phytosterols, and vitamins could also contribute to the antioxidant effect and consequently protective against colorectal carcinogenesis. However, the positive correlation between antioxidant capacity and total phenolic content is well established (Arruda, Pereira & Pastore, 2018) and our results demonstrate marolo antioxidant capacity that can be related to the content of phenolic compounds.

The scientific literature has already confirmed that the majority phenolics compounds present in marolo pulp (Table 2), including quercetin (Sheree et al., 2021); Sankaranarayanan et al., 2021), and chlorogenic acid (Villota et al., 2022; Murai & Matsuda, 2023) have shown the chemopreventive effects on colorectal carcinogenesis in rats.

4. Conclusion

The chemopreventive effect of marolo pulp against colorectal preneoplastic lesions supports its consumption. This potential could be associated with the different types of phenolic compounds presents mainly flavonoids (eg., quercertin) and phenolic acids (eg., chlorogenic acid) which may contribute to its ability to reduce free radicals, could avoid oxidative stress in cells. In addition, based on scientific literature reported the equivalent amines dose ingested in the treatments could be considered enough to suggest that the polyamine, spermidine, and the diamine, putrescine, could be supporting compounds in marolo pulp to inhibition the preneoplastic colon lesions. These results provide new perspectives in chemopreventive foods, which may lead to the improvement of already established therapies and the broadening of preventive strategies. In addition, the marolo pulp could serve as a valuable source natural antioxidant with potential application in the production of functional foods with a considerably source of bioactive amines. Therefore, it is a fruit with promising economic value, but with the need to alert consumers, especially those under MAOI drugs, of the possible risk of adverse effects related to the ingestion of TYM, from migraine up to hypertensive crises.

CRediT authorship contribution statement

Patrícia Felix Ávila: Writing – original draft, Writing – review & editing, Formal analysis. **Angélica Pereira Todescato:** Validation, Investigation, Visualization. **Mylena de Melo Carolo dos Santos:** Validation, Investigation, Visualization. **Luiz Fernando Ramos:** Validation, Investigation, Visualization. **Isabella Caroline Menon:** Validation, Investigation, Visualization. **Michele Oliveira Carvalho:** Visualization, Formal analysis. **Maysa do Vale-Oliveira:** Investigation, Methodology. **Flávia Beatriz Custódio:** Formal analysis, Writing – original draft, Writing – review & editing, Methodology. **Maria Beatriz Abreu Gloria:** Conceptualization, Writing – original draft, Writing – review & editing. **Bruno Martins Dala-Paula:** Project administration, Supervision, Conceptualization, Resources, Funding acquisition, Writing – original draft, Writing – review & editing. **Pollyanna Francielli de Oliveira:** Project administration, Supervision, Conceptualization, Resources, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was carried out with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Financing Code 001), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – APQ-00424-18 and APQ-02589-23), Fundação Cargill (Food in Focus) and Universidade Federal de Alfenas (UNIFAL-MG).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113666.

References

Almeida, M. M. B., Sousa, P. H. M., Arriaga, Â. M. C., Prado, G. M., Magalhães, C. E. C., Maia, G. A., et al. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International, 44*(7), 2155–2159. https://doi.org/10.1016/j.foodres.2011.03.051

Arruda, H. S., & Pastore, G. M. (2019). Araticum (*Annona crassiflora* Mart.) as a source of nutrients and bioactive compounds for food and non-food purposes: A comprehensive review. *Food Research International, 123*, 450–480. https://doi.org/10.1016/j.foodres.2019.05.011

Arruda, H. S., Pereira, G. A., & Pastore, G. M. (2017). Optimization of extraction parameters of total phenolics from *Annona crassiflora* Mart. (Araticum) fruits using response surface methodology. *Food Analytical Methods, 10*, 100–110. https://doi.org/10.1007/s12161-016-0554-y

