LWT - Food Science and Technology 210 (2024) 116764

Contents lists available at ScienceDirect

LWT

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

Evaluating the potential prebiotic effects of umbu-cajá (*Spondias* spp.) fruit processing by-product flour on the human intestinal microbiota

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ARTICLE INFO

Keywords:
Spondias
Fruit residues
Gut microbiota
Prebiotic properties
Functional food

ABSTRACT

This study evaluated the potential prebiotic properties of umbu-cajá (*Spondias* spp.) fruit processing by-product flour (UCF) on the human intestinal microbiota by monitoring the relative abundance of specific intestinal bacterial populations, microbial metabolic behavior, and antioxidant activity during 48 h of *in vitro* colonic fermentation using fecal inoculum donated from healthy adult individuals. UCF had a high insoluble fiber content and flavonols, especially quercetin-3-*O*-glucoside, as the most prevalent phenolic compounds, besides showing antioxidant activity. Medium with UCF increased the relative abundance of *Lactobacillus* spp./*Enterococcus* spp., *Bifidobacterium* spp., and *Clostridium histolyticum* during the colonic fermentation and decreased the relative abundance of *Bacteroides* spp./*Prevotella* spp. and *Eubacterium rectale*/*Clostridium coccoides*. Medium with UCF maintained a higher relative abundance of *Ruminococcus albus*/*R. flavefaciens* than negative control during the colonic fermentation. UCF stimulated intense metabolic activity of the intestinal microbiota during the colonic fermentation, evidenced by decreased pH values, sugar consumption, and production of several metabolites linked to host health promotion, concomitant with high antioxidant activity. The results characterize UCF as a candidate for prebiotic use and a novel value-added circular ingredient to functionalize foods and nutraceuticals.

1. Introduction

Brazil has an abundance of fruits that are of great commercial importance to the agro-food sector (Sviech, Ubbink & Prata, 2022). However, fruit processing produces great amounts of agro-industrial by-products that are typically discarded or underused (Akter & Rabeta, 2021; Banerjee et al., 2017). These by-products are commonly composed of peels, seeds, and pomace, which are sources of fermentable carbohydrates and bioactive compounds (Cangussu, P Leão, Oliveira, & Franca, 2021).

Umbu-cajá (*Spondias* spp.), a plant species native to the Brazilian Caatinga biome, stands out for having fruit with distinct sensory characteristics, such as a pleasant flavor and aroma. In addition to being consumed fresh, umbu-cajá fruit is widely processed to produce pulps, sweets, jellies, juices, popsicles, nectars, and ice creams. The transformation of umbu-cajá fruit processing by-products into flour could be a strategy aligned with a circular economy approach for its valorization since it is a relatively simple process, does not require high production costs, reduces the perishability, and increases the concentration of nutrients in the resulting dried product (Leão, Franca, Oliveira, Bastos, & Coimbra, 2017; Oliveira, Holanda, de Assis, Xavier Júnior, & de Sousa Júnior, 2024; Xavier et al., 2022).

Umbu-cajá fruit has received attention from researchers and industry due to its antioxidant, antihypertensive, and antidiabetic activity

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https://doi.org/10.1016/j.lwt.2024.116764
Received 31 May 2024; Received in revised form 15 August 2024; Accepted 12 September 2024
Available online 12 September 2024

(Barros et al., 2020; Dutra et al., 2017; Macedo et al., 2023). The effects of the umbu-cajá fruit consumption on health are mainly linked to its high amount of dietary fiber and phenolic compounds (Santos et al., 2023), which are potentially prebiotic food components. Prebiotics are substrates that provide a selective source of fermentable substrates for beneficial microorganisms, conferring a health benefit to the host due to the modulation of microbial populations (Andrade et al., 2020; Campos et al., 2020; Gibson et al., 2017).

A previous study evaluating the effects of soursop and umbu-cajá fruit pulp fermented with yeast on the intestinal microbiota of hypertensive individuals reported beneficial modulatory effects linked to the increase in the relative abundance of *Bifidobacterium* spp. and *Lactobacillus* spp. and decrease in the relative abundance of *Eubacterium rectale*, *Clostridium coccoides,* and *Clostridium histolyticum,* besides enhancing short-chain fatty acid production (Macedo et al., 2023). However, investigations on the effects of umbu-cajá fruit processing by-product flour on healthy human intestinal microbiota are still scarce. This study aimed to evaluate the potential prebiotic properties of flour produced from umbu-cajá fruit processing by-products on the human intestinal microbiota by monitoring the relative abundance of various intestinal bacterial populations, microbial metabolic profile, and antioxidant activity during an *in vitro* colonic fermentation.

2. Materials and methods

2.1. Preparation of flour from umbu-cajá fruit processing by-product

The umbu-cajá fruit by-products (approximately 20 kg) were obtained from a local fruit pulp processing company (Pomar Polpas de Frutas, Aracaju, SE, Brazil). The by-products were dried in a dryer oven with forced air circulation (Model MA 035; Marconi Equipamentos, Piracicaba, SP, Brasil) (60 ± 2 °C, approximately 8 h) until the moisture content reached <15%. After drying, the material was crushed using a domestic processor (low speed, 10 min) to produce a powder (Aderne, Anjos, Souza, & Zanuto, 2021). The umbu-cajá fruit by-product flour (UCF) was hermetically packed in polypropylene bags, vacuum sealed, kept at room temperature (25 ± 1 °C), and protected from light in a dry place to be stored for a maximum period of six months.

2.2. Determination of the physicochemical characteristics of UCF

The physicochemical characteristics of the umbu-cajá fruit by-product flour were determined according to the methods of the Association of Official Analytical Chemists (2016), namely the dry matter content (925.10), pH (981.12), ash (923.03), total titratable acidity (942.15), crude protein (920.87; N × 5.75), and fat contents (method 940.26). An enzymatic–gravimetric method was used to determine the contents of insoluble, soluble, and total dietary fiber (method 991.43). The content of carbohydrates was determined considering the difference in relation to the sum of the contents of moisture, ash, fat, and protein (Alkehayez, Alshawi, & Aljobair, 2022).

An aqueous extract was prepared to determine the contents of sugars (glucose, fructose, and maltose) and organic acids (lactic, acetic, butyric, and propionic acids). 2 g of UCF were homogenized with 10 mL of ultrapurified water (Milli-Q® Integral Water Purification, EMD Millipore Corporation, Billerica, USA) for 10 min, centrifuged (4000 × *g*, 15 min, 24 °C) (Centrifugue MPW Medical Instruments, Warsaw, Poland) and the supernatant was filtered (0.45 μm; Whatman®, GE Healthcare, Chicago, USA) (Albuquerque, Magnani, Lima, Castellano, & de Souza, 2021). A methanol extract was prepared to determine the content of phenolic compounds. 2 g of UCF were acidified with 0.1 M HCl (Neon, São Paulo, SP, Brazil) until reaching pH 2, mixed with 10 mL of methanol: water (70:30 v/v) (60 min, 25 °C), and kept protected from light. The mixture was centrifuged (4000 × *g*, 15 min, 24 °C), and the supernatant was filtered (0.45 μm; Whatman®) (Albuquerque et al., 2021).

The contents of sugars, organic acids, and phenolic compounds were determined with high-performance liquid chromatography technique using a liquid chromatograph (model 1260 Infinity LC, Agilent Technologies, St. Clara, CA, USA). The liquid chromatograph was equipped with a quaternary solvent pump (G1311C model), degasser, thermostatic column compartment (G1316A model), automatic autosampler (G1329B model), coupled with a diode array detector (DAD) (G1315D model), and refractive index detector (RID) (G1362A model), using validated analytical conditions (Coelho et al., 2018; Padilha et al., 2017) and OpenLAB CDS ChemStation EditionTM software (Agilent Technologies) to data processing. The sample peaks were identified with the comparison of their retention times with the respective standards (Sigma-Aldrich, St. Louis, MO, USA).

2.3. Determination of the antioxidant activity of UCF

The antioxidant activity was determined with $ABTS^{\bullet+}$, DPPH, and FRAP methods. The UCF extract was obtained as described in section 2.2. The ABTS radical scavenging activity was measured as previously described (Re et al., 1999). $ABTS^{\bullet+}$ radical was prepared 16 h before analysis by adding potassium persulfate and $ABTS^{\bullet+}$ stock solution. Immediately before the analysis, the radical was stabilized at 720 nm and diluted in ethanol. An aliquot (30 μL) of each extract concentration was transferred to a microplate and mixed with $ABTS^{\bullet+}$ radical (2.2′azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (300 μL) under light protection. After 6 min, the absorbance was read (wavelength: 734 nm) and the results were expressed as a percentage of reducing activity (%) using the equation:

$$\% \text{ activity: } \{[(Abscontrol - Abssample)/Abscontrol] \times 100\} \quad \text{(Eq. 2)}$$

Where: “Sample Abs” and “Control Abs” are, respectively, the absorbance observed in the sample and in the system (without antioxidant).

The $DPPH^{\bullet}$ radical scavenging activity was measured as previously described (Brand-Williams, Cuvelier, & Berset, 1995). An aliquot (50 μL) of the extract was mixed with 400 mM $DPPH^{\bullet}$ solution (2,2-diphenyl-1-picrylhydrazyl) (150 μL) diluted in methanol. After 30 min of rest, the radical reduction was determined (wavelength: 515 nm). All determinations were accompanied by a control (without antioxidant). The decrease in optical density values of the sample was correlated with that measured for the control, and the percentage of reducing activity was estimated with the equation:

$$\% \text{ activity} = \{[(Abscontrol - Abssample)/ Abscontrol] \times 100\} \quad \text{(Eq. 2)}$$

Where: “Sample Abs” and “Control Abs” are the absorbance found in the sample and the system (without antioxidant), respectively.

The ferric-reducing antioxidant power (FRAP) was determined as previously described (Oyaizu, 1986). An aliquot (9 μL) of the extract was removed and mixed with distilled water (27 μL) and FRAP reagent (270 μL; 0.3 M acetate solution, TPTZ, and ferric chloride), added to the wells, kept in an oven (37 °C, 30 min) under light protection, and readings were taken (wavelength: 595 nm). The results were expressed in μM equivalents of Trolox equivalents using the Trolox curve ($y = 0.0009x + 0.1422$; $R2 = 0.993$).

