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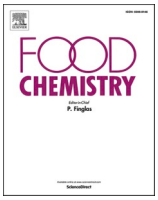

Polyphenol metabolism, short-chain fatty acids production, and microbiota changes during *in vitro* digestion and fermentation of Chilean beans (*Phaseolus vulgaris* L.)

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ABSTRACT

This study examines the effects of three Chilean boiled bean (*Phaseolus vulgaris* L.) landraces 'Negro', 'Peumo', and 'Tórtola' and their polyphenol-enriched extracts (PEE) on polyphenol release, gut microbiota, and short-chain fatty acid (SCFA) production after simulated digestion and colonic fermentation. Negro beans exhibited greater phenolic acid diversity post-digestion, while catechin bio accessibility increased in all landraces. PEE showed significant transformation, with oligomers degrading into flavan-3-ol monomers during digestion. Colonic fermentation of boiled beans produced SCFAs, confirming their prebiotic potential. Negro beans promoted beneficial bacteria, including *Catenibacterium*, *Lachnospira*, and *Bifidobacterium*, associated with fiber and polyphenol metabolism, while Tórtola beans favored *Roseburia*, an SCFA producer. These findings highlight the potential of Chilean beans and their bioactive compounds to enhance gut health.

1. Introduction

Understanding how food affects health requires searching food composition, bioactive compounds, and changes during gastrointestinal digestion and colonic fermentation. While many studies focus on food plant components, the interaction between food and gut microbiota is crucial for assessing health impacts. Simulated digestion research highlights food metabolization, absorption, and the production of health-promoting substances, such as urolithins from ellagic acid (Tomás-Barberán et al., 2017).

Bio accessibility is when an ingested component is released from the food matrix into the gastrointestinal tract and becomes accessible for absorption (Dima et al., 2020). Several steps are involved here, including gastrointestinal food digestion and solubilization of the compounds. Only compounds released from food matrices in the gastrointestinal system are considered digested. Non-digested compounds, like resistant starch and dietary fibers, reach the colon for microbiota fermentation, producing beneficial metabolites such as SCFAs (Shi et al., 2020).

Gut microbiota consists of a complex community of microorganisms in the human gastrointestinal tract. In recent years, it has been demonstrated that it plays a crucial role in shaping our diet's biochemical profile and maintaining human health through different physiological axes (Eisenstein, 2020). These microorganisms metabolize food compounds producing beneficial metabolites, reinforcing the gastrointestinal barrier, and interacting with our immune system, among other roles (Yoo et al., 2020). Different factors such as diet, age, medication, and environment influence gut microbiota. The dysbiosis or imbalance in the microbiota composition is linked to various health conditions, including inflammatory bowel disease, obesity, and mental health disorders (Li et al., 2016). To fully understand the impact of food components on human health, it is crucial to monitor changes in

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bioactive compounds during gastrointestinal digestion and fermentation, and the effect of dietary components on the gut microbiota. Most studies carried out with extracts from food sources do not consider the effects of the food matrix in the release or stability of compounds but offer a first glimpse into the structural changes following exposition to the digestive process, including the microbiota role (Barros et al., 2020).

Polyphenols are a large group of plant compounds, characterized by significant structural diversity, including phenolic acids, flavonoids, tannins, lignans, stilbenes, and coumarins. Most polyphenols in food plants occur as glycosides (Abbas et al., 2017). The health benefits of polyphenols depend on their concentration in food, as well as their bioaccessibility and bioavailability. The unabsorbed fraction of these compounds reaches the colon, where it interacts with gut microbiota. These interactions result in the metabolization of polyphenols into simpler phenolic intermediates (Mena et al., 2019). These intermediates are reabsorbed systemically and can display beneficial effects on the organism (Zeb et al., 2024). The prebiotic effects of polyphenols have been demonstrated through increased production of SCFAs and the stimulation of the growth of specific beneficial microorganisms (Parkar et al., 2013).

Common beans (*Phaseolus vulgaris* L.) are a globally important legume, particularly in Latin America, where they are a staple food. Numerous studies have examined its macronutrient content and bioactive compounds, including phenolics, saponins, and unsaponifiable lipids, as well as their health benefits (Los et al., 2018). Recent research on Chilean bean landraces revealed that cooking decreases total phenolics and saponins but enhances certain compounds, such as ferulic acid and soyasaponins Ba and Bb, altering antioxidant capacity and enzyme inhibition (Nina et al., 2023).

Beyond these findings, little is known about the changes these compounds undergo during gastrointestinal digestion and colonic fermentation. Common beans are rich in dietary fiber (Chen et al., 2016), which fosters gut bacterial growth and promotes a healthy gut environment. Polyphenols bound to fiber are unavailable in the upper intestine but reach the colon as fermentable substrates for gut microbiota, alongside undigested carbohydrates (Saura-Calixto, 2011).

This study aimed to: 1) examine changes in polyphenols during *in vitro* digestion and colonic fermentation of three Chilean bean landraces ('Negro', 'Peumo', and 'Tórtola') using boiled beans and polyphenol-enriched extracts (PEE); 2) assess polyphenol bioaccessibility after digestion of whole boiled beans; and 3) evaluate the effects of boiled beans and PEE on pH, ammonia production, gut microbiota composition, and SCFA production during colonic fermentation.

2. Material and methods

2.1. Reagents

Sodium bicarbonate, sodium carbonate, calcium chloride dihydrate, and magnesium sulfate heptahydrate were purchased from Merck (Darmstadt, Germany). Sodium chloride, potassium chloride, dibasic sodium phosphate, monobasic potassium phosphate, ammonium chloride, sodium hydroxide, Tween 80, and yeast extract were purchased from Scharlau (Barcelona, Spain). L-cysteine, vitamin K1, resazurin sodium salt, fructooligosaccharide (FOS), α-amylase, pepsin, porcine pancreatin, porcine bile extract, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and 4-methylvaleric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained using a Barnsted EasyPure water system. Peptone was purchased from BD Biosciences (San Jose, CA, USA). GENbox anaerobic sachets were purchased from BioMérieux (Marcy l' Etoile, France). Catechin, and kaempferol 3-glucoside were purchased from Phytolab (Vestenbergsgreuth, Germany).

2.2. Samples

Three Chilean bean landraces were selected based on their phenotype and chemical composition (Nina et al., 2023): 'Tórtola' (pale gray), 'Peumo' (reddish), and 'Negro' (black). The original seeds are kept at the Laboratory of Natural Products, Universidad de Talca, Chile, and the Center for Studies of Processed Foods (CEAP, Talca, Chile). In this study, the dry seeds were traditionally cooked as previously described (Nina et al., 2023). In addition, a polyphenol-enriched extract (PEE) was prepared from each cooked sample using a methanol-water solution (7:3), followed by sonication and multiple extraction steps. The extract was then defatted with hexane, purified using Amberlite XAD-7 resin to remove unwanted compounds, and finally dried and lyophilized to obtain the PEEs (Nina et al., 2023). The boiled beans (BB) and PEEs were subjected to *in vitro* digestion and colonic fermentation, as described below.

2.3. Total dietary fiber determination

The total dietary fiber content of boiled beans was determined using the commercial kit K-TDFR-200 A from Megazyme (Bray, Ireland), following the AOAC Official Method 985.29. In brief, cooked beans were finely milled to 425 μm (1 g, in duplicate) and incubated for 15 min in a water bath at 100 °C with 50 mL of 0.08 mol/L phosphate buffer (pH 6.0) and 0.1 mL of thermostable α-amylase. This process facilitated the gelatinization, hydrolysis, and depolymerization of bean starch. The total dietary fiber content was calculated as a weight/weight percentage, corrected for ash and protein content in the residue. Results are reported as the mean ± SD.

2.4. In vitro gastrointestinal digestion

Boiled beans and polyphenol-enriched extracts (PEE) were subjected to static *in vitro* gastrointestinal digestion following the INFOGEST 2.0 protocol (Brodkorb et al., 2019). This method mimics sequential oral, gastric, and intestinal digestion using simulated salivary, gastric, and intestinal fluids (SSF, SGF, and SIF) and the appropriate enzymes. Based on our previous experience (Burgos-Edwards et al., 2020), we used a polyphenol-enriched extract (PEE) dose that reflects the amount of polyphenols likely to reach the colon under normal dietary intake conditions (Bazzocco et al., 2008; Brown et al., 2015). To determine the corresponding whole bean dose, we extrapolated the polyphenol content back to the original bean matrix. Samples of boiled beans (BB, 2.5 g) and polyphenol-enriched extracts (PEEs, 25–40 mg, equivalent to 2.5 g of dry boiled beans) were subjected to the INFOGEST 2.0 digestion protocol which provides digestion volumes relative to a fixed food amount (Scheme S1). Two controls were included: a digestion control (DC) with only simulated fluids and enzymes, and a sample control (SC) with simulated fluids and samples but no enzymes.

Amylase solution (1,500 U/mL) and $CaCl_2$ (0.3 M) were added to the BB and PEE tubes for oral digestion. The final volume was adjusted to 5 mL with SSF, mixed, and incubated for 2 min at 37 °C. Gastric digestion was performed by adding 4 mL of SGF, adjusting the pH to 3.0, and introducing pepsin (80,000 U/mL, 250 μL) and $CaCl_2$ (0.3 M, 25 μL). The volume was adjusted to 10 mL with SGF, mixed, and incubated for 120 min at 37 °C.

For intestinal digestion, 4.25 mL of SIF was added, and the pH was adjusted to 7.0 with 2.0 M NaOH. Pancreatin (800 U/mL, 2.5 mL), bile salts (10 mM, 1.25 mL), and $CaCl_2$ (0.3 M, 20 μL) were added, followed by incubation for 120 min. The digested samples (GID) were cooled on ice, centrifuged (10 min, 5000 ×*g*, 4 °C), and the supernatants were frozen at −80 °C for further analysis. Solid residues were stored for colonic fermentation, with 10 % of the supernatant added to the wet residue (1.25 g) to mimic the unabsorbed fraction after digestion. The percentage of total digestibility (TD) was calculated as:

$$\%TD = \frac{(\text{dry weight starting material} - \text{dry weight after digestion})}{\text{weight of the starting material}} \text{x } 100\%$$

The %TD was corrected using the sample control (digested BB without enzymes and bile salts) as $\%TD_{corrected}$ = % BB TD - % control BB TD, and the corrected results are given as %TD.

