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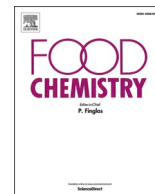
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# Effect of colonic fermentation of *uvaia* (*Eugenia pyriformis* Cambess) fractions on phenolic compounds and gut microbiota composition: An *in vitro* study using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)

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## ABSTRACT

This study evaluated the effect of colonic fermentation of *uvaia* seed and *uvaia* pulp on the phenolic compounds produced in the lumen and on the gut microbiota composition. Fermentation of *uvaia* pulp increased epigallocatechin, hippuric acid, ellagic acid, epicatechin gallate, and procyanidins in proximal colon (PC). The microbiota action on *uvaia* seed decreased hesperetin and quercetin in descending colon (DC). Fecal fermentation of *uvaia* pulp fraction showed the highest production of acetate (1469.82–5860.10 mg/L), propionate (543.72–3039.37 mg/L), and butyrate (8.58–2225 mg/L). *Firmicutes* decreased its overabundance after fermentation of both *uvaia* fractions. Feeding with *uvaia* seed reduced *Veillonella* in all the colon compartments and increased *Akkermansia* in DC. These results suggest that *uvaia* consumption can increase polyphenol concentrations in the gut lumen, primarily through the release of bound phenolic compounds from the plant cell wall during colonic fermentation, which in turn modifies the composition of the gut microbiota.

## 1. Introduction

Antioxidant dietary fiber (ADF) is an emerging concept used for describing an existing natural complex between polyphenols and dietary fiber. The name was coined by Saura-Calixto (Saura-Calixto, 1998) to indicate the complex between non-extractable polyphenols (phenolic compounds physically and/or chemically linked to components from the food matrix) and the polysaccharides forming the plant cell wall and identified as dietary fiber (Wang et al., 2024). Because of their complexity, non-extractable polyphenols normally are not released from the food matrix during mastication, acid pH in the stomach or action of digestive enzymes in the small intestine, reaching the colon almost in the native form (Pérez-Jiménez et al., 2013). Once they reach the colon, non-extractable polyphenols are transformed and metabolized by the gut microbiota leading to the breakdown of interactions between

phenolic compounds and dietary fiber, which produces free phenolic compounds and phenolic metabolites (Wang et al., 2024).

Non-extractable polyphenols are bioactive compounds with potential benefits on health including effects against dysbiosis of the gut microbiota, inflammatory bowel diseases and atopic dermatitis (Lee et al., 2023; Maurer et al., 2019), however few studies have focused on how non-extractable polyphenols are metabolized during the gut microbiota fermentation and which is the influence of this process on gut microbiome composition (Condezo-Hoyos et al., 2014; Lee et al., 2023; Low et al., 2016; Zheng et al., 2024). Besides that, many plant species which are good source of non-extractable polyphenols remain still unexplored. *Uvaia* (*Eugenia pyriformis* Cambess) is a species native to the Atlantic Rainforest considered a good source of dietary fiber and phenolic compounds (da Silva et al., 2019; da Silva et al., 2022; de Paulo Farias et al., 2020) but lacks information about its functional potential.

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We hypothesize that the occurrence of antioxidant dietary fiber in *uvaia* may modulate the gut microbiome composition and functions, suggesting that the consumption of this fruit could bring effects on health. Therefore, this study aims to evaluate the biotransformation occurring during the colonic fermentation of *uvaia* pulp and *uvaia* seed and the influence on the gut microbiota composition using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®).

## 2. Material and methods

### 2.1. Sample acquisition and preparation

Ripe *uvaia* fruits (*Eugenia pyriformis* Cambess) were harvested from a single biological sample, previously cataloged (de Paulo Farias et al., 2020), between September and October 2022. To minimize bias, fruits were selected from different parts of the tree; however, no formal randomization strategy was applied during the collection process.

After collection, the fruits were sanitized and fractionated into the edible fraction (pulp + peel, here referred to as *uvaia* pulp (PU)) and the seed (SU). These fractions were frozen, lyophilized (LIOTOP, model L101, São Carlos, Brazil), milled, and stored at  $-20\text{ }^{\circ}\text{C}$  until analysis.

### 2.2. Total phenolics

The assay was carried out mixing phenolic extract or standard with Folin-Ciocalteu reagent (10 %, v/v) and  $\text{Na}_2\text{CO}_3$  (7.5 %, w/v) followed by incubation at  $45\text{ }^{\circ}\text{C}$  for 6 min and measuring the absorbances using a microplate reader at 760 nm. Calibration curve was prepared using gallic acid and the results expressed as mg gallic acid equivalent (GAE)/L (Roesler et al., 2007).

### 2.3. Total flavonoids

The assay was performed mixing phenolic extract or standard with  $\text{NaNO}_2$  5 % and  $\text{AlCl}_3$  10 %, followed by the addition of NaOH 1 mol/L. The absorbance of the reaction was measured using a microplate reader at 510 nm (Zhishen et al., 1999). Calibration curve was prepared using catechin in different concentrations and the results were expressed as mg catechin equivalent (CE)/L.

### 2.4. Determination of phenolic compounds by LC-MS

The determination of phenolic compounds was performed according to our previous published study (de Paulo Farias et al., 2025). The phenolic extraction was performed mixing 1 mL of fecal slurry with 1 mL of MeOH/ $\text{H}_2\text{O}$  (1/1, v/v) followed by vortex agitation and filtration using 0.2  $\mu\text{m}$  PTFE filter. After extraction, the filtered samples were properly diluted and injected into a Nexera UHPLC system (Shimadzu Corporation, Kyoto, Japan) coupled with a LCMS- 8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with a Acquity UHPLC BEH C18 column (1.7  $\mu\text{m}$ ,  $2.1 \times 100\text{ mm}$ ) connected to an Acquity UHPLC BEH C18 VanGuard Pre-column (1.7  $\mu\text{m}$ ,  $2.1\text{ mm} \times 5\text{ mm}$ , Waters Chromatography, the Netherlands). The chromatographic system was set as follow: flow injection of 5  $\mu\text{L}$  and flow rate of 0.3 mL/min; and temperature of the column of  $40\text{ }^{\circ}\text{C}$ . Mobile phases were constituted of 0.1 % formic acid (solvent A) and acetonitrile with 0.1 % formic acid (solvent B) adopting the following elution gradient: (0.0 min, 5 % B), (0.5 min, 5 % B), (2.0 min, 35 % B), (7.5 min, 95 % B), (9.5 min, 95 % B), (9.6 min, 5 % B), and (13.5 min, 5 % B). The energy for the ion-spray ionization was 4.0 kV, while the temperature of electrospray ionization probe was  $300\text{ }^{\circ}\text{C}$ . The gas pressure of collision to induce the dissociation was set to 4 kPa, whereas desolvation line and heat block temperatures were set to  $250\text{ }^{\circ}\text{C}$  and  $400\text{ }^{\circ}\text{C}$ , respectively. Drying gas, nebulizer gas, and heating gas were set to 10, 3, and 10 mL/min, respectively. MS data were acquired in both positive and negative ionization modes and analyzed using LabSolutions (Shimadzu

Corporation, Kyoto, Japan) software. Calibration curve was built using a mixture with all the compounds in different concentration levels. The identification of the phenolic compounds was performed based on their retention time and MS fragments. The most abundant fragment ion was used for compound quantification, whereas the second and third fragments were used for structural confirmation according to the optimized single reaction monitoring (SRM) transition (Table S1). The signal intensity of each confirmed compound was select to perform the heatmap analysis.

### 2.5. DPPH scavenging assay

The assay was performed mixing 50  $\mu\text{L}$  of phenolic extract or standard with 250  $\mu\text{L}$  of DPPH solution (4 mg diluted into 100 mL of ethanol) followed by measuring using a microplate reader at 517 nm (Roesler et al., 2007). Calibration curve was prepared using Trolox and the results were expressed as  $\mu\text{mol}$  Trolox equivalent (TE)/L.