2.4. Simulated gastrointestinal digestion of UCF

The UCF was submitted to an *in vitro* digestion in sequential steps (oral, gastric, and intestinal), with adjustments of pH, time, temperature, rotation, and specific enzymes in each step. The final digested UCF solution was dialyzed and freeze-dried as previously described (Albuquerque et al., 2021). The powdered material was stored (5 ± 0.5 °C) in hermetically sealed polyethylene bags until use in the experiments.

2.5. Preparation of human fecal inoculum

The inoculum was prepared with fresh fecal samples from six donors (three men and three women, aged between 20 and 30). The donors declared that they were not suffering from colonic conditions, had an omnivorous diet, did not use probiotic foods or concentrated prebiotics, and did not use antibiotics during the six months before the collection of fecal samples. Donors received specific instructions for feces sample collection and a hygienic collection/storage kit (gloves, mask, 70% alcohol, and sterile kit bottle) (Guergoletto, Costabile, Flores, Garcia, & Gibson, 2016). The flasks with the feces were placed in packaging with an anaerobic generator system (AnaeroGen, Oxoid, Basingstoke England) immediately after collection. The six fecal samples were mixed (1:1:1:1:1:1, w/w), diluted (1:10, w/v) in sterile phosphate-buffered saline (PBS 0.1 M; pH 7.4), homogenized (2 min, 200 rpm), and filtered through a triple-layer gauze (Albuquerque et al., 2021).

2.6. Preparation of fermentation media

The fermentation medium (1 L) was formulated with 4.5 g of NaCl, 4.5 of KCl, 1.5 g of $NaHCO_3$, 0.69 g of $MgSO_4$, 0.8 g of L-cysteine HCl, 0.5 g of KH_2PO_4, 0.5 g of K_2HPO_4, 0.4 g of bile salt, 0.08 g of $CaCl_2$, 0.005 g of $FeSO_4$, and 1 mL de Tween 80 in distilled water. A resazurin solution (0.025%, v/v) was added to the medium as an anaerobic indicator. The pH of the basal medium was adjusted to 6.8 with HCl (1 M). The medium was autoclaved (121 °C, 1 atm, 15 min) before use in the experiments (Andrade et al., 2020). Digested UCF was used for fermentation with the pooled human fecal inoculum. The final fermentation medium volume (50 mL) was 40% basal nutrient medium (v/v), 40% human fecal inoculum (v/v), and 20% UCF (w/v). Media with fructooligosaccharides (FOS, a well-known prebiotic, 20%, w/v) and no added substrate were considered positive and negative controls, respectively. After preparation, the fermentation systems were incubated under anaerobiosis (AnaeroGen) for 48 h at 37 ± 1 °C (Albuquerque et al., 2021; Massa et al., 2022).

2.7. Quantification of intestinal bacterial populations during in vitro colonic fermentation

The relative abundance of intestinal bacterial populations during colonic fermentation was determined using flow cytometry coupled to fluorescence *in situ* hybridization technique. Oligonucleotide probes labeled with the Cy3 fluorophore to target 16S rRNA gene-specific regions of the specific bacterial populations were used: Lab 158 for *Lactobacillus* spp./*Enterococcus* spp., Bif 164 for *Bifidobacterium* spp., Rfla 729 for *Ruminococcus albus/R. flavefaciens*, Bac 303 for *Bacteroides* spp./*Prevotella* spp., Chis 150 for *Clostridium histolyticum*, and Erec 482 for *Eubacterium rectale/Clostridium coccoides* (Sigma-Aldrich, St. Louis, CA, USA). These bacterial populations were selected because are naturally present and considered the main fermenting bacterial populations in the human intestinal microbiota and often correlated with beneficial or undesirable health effects on the host (Calvigioni et al., 2023; Eastwood et al., 2023; Poveda, Pereira, Lewis, & Walton, 2020; Silva et al., 2023). SYBR Green double-stranded DNA dye (Molecular Probes, Invitrogen, Carlsbad, CA, USA) labeled the total bacterial population.

At 0, 24, and 48 h, aliquots (375 μL) of the fermentation media were collected, fixed, and hybridized with the distinct fluorescent probes as previously described (Albuquerque et al., 2021; Massa et al., 2022). The enumeration of the target bacterial populations was performed with a flow cytometer (BD Accuri C6, BD Biosciences, East Rutherford, NJ, USA) using 488 nm excitation from a blue solid-state laser. BD Accuri C6 software recorded signals as cytograms on FL1 (SYBR Green) and FL2 (Lab 158, Bif 164, Rfla 729, Bac 303, Chis 150, and Erec 482) channels. Results were expressed as the relative abundance (percentage) of cells hybridized with each probe in relation to the total bacterial population.

2.8. Evaluation of microbial metabolic activity during in vitro colonic fermentation

Indicators of microbial metabolic activity (pH, sugars, organic acids, and global metabolic profile) were evaluated in media with UCF, FOS, and NC at 0, 24, and 48 h (Albuquerque et al., 2021; Silva et al., 2023). The pH was determined using a potentiometric pH meter (Q400AS, Quimis, São Paulo, SP, Brazil). The contents of sugars and organic acids were determined as described in section 2.2. The global metabolite profiling was determined with ^{1}H and 13C Nuclear Magnetic Resonance (NMR) spectroscopic technique using Bruker equipment (model Avance Neo, Bruker, Billerica, MA, USA) operating at 500 MHz for 1H NMR and 125 MHz for 13C. The spectrum sequence was obtained using the following parameters: Ic1pngpf2; temperature 26 °C; number of scans: 64; scan dumer: 16; receiver gain: 32; acquisition time: 3.27 s. An aliquot of each sample (2 mL) was diluted (1:1) in a solution with HPLC grade methanol (CH_3OH) and deuterated water (D2O) (9:1, v/v), centrifuged (1696 × *g*, 10 min, 4 °C), and filtered (0.45 μm, Millipore®) before using in the NMR analysis. The sample data analysis consisted of peaks that vary according to the signal intensity in the different regions evaluated. Spectra processing was performed using the MestReNova® (MNova) software version 5.2.3.

2.9. Evaluation of the antioxidant activity during in vitro colonic fermentation

The antioxidant activity in media with digested UCF was evaluated at 0, 24, and 48 h with $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays described in section 2.3.

2.10. Statistical analysis

The experiments were performed in triplicate on three different occasions. The results were presented as average ± standard deviation. The Kolmogorov-Smirnov normality test checked the data normal distribution. The data were subjected to analysis of variance (ANOVA) followed by Tukey's test and unpaired Student's t-test. A P-value <0.5 was considered significant. A Principal Component Analysis (PCA) was conducted with a matrix of six lines (three lines for UCF, FOS, and negative control, and three lines for different *in vitro* colonic fermentation periods) and 12 columns (six columns for the relative abundance of the intestinal bacterial populations, two for sugar contents, and four for organic acid contents). R Software (Version 4.3.1 Ross Ihaka & Robert Gentleman, University of Auckland, Auckland, New Zealand) was used for the statistical analysis.

3. Results

3.1. Physicochemical characteristics of UCF

The UCF had low moisture content, high acidity, and high total dietary fiber content (57.46 ± 0.28%). UCF had higher contents of glucose and fructose than maltose and higher lactic acid content than succinic, malic, and formic acids. Flavonols were the most prevalent phenolic compounds in UCF. Quercetin-3-*O*-glucoside was the flavonol with the highest contents (36.73 mg/100g). UCF showed antioxidant activity when determined with the FRAP, $DPPH^{\bullet}$, and $ABTS^{\bullet+}$ methods. UCF had a higher antioxidant activity when determined with the FRAP method (Table 1).

3.2. Relative abundance of selected intestinal bacterial populations during in vitro colonic fermentation

The relative abundance of the selected intestinal bacterial populations in media with UCF, FOS, and NC during 48 h of *in vitro* colonic fermentation is shown in Table 2. The relative abundance of

Table 1
Physicochemical parameters of umbu-cajá fruit processing flour (average ± standard deviation; n = 3).

Parameters	Average ± standard deviation
Moisture (%)	7.73 ± 000
pH	2.80 ± 0.01
Ash (%)	4.48 ± 0.05
Titratable total acidity (% citric acid)	4.81 ± 0.03
Carbohydrate (%)	78.71 ± 0.18
Proteins (g/100 g)	6.42 ± 0.03
Lipids (g/100 g)	2.56 ± 0.66
Total dietary fiber (%)	63.99 ± 0.43
Soluble dietary fiber (%)	6.53 ± 0.15
Insoluble dietary fiber (%)	57.46 ± 0.28
Sugars (g/100 g)	
Glucose	1.92 ± 0.31
Fructose	3.31 ± 0.53
Maltose	0.96 ± 0.12
Organic acids (g/100 g)	
Formic acid	0.11 ± 0.28
Malic acid	0.16 ± 0.63
Succinic acid	0.18 ± 0.14
Lactic acid	0.36 ± 0.14
Phenolic compounds (mg/100 g)	
Phenolic acids	
Gallic acid	2.33 ± 0.01
Caftaric acid	3.33 ± 0.00
Chlorogenic acid	9.84 ± 0.05
p -Coumaric acid	7.82 ± 0.01
Flavone	
Hesperidin	1.55 ± 0.04
Naringenin	1.83 ± 2.59
Flavanols	
Catechin	1.19 ± 0.13
Epigallocatechin gallate	5.29 ± 0.05
Epicatechin	2.98 ± 0.96
Procyanidin A2	1.01 ± 0.01
Procyanidin B1	7.89 ± 0.09
Procyanidin B2	9.81 ± 0.02
Myricetin	3.65 ± 0.04
Quercetin 3-glucoside	36.96 ± 0.31
Kaempferol 3-glucoside	2.93 ± 0.04
Isorhamnetin	4.67 ± 4.97
Trans-resveratrol	6.00 ± 0.05
Antioxidant activity	
DPPH$^{\bullet}$ (% inhibition)	72.01 ± 3.96
ABTS$^{\bullet+}$ (% inhibition)	73.91 ± 6.85
FRAP (Trolox equivalent - μM)	758.77 ± 34.3

Abbreviations: ABTS$^{\bullet+}$ cation-2,2-azinobis (3-etilbenzo-tiazoline) -6-sulfonic acid; DPPH$^{\bullet}$, 1,1-diphenyl-2 picrylhydrazyl; FRAP, ferric-reducing ability of plasma; $FeSO_4$, ferrous sulfate.