The concentrations of the main phenolics were measured in the filtrate after digestion, representing the soluble forms of the compounds that are accessible for absorption (bioaccessible). Additionally, the percent bioaccessibility was calculated as the amount (in μg) of each compound released from 1 g of the sample during *in vitro* digestion, divided by the total amount (in μg) of the compound present in 1 g of the non-digested sample.

2.5. In vitro microbial fermentation

2.5.1. Donor participants and diet study

Human fecal samples were collected from healthy volunteers. Inclusion criteria required participants to have no chronic diseases, no ongoing medical treatments, and follow a mixed diet. Exclusion criteria included smoking, use of dietary supplements, and antibiotic treatment within at least six months before sample collection. Six volunteers (three males and three females) aged 24 to 37 participated in the study.

Written informed consent was obtained from all participants, and the protocol (stool donation, food questionnaires, and personal data collection) was approved by the Research Ethical Committee of the Hospital Clínico San Carlos, Madrid, Spain (approval number 22/418). Donor diets were assessed using a three-day dietary record completed in the three days preceding stool donation. Data from these records were analyzed using the DIAL diet analysis program V3.15 (Ortega et al., 2021) to determine the macronutrient, micronutrient, and fiber composition of the participants' diets.

2.5.2. Preparation of inoculum

Fecal samples were self-collected by donors in sterile plastic tubes containing a GENbox™ anaerobic sachet (BioMérieux, Marcy L'Etoile, France). The samples were transported in a cooler to the laboratory and processed within 30 min of collection. A fecal slurry was prepared following the methods of O'Donnell et al. (2016) and De Carvalho et al. (2021), with modifications, in an anaerobic chamber (Baker Ruskinn Concept 4000, Bridgend, UK) filled with a gaseous mixture of N_2 and H_2 (90:10).

Briefly, 10 g of feces from each donor was mixed with 100 mL of reduced PBS in a stomacher bag. After homogenization, the mixture was centrifuged at 4000 ×*g* for 10 min at 4 °C. The supernatant was discarded. The pellet was resuspended in a cryopreservation solution (30 % glycerol in reduced PBS) and stored at −80 °C.

On the day of the experiment, fecal samples from each donor were thawed under anaerobic conditions. The samples were centrifuged (4696 ×*g*, 5 min at 4 °C), and the supernatant was discarded. The pellets were washed twice with reduced PBS to remove residual glycerol. The washed pellets from each donor were resuspended in PBS (1:10 *w/v*) and combined to create a pooled fecal inoculum (10 %) from the six donors.

2.5.3. In vitro colonic fermentation

In vitro batch fermentation was conducted as described by Pérez-Burillo et al. (2021), with the basal medium prepared according to Tzounis et al. (2008). The medium and PBS were pre-reduced in an anaerobic chamber for 24–48 h. The medium was stabilized with fecal inoculum (final concentration 20 % *v*/v) for 4 h at 37 °C before fermentation initiation. The residues remaining after *in vitro* digestion simulating the small intestine of digested boiled beans (BB; 1.25 g plus 10 % of the supernatant), digested polyphenol-enriched extracts (PEE), and the digestion control (DC) were subjected to *in vitro* fermentation. A negative fermentation control (medium and inoculum) and fructooligosaccharides (FOS) as a positive control were included. Fermentation was carried out for 48 h under anaerobic conditions with continuous agitation. Aliquots for analyses were collected at 0, 8, 24, and 48 h. Each aliquot was centrifuged at 4696 ×*g* for 15 min. The supernatant was used to measure pH, ammonia content, and SCFA and branched-chain fatty acids (BCFA), while the pellets were used for bacterial DNA extraction. Both supernatants and pellets were stored at −80 °C until analysis. Three independent experiments were conducted in duplicate.

2.6. Determination of pH and ammonia content

The pH at each time point was measured using a Basic 20+ Crison pH meter (Hach Lange, Barcelona, Spain), according to Dai and Karring (2014). Ammonia content was determined as outlined by Burgos-Edwards et al. (2020), using a high-performance ammonia-selective ion electrode (Orion™, Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Analysis of SCFA and BCFA

SCFA and BCFA analyses of the fermented supernatants collected at 0, 8, 24, and 48 h were conducted using HPLC-DAD. The samples were derivatized following the methodology described by Wang et al. (2019) and analyzed on an Agilent 1100 HPLC system (Agilent Technologies, Germany), equipped with a quaternary gradient pump, an online degasser, an autosampler, a thermostatically controlled column compartment (30 °C), and a photodiode array detector (1260 DAD). Sample separation was performed on a Purospher® STAR RP-18e column (4.6 × 150 mm; 5 μm particle size) with a constant flow rate of 0.6 mL/min. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both acidified with 0.01 % formic acid. The elution gradient was as follows: 10–40 % B from 0 to 15 min, and 40–80 % B from 15 to 30 min. The injection volume was 20 μL, and the absorbance was recorded at 355 nm. SCFA and BCFA quantification was carried out using an eight-point external calibration curve (0.3–10 mM) with reference standards.

2.8. Microbiota analysis

2.8.1. Bacterial DNA extraction

Bacterial DNA was extracted from the pellets after colonic fermentation using the International Human Microbiota Standard (IHMS) Protocol H (Dore et al., 2015). The concentration and purity of bacterial DNA were determined using a NanoDrop instrument (ThermoFisher Scientific, Waltham, MA, USA).

2.8.2. Microbiota analysis by 16S rRNA gene sequencing

To study the bacterial community, a 459 bp fragment of the V3/V4 regions of the 16S rRNA gene was amplified using the primer pair 341F/785R (5′-TCGT CGGC AGCG TCAG ATGT GTAT AAGA GACA GCCT ACGG GNGG CWGCA-G3′) / (5′-GTCT CGTG GGCT CGGA GATG TGTA TAAG AGAC AGAC AGGA CTAC HVGG GTAT CTAA TCC3′) (Klindworth et al., 2013). Library construction was performed following the Illumina 16S rRNA Cod 15,044,223 RevA sequencing protocol. The primers were modified with adapter sequences to make them compatible with the Illumina Nextera XT Index kit. The V3/V4 region of 16S rRNA was sequenced on an Illumina MiSeq using 2 × 250-2 × 300 length sequencing (paired-end) to generate approximately 100,000 reads per sample. Quantitative Insights into Microbial Ecology (QIIME2) v. 2022.11 was used for bioinformatic analyses.

Raw sequencing data were processed with the DADA2 package (q2-dada2 plugin). Machine learning from the classify-sklearn library was applied for taxonomy assignment, using a specific classifier obtained from the SILVA 138 reference database. Sequences were extracted from the SILVA database (V3 and V4 regions of the 16S rRNA gene) to build the reference database according to our primers and clustered at 99 % identity. Annotation was assigned at the species level for matches with 100 % similarity and for matches with 97 % similarity or higher if there

was a difference of at least 2 % with the next highest match. The remaining sequences were annotated at the deepest possible taxonomic level.

2.9. Polyphenols and their metabolites analyses

2.9.1. Qualitative determination by UHPLC-DAD-MS/MS

The digested and fermented samples were analyzed using an Agilent 1290 Infinity Ultra High-Performance Liquid Chromatography (UHPLC) system equipped with an Agilent 1290 G4212 diode array detector and a Q-Exactive Plus Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Compounds were separated on a Zorbax Eclipse Plus C18 column (2.1 × 50 mm; 1.8 μm particle size) maintained at 40 °C. The mobile phases were water (A) and acetonitrile (B), both containing 0.2 % formic acid, delivered at a flow rate of 0.3 mL/min. The gradient elution applied was as follows: 0–1.5 min, 100 % A, 0 % B; 1.5–15 min, 85 % A, 15 % B; 15–29 min, 25 % A, 75 % B; 29–32 min, 0 % A, 100 % B; 32–36 min, 0 % A, 100 % B; 36–37 min, 100 % A, 0 % B. UV spectra were recorded between 190 and 640 nm with 2.0 nm scan steps. Mass spectrometry settings were configured for both negative mode (3500 V) and positive mode (4200 V) with a scan range of 100–1100 *m/z*, sheath gas flow at 60, auxiliary gas flow at 20, and a sample heater temperature of 380 °C. The system was operated using XCalibur 4.0 software.

2.9.2. Main phenolic compounds quantification

The supernatants from *in vitro* digestion and fermentation (samples at 8 and 24 h) were diluted with methanol (1:2 *v*/v), centrifuged at 12,298 ×*g* for 30 min, filtered through a 0.22 μm filter, and analyzed by HPLC-DAD. The analysis was conducted using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan), consisting of an LC-20AT pump, a SPD-M20A UV diode array detector, a CTO-20 AC column oven, and LabSolution software. The separation was performed on an Inertsil ODS-4 RP-18 column (4.6 × 250 mm; 5 μm particle size) maintained at 30 °C. The HPLC analysis was carried out using a linear gradient solvent system composed of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), as follows: 0–20 min, 10–35 % B; 20–50 min, 35–65 % B; 50–55 min, 65–100 % B; 55–60 min, 100 % B. The flow rate was 1.0 mL/min, and the injection volume was 20 μL. The compounds were monitored at 280 and 360 nm, with UV spectra recorded from 200 to 600 nm for peak characterization. The quantification of the main compounds was carried out using external calibration curves, with standards of catechin, ferulic acid, and kaempferol 3-O-glucoside used for calibration (concentrations from 5 to 200 μM, $r^2 = 0.9999$). Results are expressed as mean values ± standard deviation, derived from three independent fermentations and corresponding injections into the HPLC system.

2.10. Statistical analysis

Three independent experiments were conducted for each analysis. All results are presented as mean ± SD. Statistical analyses were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, Massachusetts, USA). Differences between samples, incubation times, and fermentation metabolites (pH, ammonia, SCFA, and BCFA) were assessed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, with a significance level set at $p < 0.05$. Pearson's correlation coefficient was used to evaluate the relationships among different variables. Multiple comparison analyses were adjusted with the Benjamini-Hochberg (BH) correction. The statistical differences in polyphenol changes between the sample control and digested samples during fermentation were analyzed using paired *t*-tests. A *p*-value <0.05 was considered statistically significant. For microbial analysis, β-diversity indices were analyzed using permutation-based analysis of variance (PERMANOVA). Alpha diversity was assessed using the Kruskal-Wallis test, and "Analysis of Microbiome Composition with Bias Correction" (ANCOM-BC) was applied to identify bacterial taxa differentially represented between groups (Lin & Peddada, 2020).