Arruda, H. S., Pereira, G. A., & Pastore, G. M. (2018). Brazilian Cerrado fruit araticum (*Annona crassiflora* Mart.) as a potential source of natural antioxidant compounds. *International Food Research Journal, 25*(5), 2005–2012. https://doi.org/10.1016/j.foodres.2019.05.011

Arruda, H. S., Borsoi, F. T., Andrade, A. C., Pastore, G. M., & Marostica Junior, M. R. (2023). Scientific Advances in the Last Decade on the Recovery, Characterization, and Functionality of Bioactive Compounds from the Araticum Fruit (Annona crassiflora Mart.). *Plants, 12*(7), 1536. https://doi.org/10.3390/plants12071536

Boateng, J., Verghese, M., Walker, L. T., & Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). LWT-Food. *Science and Technology, 41*(9), 1541–1547. https://doi.org/10.1016/j.lwt.2007.11.025

Boots, A. W., Veith, C., Albrecht, C., Bartholome, R., Drittij, M. J., Claessen, S. M., et al. (2020). The dietary antioxidant quercetin reduces hallmarks of bleomycin-induced lung fibrogenesis in mice. *BMC Pulmonary Medicine, 20*, 1–16. https://doi.org/10.1186/s12890-020-1142-x

Cardoso, L. M., Oliveira, D. S., Bedetti, S. F., Martino, H. S. D., & Pinheiro-Sant'Ana, H. M.. (2013). Araticum (*Annona crassiflora* Mart.) from the Brazilian Cerrado: Chemical composition and bioactive compounds. *Fruits, 68*(2), 121–134. https://doi.org/10.1051/fruits/2013058

Carvalho, N. C. C., Monteiro, O. S., da Rocha, C. Q., Longato, G. B., Smith, R. E., da Silva, J. K. R., & Maia, J. G. S. (2022). Phytochemical Analysis of the Fruit Pulp Extracts from *Annona crassiflora* Mart. and Evaluation of Their Antioxidant and Antiproliferative Activities. *Foods*, *11*(14), 2079. doi: 10.3390/foods11142079.

Chen, G.-L., Zhang, X., Chen, S.-G., Han, M.-D., & Gao, Y.-Q. (2017). Antioxidant activities and contents of free, esterified and insoluble-bound phenolics in 14 subtropical fruit leaves collected from the south of China. *Journal of Functional Foods, 30*, 290–302. doi: doi.org/10.1016/j.jff.2017.01.011.

Chondrogianni, N., Kapeta, S., Chinou, I., Vassilatou, K., Papassideri, I., & Gonos, E. S. (2010). Anti-ageing and rejuvenating effects of quercetin. *Experimental gerontology, 45*(10), 763–771. https://doi.org/10.1016/j.exger.2010.07.001

Clapper, M. L., Chang, W. C. L., & Cooper, H. S. (2020). Dysplastic aberrant crypt foci: Biomarkers of early colorectal neoplasia and response to preventive intervention. *Cancer prevention research, 13*(3), 229–240. https://doi.org/10.1158/1940-6207.CAPR-19-0316

Dala-Paula, B. M.; Tavano, O. L & Gloria, M. B. A. (2019). *In vitro* bioacessibility of bioactive amines from *Annona crassiflora* pulp. In: 13° SIMPÓSIO LATINO AMERICANO DE CIÊNCIA DE ALIMENTOS, Galoá, 4., Campinas. Anais [...].

Dala-Paula, B. M., Deus, V. L., Tavano, O. L., & Gloria, M. B. A. (2021). In vitro bioaccessibility of amino acids and bioactive amines in 70% cocoa dark chocolate: What you eat and what you get. *Food Chemistry, 343*, Article 128397. https://doi.org/10.1016/j.foodchem.2020.128397

Dala-Paula, B. M., Starling, M. F., & V., & Gloria, M. B. A.. (2021). Vegetables consumed in Brazilian cuisine as sources of bioactive amines. *Food Bioscience, 40*, Article 100856. https://doi.org/10.1016/j.fbio.2020.100856