Lactobacillus spp./*Enterococcus* spp. increased in the media with UCF and FOS during the colonic fermentation ($p \leq 0.05$). Media with UCF and FOS increased ($p \leq 0.05$) the relative abundance of *Bifidobacterium* spp. at 24 h of colonic fermentation. Medium with UCF (8.81 ± 0.37%) had the highest relative abundance of *Bifidobacterium* spp. during the colonic fermentation ($p \leq 0.05$), followed by medium with FOS (5.15 ± 0.29%). The medium with UCF had the highest relative abundance of *Lactobacillus* spp./*Enterococcus* spp. (7.39 ± 0.32%) at 48 h of colonic fermentation, followed by medium with FOS (6.51 ± 0.19%) and NC (0.12 ± 0.03%) ($p \leq 0.05$).

The media with FOS (0.64 ± 0.16%) and UCF (0.47 ± 0.13%) had a higher relative abundance of *R. albus/R. flavefaciens* compared to NC at 48 h of colonic fermentation ($p \leq 0.05$). NC had a lower relative abundance of *R. albus/R.flavefaciens* at 48 h of colonic fermentation compared to 0 h ($p \leq 0.05$). The relative abundance of *Bacteroides* spp./*Prevotella* spp. decreased ($p \leq 0.05$) at 24 and 48 h of colonic fermentation regardless of the medium, with the lowest relative abundance in the medium with UCF ($p \leq 0.05$). The relative abundance of *Bacteroides* spp./*Prevotella* spp. did not change in NC (6.99 ± 0.38% to 6.42 ± 0.26%) during the 48 h of colonic fermentation ($p > 0.05$).

Table 2
Relative abundance (%, average ± standard deviation; n = 3) of different intestinal bacterial groups in the medium with umbu-cajá fruit processing flour (UCF), fructooligosaccharides (FOS), and negative control (NC, without fermentable substrate) at 0, 24, and 48 h of *in vitro* colonic fermentation.

Bacterial groups	Fermentation medium	Time of fermentation		
		0 h	24 h	48 h
Lactobacillus spp./ *Enterococcus* spp.	UCF	0.44 ± 0.15%^{Ab}	2.67 ± 0.21%^{Bc}	7.39 ± 0.32%^{Cc}
	FOS	0.42 ± 0.18%^{Ab}	1.86 ± 0.18%^{Bb}	6.51 ± 0.19%^{Cb}
	NC	0.11 ± 0.05%^{Aa}	0.20 ± 0.08%^{Aa}	0.12 ± 0.03%^{Aa}
Bifidobacterium spp.	UCF	1.90 ± 0.14%^{Ab}	3.12 ± 0.26%^{Bb}	8.81 ± 0.37%^{Cc}
	FOS	1.33 ± 0.17%^{Aa}	3.65 ± 0.22%^{Bc}	5.15 ± 0.29%^{Cb}
	NC	1.39 ± 0.20%^{Ca}	0.11 ± 0.05%^{Aa}	0.32 ± 0.10%^{Ba}
Ruminococcus albus/R. flavefaciens	UCF	1.21 ± 0.16%^{Cb}	0.32 ± 0.04%^{Aa}	0.47 ± 0.13%^{Bb}
	FOS	0.42 ± 0.11%^{Aa}	0.53 ± 0.12%^{Ab}	0.64 ± 0.16%^{Ab}
	NC	0.31 ± 0.06%^{Ba}	0.10 ± 0.03%^{Aa}	0.21 ± 0.08%^{ABa}
Bacteroides spp./ *Prevotella* spp.	UCF	10.54 ± 1.21%^{Cc}	6.21 ± 0.33%^{Ab}	8.64 ± 0.42%^{Bc}
	FOS	8.43 ± 0.55%^{Cb}	5.45 ± 0.24%^{Ba}	3.87 ± 0.18%^{Aa}
	NC	6.99 ± 0.38%^{Ba}	5.76 ± 0.31%^{Aa}	6.42 ± 0.26%^{Bb}
C. histolyticum	UCF	2.77 ± 0.17%^{Aa}	3.99 ± 0.28%^{Ba}	5.09 ± 0.38%^{Cb}
	FOS	5.77 ± 0.25%^{Cc}	4.32 ± 0.20%^{Bab}	4.65 ± 0.24%^{Aab}
	NC	3.88 ± 0.23%^{Ab}	4.66 ± 0.31%^{Bb}	4.21 ± 0.29%^{ABa}
E. rectale/C. coccoides	UCF	5.77 ± 0.35%^{ABa}	5.87 ± 0.28%^{Bb}	5.31 ± 0.17%^{Ab}
	FOS	6.99 ± 0.42%^{Cb}	5.09 ± 0.22%^{Ba}	3.10 ± 0.14%^{Aa}
	NC	6.43 ± 0.30%^{Ab}	6.21 ± 0.34%^{Ab}	6.64 ± 0.26%^{Ab}

A – C: Different superscript capital letters in the same row denote differences ($p \leq 0.05$), based on Tukey's test; a-c: different superscript small letters in a column for the same bacterial population denote difference ($p \leq 0.05$), based on Tukey's test.

The relative abundance of *C. histolyticum* increased in the medium with UCF during the 48 h of colonic fermentation ($p \leq 0.05$), while it increased until 24 h of colonic fermentation ($p \leq 0.05$) and did not change at 48 h of colonic fermentation in the medium with FOS and NC ($p > 0.05$). The relative abundance of *E. rectale/C. coccoides* decreased in the media with UCF and FOS compared to time zero (5.87 ± 0.28 to 5.31 ± 0.17% and 6.99 ± 0.42 to 5.09 ± 0.22%, respectively) at 48 h of colonic fermentation ($p \leq 0.05$), while it did not change in NC (6.43 ± 0.30 to 6.64 ± 0.26%) ($p > 0.05$).

3.3. Intestinal microbial metabolic activity during in vitro colonic fermentation

The pH values decreased in the media with UCF, FOS, and NC during the 48 h of *in vitro* colonic fermentation ($p \leq 0.05$). The medium with

UCF had a lower pH (3.40 ± 0.04) at 48 h of colonic fermentation compared to the medium with FOS (4.81 ± 0.02) and NC (4.77 ± 0.02) ($p \leq 0.05$) (Supplementary material data, S2).

The results of the contents of sugars, lactic acid, and short-chain fatty acids (SCFA) in the media with UCF, FOS, and NC during the 48 of *in vitro* colonic fermentation are shown in Fig. 1. Fructose and glucose contents were below the limit of detection in the medium with UCF at 24 and 48 h of colonic fermentation, and fructose content decreased in the medium with FOS during the 48 h of colonic fermentation ($p \leq 0.05$). The contents of lactic acid and SCFA overall increased in the media with UCF and FOS during the 48 h of colonic fermentation. Propionic acid was the organic acid with the highest contents in the medium with UCF during the 48 h of colonic fermentation, followed by acetic, butyric, and lactic acids. Only lactic acid was detected in NC during the 48 h of colonic fermentation.

The global metabolic profiling analysis in the media with UCF and FOS at time 0 and 48 h of *in vitro* colonic fermentation detected 57 different chemical constituents (Fig. 2; supplementary material data, S2). Several metabolites were identified in the media with UCF and FOS, including amino acids and amino-acid-derived metabolites [leucine, isoleucine, valine, threonine, alanine, lysine, ornithine, glycine, putrescine, glutamate, proline, methylamine, phenylalanine, 2-oxo isovalerate, 2- methyl butyrate, gamma-aminobutyric acid (GABA), uracil, 5-amino pentanoate, asparagine, methionine]; and energy metabolism derived-metabolites (lactate, acetate, succinate, fructose, aspartate, formate, isocaproate, ketoisovalerate, n-butyrate, propionate, citrate, valerate, malonate, 3-methyl-2- oxo isovalerate, phenylacetate, α-xylose, β-xylose, β-glucose, α-glucose, D-galactose, UDP- glucuronate, dihydroxyacetone, and 5-aminosalicylate). Additionally, choline-derived metabolite (trimethylamine - TMA), ketone-derived metabolite (3-hydroxybutyrate), microbial-derived metabolites (isobutyrate, p-cresol, 3, and N- acetyl-5-aminosalicylate), and a variety of other metabolites, such as bile salts, ethanol, and total lipids, were detected. Proline and methylamine lost signal intensity in the medium with UCF at 48 h of colonic fermentation. Some metabolites were only detected in the medium with UCF at 48 h of colonic fermentation, including 3-hydroxyphenylacetate and asparagine.

3.4. Antioxidant activity during in vitro colonic fermentation

The medium with UCF showed antioxidant activity during the 48 h of colonic fermentation when measured with the ABTS•+, DPPH•, and FRAP methods (Fig. 3). The results showed that the antioxidant activity of UCF is high and remains significant throughout the *in vitro* colonic fermentation ($p \leq 0.05$). Using the DPPH method, the antioxidant activity was 40.16 ± 2.33% at 0 h, 16.27 ± 2.64% at 24 h, and increases to 22.96 ± 3.01% at 48 h. Using the ABTS method, the antioxidant activity started at 84.69 ± 1.11% at 0 h, decreased to 53.65 ± 1.20% at 24 h, and increased to 62.17 ± 2.04% at 48 h. Using the FRAP method, the antioxidant capacity was 327.95 ± 36.38 μM at 0 h, reduced to 78.76 ± 9.40 μM at 24 h, and increased to 136.22 ± 14.52 μM at 48 h. Despite the drop at the beginning, there was a significant recovery of the antioxidant activity at 48 h of colonic fermentation.

3.5. Chemometric analysis

The PCA evaluated the differences between the examined media considering the relative abundance of the measured bacterial populations, contents of sugars and organic acids (including SCFA) during the 48 h of *in vitro* colonic fermentation (Fig. 4). The PCA analysis explained the data with a total variance of 66.99% (Dim 1: 50.36% and Dim 2: 16.63%). The PCA map reinforced the effects of UCF in increasing the relative abundance of Lactobacillus spp./*Enterococcus* spp., Bifidobacterium spp., and *R. albus/R. flavefaciens*, as well as the production of acetic, butyric, lactic, and propionic acids *during the colonic fermentation*. NC located at the lower quadrants, with the lower results at 0, 24, and 48 h of colonic fermentation.