3. Results and discussion

The Chilean bean landraces 'Negro', 'Peumo', and 'Tórtola' were submitted to *in vitro* gastrointestinal digestion and colonic fermentation. Two different bean substrates were evaluated: boiled beans (BB) and polyphenol-enriched extracts from boiled beans (PEE). The percentage of total digestibility (TD) was calculated for each BB. The highest *in vitro* digestibility was found in Tórtola with 29.4 %, followed by Peumo and Negro with 23.3 % and 23.0 %, respectively.

3.1. Physical characteristics and diet of volunteers

The mean age of the individuals in the study was 33 ± 4 years, and all were in the normal weight category for body mass index (BMI) (Table 1). The dietary patterns of the volunteers could have influenced the results obtained (Paukkonen et al., 2024). Therefore, the main characteristics of the volunteers' diet were estimated (Table 1). The volunteers' diet was characterized by a low intake of carbohydrates and fiber, and a high intake of fat and protein, according to the dietary reference values of the European Food Safety Authority (EFSA (European Food Safety Authority), 2017).

3.2. Changes in main polyphenols after simulated digestion and colonic fermentation

The changes and/or release of polyphenols and their metabolites from BB and PEEs after *in vitro* gastrointestinal digestion and colonic fermentation are detailed in Tables 2 and 3, respectively. After gastrointestinal digestion, the analysis of the supernatant revealed the presence of 22 compounds from BB, including organic acids, phenolic acids, and flavonoids. Interestingly, variations were observed in the release of these compounds among the different bean landraces. Phenolic acids and their derivatives were predominantly detected in Negro beans, while Tortola beans exhibited a lower release of compounds. Peumo beans released 18 compounds, including catechin, kaempferol, quercetin, and their corresponding derivatives. Compounds **1** (citric acid, *m/z* 191.0190), **11** (feruloylglucaric acid, *m/z* 385.0779), **13** (coumaroylglucaric acid, *m/z* 355.0670), **18** (catechin, *m/z* 289.0719), and **27** (phaseolic acid, *m/z* 261.1341), were identified across all landraces. The observations agree with the polyphenol composition and phenotypic characteristics of the landraces selected. Peumo and Negro beans, which are darker, are rich in phenolic compounds, while the pale gray Tórtola beans, have a lower phenolic content (Nina et al., 2023). These findings support the polyphenol content and composition of the landraces analyzed.

The simulated digestion of PEE allowed to understand the polyphenol change during the process. Several compounds remained intact throughout digestion. Compound **8**, present in all PEEs, was identified as catechin hexoside, with a [M-H] at *m/z* 451.1246, and a neutral loss of hexose (162 Da). After digestion, compound **8** was detected only in

Table 1
Characteristics and nutritional intake of fecal donors.

Characteristic	Male (n = 3)	Female (n = 3)
Age, years (range)	33 (29–37)	31 (24–35)
Body weight, Kg (range)	65.7 (60–74)	56.7 (51–61)
BMI kg/m^2, (range)	23.1 (21.8–24.8)	22.1 (19.7–23.8)
Energy intake, kcal (range)	1998 (1557–2506)	1413 (1109–1705)
Total carbohydrates, %E (range)	36.2 (31.1–44.8)	37.9 (26.0–46.9)
Total fats, %E (range)	40.6 (33.3–46.1)	44.8 (35.4–59.6)
Total protein, %E (range)	20.7 (20.3–21.1)	15.7 (13.1–20.8)
Dietary fiber, g/day (range)	13.7 (10.0–17.0)	10.4 (7.0–16.1)

%E: Percentage of energy intake.

Table 2
Tentative identification by HPLC-ESI-QTOF-MS of metabolites after *in vitro* gastrointestinal digestion of BB and PEE of Chilean bean landraces. The base peak is bold.

Peak N°	Rt (min)	[M-H]⁻	Molecular formula	Error (ppm)	MS/MS fragmentation	Tentative identification	PEE extract	PEE digested	BB digested
1	2.04	191.0190	$C_6H_7O_7$	−0.57	191.0185, 129.0184, **111.0075**, 87.0074	Citric acid	*Ne*		*Ne, Pe, To*
2	3.28	183.0290	$C_8H_7O_5$	1.15	**183.0290,** 139.0390, 109.0283	Dihydroxy methoxybenzoic acid	*Ne*		
3	4.30	169.0133	$C_7H_5O_5$	1.13	**125.0233**	Gallic acid	*Ne*		*Ne*
4	4.37	345.0827	$C_{14}H_{17}O_{10}$	3.03	**345.0827,** 125.0232	Methyl galloyl hexoside	*Ne*		
5	6.34	167.0341	$C_8H_7O_4$	0.51	123.0440, 109.0282	Homogentisic acid	*Ne, Pe*		
6	6.54	153.0182	$C_7H_5O_4$	0.62	**109.0284**	Protocatechuic acid	*Ne, To*	*Pe, Ne*	*Ne, Pe*
7	7.96	329.0877	$C_{14}H_{17}O_9$	3.13	**167.0340,** 152.0105, 123.0439, 108.0204	Vanillic acid hexoside	*Ne, To*		
8	8.50	451.1246	$C_{21}H_{23}O_{11}$	1.38	**289.0718**, 137.0232, 109.0283	Catechin hexoside	*Ne, Pe, To*	*Pe*	*Pe*
9	8.51	305.0667	$C_{15}H_{13}O_7$	3.50	**305.0668,** 179.0340, 137.0232, 125.0233, 109.0283	(epi)gallocatechin	*Ne*		
10	8.60	385.0779	$C_{16}H_{17}O_{11}$	2.45	209.0297, 191.0290, 147.0288, 129.00182, **85.0282**	Feruloylglucaric acid	*Ne, To*	*Ne*	*Ne, Pe, To*
11	8.87	431.1194	$C_{18}H_{23}O_{12}$	2.27	431.1194, 299.0772, 179.0239, 137.0223, **93.0333**	Apiosyl hexosyl 4-hydroxybenzoate	*Ne, To*		*Ne*
12	9.40	355.0670	$C_{15}H_{15}O_{10}$	0.28	209.0294, 191.0190, 163.0388, 147.0289, 129.0183, **85.0282**	Coumaroylglucaric acid	*Ne, Pe, To*	*Ne*	*Ne, To*
13	9.78	183.0291	$C_8H_7O_5$	1.37	183.0291, 168.0055, 139.0390, **124.0155**, 95.0126	Methyl gallate	*Ne*	*Ne*	*Ne*
14	9.81	137.0233	$C_7H_5O_3$	−0.0	**137.0233,** 108.0202, 93.0332	Hydroxybenzoic acid	*Ne, To*	*Ne*	*Ne*
15	10.37	577.1356	$C_{30}H_{25}O_{12}$	2.51	451.1035, 425.0881, 407.0767, **289.0718,** 161.0232, 125.0232	Procyanidin B2	*Ne, Pe*	*Pe*	
16	11.62	561.1403	$C_{30}H_{25}O_{11}$	2.11	435.1115, 407.0775, **289.0719,** 137.231, 125.0232, 109.0284	Propelargonidin dimer	*Pe*		
17	11.36	289.0719	$C_{15}H_{13}O_6$	3.65	245.0820, 203.0710, 151.0391, 137.0233, 123.0439, 109.0284	Catechin*	*Ne, Pe, To*	*Pe, Ne*	*Ne, Pe, To*
18	13.29	179.0341	$C_9H_7O_4$	0.18	179.0341, **135.0441**	Caffeic acid	*To*		
19	14.72	449.1092	$C_{21}H_{21}O_{11}$	3.44	**449.1091**, 287.0561, 259.0609, 151.0029, 125.0232	Eriodyctiol/aromadendrin hexoside	*Ne, Pe, To*	*Pe*	*Pe*
20	14.76	289.0718	$C_{15}H_{13}O_6$	3.86	**289.0718,** 245.0816, 203.0713, 179.0342, 109.0283	(epi)catechin		*Ne, Pe*	*Pe*
21	14.93	319.0458	$C_{15}H_{11}O_8$	3.12	**301.0352**, 257.0457, 193.0135, 125.0232	Dihydromyricetin	*Ne*		
22	15.00	193.0135	$C_9H_5O_5$	1.97	**193.0135,** 137.0233, 109.0283	Ferulic acid*	*Ne, To*		
23	15.40	577.1352	$C_{30}H_{25}O_{12}$	2.51	451.1014, 425.0883, 407.0771, **289.0718**, 245.0450, 161.0238, 125.0232	Procyanidin B1	*Ne, Pe*		
24	15.41	191.0343	$C_{10}H_7O_4$	2.17	146.9377, 102.9475, 81.0333, 63.0227	Scopoletin	*Ne, Pe, To*		*Pe*
25	15.78	163.0392	$C_9H_7O_3$	1.10	163.0388, **119.0491**	*p*-coumaric acid	*To*	*To*	
26	17.72	261.1341	$C_{12}H_{21}O_6$	2.8	261.1340, 187.0967, **125.0959**	Phaseolic acid			*Ne, Pe, To*
27	18.38	181.0498	$C_9H_9O_4$	1.30	**181.0497**, 166.0262	Dimethoxybenzoic acid		*To*	
28	18.51	595.1309	$C_{26}H_{27}O_{16}$	−0.67	463.0872, 300.0275, 271.0247, 255.0296	Quercetin hexoside pentoside	*Pe*		
29	18.82	479.0830	$C_{21}H_{19}O_{13}$	2.01	479.0830, **317.0302**, 287.0199, 271.0247	Myricetin hexoside	*Ne*		
30	19.05	177.0184	$C_9H_5O_4$	1.04	**177.0184**, 133.0284, 109.0283	5,7-Dihydroxy-4H-1-benzopyran-4-one		*Ne*	
31	19.85	579.1356	$C_{26}H_{27}O_{15}$	2.13	284.0322, 255.0297, 227.0346	Kaempferol hexoside pentoside	*Ne, Pe*	*Pe*	*Pe*
32	20.19	287.0562	$C_{15}H_{11}O_6$	4.16	**287.0562**, 259.0611, 243.0660, 177.0549, 151.0028, 125.0233	Dihydrokaempferol/ aromadendrin		*Ne, Pe, To*	*Pe*
33	20.23	463.0882	$C_{21}H_{19}O_{12}$	2.56	**463.0882**, 301.0353, 271.0247, 255.0296, 243.0294, 151.0028	Quercetin hexoside	*Ne, Pe, To*	*Ne, Pe, To*	*Pe*
34	20.82	269.0455	$C_{15}H_9O_5$	3.79	**269.0455,** 255.0553, 181.0649, 133.0285	Genistein	*Ne*	*Ne*	
35	21.41	549.0886	$C_{24}H_{21}O_{15}$	1.61	**505.0992,** 301.0351, 271.0245, 225.0286, 227.0803	Quercetin malonyl hexoside	*Ne*		
36	21.43	609.1467	$C_{27}H_{29}O_{16}$	−0.98	447.0936, **285.0409**, 255.0296, 227.0348	Kaempferol dihexoside	*Ne, Pe*		*Pe*
37	21.59	167.0340	$C_8H_7O_4$	0.87	**167.0340**, 123.0440, 81.0333	Vanillic acid	*Ne*		
38	21.63	447.0933	$C_{21}H_{19}O_{11}$	2.40	**447.0934**, 285.0393, 255.0294, 227.0341	Kaempferol 3-*O*- glucoside*	*Ne, Pe, To*	*Ne, Pe, To*	*Pe*
39	21.91	303.0509	$C_{15}H_{11}O_7$	3.30	303.0510, **151.0390,** 109.0126	Taxifolin	*Ne*		
40	22.75	253.0504	$C_{15}H_9O_4$	3.30	**253.0506,** 195.0446, 132.0207	Chrysin		*Ne, Pe, To*	
41	22.82	191.0342	$C_{10}H_7O_4$	2.2	**191.0343**, 146.9376, 123.0454	4-methylaesculetin	*To*	*Ne, To*	
42	22.86	287.0560	$C_{15}H_{11}O_6$	3.43	287.0560, 161.0234, **125.0232**	Eriodictyol		*Ne*	
43	23.04	317.0302	$C_{15}H_9O_8$	3.02	**317.0302,** 151.0027, 137.0232, 109.0282	Myrycetin	*Ne*		