Damiani, C., Vilas Boas, E. V. B., Asquieri, E. R., Lage, M. E., Oliveira, R. A., Silva, F. A., et al. (2011). Characterization of fruits from the savanna: Araça (*Psidium guinnensis* Sw.) and Marolo (*Annona crassiflora* Mart.). *Food Science and Technology, 31*(3), 723–729. https://doi.org/10.1590/S0101-20612011000300026

Demir, S. C., Yildirim, A. B., Turker, A. U., & Eker, I. (2022). Seasonal variation in alkaloid content, phenolic constituent and biological activities of some *Leucojum aestivum* L. populations in Turkey. *South African Journal of Botany, 147*, 713–723. https://doi.org/10.1016/j.sajb.2022.03.004

De Sousa, A. C., Gattass, C. R., Alviano, D. S., Alviano, C. S., Blank, A. F., & Alves, P. B. (2004). Melissa officinalis L. essential oil: Antitumoral and antioxidant activities. *Journal of pharmacy and pharmacology, 56*(5), 677–681. https://doi.org/10.1211/0022357023321

Dragano, N. R. V., de Venancio, V. P., Paula, F. B. D. A., Della Lucia, F., Fonseca, M. J. D. O., & Azevedo, L. (2010). Influence of marolo (Annona crassiflora Mart.) pulp intake on the modulation of mutagenic/antimutagenic processes and its action on oxidative stress in vivo. *Plant foods for human nutrition, 65*, 319–325. https://doi.org/10.1007/s11130-010-0191-3

EFSA, European Food Safety Authority. (2011). Scientific opinion on risk-based control of biogenic amine formation in fermented foods. EFSA, Panel on Biological Hazards (BIOHAZ). *EFSA Journal, 9*, 2393.

Encalada, M. A., Hoyos, K. M., Rehecho, S., Berasategi, I., de Ciriano, M. G.Í., Ansorena, D., et al. (2011). Anti-proliferative effect of Melissa officinalis on human colon cancer cell line. *Plant Foods for Human Nutrition*, *66*, 328–334. https://doi.org/10.1007/s11130-011-0256-y

Formagio, A. S. N., Ramos, D. D., Vieira, M. C., Ramalho, S. R., Silva, M. M., Zárate, N. A. H., et al. (2015). Phenolic compounds of Hibiscus sabdariffa and influence of organic residues on its antioxidant and antitumoral properties. *Brazilian Journal of Biology, 75*, 69–76. https://doi.org/10.1590/1519-6984.07413

Gadkaria, P. V. & Balaraman, M. (2015). Catechins: Sources, extraction and encapsulation: A review. *Food and Bioproducts Processing*, 93, 122–138. doi: dx.doi.org/10.1016/j.fbp.2013.12.004.

Ko, H. J., Ahn, E. K., & Oh, J. S. (2015). N-trans-ρ-caffeoyl tyramine isolated from *Tribulus terrestris* exerts anti-inflammatory effects in lipopolysaccharide-stimulated RAW 264.7 cells. *International Journal of Molecular Medicine, 36*, 1042–1048.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The scientific world journal, 2013*. https://doi.org/10.3892/ijmm.2015.2301

Kurzawa-Zegota, M., Najafzadeh, M., Baumgartner, A., & Anderson, D. (2012). The protective effect of the flavonoids on food-mutagen-induced DNA damage in peripheral blood lymphocytes from colon cancer patients. *Food and chemical toxicology, 50*(2), 124–129. https://doi.org/10.1016/j.fct.2011.08.020

Lamichhane, P., Maiolini, M., Alnafoosi, O., Hussein, S., Alnafoosi, H., Umbela, S., et al. (2020). Colorectal cancer and probiotics: Are bugs really drugs. *Cancers, 12*(5), 1162. https://doi.org/10.3390/cancers12051162

Lee, F., & Lawrence, D. A. (2018). From infections to anthropogenic inflicted pathologies: Involvement of immune balance. *Journal of Toxicology and Environmental Health, Part B, 21*(1), 24–46. https://doi.org/10.1080/10937404.2017.1412212