4. Discussion

The UCF had low moisture content, below the maximum limit of current Brazilian legislation for edible vegetable flours (i.e., ≤15%). The low moisture and high acidity of UCF are intrinsic characteristics limiting microbial growth and enzymatic activity, allowing greater stability and safety during storage (Alp & Bulantekin, 2021). The presence of soluble and insoluble dietary fiber in UCF indicates the presence of carbohydrates resistant to digestion and absorption in the small intestine, reaching the colon as substrates for colonic fermentation (Ioniță-Mîndrican et al., 2022). The variety of phenolic compounds in UCF, mainly flavonols, could be linked to its antioxidant activity (Kamal et al., 2023).

The medium with UCF induced an increase in bacterial populations associated with beneficial effects on the human intestinal microbiota, such as *Lactobacillus* spp./*Enterococcus* spp., *Bifidobacterium* spp., and *R. albus/R. flavefaciens* during the 48 h of colonic fermentation (Sanders, Merenstein, Reid, Gibson, & Rastall, 2019; Zhang, Hu, Wang, Liu, & Pan, 2018). In the colonic environment, these bacteria exert a protective effect against pathogens, increase the permeability of the membrane of target cells, which polarizes the membrane and cause cell death, besides producing mucin that forms a physical barrier against invading agents, and strengthens innate and acquired immunity (Aleman & Yadav, 2024). The increase in the populations of beneficial intestinal bacteria concomitant with the decrease in pH and increased production of lactic acid and SCFA, namely acetic, butyric, and propionic acids, indicates intense intestinal microbial metabolic activity (Markowiak-Kopeć & Śliżewska, 2020). Once produced, SCFA can migrate to other organs via the so-called "gut-organ axis", playing an important role in inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), obesity and metabolic syndrome, type 2 diabetes, cardiovascular diseases, cancer, neurological diseases, allergies, and asthma (Zhang et al., 2023).

An overview of the data obtained in this study summarized by PCA analysis showed that UCF colonic fermentation generated satisfactory results in several analyses, reinforcing the capability of the bioactive compounds typically found in fruit by-products to exert beneficial effects on the human intestinal microbiota (Massa et al., 2022). The restricted growth of the beneficial intestinal bacterial populations in NC during the colonic fermentation indicates a lack of nutrients to support the proliferation of these microorganisms. Therefore, any increase in beneficial intestinal bacterial populations in the medium with UCF during the colonic fermentation could be associated with its capacity to selectively foster bacterial growth (Melo et al., 2020).

The contents of glucose and fructose in the medium with UCF were quickly consumed during the colonic fermentation. It indicates the use of UCF as a fermentable substrate for beneficial bacteria since intestinal bacteria, such as *Lactobacillus* spp., *Enterococcus* spp., and *Bifidobacterium* spp., have a strictly fermentative metabolism and obtain energy through the anaerobic breakdown of carbohydrates resistant to digestion and absorption in the upper gastrointestinal tract, with the production of organic acids, such as lactic acid and SCFA (Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016). *Lactobacillus* and *Bifidobacterium* can convert lactic and acetic acids to butyric acid through cross-feeding, the preferred energy source for colonocytes (Moens, Verce, & De Vuyst, 2017). The butyric acid production, in parallel with the decrease in pH during the colonic fermentation, could be related to the increased relative abundance of *Lactobacillus* spp. and *Bifidobacterium* spp. in the medium with UCF. Increased intestinal production of SCFA has been associated with anti-inflammatory, immunoregulatory, anti-obesity, anti-diabetes, anticancer, hepatoprotective, cardiovascular protective, and neuroprotective effects (Xiong et al., 2022).

The presence of flavonoids in UCF could have contributed to the increase in the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. during the colonic fermentation (Rha et al., 2019). Flavonols can occur

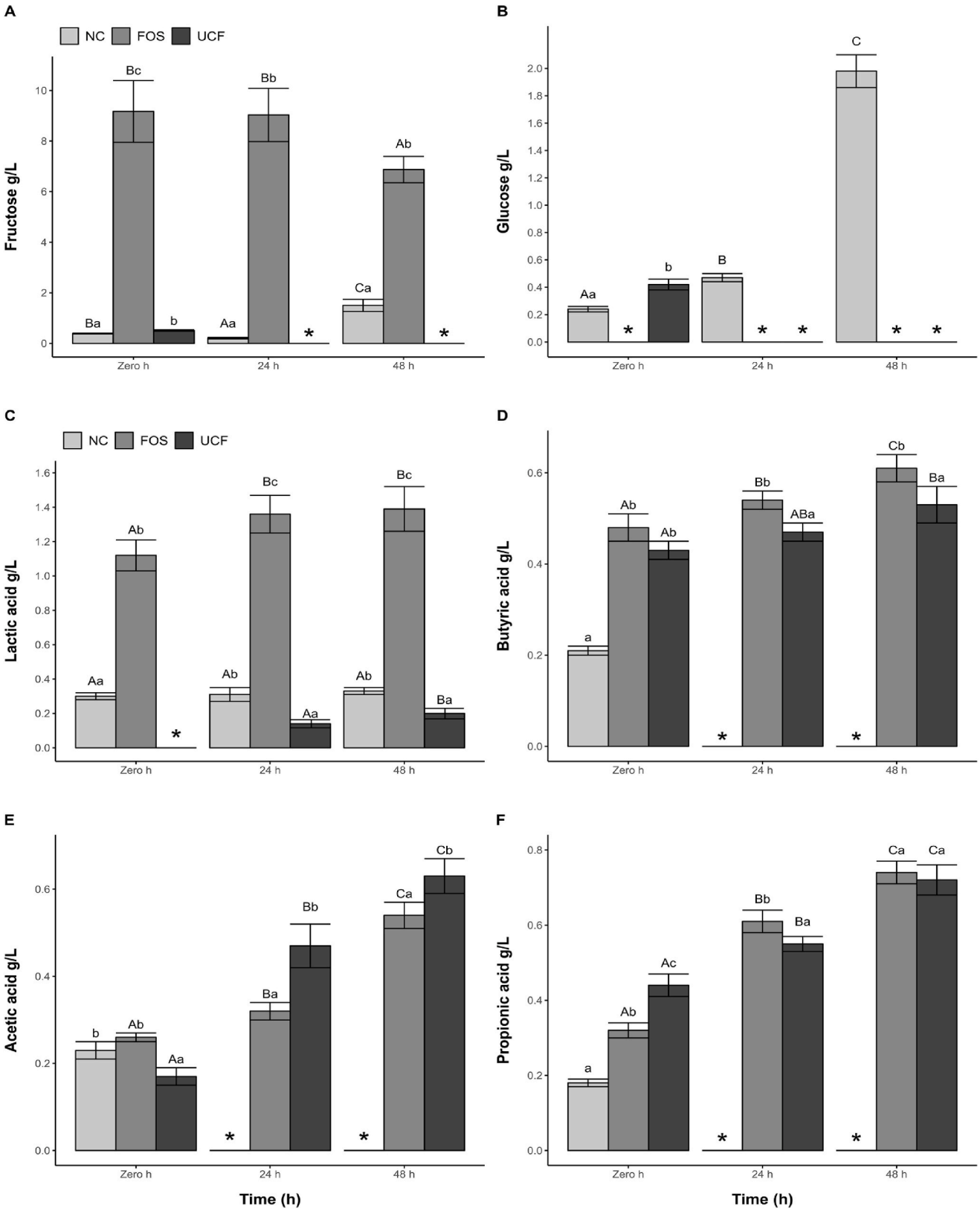

Fig. 1. Sugars, lactic acid, and short-chain fatty acid (SCFA; g/L; average ± standard deviation; n = 3) in medium with digested umbu-cajá flour (UCF), fructo-ligosaccharides (FOS), and negative control (NC; without fermentable substrate) at time zero, 24, and 48 h of *in vitro* colonic fermentation. * <LOD: below the limit of detection.

A–C: Different superscript capital letters for the same medium at different colonic fermentation times denote differences ($p \leq 0.05$), based on Tukey's test; a–c: Different superscript small letters for different media at the same colonic fermentation time denote differences ($p \leq 0.05$), based on Tukey's test.