### 2.6. Trolox equivalent antioxidant capacity (TEAC)

This assay was performed according to Re et al. (1999). Briefly, 50  $\mu\text{L}$  of sample or standard were mixed with 250  $\mu\text{L}$  of ABTS<sup>+</sup> solution followed by measuring using a microplate reader at 734 nm. The radical solution was obtained by mixture of 5 mL of ABTS (7 mmol) with 88  $\mu\text{L}$  of potassium persulfate (140 mmol). This solution was kept overnight, and its absorbance was adjusted to 0.70 at 734 nm. Trolox was used to prepare the calibration curve, and the results were expressed as  $\mu\text{mol}$  TE/L.

### 2.7. Colonic fermentation

The study of human microbiome interactions *in vivo* has some limitations, including ethical concerns and challenges in obtaining representative gut samples (Cárdenas-Castro et al., 2019). For this reason, several systems have been developed to simulate the functioning of the human colon microbiome ecosystem. Among them, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) is considered the most robust for evaluating the effects of various ingredients on modulating the composition of gut microbiota (Roupar et al., 2021).

Unlike other models, SHIME is designed to provide a more complete simulation of the human gastrointestinal tract. It encompasses the stomach, small intestine, and large intestine, offering a more comprehensive view of the interaction between microbiota and food across different segments of the tract (Roupar et al., 2021). Additionally, SHIME is particularly valuable for evaluating microbial effects and fermentative processes in the large intestine, providing a robust representation of human physiological conditions. In this study, colonic fermentation was conducted using the SHIME® system (Prodigest, Belgium).

SHIME inoculum was provided from the previous SHIME study, in which fecal samples were collected from two healthy, non-smoking donors, aged between 27 and 40 years, and without any non-communicable disease. This experiment was performed at Wageningen University & Research/Food Quality and Design group (The Netherlands).

The SHIME was set up following the protocol of Koper et al. (2019), with some modifications. The gut microbiota stabilization was performed for two weeks using the SHIME® basal media, which contains starch (4.0 g/L), pectin (2.0 g/L), arabinogalactan (1.2 g/L), xylan (0.5 g/L), glucose (0.4 g/L), yeast extract (3.0 g/L), mucin (3.0 g/L), peptone (1.0 g/L), and L-cysteine-HCl (0.5 g/L). Approximately 224 mL of basal media was added every 8 h, followed by small intestinal digestion by adding 96 mL of pancreatic juice ( $\text{NaHCO}_3$  (12.5 g/L) Oxgall (6 g/L), and pancreatin (0.9 g/L) from porcine with activity equivalent to 3xUSP) (Koper et al., 2019). The digestion occurred for 90 min, after which the total volume was transferred first to the proximal portion (PC)

and then to the distal portion (DC) within 60 min.

The treatment was started after 2 weeks of gut microbiota stabilization and was performed for 13 days, and the only difference was because instead using 4.0 g/L as used in the basal media, it was used 2.0 g/L of starch plus the equivalent amount of sample to have more 2.0 g/L of fiber (based on total fiber content found in the sample), reaching the total fiber content of 7.7 g/L. Samples were taken before starting the treatment (basal period, day 0) and every single day during 13 days at the same time of the day. After taking the samples, aliquots were centrifuged at 9000g, 4 °C for 5 min. Supernatant was used to determine short chain fatty acids (SCFAs) production, phenolics and their metabolites, and antioxidant capacity while pellet was used to perform microbiome characterization. For microbial characterization, only the days 0, 7 and 13 were used. Supernatants and pellets were stored separately at -20 °C until further analysis.

## 2.8. SCFAs production

The SCFAs production was performed mixing 75 µL of 2-ethylbutyric acid (prepared at 450 mg/L and diluted in 0.3 M HCl + 0.9 mol/L oxalic acid solution) with 150 µL of filtered sample (0.2 µm RC filter). The injection was carried out using a gas chromatography system (GC-2014, Shimadzu, Hertogenbosch, Netherlands) equipped with flame ionization detector and a capillary fatty-acid-free Stabilwax-DA column (1 µm × 0.33 mm × 30 m, Restek, Bellefonte, PA, U.S.A.) (Guo et al., 2020). Calibration curve was prepared from a mix containing all the analyzed compounds (acetic, propionic, isobutyric, butyric, valeric, and isovaleric acids) at different concentration levels (14.06–450 mg/L). The results were expressed as mg/L.

## 2.9. Microbial analysis

### 2.9.1. Oxford nanopore sequencing

Crude samples were extracted with ZymoBIOMICS™ DNA Miniprep kit (Zymo Research). The samples were quantified and analyzed for quality and quantity. The DNA samples were subjected to amplification

of the entire 16S rRNA gene using primers 27F and 1492R (fragment of ~1.6 kb). Amplicons were visualized on agarose gel and quantified. The resulting fragments were used to construct sequencing libraries and sequenced on the MinION platform (Oxford Nanopore) using Flongles Flow Cell (FLO-FLG114).

### 2.9.2. 16S microbiota analysis

After sequencing, the resulting reads were subjected to basecalling using Guppy Basecaller (v 6.0.7) GPU version. Produced reads were filtered for quality control in Q10 using NanoFilt (v2.3.0) and demultiplexed by Porechop (v0.2.4). The reads were mapped against the 16S reference database using the KMA tool (v1.4.3). Data analyses were generated using Python 3.7.

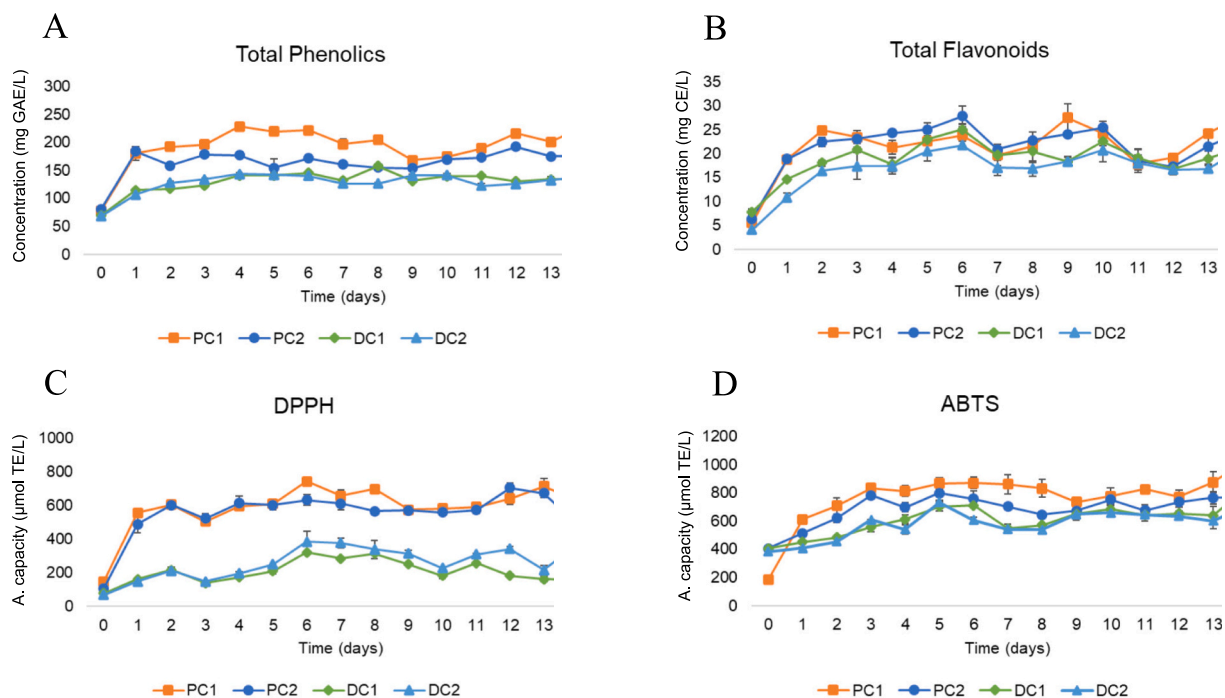
## 2.10. Statistical analysis

The analyses were performed in triplicate and the results were expressed as mean ± standard deviation. The data were tested by analysis of variance (ANOVA), followed by the Tukey's test considering  $p \leq 0.05$ . Data processing and heatmap analyses were performed using R studio software (RStudio Team, USA).