Lucia, F. D., de Elias, H. H., & S., da Silva, E. P., Xiusto, A. L. R. P., Cardoso, A. F. L., de Lima, J. P., Ferreira, E. B. & Boas, E. V. de B. V.. (2023). Postharvest quality of marolo fruit (*Annona crassiflora* mart) along storage. *Journal of Food and Nutrition Research, 11*(6), 393–403. https://doi.org/10.12691/jfnr-11-6-1

Madeo, F., Eisenberg, T., Pietrocola, F., & Kroemer, G. (2018). Spermidine in health and disease. *Science, 359*, 410. https://doi.org/10.1126/science. aan2788

Madeo, F., Hofer, S. F., Pendl, T., Bauer, M. A., Eisenberg, T., Carmona-Gutierrez, D., et al. (2020). Nutritional aspects of spermidine. *Annual Review of Nutrition, 40*, 135–159. https://doi.org/10.1146/annurev-nutr-120419-015419

Margraf, T., Santos, É. N. T., de Andrade, E. F., van Ruth, S. M., & Granato, D. (2016). Effects of geographical origin, variety and farming system on the chemical markers and in vitro antioxidant capacity of Brazilian purple grape juices. *Food Research International, 82*, 145–155. https://doi.org/10.1016/j.foodres.2016.02.003

Moo-Huchin, V. M., Estrada-Mota, I., Estrada-León, R., Cuevas-Glory, L., Ortiz-Vázquez, E., & y Vargas, M. D. L. V., & Sauri-Duch, E.. (2014). Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food chemistry, 152*, 508–515. https://doi.org/10.1016/j.foodchem.2013.12.013

Murai, T., & Matsuda, S. (2023). The Chemopreventive Effects of Chlorogenic Acids, Phenolic Compounds in Coffee, against Inflammation, Cancer, and Neurological Diseases. *Molecules, 25*(5), 2381. https://doi.org/10.3390/molecules28052381

Nascimento, A. L. A. A., Brandi, I. V., Durães, C. A. F., Lima, J. P. D., Soares, S. B., & Mesquita, B. M. A. D. C. (2020). Chemical characterization and antioxidant potential of native fruits of the Cerrado of northern Minas Gerais. *Brazilian Journal of Food Technology, 23*. https://doi.org/10.1590/1981-6723.29619

Nkhata Malunga, L., Ames, N., Mitchell Fetch, J., Netticadan, T., & Joseph Thandapilly, S. (2022). Genotypic and environmental variations in phenolic acid and avenanthramide content of Canadian oat (*Avena sativa*). *Food Chemistry, 388*, Article 132904. https://doi.org/10.1016/j.foodchem.2022.132904

Oliveira, F. P., Leandro, L. F., Furtado, R. A., Ferreira, N. H., Pauletti, P. M., Barbosa Araujo, A. R., et al. (2021). Styrax camporum, a typical species of the Brazilian cerrado, attenuates DNA damage, preneoplastic lesions and oxidative stress in experimental rat colon carcinogenesis. *Journal of Toxicology and Environmental Health, Part A, 84*(14), 582–592. https://doi.org/10.1080/15287394.2021.1910090

Oliveira, C. S., Baptistella, M. M., Siqueira, A. P., Carvalho, M. O., Ramos, L. F., de Souto, B. S., et al. (2023). Combination of vitamin D and probiotics inhibits chemically induced colorectal carcinogenesis in Wistar rats. *Life Sciences, 121617*. https://doi.org/10.1016/j.lfs.2023.121617

Patel, A., Thompson, A., Abdelmalek, L., Adams-Huet, B., & Jialal, I. (2019). The relationship between tyramine levels and inflammation in metabolic syndrome. *Hormone Molecular Biology and Clinical Investigation, 6*, 1–7. https://doi.org/10.1515/hmbci-2019-0047