(*continued on next page*)

Table 2 (*continued*)

Peak N°	Rt (min)	[M-H]⁻	Molecular formula	Error (ppm)	MS/MS fragmentation	Tentative identification	PEE extract	PEE digested	BB digested
44	23.46	285.0404	$C_{15}H_9O_6$	3.42	285.0404, 217.0502, 175.0393, 133.0285	Luteolin		*Ne*	
45	24.91	489.1036	$C_{23}H_{21}O_{12}$	1.81	**489.1040**, 285.0403, 255.0297, 227.0347	Kaempferol acetyl hexoside	*Ne, Pe, To*	*Ne, Pe*	*Pe*
46	24.04	533.0939	$C_{24}H_{21}O_{14}$	2.42	489.1038, **285.0403**, 255.0296, 227.0345, 165.0905	Kaempferol malonyl hexoside	*Ne, Pe, To*	*Pe*	*Pe*
47	25.78	301.0354	$C_{15}H_9O_7$	1.15	**301.0354**, 178.9974, 155.0027	Quercetin	*Ne, Pe, To*		*Pe*
48	27.55	285.0405	$C_{15}H_9O_6$	1.11	**285.0405,** 255.0300, 239.0345	Kaempferol	*Ne, Pe, To*		

PEE: polyphenol enriched extract, Pe: Peumo, Ne: Negro, To: Tórtola.

Table 3
Characterization of fermentation metabolites of BB after *in vitro* colonic fermentation by UPLC-ESI-QTOF-MS. The base peak is in bold.

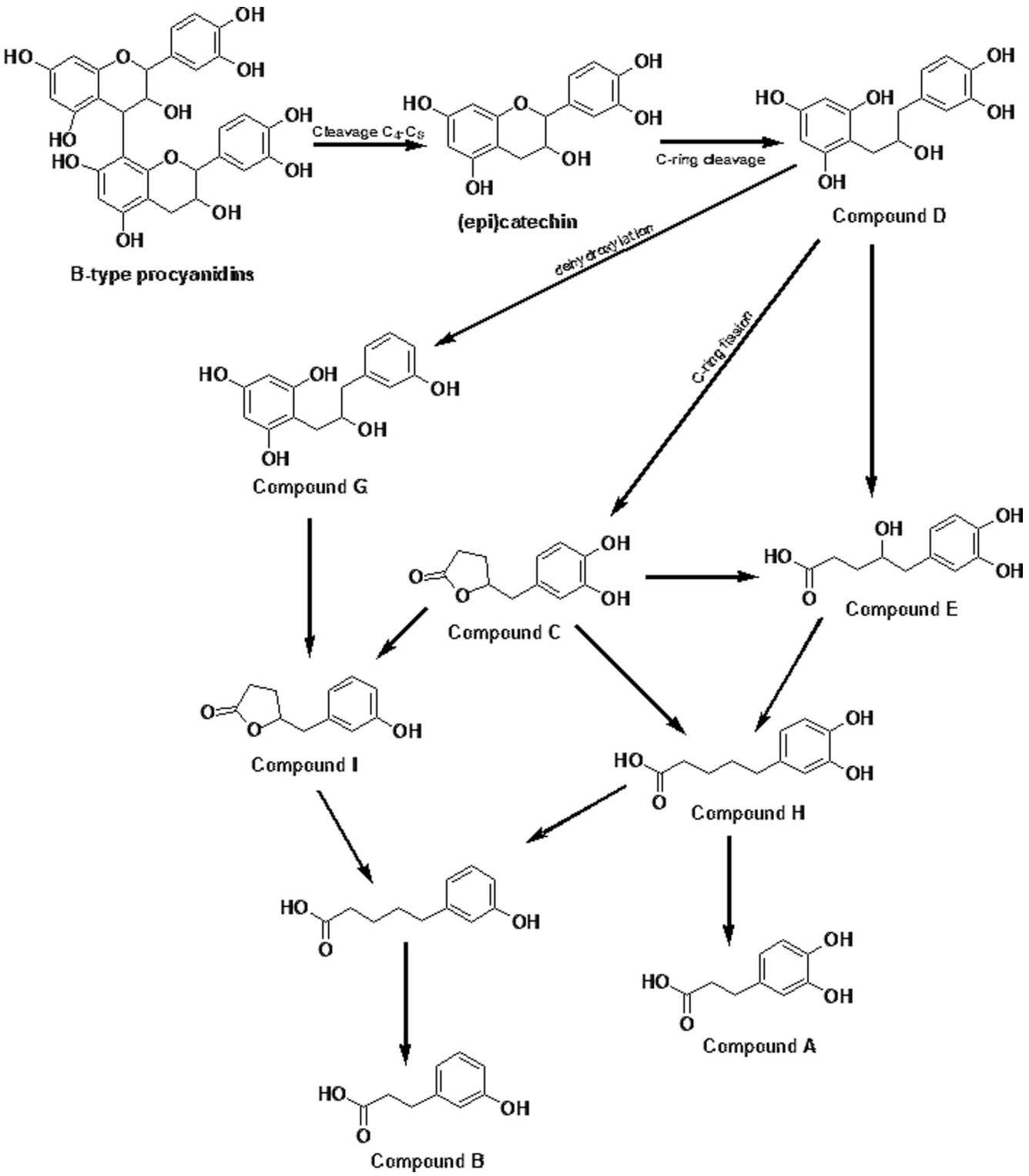
Peak	Rt (min)	[M-H]⁻	Formula molecular	Error (ppm)	MS fragmentation	Compound	Fermentation time	
							8 h	24 h
A	7.94	181.0498	$C_9H_9O_4$	1.68	163.0390, **136.9088,** 92.9187	Dihydroxyphenyl propionic acid	*Ne, Pe, To*	
B	12.81	165.0548	$C_9H_9O_3$	0.84	165.0549, **147.0443**, 119.0490, 72.0918	Hydroxyphenyl propionic acid	*Ne, Pe, To*	
C	13.06	207.0657	$C_{11}H_{11}O4$	2.05	**207.0657,** 163.0754, 122.0361	Dihydroxyphenyl γ-valerolactone	*Pe, To*	*Ne, Pe*
D	14.31	291.0874	$C_{15}H_{15}O_6$	3.52	**291.0874,** 247.0974, 167.0341, 135.0441, 123.0439	1-(3′,4′-dihydroxy phenyl)-3-(2″,4″,6″-trihydroxy phenyl) propan-2-ol	*Pe, To*	*Pe*
E	14.34	225.0764	$C_{11}H_{13}O_5$	1.78	225.1125, 180.6425, 141.9114, **59.0126**	4-Hydroxy-5-(3′,5′-dihydroxyphenyl)-valeric acid	*Ne, To*	*Pe*
F	15.21	287.0560	$C_{15}H_{11}O_6$	0.35	287.0559, 259.0610, 243.0664, 177.0547, 125.0232	Dihydrokaempferol	*Pe*	
G	16.43	275.0924	$C_{15}H_{15}O_5$	1.1	275.0927, 231.1019, 191.0703	1-(hydroxy phenyl)-3-(2″,4″,6″-trihydroxy phenyl) propan-2-ol	*Pe*	*Pe*
H	18.21	209.0790	$C_{11}H_{13}O_4$	−8.8	209.0790, 141.0911	Dihydroxyphenyl valeric acid	*Ne, To*	*Ne*
I	18.38	191.0742	$C_{11}H_{11}O_3$		146.9376, 102.9475, 87.9240	Hydroxyphenyl γ-valerolactona	*Ne, Pe, To*	*Pe*
J	26.63	181.0496	$C_9H_9O_4$	0.58	181.0496, 166.0262, 136.9088, **112.9844**	Homovanillic acid	*Ne, Pe, To*	*Ne, To*

Pe: Peumo, Ne: Negro, To: Tórtola.