## 3. Results and discussion

### 3.1. Chemical modification induced by uvaia fractions gut fermentation

In Fig. 1 the data of total phenolics and total flavonoids content after SHIME fermentation of *uvaia* pulp of the two donors and in different colon portions are reported. Data showed that proximal colon donor 1 (PC1) had the greatest content of phenolic compounds. The peak was achieved after 4 days of fermentation which was approximately 3-fold increase respect to the time zero (Fig. 1A). Descending colon (DC) showed lower values for total phenolics, but donor 1 (DC1) presented higher concentration after 8 days of fermentation (increased by 107 %, respect to time zero, Fig. 1A). Similarly, the content of total flavonoid was higher in PC1 after 9 days of fermentative process (around 5-fold



**Fig. 1.** Bioactive compounds and antioxidant capacity during SHIME fermentation of *uvaia* pulp. A: Total phenolics (mg gallic acid equivalent (GAE)/L). B: Total flavonoids (mg catechin equivalent (CE)/L). C: Antioxidant capacity by DPPH assay (µmol Trolox equivalent (TE)/L). D: Antioxidant capacity by ABTS assay (µmol TE/L). PC1: Proximal colon donor 1; PC2: Proximal colon donor 2; DC1: Descending colon donor 1; DC2: Descending colon donor 2.

increase, Fig. 1B).

Table S2 is showing the total phenolics and total flavonoid content of *uvaia* seed after SHIME fermentation of the two donors and colon portions. Total phenolic compounds significantly decreased for all the colon portions after 2–3 days, increased after 4–7 days, and decreased again after 8–13 days (Table S2). In this regard, PC1 had the greatest total phenolics content which increased 2-fold in the first day after feeding and decreased 23.7 % after 13 days of gut microbiota fermentation (Table S2). All the colon portions increased the total flavonoids content after *uvaia* seed feeding and the highest concentration was registered for PC donor 2 (PC2) after 4 days of SHIME fermentation (around 76 mg CE/L), but this amount decreased along the process and PC1 showed higher concentration after 13 days (64.5 mg CE/L) (Table S2). It is important to consider the natural biological variability among donors, including differences in genetic composition, metabolism, immune characteristics, and even variations in sample quality, all of which could have significantly influenced the experimental outcomes in this study.

The antioxidant capacity after SHIME fermentation of both *uvaia* fractions is shown in Fig. 1 and Tables S2–S3. The antioxidant capacity of *uvaia* pulp by DPPH assay showed a very clear difference between PCs and DCs. In this regard, PC1 obtained the highest antioxidant potential, mainly after 6 days of fermentation (5-fold increase, Fig. 1C and Table S3). The antioxidant potential by ABTS assay also increased after

feeding with *uvaia* pulp, but the difference between PCs and DCs were tighter (Fig. 1D and Table S3). Regarding the *uvaia* seed, the antioxidant capacity of proximal colon was higher than descending colon for both assays (Table S2). PC2 showed higher content for DPPH and increased 97.6 % the antioxidant potential after 2 days of fermentation (Table S2), whereas PC1 had greater content for ABTS which increased 3.4-fold after 11 days of gut microbiota fermentation (Table S2). Differently from the results we found in *uvaia* fractions, the total phenolic content of sea buckthorn berry juice increased around 31.84-fold during SHIME fermentation (Attri & Goel, 2018). Recently, it was demonstrated that the antioxidant performance (DPPH, ABTS e FRAP) of soy isoflavones during SHIME digestion decreased significantly after 12–24 h (Chen et al., 2022).

In Fig. 2 the content of individual phenolics after SHIME fermentation of *uvaia* pulp is shown. The data showed that the content of some individual phenolics increased during the SHIME fermentation mainly in the PCs, confirming they were released from this *uvaia* fraction. PC1 showed higher content of epigallocatechin, ellagic acid, caffeic acid, epicatechin gallate, procyanidin B2, procyanidin C1, kaempferol, and catechin during the process (Fig. 2A). In turn, epigallocatechin, hippuric acid, ellagic acid, epicatechin gallate, procyanidin B2, procyanidin C1, and salicylaldehyde were the most predominant phenolic compounds in PC2 (Fig. 2B). In contrast, epigallocatechin, hesperetin and quercetin

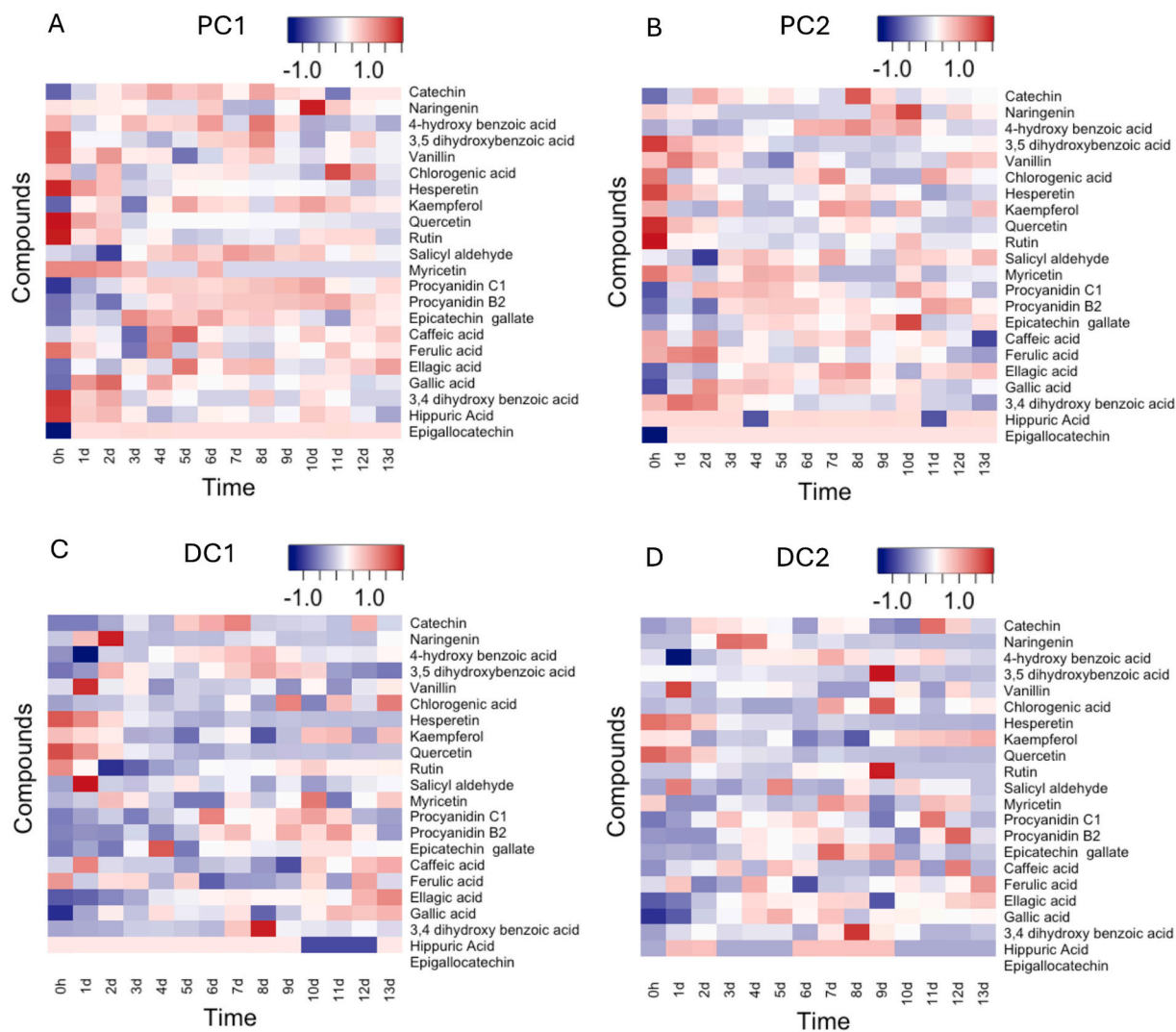


Fig. 2. Heatmap of confirmed phenolic compounds after SHIME fermentation of *uvaia* pulp. A: Phenolic compounds identified in the proximal colon donor 1 (PC1); B: Phenolic compounds identified in the proximal colon donor 2 (PC2). C: Phenolic compounds identified in the descending colon donor 1 (DC1); D: Phenolic compounds identified in the descending colon donor 2 (DC2).