Prado, L. G., Arruda, H. S., Araujo, N. M. P., de Oliveira Braga, L. E., Banzato, T. P., Pereira, G. A., et al. (2020). Antioxidant, antiproliferative and healing properties of araticum (Annona crassiflora Mart.) peel and seed. *Food research international, 133*, Article 109168. https://doi.org/10.1016/j.foodres.2020.109168

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (Camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients, 11*(2), 474. https://doi.org/10.3390/nu11020474

Ramos, A. L. C. C., Minighi, E. C., Soares, I. I. C., Ferreira, R. M. S. B., Sousa, I. M. S., Augusti, R., Labanca, R. A., de Araujo, R.L.B. & Melo, J. O. F. (2023). Evaluation of the total phenolic content, antioxidative capacity, and chemical fingerprint of *Annona crassiflora* Mart. Bioaccessible molecules. *Food Research International*, *165*, 112514. doi: doi.org/10.1016/j.foodres.2023.112514.

Rashidi, R., Rezaee, R., Shakeri, A., Hayes, A. W., & Karimi, G. (2022). A review of the protective effects of chlorogenic acid against different chemicals. *Journal of Food Biochemistry, 46*, e14254.

Reis, G. C., Dala-Paula, B. M., Tavano, O. L., Guidi, L. R., Godoy, H. T., & Gloria, M. B. A. (2020a). In vitro digestion of spermidine and amino acids in fresh and processed *Agaricus bisporus* mushroom. *Food Research International*, *137*, 109616. doi: 10.1016/j.foodres.2020.109616.

Reis, G. C., Custódio, F. B., Botelho, B. G., Guidi, L. R., & Gloria, M. B. A. (2020b). Investigation of biologically active amines in some selected edible mushrooms. *Journal of Food Composition and Analysis*, *86*, 103375. doi.org/10.1016/j.jfca.2019.103375.

Rodrigues, M. A. M., Silva, L. A. G., Salvadori, D. M. F., De Camargo, J. L. V., & Montenegro, M. R. (2002). Aberrant crypt foci and colon cancer: Comparison between a short-and medium-term bioassay for colon carcinogenesis using dimethylhydrazine in Wistar rats. *Brazilian Journal of Medical and Biological Research, 35*, 351–355. https://doi.org/10.1590/S0100-879X2002000300010

Roesler, R., Catharino, R. R., Malta, L. G., Eberlin, M. N., & Pastore, G. (2007). Antioxidant activity of Annona crassiflora: Characterization of major components by electrospray ionization mass spectrometry. *Food Chemistry, 104*(3), 1048–1054. https://doi.org/10.1016/j.foodchem.2007.01.017

Rufino, M. S. M., Alves, R. E., Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry, 121*(4), 996–1002. https://doi.org/10.1016/j.foodchem.2010.01.037

Sánchez-Pérez, S., Comas-Basté, O., Rabell-González, J., Veciana-Nogués, M. T., Latorre-Moratalla, M. L., & Vidal-Carou, M. C. (2018). Biogenic amines in plant-origin foods: Are they frequently underestimated in low-histamine diets. *Foods, 7*(12), 205. https://doi.org/10.3390/foods7120205

Sankaranarayanan, R., Sekhon, P. K., Ambat, A., Nelson, J., Jose, D., Bhat, G. J., et al. (2021). Screening of Human Gut Bacterial Culture Collection Identifies Species That Biotransform Quercetin into Metabolites with Anticancer Properties. *International Journal of Molecular Sciences, 22*(13), 7045. https://doi.org/10.3390/ijms22137045

Santiago-Silva, P., Labanca, R. A., & Gloria, M. B. A. (2011). Functional potential of tropical fruits with respect to free bioactive amines. *Food Research International, 44*(5), 1264–1268. https://doi.org/10.1016/j.foodres.2010.11.026

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., et al. (2011). *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmaceutics, 403*, 136–138. https://doi.org/10.1016/j.ijpharm.2010.09.035

Silva, E. P., Vilas Boas, E. V. B., & Xisto, A. L. P. R. (2013). Characterization and development of marolo (*Annona crassiflora*, Mart.). *Food Science and Technology, 33*(4), 666–675. https://doi.org/10.1590/S0101-20612013000400011

Siqueira, E. M. D. A., Rosa, F. R., Fustinoni, A. M., de Sant'Ana, L. P., & Arruda, S. F. (2013). Brazilian savanna fruits contain higher bioactive compounds content and higher antioxidant activity relative to the conventional red delicious apple. *PloS one, 8*(8), e72826.