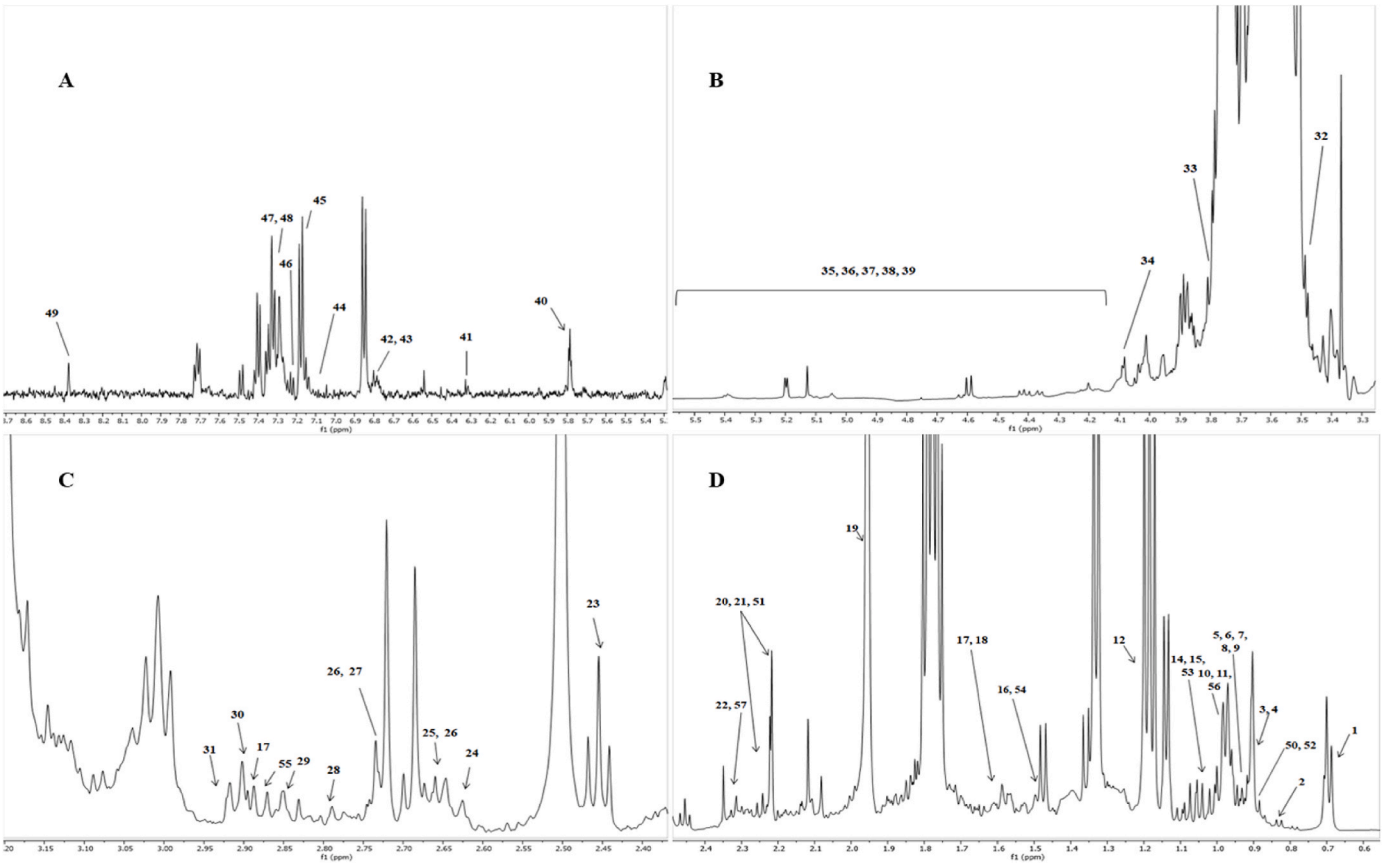

Fig. 2. Representative ^{1}H NMR spectra in the medium with umbu-cajá fruit processing flour (UCF), fructooligosaccharides (FOS), and negative control (NC; without fermentable substrate) at 0 and 48 h of *in vitro* colonic fermentation. A: 5.3–8.7 ppm; B: 3.3–5.5 ppm; C: 2.4–3.2 ppm; D: 0.6–2.4 ppm. 1: biliary salts; 2: methylbutyrate; 3: valerate; 4; 5: n-butyrate; 6: isoleucine; 7: valine; 8: propionate; 9: isobutyrate; 10: 3-methyl-2-oxoisovalerate; 11: 2-oxoisovalerate; 12: ethanol; 13: 3-hydroxybutyrate; 14: threonine; 15: lactate; 16: alanine; 17: lysine18: ornithine; 19: acetate; 20: proline; 21: glutamate; 22: 5-aminopentanoate; 23: succinate; 24: methylamine; 25: methionine; 26: citrate; 27: aspartate; 28: asparagine; 29: trimethylamine; 30: putrescine; 31: malonate; 32: glycine; 33: fructose; 34:dihydroxyacetone; 35: α-Xylose; 36: β-xylose; 37:β-glucose; 38: α-glucose; 39: D-galactose; 40: UDP-glucuronate; 41: 3 hydroxyphenylacetate; 42: p-cresol; 43: tyrosine; 44: 5-aminosalicylate; 45: phenylalanine; 46:uracil; 47: N-acetyl-5-aminosalicylate; 48: phenylacetate; 49: formate; 50: caprylate; 51: isocaproate; 52: isovalerate; 53: 3-hydroxyisovalerate; 54: total lipids; 55: gamma-aminobutyric acid (GABA); 56: Ketoisovalerate and 57: acetone.

in foods bound to sugars, called glycosides, which are more stable than free flavonoids (Šamec, Karalija, Šola, Vujčić Bok, & Salopek-Sondi, 2021). The interaction of flavonols with the intestinal microbiota could increase the populations of beneficial bacteria, especially of *Lactobacillus* spp. and *Enterococcus* spp., and decrease the populations of potentially pathogenic bacteria, such as *C. coccoides* and *E. rectale* (Pan et al., 2023).

The medium with UCF increased the production of organic acids during the colonic fermentation, standing out the contents of propionic acid. Members of the Bacteroidetes phylum can mostly produce propionic acid in the intestine via the succinic acid pathway (Reichardt et al., 2014). The increase in propionic acid production agrees with the results showing the higher relative abundance of *Bacteroides* spp./*Prevotella* spp. in the medium with UCF at 24 h of colonic fermentation, indicating that this SCFA could have been mostly produced by Bacteroidaceae and Prevotellaceae members. *C. histolyticum* can also produce propionic acid (Hughes, Shewry, Gibson, McCleary, & Rastall, 2008), and it could explain the increase in propionic acid contents in the medium with UCF during the 48 h of colonic fermentation, concomitant with the increase in the relative abundance of *Bacteroides* spp./*Prevotella* spp. and *C. histolyticum* populations, suggesting a cross-feeding interaction (Peterson, Perez Santiago, Iablokov, Chopra, Rodionov, & Peterson, 2022). These bacteria are saccharolytic species capable of producing high concentrations of SCFA from available sugars (Rowland et al., 2018). Propionic acid in the intestine promotes anti-inflammatory effects and increases mineral absorption, besides reducing colonic pH (Haghikia et al., 2022; Yan et al., 2022).

The increase in the relative abundance of *R. albus*/*R. flavefaciens* in the medium with UCF at 48 h of colonic fermentation compared with 24 h could be linked to the increase in the contents of acetic acid. *Ruminococcus* species are found abundantly in the human intestinal microbiota. *R. albus* and *R. flavefaciens* are cellulolytic species that produce SCFA, especially acetic acid, from carbohydrate fermentation (La Reau & Suen, 2018). Acetic acid is a substrate that produces fatty acids in the liver and is an energy source for muscle and brain tissues (Liu, Fu, & Li, 2019). The high contents of insoluble dietary fibers in UCF could direct the higher acetic acid production by *R. albus* and *R. flavefaciens* during colonic fermentation (Yeoman et al., 2021).

E. rectale/*C. coccoides* are butyric acid producers (Singh et al., 2023; Abbeele et al., 2012). The relative abundance of *E. rectale*/*C. coccoides* did not change in the medium with UCF during the colonic fermentation, being lower than the relative abundance of *Lactobacillus* spp./*Enterococcus* spp. found therein. These results indicate that butyric acid production resulted mostly from *Lactobacillus* spp./*Enterococcus* spp. and *Bifidobacterium* spp. metabolism. The presence of flavonols in UCF could also be an influential factor in decreasing the relative abundance of *E. rectale*/*C. coccoides* during the colonic fermentation since these compounds are inhibitory for intestinal pathogenic bacteria (Song, Shen, Deng, Zhang, & Zheng, 2021). The presence of phenolic compounds in UCF could have contributed to the antioxidant activity in the

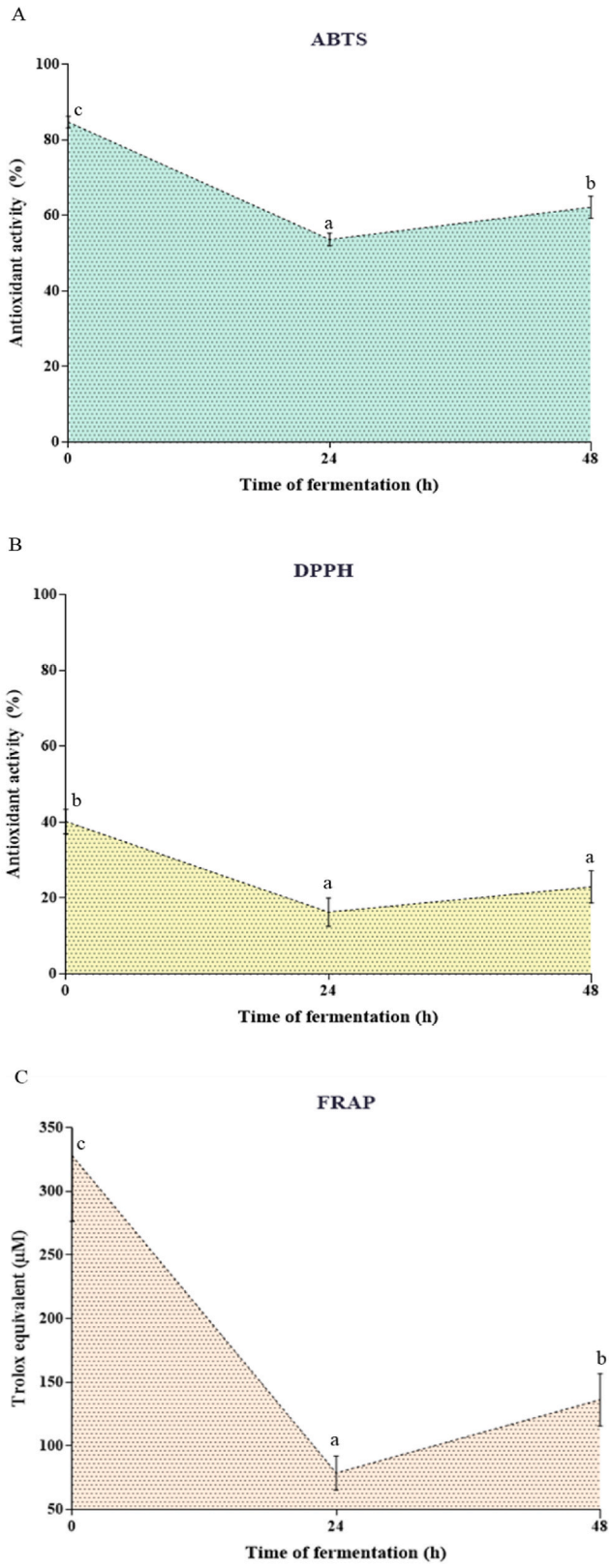

Fig. 3. Antioxidant activity (average ± standard deviation; n = 3) determined with ABTS (A), DPPH (B), and FRAP (C) methods in the medium with umbucajá fruit processing flour (UCF) at 0, 24, and 48 h of *in vitro* colonic fermentation.

a–c: Different superscript small letters at different colonic fermentation times denote differences ($p \leq 0.05$), based on Tukey's test.

medium containing UCF during the colonic fermentation (Xavier et al., 2022). It is an important result since keeping antioxidant activity in the colonic environment has been typically associated with beneficial alterations in the composition and function of intestinal microbiota (Ma et al., 2022). Furthermore, the decrease in the antioxidant activity in the medium with UCF at 24 and 48 h of colonic fermentation compared to time 0 h could be linked to the consumption of phenolic compounds initially available in the medium by the intestinal microbiota.