were highly metabolized during colonic fermentation in DC1 and DC2 (Figs. 2C–D). Indeed, *uvaia pulp* contains 47 % of non-extractable phenolic compounds (de Paulo Farias et al., 2025), which can be released during the colonic fermentation process by the action of enzymes produced by the gut microbiota. This liberation occurred mainly in the PCs because is the first portion of the colon where this *uvaia* fraction meets the gut microbiota, leading to the breakdown of the ADF complex and release of phenolic compounds that were bound to the cell wall. Once released from this portion, the now free phenolic compounds are metabolized by the gut microbiota present in the other portions of the colon, producing phenolic metabolites. This helps to explain the fact that some phenolic compounds reduce their content after fermentation of *uvaia* edible fraction in DCs. In contrast, *uvaia* seed contains 99.73 % of soluble phenolic compounds (de Paulo Farias et al., 2025), which means that they are readily available to be metabolized by the gut microbiota already in the first portion of the colon, which could justify the reduction in the content of kaempferol, rutin, procyanidin B2, gallic acid, hippuric acid, and epigallocatechin following colonic fermentation of *uvaia* seed in PCs (data not shown).

In our previous study, the undigested fractions of *uvaia* from the same batch of fruit used in the present investigation contained significant amounts of epigallocatechin (12.04–38.60 mg/100 g), 3,4-dihydroxycinnamic acid (0.11–0.36 mg/100 g), epicatechin (0.16–14.41 mg/100 g), procyanidin B2 (4.18–12.94 mg/100 g), myricetin (0.18 mg/100 g), ferulic acid (0.19 mg/100 g), and salicyl aldehyde (14.84–132.03 mg/100 g) (de Paulo Farias et al., 2025). After INFOGEST gastrointestinal digestion, epigallocatechin, epicatechin, myricetin, and ferulic acid increased in *uvaia* pulp, while epicatechin and myricetin levels rose in *uvaia* seed. Following colonic fermentation in a static model, the phenolic compounds in both *uvaia* fractions were extensively metabolized by the gut microbiota (de Paulo Farias et al., 2025). However, the present study revealed a different phenolic metabolism profile, with phenolic release in the PCs and phenolic degradation in the DCs (Fig. 2). Therefore, the dynamic model demonstrated that the metabolism of phenolic compounds in *uvaia* fractions was also colon portion-dependent.

It is important to highlight that the *in vitro* dynamic model applied in this study did not include a simulated gastric phase with pepsin and acidic conditions. This limitation may have influenced the release, chemical transformation, and subsequent bioaccessibility of phenolic compounds from *uvaia*. The acidic environment and enzymatic activity in the stomach can significantly alter the structure of polyphenols, potentially affecting their intestinal absorption and microbial metabolism in the colon. Therefore, the absence of this digestive stage might have impacted the profile and quantity of phenolic compounds available for fermentation in the SHIME system. Future studies incorporating a complete gastrointestinal simulation, including the gastric phase, are essential to better understand its impact on the bioaccessibility and microbiota-modulating effects of phenolic compounds from *uvaia* fractions.

The increase in the content of individual phenolic compounds found for *uvaia* pulp mainly in the PCs is in parallel with the highest content of total phenolic compounds and antioxidant potential showed in Fig. 1 and Table S2. In addition, this suggests the presence of non-extractable phenolic compounds in these colon portions which were converted into free phenolic compounds through deglycosylation process by glycosidases produced by the gut microbiota (Wang et al., 2024). Afterwards, these free phenolic compounds were extensively metabolized in DCs by gut microbiota during SHIME fermentation. Indeed, scientific evidence show that phenolic compounds can be metabolized by the gut microbiome which convert these compounds into different phenolic compounds-derived metabolites by the action of specific enzymatic reactions including esterification, decarboxylation, hydroxylation, etc. (Wang et al., 2024).

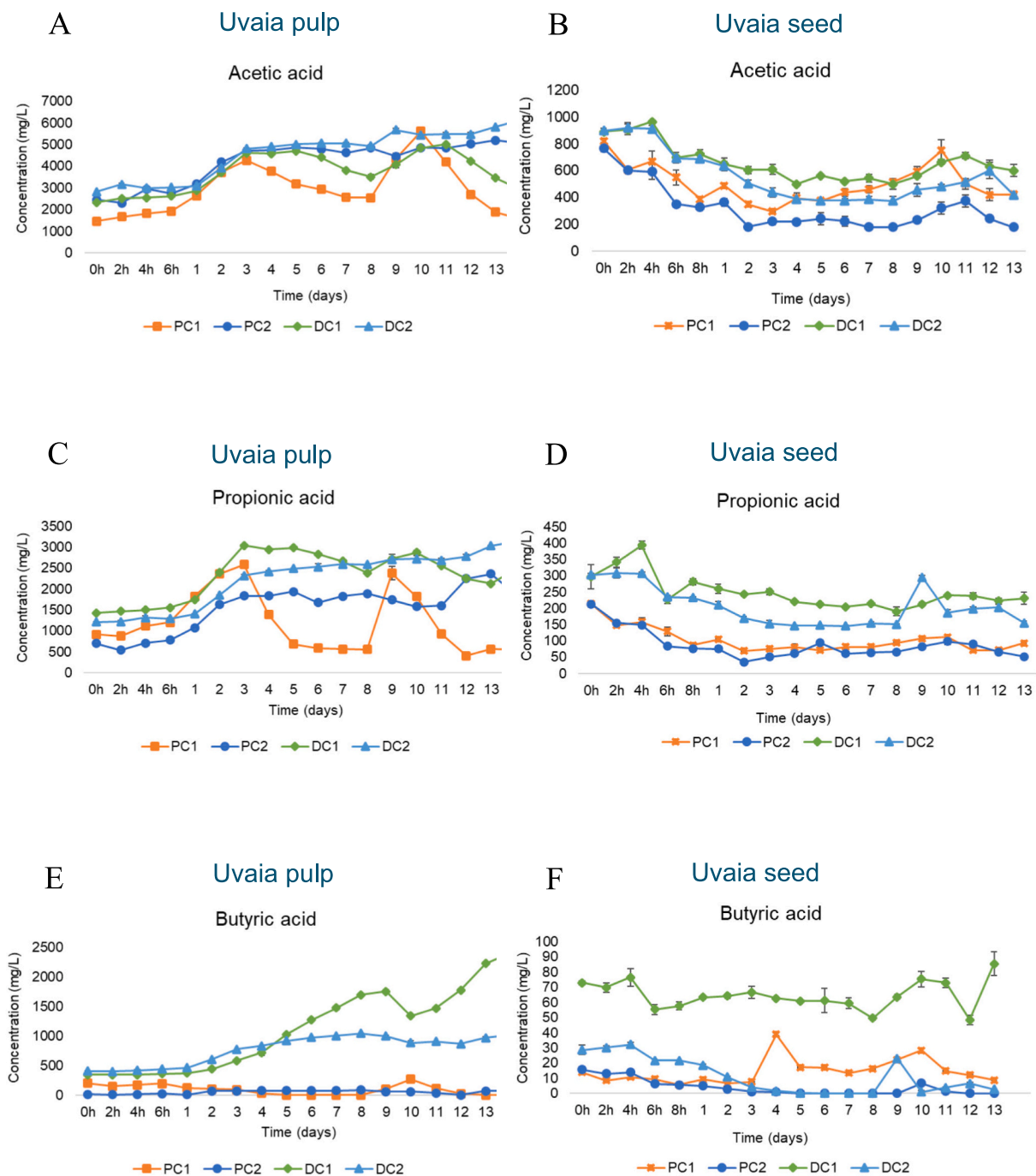
For example, flavan-3-ol compounds from red wine were metabolized by *Lactobacillus plantarum* into different compounds during SHIME