Peumo, suggesting that the glycoside was hydrolyzed to catechin or *epi*catechin in Negro and Tórtola beans. The procyanidin dimers (**15**, **16**, and **23**) with a base peak at *m/z* 289.0718 are hydrolyzed during simulated digestion to mixtures of (*epi*)catechin monomers, enhancing their potential for absorption in the small intestine. Compound **11**, identified as a hydroxybenzoic acid diglycoside (*m/z* 431.1194) in PEE from Negro beans, yielded hydroxybenzoic acid (compound **14**, *m/z* 137.0233) after hydrolysis. Dihydrokaempferol or aromadendrin (*m/z* 287.0562) in all samples after digestion suggests hydrolysis of compound **19** (*m/z* 449.1092). Most flavonoids in common beans are glycosylated, and our results showed they can reach the small intestine unchanged. Absorption can be improved by hydrolysis to aglycones due to human endogenous enzymes, such as lactase phlorizin hydrolase and cytosolic β-glucosidase in the intestine lumen (Ketnawa et al., 2022). Moreover, only unabsorbed flavonoids pass to the colon and can undergo biotransformation by microbiota. Our results suggest that polyphenols bonded to the fiber/food matrix can be released slowly and made available for absorption in the small intestine or metabolized in the colon by the gut microbiota.

After colonic fermentation, ten compounds were detected as end products of the degradation of phenolic compounds released from BB (Table 3). Most of them are detected at 8 h of fermentation. The metabolism of polyphenols is time and concentration-dependent, associated with the following metabolic events after cleavage of the ester or glycosidic bond: reduction of the aliphatic double bond, dehydroxylation, α and β-oxidation and C-ring fission in flavonoids (Xie et al., 2022). Simple phenolic acids are produced after the microbial metabolism of polyphenols. According to Stoupi et al. (2010) and Ou et al. (2014), there are characteristic metabolites of the microbial degradation of epicatechin/catechin and procyanidin B2. They were detected in our samples, including 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (compound **C**, *m/z* 207.0657), 5-(3′-hydroxyphenyl)-γ-valerolactone (compound **I**, *m/z* 191.0742) and dihydroxyphenyl valeric acid (compound **H**, *m/z* 209.0790). Compound **C** was detected in Peumo at 8 and 24 h, but not in Negro until 24 h of fermentation. Meanwhile, compounds **I** and **J** were detected at 8 h in Negro and Tórtola and after 24 h in Negro beans. Two species of bacteria isolated from human feces could be implicated in the degradation of epicatechin/catechin, namely *Eggerthella lenta* and *Flavonifractor plautii* (Kutschera et al., 2011). *E. lenta*, cleaved the heterocyclic C-ring of both monomers giving rise to 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol (compound **D**, *m/z* 291.0874) and *F. plautii* further converted **D** to compound **C** and 4-hydroxy-5-(3,4-dihydroxyphenyl)valeric acid (compound **E**, *m/z* 225.0764). The loss of one hydroxyl group in the B-ring of compound **D** led to compound **G** with *m/z* 275.0924 (1-(hydroxy phenyl)-3-(2″,4″,6″-trihydroxy phenyl) propan-2-ol), followed by compound **J** (Stoupi et al., 2010). The proposed metabolic biotransformation of related procyanidin compounds is shown in Scheme 1.

Flavan-3-ols, flavonols and hydroxycinnamic acids lead to 3-(3′,4′-dihydroxyphenyl)-propionic acid, 3-(3′-hydroxyphenyl) propionic acid, and 3-(4′-hydroxyphenyl) propionic acid, which means some enzymes and metabolic pathways are quite common among bacteria (Makarewicz et al., 2021). The three bean landraces showed dihydroxyphenyl propionic acid (compound **A**, *m/z* 181.0948) and



Scheme 1. Proposed biotransformation of B-type procyanidins and (epi)catechin in landrace beans

hydroxyphenyl propionic acid (compound **B**, *m/z* 165.0548) after 8 h of fermentation. They could be produced by the degradation of hydroxycinnamic acids, feruloylglucaric acid (**10**), coumaroylglucaric acid (**12**), and phaseolic acid (**26**). Peumo beans are rich in kaempferol derivatives. The route of degradation involves sugar loss and reduction of kaempferol to dihydrokaempferol (compound **F**, *m/z* 287.0560), detected after 8 h of fermentation.

Homovanillic acid (compound **J**, *m/z* 181.0496) differs from compound **A**, due to the methoxy group in the phenyl ring, showing lower polarity and elutes last. Homovanillic acid was detected as a quercetin or quercetin glycosides fermentation product, and was the exclusive quercetin fermentation metabolite, according to Serra et al. (2012). Quercetin glycosides were detected after digestion only in Peumo beans. The occurrence of compound **J** suggests that some quercetin or quercetin derivatives were released during colonic fermentation and made available for microbial metabolism. Flavonoids and phenolic

acids could be released and metabolized in the colon but were not detected in the fermented samples. Fewer end-product compounds were detected in PEE samples after colonic fermentation at 8 h. The concentrations available for the microbiota were probably low, and no effects were observed during the fermentation.

Polyphenol catabolites have demonstrated various effects both *in vitro* and *in vivo* assays. These include anti-inflammatory regulation by reducing pro-inflammatory mediators, significant inhibition of growth in intestinal epithelial cancer cells, and prevention of urinary tract infections (UTIs) by blocking the adherence of uropathogenic *Escherichia coli* (UPEC) to bladder epithelial cells (Mena et al., 2019). Additionally, these metabolites can mitigate weight gain, reduce oxidative stress imbalance, and improve glucose and lipid metabolism disorders caused by a high-fat diet in mice (Chen et al., 2024).

3.3. Quantification of main phenolic compounds and bioaccessibility

The HPLC-DAD analysis allowed us to quantify the main phenolic released from the plant matrix after *in vitro* gastrointestinal digestion and compare it with the phenolic content in the undigested beans to estimate their bioaccessibility, expressed as a percentage (Table 4). The main compounds reported in Chilean beans, according to Nina et al. (2023), were catechin, catechin glucoside, kaempferol glucoside, ferulic acid, and anthocyanins in Negro or black beans. Thermal processing significantly affected the content of anthocyanins in black beans, decreasing by 10-fold after cooking. However, the total content of proanthocyanidins increased (Nina et al., 2023), which explains the occurrence of catechin/epicatechin after simulated digestion in Negro beans. The components of the samples submitted to *in vitro* digestion were compared to the parallel simulated digestion of the sample without adding digestive enzymes and bile salts (sample control, SC). Catechin, ferulic acid, and kaempferol 3-glucoside were quantified as markers of phenolics released from the BB after *in vitro* gastrointestinal digestion. The catechin content was 975.40, 829.95, and 1668.05 μg/g dry weight for Peumo, Negro, and Tórtola, respectively. In Negro and Tórtola, ferulic acid content was 5.34 and 4.46 μg/g dry weight, respectively. Kaempferol 3-glucoside occurs in Peumo (110.99 μg/g dry weight), but it is not statistically significant from its SC (Table 4). The information obtained supports the release of catechin and ferulic acid from the matrix. Catechin was released from the matrix after gastrointestinal digestion in all BB samples, achieving notable bio accessibility, especially in Tórtola beans. Similar results were reported by Chen et al. (2015) for two cultivars of cranberry beans (*P. vulgaris*). The phenolic-dietary fiber interaction mainly depends on the molecular weight, degree of hydroxylation, methoxylation, methylation, esterification, hydrogenation, and glycosylation of phenolic compounds (Rocchetti et al., 2022). Enzymes and pH conditions may release phenolics from the food matrix in the gastrointestinal tract (Jakobek & Matić, 2019). Thermal processing induces changes to the food matrix that facilitates the release of polyphenols (Singh et al., 2010).

During the digestion of the PEEs, the catechin concentration increased significantly ($p < 0.05$) in all landraces, suggesting degradation of procyanidin dimers to monomers (Zhang et al., 2016). B-type procyanidins were detected in the PEEs before *in vitro* digestion, but not in the digested or fermented samples. No significant differences were observed in the concentrations of kaempferol 3-glucoside (K3G) between the sample control and digested samples. This observation suggests that K3G is stable during digestion (Table 4). Giusti et al. (2019) reported that free phenolic extracts of Pinto and black beans after simulated digestion did not show changes in the phenolic concentration. Remanent catechin is released from the matrix and transformed/metabolized by the gut microbiota in the colon. This metabolite was detected and quantified after 8 h of fermentation. Tórtola beans showed a higher concentration (49.42 μg/mL) compared to Peumo and Negro, with 35.59 and 35.69 μg/mL, respectively. The released compound becomes available for microbial degradation into smaller molecules.

3.4. pH, Ammonia production and fiber bean content

Fecal pH and ammonia content are parameters related to colon health and indirect indicators of bacterial fermentation and metabolite production. pH and ammonia were monitored during the *in vitro* fermentation at 0, 8, 24, and 48 h (Fig. 1). As expected, during the first 8 h, FOS (positive prebiotic control) significantly decreased the pH of the medium compared to the negative control. The digestion control exhibited results comparable to the negative control across all parameters. The fermentation of the digested BB also induced a significant decrease of 1.3–1.6 pH units ($p < 0.05$). No significant differences were found among the landraces. On the other hand, the pH was not affected by the metabolization of the PEEs by gut microbiota (Fig. 1A). The decrease in pH during the fermentation process could be due to an increase in the production of acid metabolites by the microbiota, specifically SCFAs. Common beans are important sources of dietary fiber, which serve as substrates for microbiota that produce SCFAs. In fact, in our samples, the total dietary fiber content was 23.97 %, 21.11 %, and 21.18 % for Negro, Peumo, and Tórtola, respectively, values like those reported previously in raw beans (23 % total dietary fiber) that did not undergo any significant change after soaking and cooking (Liu et al., 2024). Changes in pH due to fiber fermentation could significantly influence colonic enterocyte health (Campos-Vega et al., 2012). An acidic

Table 4
Quantification of main phenolic compounds and bioaccessibility from boiled beans and changes in phenolics in PEE during *in vitro* gastrointestinal digestion. Concentration is expressed as μg/g DW of boiled seeds.