The results of the global metabolic profiling showed a large number and variety of metabolites in the medium with UCF during the colonic fermentation. Most of the detected metabolites are detected in human fecal samples and reinforce the stimulatory effects of UCF on the metabolic activity of intestinal microbiota, agreeing with the results showing the sugar consumption and organic acid production during the colonic fermentation. Specifically, the medium with UCF lost the signal intensity of proline at 48 h of colonic fermentation. Proline synthesis occurs from glutamate (Jones et al., 2021), and the non-detection of proline in the medium with UCF indicates that this amino acid and/or its precursor were degraded by intestinal microbiota during the colonic fermentation (Ji, Guo, Yin, Blachier, & Kong, 2018).

Asparagine is synthesized from aspartate (Lomelino, Andring, McKenna, & Kilberg, 2017). The medium with UCF lost the signal intensity of aspartate at 24 h of colonic fermentation, besides increasing the signal intensity of asparagine during 48 h of colonic fermentation, indicating that aspartate was metabolized by intestinal microbiota to produce asparagine. It is an important result since asparagine can protect the intestinal barrier and promote intestinal microbiota homeostasis (Zhu, Pi, & Leng, 2017). 3-hydroxyphenylacetate was detected only in the medium with UCF at 48 h of colonic fermentation. 3-hydroxyphenylacetate is the main metabolite derived from quercetin. The quercetin degradation pathway begins with the formation of dihydroquercetin, which is degraded into 3-(3,4-dihydroxyphenyl) and 3,4-dihydroxyphenylacetate, and, after successive degradations, produces 3- hydroxyphenylacetate (Dias, Pourová, Vopršalová, Nejmanová, & Mladěnka, 2022). The presence of 3-hydroxyphenylacetate in the medium with UCF indicates the metabolization of phenolic compounds by the intestinal microbiota during the colonic fermentation. Various branched-chain fatty acids were detected in the medium with UCF during the colonic fermentation. Some bacteria forming the intestinal microbiota produce branched-chain fatty acids (BCFA), such as isobutyric, isovaleric, and 2-methylbutyric acids, from the catabolism of valine, leucine, and isoleucine (Ezzine et al., 2022), indicating amino acid consumption during the colonic fermentation. BCFA affect gastrointestinal activity through several mechanisms, including increased satiety (Tian et al., 2019).

The use of *in vitro* colonic fermentation systems and fluorescence *in situ* hybridization coupled with flow cytometry technique has been recognized as an effective analytical protocol for investigating the putative modulatory effects of several foods and food ingredient candidates for prebiotic use (Albuquerque et al., 2021; Macedo et al., 2023; Massa et al., 2022; Medeiros et al., 2021; Menezes et al., 2021; Sampaio, Alves, et al., 2022; Sampaio, Nascimento, Garcia, & Souza, 2022; de Oliveira et al., 2023; Sampaio, Nascimento, Garcia, & Souza, 2022). These results become more robust when this integrated investigative protocol uses a validated *in vitro* gastrointestinal digestion procedure, a fresh pooled fecal inoculum, an anaerobic generating system, and measurement of representative target bacterial populations forming the human intestinal microbiota, as carefully applied in this study (de Oliveira et al., 2023; Martins et al., 2023). However, a few limitations of this study could be considered: i) difficulties in simulating some physicochemical conditions and physiological events occurring in the human gastrointestinal digestion; ii) difficulties in accurately estimating the complexity of the host-intestinal microbiota interactions; iii) difficulties in maintaining a continuous and anaerobic fermentation system; and iv) the use of more probes to measure other important bacterial populations commonly found in the human intestinal microbiota (*e.g., Akkermansia*

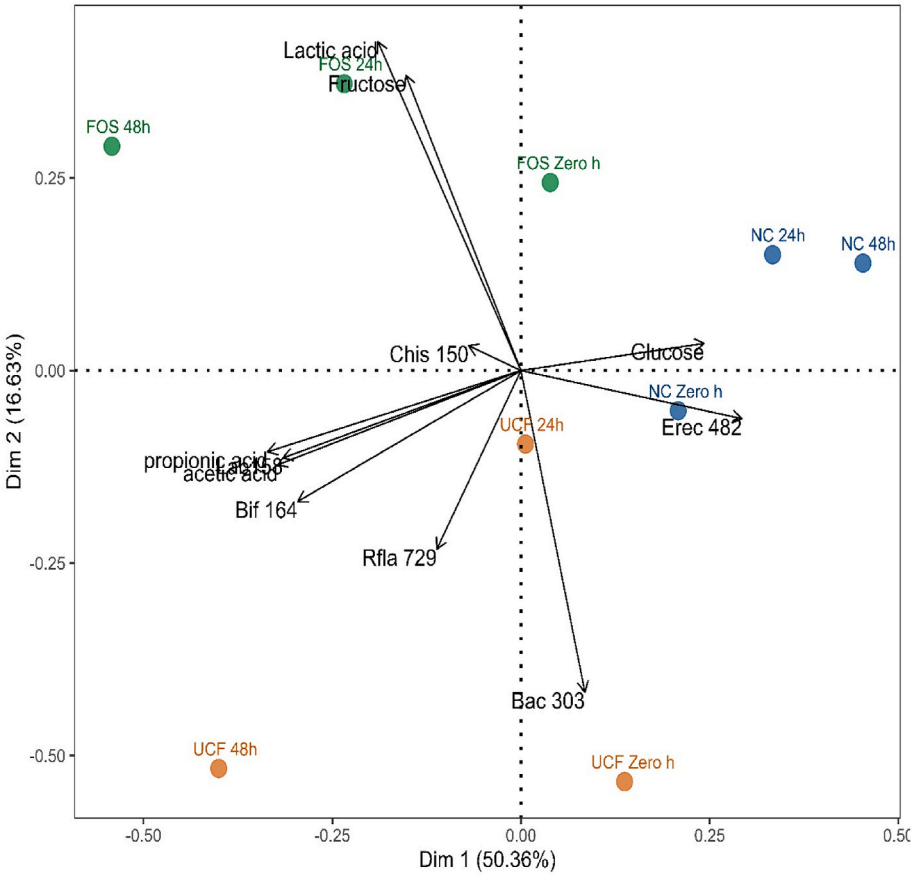

Fig. 4. Principal component analysis (PCA) considering measured variables in the medium with umbu-cajá fruit processing flour (UCF), fructooligosaccharides (FOS), and negative control (NC; without fermentable substrate) at 0, 24, and 48 h of *in vitro* colonic fermentation. Variables: Relative abundance of selected intestinal bacterial groups, and contents of sugars and short-chain fatty acids. Lab 158: *Lactobacillus* spp./*Enterococcus* spp.; Bif 164: *Bifidobacterium* spp.; Rfla 729: *Ruminococcus albus/R. flavefaciens;* Bac 303: *Bacteroides* spp./*Prevotella* spp.; Chis 150: *Clostridium histolyticum* and Erec 482: *Eubacterium rectal/Clostridium coccoides*.

muciniphila, Clostridium leptum, and *Faecalibacterium prausnitzii*). Although with these possible limitations, the results of this study are important and innovative to direct future *in vivo* experimental investigations with advanced molecular techniques, such as next-generation sequencing (NGS), to confirm the prebiotic effects of UCF on human intestinal microbiota.

5. Conclusion

UCF increased the relative abundance of populations of beneficial intestinal bacteria during the colonic fermentation, besides decreasing or not affecting the relative abundance of populations of not beneficial intestinal bacteria. Additionally, UCF stimulated the metabolic activity of intestinal microbiota during the colonic fermentation, reducing the pH, increasing the consumption of sugars and production of lactic acid and SCFA, changing the metabolic profile, and keeping the antioxidant activity. These results demonstrate the potential use of a low-cost flour derived from a typically discarded fruit processing by-product as a promising candidate for developing sustainable prebiotic formulations. However, further *in vivo* studies could advance in confirming the beneficial effects of UCF on human intestinal microbiota and parameters associated with intestinal and systemic health.

Ethical statement

The Ethics Committee for Research with Human Beings of the Federal University of Sergipe (São Cristóvão, SE, Brazil) granted ethical approval (protocol number 6.419.531; CAAE 7075823.0.0000.5546) since this study involved human subjects.

CRediT authorship contribution statement

Jordana Nunes de Oliveira: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Thatyane Mariano Rodrigues de Albuquerque:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Marcos dos Santos Lima:** Methodology, Investigation. **Elma Regina Silva de Andrade Wartha:** Methodology, Investigation, Funding acquisition, Conceptualization. **Mariana Monteiro:** Methodology, Investigation. **Yuri Mangueira do Nascimento:** Visualization, Methodology, Investigation. **Josean Fechine Tavares:** Visualization, Methodology, Investigation. **Marcelo Sobral da Silva:** Methodology, Investigation, Funding acquisition. **Evandro Leite de Souza:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation. **Jane de Jesus da Silveira Moreira:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors of the paper "**Evaluating the potential prebiotic effects of umbu-cajá (*Spondias* spp.) fruit processing by-product flour on the human intestinal microbiota"** submitted to LWT – Food Science and Technology 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

Data will be made available on request.

Acknowledgments

The authors thank FAPITEC - SE (Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil; Finance code 001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil; Process number 441379/2023-0) for funding this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116764.