Sheree, A., Islam, J., & Sultana, S. (2021). Quercetin ameliorates reactive oxygen species generation, inflammation, mucus depletion, goblet disintegration, and tumor multiplicity in colon cancer: Probable role of adenomatous polyposis coli, β-catenin. *Phytotherapy Research, 35*(4), 2171–2184. https://doi.org/10.1002/ptr.6969

Soda, K. (2018). Polyamine metabolism and gene methylation in conjunction with one-carbon metabolism. *International journal of molecular sciences, 19*(10), 3106. https://doi.org/10.3390/ijms19103106

Soda, K. (2022). Overview of polyamines as nutrients for human healthy long life and effect of increased polyamine intake on DNA methylation. *Cells, 11*(1), 164. https://doi.org/10.3390/cells11010164

Soda, K., Kano, Y., Chiba, F., Koizumi, K., & Miyaki, Y. (2013). Increased polyamine intake inhibits age-associated alteration in global DNA methylation and 1, 2-dimethylhydrazine-induced tumorigenesis. *PLoS One, 8*(5), e64357.

Souza, V. R., Pereira, P. A. P., Queiroz, F., Borges, S. V., & Carneiro, J. D. D. S. (2012). Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. *Food chemistry, 134*(1), 381–386. https://doi.org/10.1016/j.foodchem.2012.02.191

Tauchen, J., Bortl, L., Huml, L., Miksatkova, P., Doskocil, I., Marsik, P., et al. (2016). Phenolic composition, antioxidant and anti-proliferative activities of edible and medicinal plants from the Peruvian Amazon. *Revista Brasileira de Farmacognosia, 26*, 728–737. https://doi.org/10.1016/j.bjp.2016.03.016

Venkatachalam, K., Vinayagam, R., Anand, A. V., & M., Isa, N. M., & Ponnaiyan, R.. (2020). Biochemical and molecular aspects of 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis: A review. *Toxicology research, 9*(1), 2–18. https://doi.org/10.1093/toxres/tfaa004

Vieira, S. M., Theodoro, K. H., & Gloria, M. B. A. (2006). Profile and levels of bioactive amines in orange juice and orange soft drink. *Food Chemistry, 100*(895–903), 2007. https://doi.org/10.1016/j.foodchem.2005.10.050

Villota, H., Santa-González, G. A., Uribe, D., Henao, I. C., Arroyave-Ospina, J. C., Barrera-Causil, C. J., et al. (2022). Modulatory Effect of Chlorogenic Acid and Coffee Extracts on Wnt/β-Catenin Pathway in Colorectal Cancer Cells. *Nutrients, 14*(22), 4880. https://doi.org/10.3390/nu14224880

Wada, M., Funada-Wada, U., Mano, H., Higashiguchi, M., Haba, R., Watanabe, S., & Udaka, S. (2002). Effects of dietary polyamines on the promotion of mammary tumor in rats. *Journal of health science, 48*(4), 376–380. https://doi.org/10.1248/jhs.48.376

Wang, Z. X., Lin, Q. Q., Tu, Z. C., & Zhang, L. (2020). The influence of in vitro gastrointestinal digestion on the Perilla frutescens leaf extract: Changes in the active compounds and bioactivities. *Journal of Food Biochemistry, 44*(12), e13530.

WORLD HEALTH ORGANIZATION et al. Fruit and vegetables for health: report of the Joint FAO. 2005.