Compounds	Catechin (μg/g)	% bioaccessibility	Ferulic acid (μg/g)	% bioaccessibility	K 3-*O*-glucoside (μg/g)	% bioaccessibility
Boiled Beans						
Peumo control	145.27 ± 6.78^{a}		–		96.70 ± 0.01^{a}	
Peumo digested	975.40 ± 19.55^{b}	277	–		110.99 ± 0.78^{a}	10
Negro control	144.36 ± 5.84^{a}		2.85 ± 0.01^{a}		–	
Negro digested	829.95 ± 2.62^{b}	376	5.34 ± 0.08^{b}	21	–	
Tortola control	200.76 ± 7.35^{a}		–		–	
Tortola digested	1668.05 ± 62.67^{b}	1921	4.46 ± 0.66	50	–	
Phenolic Enriched Extract						
Peumo control	110.00 ± 7.10^{a}				550.00 ± 6.21^{a}	
Peumo digested	169.66 ± 8.90^{b}				557.70 ± 7.10^{a}	
Negro control	90.00 ± 5.66^{a}		8.78 ± 0.01^{a}		–	
Negro digested	304.35 ± 6.02^{b}		13.07 ± 0.0^{b}		–	
Tortola control	60.00 ± 0.01^{a}		5.51 ± 0.01^{a}		20.00 ± 0.01^{a}	
Tortola digested	156.96 ± 5.63^{b}		6.18 ± 0.11^{a}		20.96 ± 0.27^{a}	

Values are the mean ± SD. Different superscript letters (a-b) in the same column show significant differences between the control sample and digested sample according to the paired t-test ($p < 0.05$).

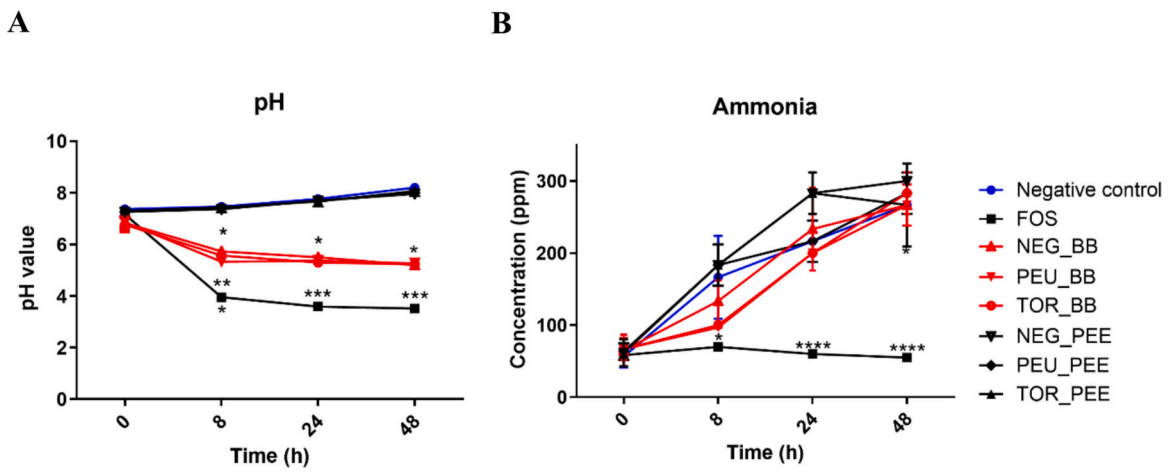


Fig. 1. A) pH and B) ammonia content during *in vitro* colonic fermentation of BB and PEEs. The results are expressed as mean ± SD ($n = 3$). Significant differences $p < 0.001$ and $p < 0.05$ between treatments and control are indicated in the graphics (***) and (*), respectively, according to ANOVA followed by Tukey's test.

pH has several health benefits, including inhibition of pathogenic bacteria and increased mineral absorption, such as calcium (Yamamura et al., 2023). Other studies, such as those by Chen et al. (2020), investigated the *in vitro* digestion and fermentation of soybeans and common beans using pig feces and observed a decrease in pH values during the first 6 h of fermentation.

Beans also contain proteins. In a recent study, the protein content of Chilean beans was analyzed, revealing that the three accessions under investigation contained between 22.6 and 23.2 g of protein per 100 g (Márquez et al., 2024). Protein fermentation mainly occurs in the distal colon when carbohydrates are depleted and results in the production of potentially toxic metabolites such as ammonia, amines, phenols, and sulfides (Windey et al., 2012). Ammonia levels were measured to test if the protein content of the beans increased ammonia production. An increase in ammonia levels was observed for the negative control, PEE samples, and BB samples, except for the positive prebiotic control FOS (Fig. 1B). The increase in ammonia in BB Peumo and Tórtola was lower than that observed in the negative control ($p < 0.05$) after 8 h of fermentation, indicating that bacteria were still using carbohydrates from these BB as an energy source. Thus, common beans would be a

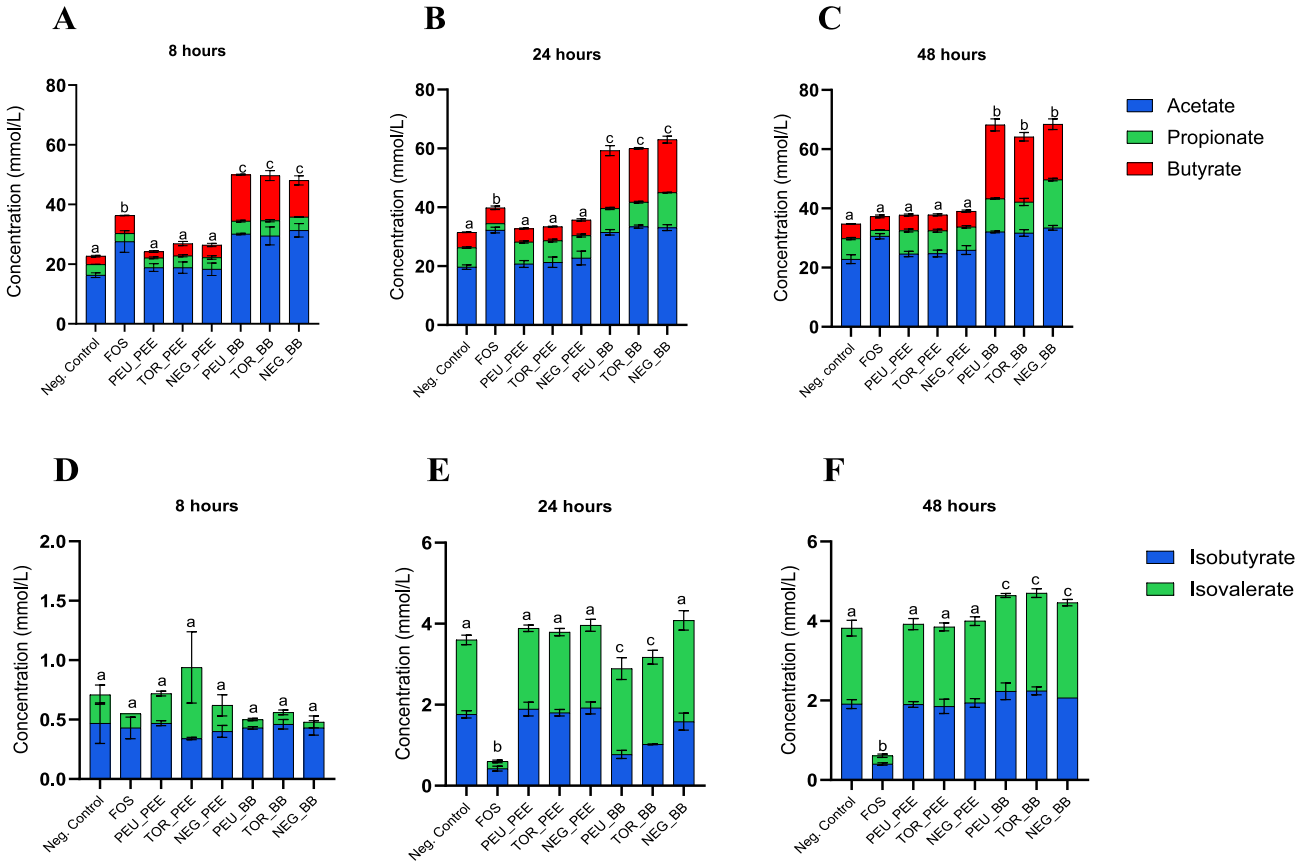


Fig. 2. Production of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) during *in vitro* colonic fermentation with BB and PEE. The results are shown as mean ± SD (n = 3). Different letters (a-c) in the bars show significant differences between treatments and control, according to ANOVA followed by Tukey's test ($p < 0.05$).

good protein source in the diet and decrease ammonia production compared to animal protein (Xiao et al., 2021). No significant differences were found in PEEs and the negative control.

3.5. SCFA and BCFA analysis

The main fermentation products of complex carbohydrate breakdown by the gut microbiota are SCFAs. The production of SCFAs during the *in vitro* colonic fermentation of BB and PEEs was analyzed at 8, 24, and 48 h (Fig. 2). After 8 h of fermentation, there was a significant increase in acetate production by the microbiota when fermenting all three BB and the FOS positive control (Fig. 2A). Acetate concentration from fermented BB ranged from 29.47 to 31.33 mmol/L after 8 h of fermentation, which was comparable to the positive control, indicating a similar fermentative response. Acetate production at 24 h slightly increased, reaching significant concentrations between 31.50 and 33.41 mmol/L for all three BB and FOS (Fig. 2B), and remained steady at 48 h for all three BB, with FOS-induced SCFA production decreasing to control levels (Fig. 2C). This suggests that the effect of the three BB on SCFA production is more persistent over time than FOS. When landraces were compared, Negro BB significantly increased acetate production more than the other two BB landraces and FOS at 8 h of fermentation (Fig. 2A). No statistically significant differences were observed between Peumo and Tórtola BB and the positive control FOS. The presence of PEEs in the fermentation medium did not influence acetate production, with acetate values not significantly different from those of the negative control. In a similar study using whole boiled black beans, acetate production at 24 h was in line with our results (34.09 mmol/L), with a slight increase observed after 48 h of fermentation (Teixeira-Guedes et al., 2020). Chen et al. (2020) reported a significant increase in acetate levels during the initial 12 h of fermentation, followed by a lower rate of acetate generation in soluble and insoluble residues of Pinto beans until 24 h of fermentation. Acetate is a key compound regulating glucose and lipid metabolism, controlling appetite, and reducing inflammation. It acts as a signaling molecule that influences energy homeostasis, modulates insulin sensitivity, and impacts inflammatory responses, contributing to overall metabolic health (Canfora et al., 2015), and potentially acting as a mediator of the beneficial effects of consuming the studied beans on health.