fermentation, including three phenylpropionic acids and phenylacetic acids derivatives. In addition, *L. plantarum* increased the conversion of monomeric flavan-3-ols and their intermediate metabolites into phenylpropionic acids (mainly 3-(3'-hydroxyphenyl) propionic acid) (Barroso et al., 2014). In one study evaluating the *in vitro* fermentation of cranberry and grape seed polyphenols using SHIME was possible to identify and quantify 27 compounds, including phenylacetic acids, phenylpropionic acids, benzoic acids, cinnamic acids, phenols, and metabolites derived from the catabolism of flavan-3-ols, being 4-hydroxy-5-(3'-hydroxyphenyl)-valeric acid, benzoic acid, and phenylacetic acid the main compounds found in the samples (Sánchez-Patán et al., 2015). Also, hesperetin from orange juice was metabolized into three propionic acids, namely 3-(3'-hydroxyphenyl) propionic acid, 3-(3'-hydroxy-4'-methoxyphenyl) propionic acid, and 3-(3',4'-dihydroxyphenyl) propionic acid (Pereira-Caro et al., 2015). Recently, it was demonstrated that nanoliposomes coated with chitosan and carrageenan retained 76 % of the hesperetin after gastrointestinal digestion and increased the intestinal epithelium uptake of hesperetin, which might be an alternative to ensure the bioactive potential of this flavanone (Meng et al., 2024). Although the results obtained in this study provide important information on the polyphenolic profile of *uvaia* and its interaction with the gut microbiota, the analysis of microbial catabolites generated during *in vitro* fecal fermentation was not performed. Therefore, future research should also consider this aspect.

The SCFAs production after SHIME fermentation of both *uvaia* fractions is shown in Fig. 3 and Tables S2–S3. It was verified that acetic acid was the most produced SCFA during SHIME fermentation of *uvaia* pulp and increased 21.54 % its content in DC2 after 13 days of gut microbiota fermentation (Fig. 3A). DC1 presented the maximum concentration of propionic acid after 3 days of fermentation ( $3039.37 \pm 10.59$  mg/L), but this amount decreased and DC2 had similar concentration after 13 days of treatment ( $3032.39 \pm 3.39$  mg/L) (Fig. 3C). In turn, the production of butyric acid was higher in both DC1 and DC2, being DC1 the main butyric acid-producer ( $2225 \pm 29.50$  mg/L after 13 days) (Figs. 3E). Similarly, acetic acid was also the main SCFA produced after SHIME fermentation of *uvaia* seed, but its content was lower than *uvaia* pulp and decreased throughout process (Fig. 3B). In general, DC1 showed the highest production for 0–9 days and 11–13 days (with higher production of  $892.16 \pm 23.18$  mg/L in the basal period, Fig. 5B). DC1 and DC2 clearly showed higher production of propionic acid, but this content was lower comparing to the basal period (0 h). Considering the period between 1 and 13 days, the greatest production of propionic acid was registered after 9 days for DC2 ( $295.52 \pm 5.16$  mg/L), despite DC1 had maintained higher production in the rest of days (Fig. 3D). In terms of production of butyric acid, DC1 showed the biggest production, mainly after 13 days of SHIME fermentation ( $85.56 \pm 7.63$  mg/L) (Fig. 3F).

Similarly to the results found to *uvaia* fractions, fresh orange juice also showed higher concentration of acetic acid ( $179.73 \pm 16.19$  mmol/L) and butyric acid ( $81.77 \pm 3.35$  mmol/L) in the descending colon (comparing to the ascending colon), whereas pasteurized orange juice had maximum production of propionic acid in the descending part ( $22.89 \pm 0.97$  mmol/L) (Duque et al., 2016). In another study, it was demonstrated that the production of acetic, propionic, and butyric acids increased in PC and decreased in DC, after 48 h of SHIME fermentation of cranberry and grape seed polyphenols (Sánchez-Patán et al., 2015).

In the present study, the highest production of SCFAs observed in the DCs, in particular propionic and butyric acids, can be associated to the previous release of phenolic compounds from the dietary fiber (degradation of the ADF complex) mainly observed in the PCs. The degradation of the phenolic compounds released from the ADF complex (which was more evident in the DCs) will produce phenolic metabolites, whereas the microbial metabolism of dietary fiber will produce monosaccharides which could be converted into acetate, propionate and butyrate through different biochemical pathways. Acetate can be produced from pyruvate via acetyl-CoA or by Wood-Ljungdahl pathway, while propionate can be produced from phosphoenolpyruvate (succinate pathway), reduction of



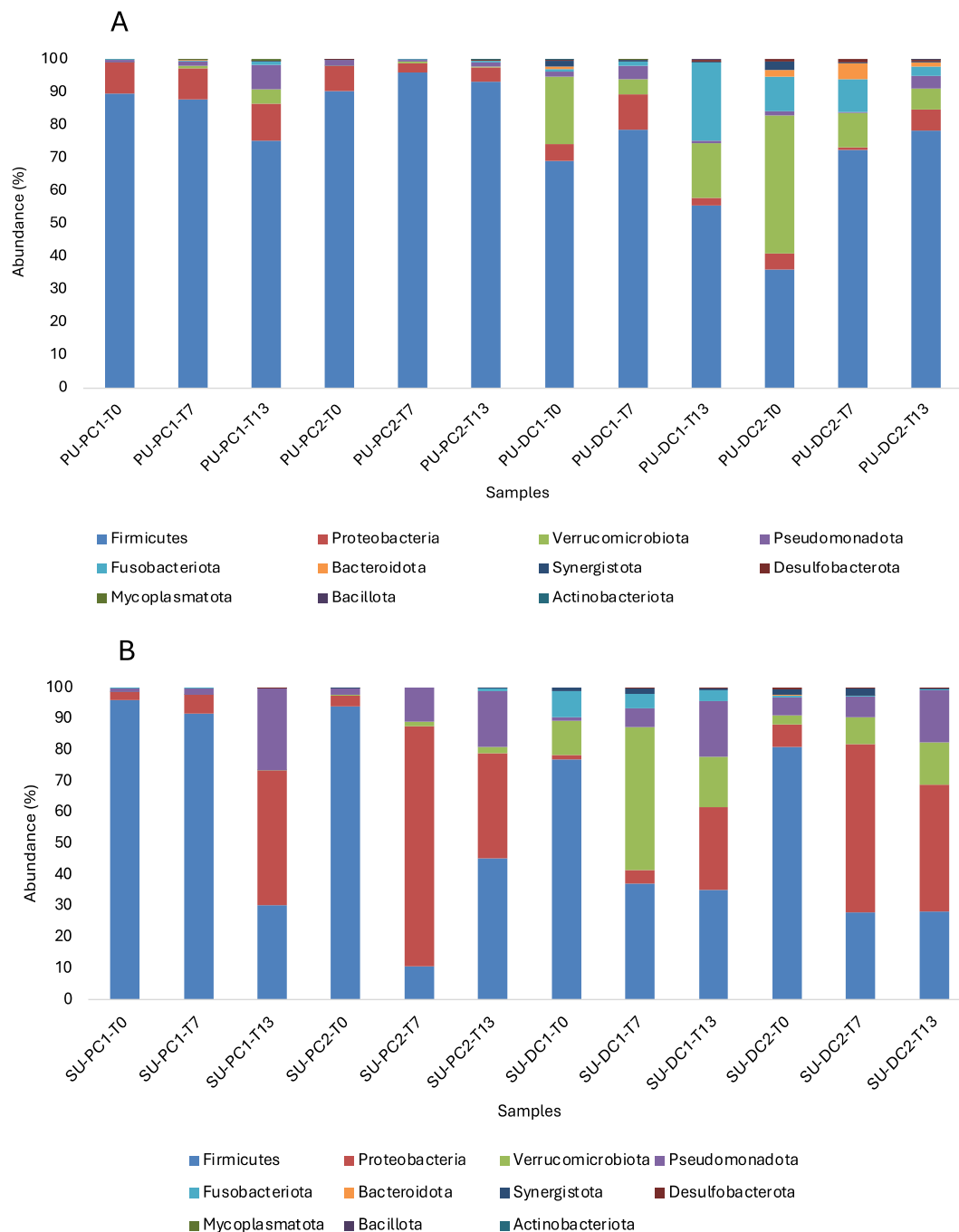
**Fig. 3.** SCFAs production after SHIME fermentation of both *uvaia* fractions. Figure 3A, C, and E mean respectively the production of acetic, propionic and butyric acids after SHIME fermentation of *uvaia* pulp. Figure 3B, D and F mean respectively the production of acetic, propionic and butyric acids after SHIME fermentation of *uvaia* seed. PC1: Proximal colon donor 1; PC2: Proximal colon donor 2; DC1: Descending colon donor 1; DC2: Descending colon donor 2.