References

Abbeele, P. V., Belzer, C., Goossens, M., Kleerebezem, M., De Vos, W. M., Thas, … Van de Wiele, T. (2012). Butyrate-producing *Clostridium cluster* XIVa species specifically colonize mucins in an *in vitro* gut model. *The ISME Journal, 7*(5), 949–961. https://doi.org/10.1038/ismej.2012.158

Aderne, I., Anjos, D. A., Souza, C. C. E., & Zanuto, M. E. (2021). Nutritional and functional potential of umbu (*Spondias tuberosa Arruda* Cam.) peel flour. *Revista Brasileira de Agrotecnologia, 11*(2), 964–974. https://doi.org/10.18378/REBAGRO.V12I2.8883. In Portuguese.

Akter, B., & Rabeta, M. S. (2021). Synbiotic and antioxidant activity of fruit by-products and their effect on human health. *Food Research, 5*(1), 24–35. https://doi.org/10.26656/fr.2017.5(1).40

Albuquerque, T. M. R., Magnani, M., Lima, M. S., Castellano, L. R. C., & de Souza, E. L. (2021). Effects of digested flours from four different sweet potato (*Ipomoea batatas* L.) root varieties on the composition and metabolic activity of human colonic microbiota *in vitro*. *Journal of Food Science, 86*, 3707–3719. https://doi.org/10.1111/1750-3841.15852

Aleman, R. S., & Yadav, A. (2024). Systematic review of probiotics and their potential for developing functional nondairy foods. *Applied Microbiology, 4*(1), 47–69. https://doi.org/10.3390/applmicrobiol4010004

Alkehayez, N. M., Alshawi, A. H., & Aljobair, M. O. (2022). The physicochemical composition and sensory attributes of sponge cake fortification with date powder. *Food Science and Technology, 42*. https://doi.org/10.1590/fst.92522. Article e92522.

Alp, D., & Bulantekin, O. (2021). The microbiological quality of various foods dried by applying different drying methods: A review. *European Food Research and Technology, 247*, 1333–1343. https://doi.org/10.1007/s00217-021-03731-z

Andrade, R. M. S. de, Silva, S., Costa, C. M. D. S. F., Veiga, M., Costa, E., Ferreira, M. S. L., et al. (2020). Potential prebiotic effect of fruit and vegetable byproducts flour using *in vitro* gastrointestinal digestion. *Food Research International, 137*. https://doi.org/10.1016/j.foodres.2020.109354. Article 109354.

Association of Official Analytical Chemists (AOAC). (2016). Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics, 20th ed. Official Methods of Analysis of the AOAC International. Rockville, MD, USA. ISBN: 97-8-093558487-5 *Nature Reviews Gastroenterology & Hepatology, 14*, 491–502. https://doi.org/10.1038/nrgastro.2017.75.

Banerjee, J., Singh, R., Vijayaraghavan, R., MacFarlane, D., Patti, A. F., & Arora, A. (2017). Bioactives from fruit processing wastes: Green approaches to valuable chemicals. *Food Chemistry, 225*, 10–22. https://doi.org/10.1016/j.foodchem.2016.12.093

Barros, R. G. C., Pereira, U. C., Andrade, J. K. S., de Oliveira, C. S., Vasconcelos, S. V., & Narain, N. (2020). *In vitro* gastrointestinal digestion and probiotics fermentation impact on bioaccessbility of phenolics compounds and antioxidant capacity of some native and exotic fruit residues with potential antidiabetic effects. *Food Research International, 136*, Article 109614. https://doi.org/10.1016/j.foodres.2020.109614

Brand-Williams, W., Cuvelier, M., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science & Technology, 28*, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Calvigioni, M., Panattoni, A., Biagini, F., Donati, L., Mazzantini, D., Massimino, et al. (2023). Development of an *in vitro* model of the gut microbiota enriched in mucus-adhering bacteria. *Microbiology Spectrum, 11*(4), Article e0033623. https://doi.org/10.1128/spectrum.00336-23

Campos, D. A., Coscueta, E. R., Vilas-Boas, A. A., Silva, S., Teixeira, J. A., Pastrana, L. M., et al. (2020). Impact of functional flours from pineapple by-products on human intestinal microbiota. *Journal of Functional Foods, 67*. https://doi.org/10.1016/j.jff.2020.103830. Article 103830.

Cangussu, L. B., P Leão, D., Oliveira, L. S., & Franca, A. S. (2021). Profile of bioactive compounds in pequi (*Caryocar brasilense* Camb.) peel flours. *Food Chemistry, 350*, Article 129221. https://doi.org/10.1016/j.foodchem.2021.129221

Coelho, E. M., Padilha, C. V. S., Miskinis, G. A., Sá, A. G. B., Pereira, G. E., Azevedo, L. C., et al. (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *Journal of Food Composition and Analysis, 66*, 160–167. https://doi.org/10.1016/j.jfca.2017.12.017

de Oliveira, S. P. A., Albuquerque, T. M. R., Massa, N. M. L., Rodrigues, N. P. A., Sampaio, K. B., do Nascimento, H. M. A., et al. (2023). Investigating the effects of conventional and unconventional edible parts of red beet (*Beta vulgaris* L.) on target bacterial groups and metabolic activity of human colonic microbiota to produce novel and sustainable prebiotic ingredients. *Food Research International, 171*, Article 112998. https://doi.org/10.1016/j.foodres.2023.112998

Dias, P., Pourová, J., Vopršalová, M., Nejmanová, I., & Mladěnka, P. (2022). 3-Hydroxyphenylacetic acid: A blood pressure-reducing flavonoid metabolite. *Nutrients, 14*(2), 328. https://doi.org/10.3390/nu14020328

Dutra, R. L. T., Dantas, A. M., Marques, D. A., Batista, J. D. F., Meireles, B. R. L. A., de Magalhães Cordeiro, Â. M. T., et al. (2017). Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. *Food Research International, 100*(Pt 1), 650–657. https://doi.org/10.1016/j.foodres.2017.07.047

Eastwood, J., Van Hemert, S., Poveda, C., Elmore, S., Williams, C., Lamport, D., et al. (2023). The effect of probiotic bacteria on composition and metabolite production of faecal microbiota using in vitro batch cultures. *Nutrients, 15*(11), 2563. https://doi.org/10.3390/nu15112563

Ezzine, C., Loison, L., Montbrion, N., Bôle-Feysot, C., Déchelotte, P., Coëffier, M., et al. (2022). Fatty acids produced by the gut microbiota dampen host inflammatory responses by modulating intestinal SUMOylation. *Gut Microbes, 14*(1). https://doi.org/10.1080/19490976.2022.2108280. Article 2108280.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The international scientific. https://doi.org/10.1038/nrgastro.2017.75.

Haghikia, A., Zimmermann, F., Schumann, P., Jasina, A., Roessler, J., Schmidt, D., … Andmesser, U. (2022). Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. *European Heart Journal, 43*(6), 518–533. https://doi.org/10.1093/eurheartj/ehab644

Hughes, S. A., Shewry, P. R., Gibson, G. R., McCleary, B. V., & Rastall, R. A. (2008). *In vitro* fermentation of oat and barley derived beta-glucans by human faecal microbiota. *FEMS Microbiology Ecology, 64*(3), 482–493. https://doi:10.1111/j.1574-6941.2008.00478.x.

Ioniță-Mîndrican, C. B., Ziani, K., Mititelu, M., Oprea, E., Neacșu, S. M., Moroșan, E., … Negrei, C. (2022). Therapeutic benefits and dietary restrictions of fiber intake: A state of the art review. *Nutrients, 14*(13), 2641. https://doi.org/10.3390/nu14132641

Ji, Y., Guo, Q., Yin, Y., Blachier, F., & Kong, X. (2018). Dietary proline supplementation alters colonic luminal microbiota and bacterial metabolite composition between days 45 and 70 of pregnancy in Huanjiang mini-pigs. *Journal of Animal Science and Biotechnology, 9*, 18. https://doi.org/10.1186/s40104-018-0233-5

Jones, B., Balasubramaniam, M., Lebowitz, J. J., Taylor, A., Villalta, F., Khoshbouei, H., … Pandhare, J. (2021). Activation of proline biosynthesis is critical to maintain glutamate homeostasis during acute methamphetamine exposure. *Scientific Reports, 11*, 1422. https://doi.org/10.1038/s41598-020-80917-7

Kamal, M., Chowdhury, G. F., Shishir, M. R. I., Sabuz, A. A., Islam, M., & Khan, H. H. (2023). Impacts of drying on physicochemical properties, bioactive compounds, antioxidant capacity, and microstructure of jackfruit seed flour. *Biomass Conversion Biorefinery*. https://doi.org/10.1007/s13399-023-04763-z

La Reau, A. J., & Suen, G. (2018). The ruminococci: Key symbionts of the gut ecosystem. *Journal of Microbiology, 56*(3), 199–208. https://doi.org/10.1007/s12275-018-8024-4

Leão, D. P., Franca, A. S., Oliveira, L. S., Bastos, R., & Coimbra, M. A. (2017). Physicochemical characterization, antioxidant capacity, total phenolic and proanthocyanidin content of flours prepared from pequi (*Caryocar brasilense* Camb.) fruit by-products. *Food Chemistry, 225*, 146–153. https://doi.org/10.1016/j.foodchem.2017.01.027

Liu, L., Fu, C., & Li, F. (2019). Acetate affects the process of lipid metabolism in rabbit liver, skeletal muscle and adipose tissue. *Animals, 9*(10), 799. https://doi.org/10.3390/ani9100799

Lomelino, C. L., Andring, J. T., McKenna, R., & Kilberg, M. S. (2017). Asparagine synthetase: Function, structure, and role in disease. *Journal of Biological Chemistry, 292*(49), 19952–19958. https://doi.org/10.1074/jbc.R117.819060

Ma, H., Hou, A., Tang, J., Zhong, A., Li, K., Xiao, Y., et al. (2022). Antioxidant activity of *Vitis davidii* foex seed and its effects on gut microbiota during colonic fermentation after *in vitro* simulated digestion. *Foods, 11*(17). https://doi.org/10.3390/foods11172615. Article 2615.

Macedo, E., L, C., Pimentel, T. C., Morais, J. S., Melo, D. S., Souza, A. C., … Magnani, M. (2023). Effects of yeast fermented soursop and umbu-cajá pulps on the colonic microbiota of middle-aged hypertensive adults. *Food Bioscience, 51*. https://doi.org/10.1016/j.fbio.2022.102309. Article 102309.