Propionate was not produced during the fermentation of FOS. However, small but significant increases ($p < 0.05$) were detected in propionate production during the fermentation of all BB, particularly at 24 and 48 h, with values ranging from 8.11 to 11.92 mmol/L and 10.46 to 16.32 mmol/L, respectively (Fig. 2B and C). Among the bean landraces, Negro showed the highest and most significant propionate production ($p < 0.001$), with 11.92 and 16.32 mmol/L at 24 and 48 h, respectively. When black beans were fermented by microbiota, Teixeira-Guedes et al. (2020) also reported lower propionate production compared to other SCFAs, but propionate after 48 h of fermentation of black beans cultivated in Portugal was lower (5.80 mmol/L) compared to our results. In line with our observations, the soluble fraction of Pinto beans fermentation increased propionate production after 24 h of fermentation (Chen et al., 2020). The presence of PEEs in the fermentation medium did not induce propionate production at any time.

Regarding butyrate, the highest increase in butyrate production was induced by the fermentation of BB, ranging from 12.28 to 15.55 mmol/L at 8 h of fermentation (Fig. 2A), from 17.99 to 19.66 mmol/L at 24 h, and from 18.70 to 24.84 mmol/L after 48 h of fermentation (Fig. 2B and C). The fermentation of FOS only increased butyrate production at 8 h (Fig. 2A), reaching a concentration of 6.04 mmol/L, while the negative control registered 2.75 mmol/L. No changes in butyrate levels were observed for the PEEs. According to our work, the butyrate production by the fermentation of black beans from Portugal was also more pronounced in the first h, with quantities like those produced by the fermentation of inulin (Teixeira-Guedes et al., 2020). However, in our study, butyrate production was maintained over time for BB except for FOS (Fig. 2). According to Chen et al. (2020), after 24 h of fermentation, the soluble fiber of Pinto beans showed higher concentrations of butyrate compared to the insoluble fiber, emphasizing the importance of dietary fiber in butyrate production. Additionally, Hernández-Salazar et al. (2010) reported that the indigestible fraction of black beans produced more butyrate (5.6 mmol/L) than the positive control raffinose after 24 h of fermentation. It is important to note that the results of these three studies cannot be directly compared due to different sources of beans and total dietary fiber content among the accessions (Los et al., 2018). The higher butyrate production observed in our samples could be attributed to their higher resistant starch content. It has been shown that resistant starch produces higher levels of butyrate than other indigestible polysaccharides (De Martino & Cockburn, 2020; Liu et al., 2020).

When considering the concentration of total SCFAs, we can conclude that the primary and significant ($p < 0.05$) induction of SCFAs occurs during the first 8 h for both FOS and BB samples. In the case of FOS, the levels of SCFAs are maintained at 24 h of fermentation, with no changes observed after 48 h in comparison to the negative control (Fig. 2). In contrast, the total concentration of SCFA production by BB fermentation continued to increase until 48 h. No changes in total SCFA production were detected for PEE samples. An increased production of SCFAs contributes to colon health. Beans are rich in polysaccharides and soluble fibers, whose bioavailability increases after cooking (Campos-Vega et al., 2009). Prebiotic fibers can be fermented selectively in the colon, providing an energy source for diverse beneficial bacterial groups. As a result of this process, bacteria produce health-promoting SCFA compounds, such as acetate, propionate, and butyrate (Fernández et al., 2016). They constitute the main energy source of colonocytes and play a crucial role in regulating the growth and proliferation rates of normal cells. SCFAs contribute to the maintenance of intestinal barrier function through several mechanisms, including stimulation of mucus synthesis and quality, synthesis of antimicrobial peptides, and reducing epithelial permeability by targeting the integrity of tight junction complexes (Feng et al., 2018). Additionally, SCFAs may regulate glucose and lipid metabolism. Among the SCFAs, butyrate is relevant in maintaining intestinal immune homeostasis and protecting against inflammation and carcinogenesis (Blaak et al., 2020; Nogal et al., 2021).

Branched-chain fatty acids (BCFAs), including isobutyrate and isovalerate, are end-products of protein fermentation (Fig. 2D-F). After 8 h of fermentation, no changes were observed in isobutyrate production across all samples (Fig. 2D). The negative control, PEEs, and BB Negro showed a significant increase ($p < 0.05$) in isobutyrate production at 24 h of fermentation (Fig. 2E). Meanwhile, FOS did not produce isobutyrate, and BB landraces Peumo and Tórtola showed lower production than the negative control. Peumo and Tórtola BB showed a significant increase ($p < 0.05$) in isobutyrate production compared to the negative control after 48 h of fermentation (Fig. 2F). The production of isovalerate followed a similar pattern to isobutyrate after 8 h of fermentation (Fig. 2D). Both negative control and samples showed an increase in isovalerate production after 24 h, sustained to 48 h of fermentation (Fig. 2E and F). However, no changes in isovalerate levels were observed throughout the fermentation process with FOS. This may be attributed to the absence of a protein source for fermentation in FOS, resulting in the lack of production of BCFAs. Common beans and other legumes are also rich in proteins. When hydrolyzed soy protein was submitted to colonic fermentation, isobutyrate, and isovalerate production at 24 h was 9.79 and 9.89 mmol/L, respectively (Ashaolu et al., 2019). BCFA concentrations are usually associated with dietary protein intake and the type of amino acids available in the colon. Amino acids such as valine, isoleucine, and leucine are fermented to isobutyrate, methyl butyrate, and isovalerate, respectively (Verbeke et al., 2015).

3.6. Correlations coefficients between pH, ammonia, SCFA, and BCFA

Pearson's correlation analyses were performed between the different metabolites produced by colonic fermentation to evaluate their

relationships (Fig. 3). A significant negative correlation was found between pH and acetate across the fermentation time (8 h: $r = -0.88$, $p < 0.05$; 24 h: $r = -0.91$, $p < 0.05$; 48 h: $r = -0.88$, $p < 0.05$), confirming the low pH in FOS and BB with high acetate content. Ammonia content presented a significant positive correlation with isovalerate at 8 h of fermentation ($r = 0.73$, $p < 0.05$), while isobutyrate and isovalerate at 24 and 48 h showed r values of 0.83 and 0.94 ($p < 0.05$), respectively. Ammonia is a product of the catabolism of proteins and amino acids, and BCFAs are typically products of the fermentation of branched-chain amino acids such as valine, leucine, and isoleucine. These correlations further support our findings on the increase in BCFA production when the availability of carbohydrates as substrates becomes depleted during the fermentation process of boiled beans. SCFAs exhibited significant correlations among themselves. Acetate showed a robust positive correlation with butyrate from 8 h ($r = 0.93$, $p < 0.05$) to 48 h ($r = 0.79$, $p < 0.05$). Similarly, propionate showed a strong positive correlation with isovalerate ($r = 0.93$, $p < 0.05$) at 24 h and significant positive relationships with butyrate ($r = 0.72$, $p < 0.05$), isobutyrate ($r = 0.75$, $p < 0.05$), and isovalerate ($r = 0.81$, $p < 0.05$) at 48 h. These results indicate that SCFA production is highly interrelated, with specific correlations varying over the fermentation period. The strong association between acetate and butyrate suggests a potential interaction in their production pathways. At the same time, the correlation of propionate with isovalerate and isobutyrate may reflect complementary or shared metabolic processes during the later stages of fermentation, primarily protein metabolism.

3.7. Effect of Chilean landrace beans on gut microbiota

3.7.1. Diversity analysis

The changes in the microbiota induced by the fermentation of three BB and their respective polyphenol-enriched extracts were analyzed through 16S rRNA sequencing at 8 h of fermentation. Regarding beta-diversity indexes, Bray-Curtis, Jaccard, and both Weighted and Unweighted indexes did not show significant changes (Supplementary Fig. S1). The alpha diversity analysis also did not show any significant changes with any of the treatments for the Shannon index, observed features, Faith's phylogenetic diversity index, and evenness (Supplementary Fig. S2).

3.7.2. Genera differential abundance analysis

After 8 h of fermentation, the gut microbiota composition at the genus level was significantly changed. The Negro BB and PEE had the most significant impact on the microbiota. The FOS treatment promoted the presence of different bacterial genera (Fig. 4A), among which the following are worth mentioning: *Catenibacterium*, *Lachnospira*, *Ruminococcaceae* UCG-010, CAG-56, and *Bifidobacterium*.

Catenibacterium is a Gram-positive genus whose presence is promoted by various types of fiber *in vitro* (Sáyago-Ayerdi et al., 2020; Loo et al., 2022; Gutiérrez-Sarmiento et al., 2020) and *in vivo* (He et al., 2018; Jarett et al., 2019) or has been correlated with fiber intake (Ma et al., 2021). Its primary metabolites are acetate, butyrate, lactate, and small amounts of isobutyrate (Kageyama & Benno, 2000). Also, *Lachnospira* is a genus whose presence increases with fiber fermentation (Sun et al., 2023; Yamamoto et al., 2024) and correlates with fiber intake (Di Iorio et al., 2019), as well as *Ruminococcaceae* UCG-010, which has been associated with polysaccharide intake (He et al., 2024). The CAG-56 genus belongs to the *Lachnospiraceae* family but has been much less studied than the *Lachnospira* genus. The health-promoting *Bifidobacterium* is renowned for its growth stimulated by FOS (Dou et al., 2022).