lactate to propionate (acrylate pathway), or from deoxyhexose sugars (propanediol pathway). In turn, butyrate may be produced from the reduction of two condensed molecules of acetyl-CoA to butyryl-CoA and conversion of this last one to butyrate by phosphotransbutyrylase and butyrate kinase or *via* butyryl-CoA:acetate CoA-transferase route (Koh et al., 2016).

### 3.2. Influence of fermentation of *uvaia* fractions on gut microbiota composition

In Fig. 4, the bacterial composition at phylum level after SHIME fermentation of both *uvaia* fractions is shown. In general, the main

phylum found in fermented *uvaia* pulp was *Firmicutes*, mainly in PC1 and PC2 (Fig. 4A). However, fermentation of *uvaia* pulp in DC1 decreased *Firmicutes* after 13 days (from 69.13 to 55.43 %) and increased *Fusobacteriota* (from 0.78 to 23.81 %) (Fig. 4A). Likewise, fermentation of *uvaia* seed for 7–13 days in both PCs decreased *Firmicutes* and increased the abundance of *Proteobacteria* and *Pseudomonadota* (Fig. 4B). *Firmicutes* also decrease in both DCs and there was an increase in the abundance of *Verrucomicrobiota*, *Proteobacteria*, and *Pseudomonadota* (Fig. 4B). *Firmicutes* is usually the most dominant phylum in the human gut microbiome, but its overabundance has been associated with the development of diseases related to metabolic dysfunctions in patents such as obesity and type II diabetes (Ley et al., 2006; Palmas et al., 2021;



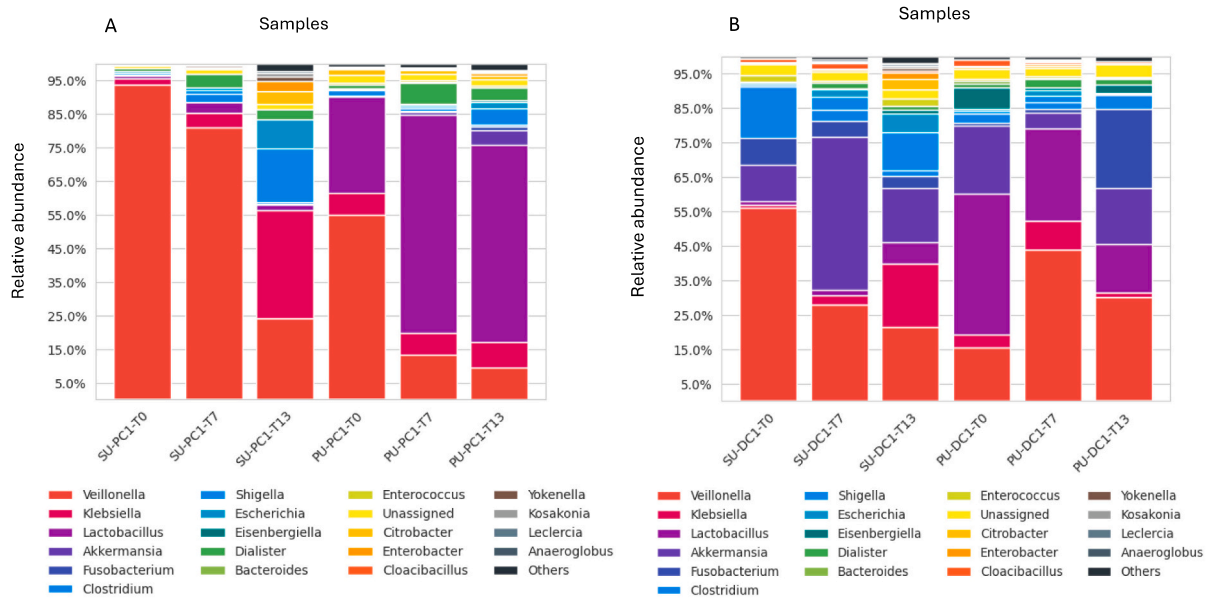
**Fig. 4.** Bacterial composition at phylum level after SHIME fermentation of both *uvaia* fractions. A: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU) in the proximal colon for donor 1 (PC1), proximal colon for donor 2 (PC2), descending colon for donor 1 (DC1), and descending colon for donor 2 (DC2). B: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* seed (SU) in the proximal colon for donor 1 (PC1), proximal colon for donor 2 (PC2), descending colon for donor 1 (DC1), and descending colon for donor 2 (DC2).

Wang et al., 2012).