Markowiak-Kopeć, P., & Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients, 12*(4), 1107. https://doi.org/10.3390/nu12041107

Martins, A. C. S., Medeiros, G. K. V. V., de Oliveira, S. P. A., Albuquerque, T. M. R., Sampaio, K. B., Lima, M., et al. (2023). Unrevealing the *in vitro* impacts of *Cereus jacamaru* DC. cladodes flour on potentially probiotic strains, selected bacterial populations, and metabolic activity of human intestinal microbiota. *Food Research International, 174*, Article 113658. https://doi.org/10.1016/j.foodres.2023.113658

Massa, N. M. L., de Oliveira, S. P. A., Rodrigues, N. P. A., Menezes, F. N. D. D., Lima, M. S., Magnani, M., et al. (2022). *In vitro* colonic fermentation and potential

prebiotic properties of pre-digested jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg) by-products. *Food Chemistry, 388*. https://doi.org/10.1016/j.foodchem.2022.133003. Article 133003.

Medeiros, V. P. B., de Souza, E. L., Albuquerque, T. M. R., Sassi, C. F. C., Lima, M. S., Sivieri, K., et al. (2021). Freshwater microalgae biomasses exert a prebiotic effect on human colonic microbiota. *Algal Research, 60*, Article 102547. https://doi.org/10.1016/j.algal.2021.102547

Melo, F. H. C., Menezes, F. N. D. D., Souza, J. M. B., Lima, M. S., Borges, G. S. C., de Souza, E. L., et al. (2020). Prebiotic activity of monofloral honeys produced by stingless bees in the semi-arid region of Brazilian Northeastern toward *Lactobacillus acidophilus* LA-05 and *Bifidobacterium lactis* BB-12. *Food Research International, 18*. https://doi.org/10.1016/j.foodres.2019.108809. Article 108809.

Menezes, F. N. D. D., Melo, F. H. C., Vieira, A. R. S., Almeida, E. T. C., Lima, M. S., Aquino, J. S., et al. (2021). Acerola (*Malpighia glabra* L.) and guava (*Psidium guayaba* L.) industrial processing by-products stimulate probiotic *Lactobacillus* and *Bifidobacterium* growth and induce beneficial changes in colonic microbiota. *Journal of Applied Microbiology, 130*, 1323–1336. https://doi.org/10.1111/jam.14824

Moens, F., Verce, M., & De Vuyst, L. (2017). Lactate- and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans. *International Journal of Food Microbiology, 241*, 225–236. https://doi.org/10.1016/j.ijfoodmicro.2016.10.019

Oliveira, F. C. M., Holanda, T. M. V., de Assis, C. F., Xavier Júnior, F. H., & de Sousa Júnior, F. C. (2024). Flours from *Spondias mombin* and *Spondias tuberosa* seeds: Physicochemical characterization, technological properties, and antioxidant, antibacterial, and antidiabetic activities. *Journal of Food Science*. https://doi.org/10.1111/1750-3841.16882, 10.1111/1750-3841.16882.

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307

Padilha, C. V. S., Miskinis, G. A., de Souza, M. E. A. O., Pereira, G. E., de Oliveira, D., Bordignon -Luiz, M. T., et al. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian grape varieties. *Food Chemistry, 228*, 106–115. https://doi.org/10.1016/j.foodchem.2017.01.137

Pan, L., Ye, H., Pi, X., Liu, W., Wang, Z., Zhang, Y., et al. (2023). Effects of several flavonoids on human gut microbiota and its metabolism by *in vitro* simulated fermentation. *Frontiers in Microbiology, 14*, Article 1092729. https://doi.org/10.3389/fmicb.2023.1092729

Peterson, C. T., Perez Santiago, J., Iablokov, S. N., Chopra, D., Rodionov, D. A., & Peterson, S. N. (2022). Short-chain fatty acids modulate healthy gut microbiota composition and functional potential. *Current Microbiology, 79*(5), 128. https://doi.org/10.1007/s00284-022-02825-5

Poveda, C., Pereira, D. I. A., Lewis, M. C., & Walton, G. E. (2020). The impact of low-level iron bowel syndrome on healthy donors using *in vitro* batch cultures. *Nutrients, 12*(12), 3819. https://doi.org/10.3390/nu12123819

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*(9–10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., … Louis, P. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *The ISME Journal, 8*(6), 1323–1335. https://doi.org/10.1038/ismej.2014.14

Rha, C.-S., Seong, H., Jung, Y. S., Jang, D., Kwak, J.-G., Kim, D.-O., et al. (2019). Stability and fermentability of green tea flavonols in *in-vitro*-simulated gastrointestinal digestion and human fecal fermentation. *International Journal of Molecular Sciences, 20*, 5890. https://doi.org/10.3390/ijms20235890

Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). Bifidobacteria and butyrate-producing colon bacteria: Importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology, 7*, 979. https://doi.org/10.3389/fmicb.2016.00979

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., et al. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition, 57*(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8

Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., & Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants, 10*(1), 118. https://doi.org/10.3390/plants10010118

Sampaio, K. B., Alves, J. L. B., Nascimento, Y. M., Tavares, J. F., Silva, M. S., Nascimento, D. S., et al. (2022). Nutraceutical formulations combining *Limosilactobacillus fermentum*, quercetin, and or resveratrol with beneficial impacts on the abundance of intestinal bacterial populations, metabolite production, and antioxidant capacity during colonic fermentation. *Food Research International, 161*, Article 111800. https://doi.org/10.1016/j.foodres.2022.111800

Sampaio, K. B., Nascimento, D. S., Garcia, E. F., & Souza, E. L. (2022). An outlook on fluorescent in situ hybridization coupled to flow cytometry as a versatile technique to evaluate the effects of foods and dietary interventions on gut microbiota. *Archives of Microbiology, 204*, 469. https://doi.org/10.1007/s00203-022-03090-7

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: From biology to the clinic. *Nature Reviews Gastroenterology & Hepatology, 16*(10), 605–616. https://doi.org/10.1038/s41575-019-0173-3

Santos, É. M. D., Ataide, J. A., Coco, J. C., Fava, A. L. M., Silvério, L. A. L., Sueiro, A. C., … Mazzola, P. G. (2023). *Spondias* sp: Shedding light on its vast pharmaceutical potential. *Molecules, 28*(4), 1862. https://doi.org/10.3390/molecules28041862

Silva, J. Y. P., Nascimento, H. L. A., Albuquerque, T. M. R., Sampaio, K. B., Lima, M. S., Monteiro, M., et al. (2023). Revealing the potential impacts of nutraceuticals formulated with freeze-dried jabuticaba peel and *Limosilactobacillus fermentum* strains candidates for probiotic use on human intestinal microbiota. *Probiotics and Antimicrobial Proteins*. https://doi.org/10.1007/s12602-023-10134-x

Singh, V., Lee, G., Son, H., Koh, H., Kim, E. S., Unno, T., et al. (2023). Butyrate producers, "The Sentinel of Gut": Their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. *Frontiers in Microbiology, 13*, Article 1103836. https://doi.org/10.3389/fmicb.2022.1103836

Song, H., Shen, X., Deng, R., Zhang, Y., & Zheng, X. (2021). Dietary anthocyanin-rich extract of açai protects from diet-induced obesity, liver steatosis, and insulin resistance with modulation of gut microbiota in mice. *Nutrition, 86*, Article 111176. https://doi.org/10.1016/j.nut.2021.111176

Sviech, F., Ubbink, J., & Prata, A. S. (2022). Potential for the processing of Brazilian fruits - a review of approaches based on the state diagram. *Food Science and Technology, 156*, Article 113013. https://doi.org/10.1016/j.lwt.2021.113013

Tian, M., Heng, J., Song, H., Zhang, Y., Chen, F., Guan, W., et al. (2019). Branched chain amino acids stimulate gut satiety hormone cholecystokinin secretion through activation of the umami taste receptor T1R1/T1R3 using an *in vitro* porcine jejunum model. *Food & Function, 10*(6), 3356–3367. https://doi.org/10.1039/c9fo00228f

Xavier, V. L., Feitoza, G. S., Barbosa, J. M. L., Araújo, K. S. D., Silva, M. V. D., Correia, M. T. S., et al. (2022). Nutritional and technological potential of Umbu (*Spondias tuberosa* Arr. Cam.) processing by-product flour. *Anais da Academia Brasileira de Ciências, 94*(1). https://doi.org/10.1590/0001-3765202220200940. Article e20200940.

Xiong, R.-G., Zhou, D.-D., Wu, S.-X., Huang, S.-Y., Saimaiti, A., Yang, Z.-J., … Li, H. B. (2022). Health benefits and side effects of short-chain fatty acids. *Foods, 11*, 2863. https://doi.org/10.3390/foods11182863

Yan, J., Pan, Y., Shao, W., Wang, C., Wang, R., He, Y., … Dong, S. (2022). Beneficial effect of the short-chain fatty acid propionate on vascular calcification through intestinal microbiota remodelling. *Microbiome, 10*(1), 195. https://doi.org/10.1186/s40168-022-01390-0

Yeoman, C. J., Fields, C. J., Lepercq, P., Ruiz, P., Forano, E., White, B. A., et al. (2021). *In vivo* competitions between *Fibrobacter succinogenes, Ruminococcus flavefaciens*, and *Ruminoccus albus* in a gnotobiotic sheep model revealed by multi-omic analyses. *mBio, 12*(2), Article e03533. https://doi.org/10.1128/mBio.03533-20, 20.

Zhang, S., Hu, H., Wang, L., Liu, F., & Pan, S. (2018). Preparation and prebiotic potential of pectin oligosaccharides obtained from citrus peel pectin. *Food Chemistry, 244*, 232–237. https://doi.org/10.1016/j.foodchem.2017.10.071

Zhang, D., Jian, Y.-P., Zhang, Y., Yao, L., Gu, L., Sun, H., et al. (2023). Short-chain fatty acids in diseases. *Cell Communication and Signaling, 21*(1). https://doi.org/10.1186/s12964-023-01219-9

Zhu, H., Pi, D., & Leng, W. (2017). Asparagine preserves intestinal barrier function from LPS-induced injury and regulates CRF/CRFR signaling pathway. *Innate Immunity, 23*(6), 546–556. https://doi.org/10.1177/1753425917721631