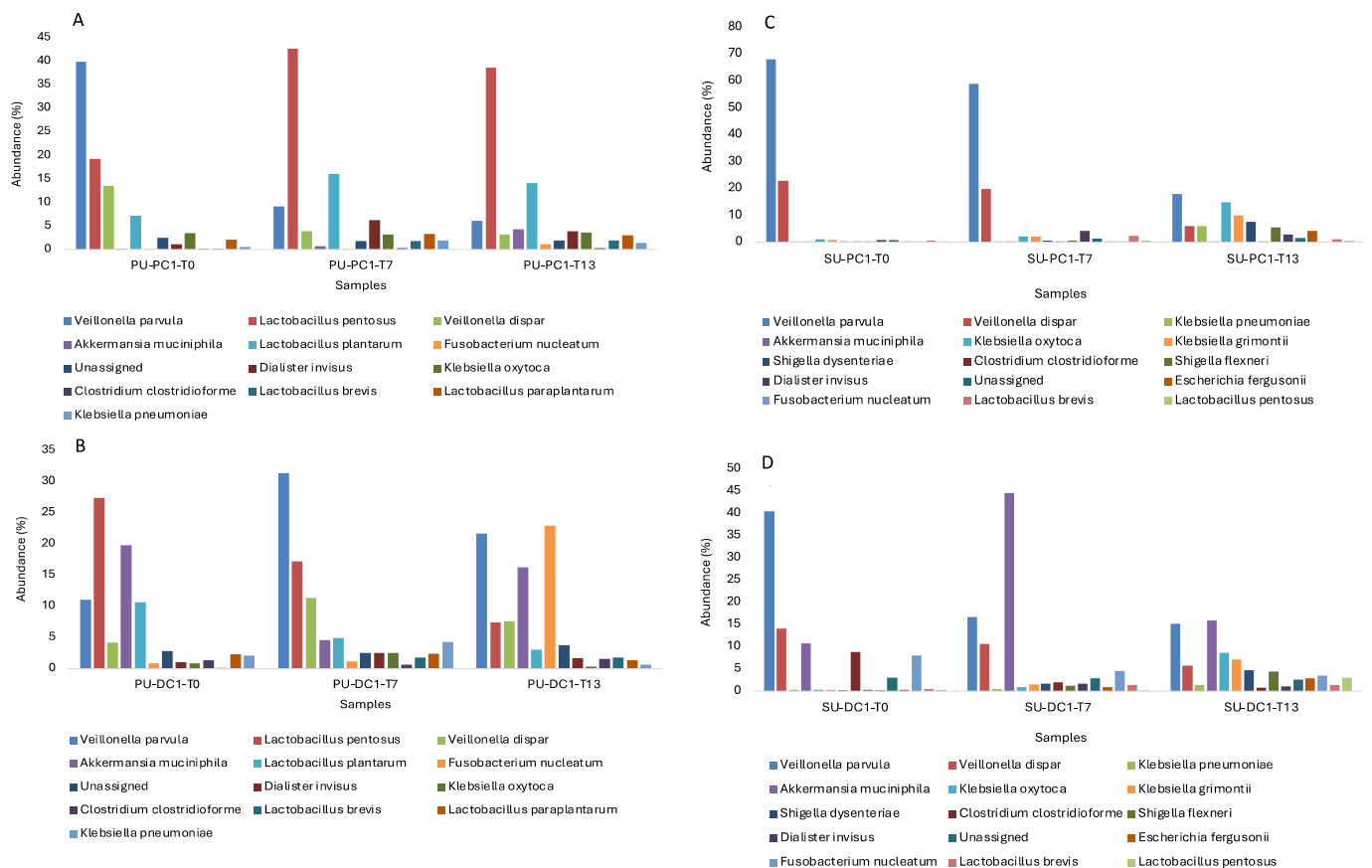
The Fig. 5 is showing the bacterial composition at genus level after SHIME fermentation of both *uvaia* fractions in the proximal and descending colons. Fermentation of *uvaia* seed for 13 days decreased 74 % the overabundance of *Veillonella* but increased the abundance of other pathogenic bacteria such as *Klebsiella* and *Shigella* in PC1 (Fig. 5A). Feeding with *uvaia* pulp also decreased significantly the abundance of *Veillonella* in PC1 after 13 days of SHIME fermentation (−82.7 %) and increased the abundance of *Lactobacillus* in PC1 after 7 and 13 days of fermentation (+126 and 105 %, respectively) (Fig. 5A). The treatment with *uvaia* seed for 7 days decreased 50 % the abundance of *Veillonella*

and increased 4-fold the abundance of *Akkermansia* in DC1 (Fig. 5B). In turn, feeding with *uvaia* pulp for 13 days decreased *Lactobacillus*, increased *Fusobacterium* and restored the abundance of *Akkermansia* in DC1 (Fig. 5B).

This is in line with other studies that have demonstrated the effect of the food matrix on altering the composition of the host microbiota. For example, Duque et al. (2016) evaluating the feeding of the SHIME with fresh orange juice (FOJ) for 14 days also verified an increase in the abundance of *Lactobacillus* spp., in addition to decrease the concentration of ammonium, which is good for intestinal health (Duque et al., 2016). An increase in the abundance of *Klebsiella* and *Akkermansia* was



**Fig. 5.** Bacterial composition at genus level after SHIME fermentation of both *uvaia* fractions. A: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU) and seed (SU) in the proximal colon for donor 1 (PC1). B: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU) and seed (SU) in the descending colon for donor 1 (DC1).



**Fig. 6.** Bacterial composition at specie level after SHIME fermentation of both *uvaia* fractions. A: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU) in the proximal colon for donor 1 (PC1). B: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU) in the descending colon for donor 1 (DC1). C: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* seed (SU) in the proximal colon for donor 1 (PC1). D: Bacterial composition after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* seed (SU) in the descending colon for donor 1 (DC1).

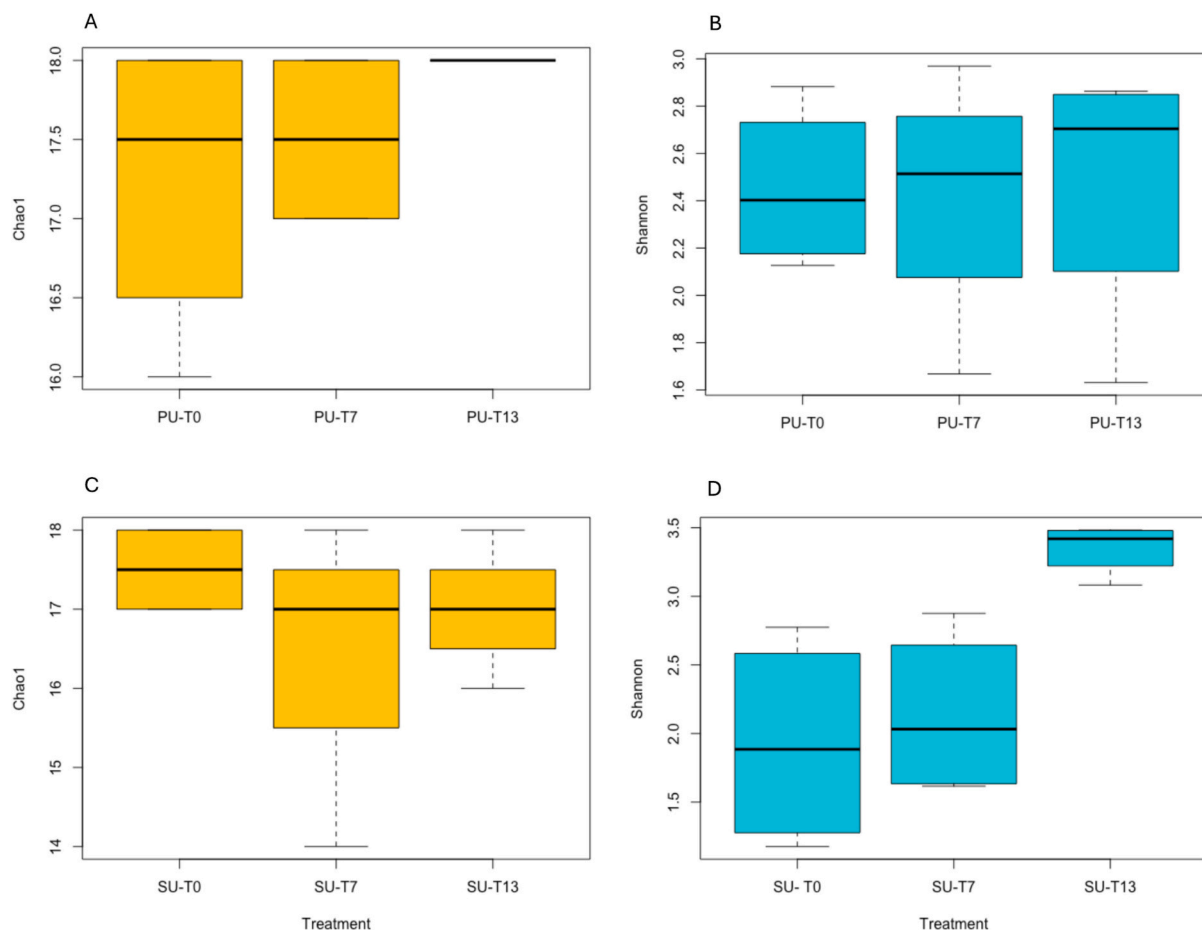
also reported after SHIME fermentation of black tea and red wine grape extract (Kemperman et al., 2013). In another study using SHIME, the administration of Aronia juice (*Aronia melanocarpa*, 6.5 g/L) for 2 weeks increased the abundance of *Firmicutes*, proteobacteria, and *Akkermansia*, whereas increased the content of propionate and butyrate, and prevented inflammatory stress in endothelial cells by reducing the levels of molecular markers linked to proinflammatory response (Wu et al., 2018).

Recently, it was demonstrated that feeding with olive-derived ADF modulated the gut microbiota composition, improved the immune response and the butyrate production, resulting in the attenuation of the atopic dermatitis like inflammation in female BALB/c mice (Lee et al., 2023). Other studies also showed that the fermentation of ADF increased the abundance of commensal bacterial such as *Akkermansia* and *Lactobacillus*, besides reducing *Firmicutes/Bacteroidetes* ratio and the abundance of harmful bacteria communities such as *Escherichia* and *Enterobacteriaceae* (Dong et al., 2020; Zhang et al., 2019). In the present study, the increase in the content of phenolic compounds observed in PC1 during SHIME fermentation of *uvaia* pulp may be related to the increase in the abundance of *Lactobacillus* which has been reported to produce feruloyl esterase which is related to the release of phenolic compounds from the dietary fiber and therefore decomposing the ADF complex (Li et al., 2024).

Fig. 6 is showing the bacterial composition at species level after SHIME fermentation of both *uvaia* fractions in PC1 and DC1. The fermentation of *uvaia* pulp in PC1 decreased *Veillonella parvula* (−85 % after 13 days) and increased the abundance of *Lactobacillus pentosus* and

*Lactobacillus plantarum* (+120 and 114 % after 7 days, respectively) (Fig. 6A). On the other hand, feeding with *uvaia* pulp for 13 days decreased 73 % de abundance of *Lactobacillus pentosus*, whereas increased 185 % *Veillonella parvula* and 30-fold *Fusobacterium nucleatum* after 7 and 13 days of SHIME fermentation in DC1, respectively (Fig. 6B). Feeding with *uvaia* seed decreased *Veillonella parvula* in PC1 and increased *Akkermansia muciniphila* in DC1 (Figs. 6C–D). We have previously determined the content of dietary fiber in the *uvaia* fractions (de Paulo Farias et al., 2025). In this regard, *uvaia* pulp contained 10.92 % insoluble fiber and 4.91 % soluble fiber, whereas *uvaia* seed had 34.04 % insoluble fiber and 1.17 % soluble fiber. These differences in fiber composition are likely associated with the observed increase in the abundance of *Lactobacillus* and *Akkermansia*. The higher content of insoluble fiber in the seed may favor the growth of these probiotic strains, which thrive on specific types of dietary fiber.

In Fig. 7 the alpha diversity of the microorganisms after SHIME fermentation of *uvaia* fractions is shown. According to Xu et al. (2024), the Chao1 index reflects the community richness, while Shannon index is related to the community diversity. Taking this into consideration, it was possible to verify that the feeding with *uvaia* pulp for 13 days partially changed the abundance and diversity of the gut microbiota (Figs. 7A–B). On the other hand, the supplementation of *uvaia* seed slightly decreased the abundance (especially after 7 days) and increased the community diversity after 13 days of SHIME fermentation (Figs. 7C–D). Anthocyanins from *Lycium ruthenicum* Murray fruits also increased the community abundance during fermentation using gut microbiota from healthy human (Peng et al., 2021), while blueberry



**Fig. 7.** Alpha diversity after SHIME fermentation of both *uvaia* fractions. A: Chao1 index after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU). B: Shannon index after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* pulp (PU). C: Chao1 index after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* seed (SU). D: Shannon index after basal period (T0), 7 (T7) and 13 (T13) days of SHIME fermentation of *uvaia* seed (SU).

anthocyanin extracts increased the community richness and diversity (Xu et al., 2024).

The study of the gut microbiota composition and its interactions is very important given the role played by the gut microbiota on body homeostasis and its repercussions on host health. Thus, the knowledge about gut microbiota composition specially in terms of species might be very useful to understand microbiome diversity and functions, besides identifying potential key harmful or beneficial microorganisms for the human health. In this regard, it was demonstrated that the overabundance of *Veillonella parvula* promoted intestinal inflammation in the colon of mice by activating macrophages via lipopolysaccharide (LPS)-Toll-like receptor 4 (TLR4) pathway (Zhan et al., 2022). The overabundance of *Veillonella* was also correlated with primary sclerosing cholangitis and ulcerative colitis in children and adolescents (aged 3 to 19 years) from Brazil (Cortez et al., 2021).

The increase in the abundance of probiotic strains such as *L. plantarum*, *L. pentosus*, and *A. muciniphila* after SHIME fermentation of both *uvaia* fractions suggests their prebiotic-like effects (Fig. 6). *L. plantarum* has been reported by improving the intestinal barrier function and modulating the gut microbiota composition, alleviating the reduction in tight junction proteins, and downregulating proinflammatory cytokines (Wang et al., 2018). In another study, a dietary intervention with overweight and obese human volunteers showed that the daily oral supplementation of 10<sup>10</sup> pasteurized *A. muciniphila* for three months improved insulin sensitivity, and reduced insulinemia, plasma total cholesterol, and anthropometric parameters compared to the placebo group (Depommier et al., 2019).

#### 4. Conclusion

This study demonstrates that the fermentation of *uvaia* pulp and seed influences the metabolism of phenolic compounds and the composition of gut microbiota, with effects dependent on both the colon region and the donor. These differences are likely due to the interaction between dietary fiber and gut microbiota, which modulates the release and metabolism of phenolic compounds.

Moreover, fermentation of both *uvaia* fractions promoted the production of beneficial short-chain fatty acids (SCFAs), such as propionic and butyric acids, especially in the distal colon (DC), through the degradation of the ADF complex. Additionally, changes in microbiota composition were observed, with both *uvaia* fractions influencing the abundance of key probiotic strains.

These findings highlight the potential of *uvaia* to modulate the composition of gut microbiota. While the concept of microbiota modulation by fermentable, indigestible materials is well established, we believe the contribution of our study lies in identifying *uvaia* as a potentially promising ingredient for future applications in functional foods, especially given its unique profile of bioactive compounds.

Therefore, the study contributes to the understanding of the functional properties of *uvaia* and supports its potential use as an ingredient in the development of functional foods. However, further studies are needed to establish its role as a prebiotic ingredient. Specifically, future studies should compare *uvaia* fractions with established prebiotic sources to assess their potential health benefits. Finally, the low molecular weight phenolic metabolites formed after the colonic fermentation of the fractions should also be considered in future investigations.

#### CRedit authorship contribution statement

**David de Paulo Farias:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fábio Fernandes de Araújo:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zongyao Huyan:** Methodology, Investigation, Formal analysis, Conceptualization. **Juliana Villasante:** Writing – review & editing, Visualization, Software. **Thisun**

**Ranpatabendi:** Methodology, Investigation. **Vincenzo Fogliano:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Glaucia Maria Pastore:** Supervision, Resources, Project administration, Funding acquisition.

#### Ethical statements

The Medical Ethical Committee (METC) of Wageningen University (WU) declares that research with SHIME do not require a positive opinion from the METC-WU or another recognized medical ethics review committee because the research participants are not subjected to acts regulated by METC-WU and therefore it does not fit into it. This study was exempted from the Medical Ethics Committee of the University of Campinas (Brazil).

All participants were fully informed about the objectives, methods, benefits, and data processing policy of the study before agreeing to participate. Participants who donated feces voluntarily agreed to take part in the research, fully aware of all aspects involved.

#### 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.

#### Acknowledgments

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.144819>.

#### Data availability

Data will be made available on request.

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