Lachnospira was promoted by Negro BB and Negro PEE (Fig. 4B). Other studies have shown that foods rich in polyphenols, such as cocoa and walnuts, increase the *Lachnospira* genus (Tabone et al., 2022; Creedon et al., 2020). Other bacterial genera promoted by Negro beans' PEE were *Terrisporobacter* and *Ruminococcaceae* UCG-010. Bacteria in the *Ruminococcaceae* family are generally involved in the fermentation of plant material and fiber degradation in the gastrointestinal tract, particularly in herbivores, where they play a crucial role in breaking down complex carbohydrates like cellulose. These bacteria help produce SCFAs like acetate, propionate, and butyrate, which have important health benefits, including promoting gut health and serving as an energy source for colonocytes (Kim et al., 2024). *Terrisporobacter* has been previously associated with intake of hydroxycinnamic acids, including caffeic acid (Wen et al., 2024), and quercetin (Mao et al., 2024), whose derivatives occurs in Negro PEE. *Allisonella* decreased in the PEE of Negro beans, in line with results obtained from mulberry leaf powder (rich in flavonoids) on piglets' microbiota (Ma et al., 2023) and with those after gut microbiota fermentation with black rice rich in fiber and anthocyanins (Zhang et al., 2023). The fermentation of boiled Negro beans decreased *Eubacterium ventriosum* group, *Odoribacter*, and *Sutterella* genera. These results contrast with those where *Eubacterium ventriosum* and *Sutterella* are proposed as biomarkers of daily polyphenol consumption (Vita et al., 2024) and the increase in *Odoribacter* after anthocyanin intake in mice (Peng et al., 2020). *Eubacterium ventriosum* and *Odoribacter* are fiber-fermenting bacteria and producers of SCFAs. Therefore, we could hypothesize that their presence in the gut may be diminished due to competition with the genus *Lachnospira*, which has a strong and broad capacity for fiber fermentation (Vacca et al., 2020). The fermentation of Tórtola BB promoted *Staphylococcus*, *Roseburia*, and *Gemella* genera. It did not cause any decrease in microbiota genera (Fig. 4C). *Roseburia* is known for being modulated by diets rich in legumes, which influence the production of SCFAs, confirming our results

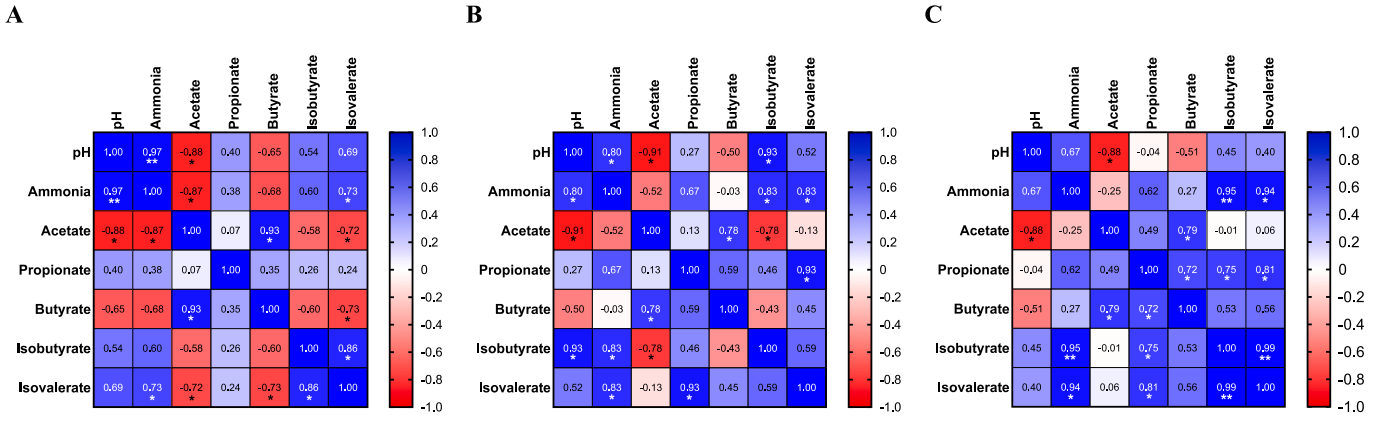


Fig. 3. Pearson correlation analysis between pH, ammonia, short-chain fatty acids (SCFA), and branched-chain fatty acids (BCFA) at 8, 24, and 48 h (A, B y C). The correlation coefficients (r) are displayed in the box. The intensity of the color gradient indicates strong positive (blue) or negative (red) correlations. * ($p < 0.05$) ** ($p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

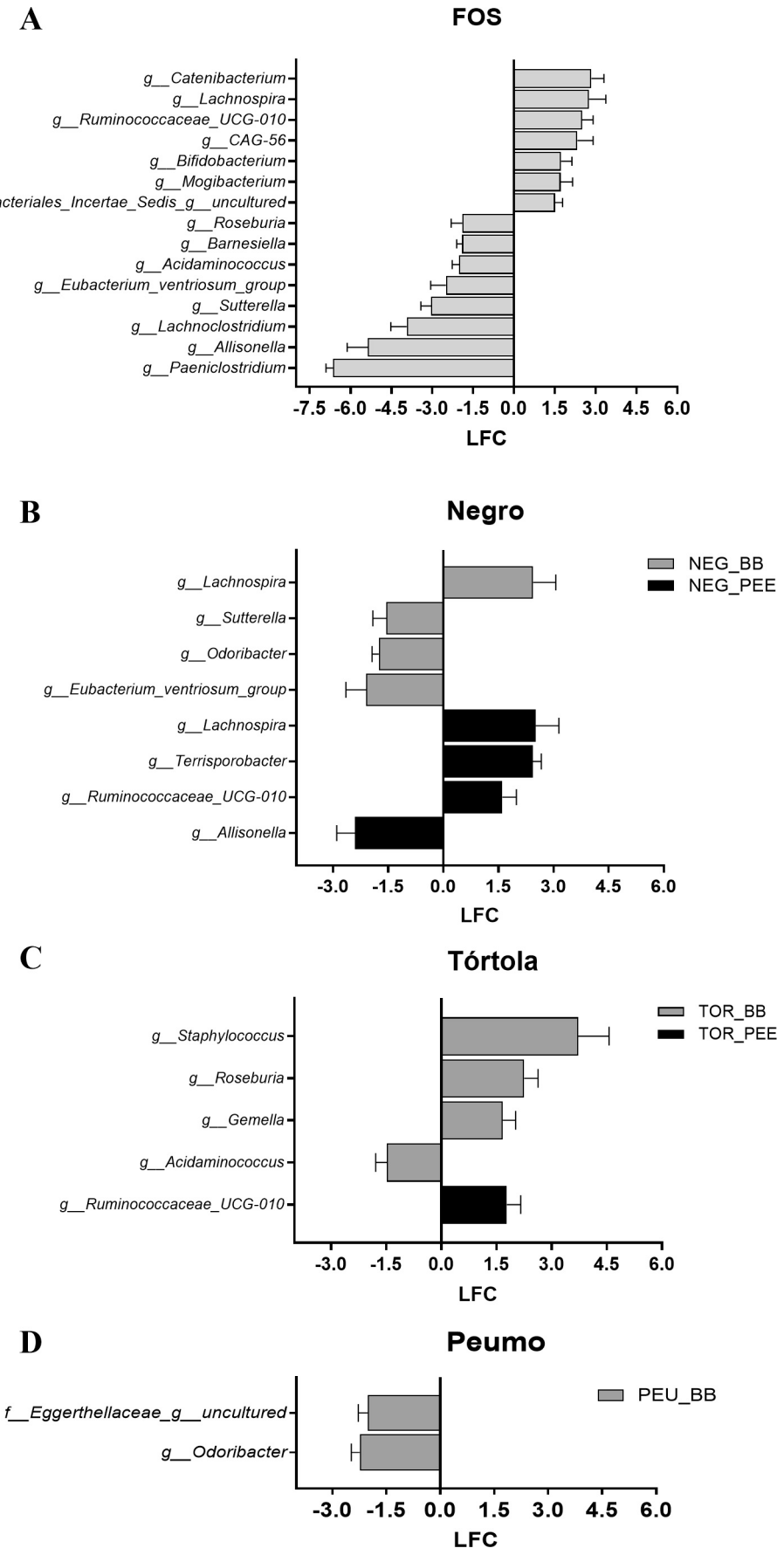


Fig. 4. Log fold change (LFC) of the differential abundance of bacteria after *in vitro* fermentation of boiled beans and enriched extracts.

in the fermentation of Tórtola beans (Ferreira et al., 2024; Vacca et al., 2020). The cause of the increase of the pathogenic *Staphylococcus* and *Gemella* genera in the microbiota is unknown to us, and we did not find results in scientific literature that could explain or support our findings. The PEE of Tórtola beans did not significantly change microbiota. The fermentation of Peumo BB caused a decrease in an unknown genus of the *Eggerthellaceae* family, as well as in the *Odoribacter* genus (Fig. 4D). Peumo PEE did not significantly alter the composition of the microbiota during *in vitro* fermentation.

We also performed a correlation analysis between the observed microbiota changes and SCFA concentrations, but after applying the BH correction, no statistically significant correlations were found. The BH method helps control the false discovery rate in multiple comparisons but can also lower statistical power, potentially masking real associations. Larger sample sizes or complementary methods may therefore be necessary to confirm any underlying correlations.

4. Conclusions

The conclusions of this study clearly demonstrate the differential impact of the food matrix on polyphenol bioaccessibility, fermentation, and the production of beneficial metabolites by gut microbiota, highlighting the importance of evaluating foods in their actual consumable forms. Experimental results showed that boiled Chilean beans, particularly the Negro landrace, favored greater diversity of phenolic acids and significant release of catechins after simulated gastrointestinal digestion. These bioactive compounds were progressively released from the food matrix, enhancing their potential absorption in the small intestine and subsequent biotransformation into bioactive metabolites by colonic microbiota.

During *in vitro* colonic fermentation, a significant increase in SCFA production, including acetate, propionate, and butyrate, was observed, particularly with Negro beans, indicating a prebiotic effect. Additionally, microbiota analyses revealed an increased abundance of beneficial bacteria such as *Ruminococcaceae-UGC-10*, *Lachnospira*, and *Roseburia*, linked to fiber and polyphenols metabolism.

The fermentation of PEE showed rapid transformation without significantly affecting SCFA production, emphasizing the critical role of the intact food matrix in modulating microbiological activity and beneficial metabolite production.

Overall, these findings provide robust evidence of how thermal processing influences certain bioactive compounds and how the whole food matrix is essential for maximizing intestinal health benefits. These findings emphasize the importance of comparing raw and processed food samples and exploring the effects of the food matrix *versus* extracts from the same source.

CRediT authorship contribution statement

Nélida Nina: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Carlo Bressa:** Software, Methodology, Formal analysis, Data curation. **Beatriz de Lucas:** Methodology, Investigation. **Isabel Martin de la Torre:** Methodology, Investigation. **Felipe Jiménez-Aspee:** Writing – original draft, Validation, Methodology, Investigation, Data curation. **Guillermo Schmeda-Hirschmann:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Mar Larrosa:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

Statements and declarations

The authors declare no conflict of interest.

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.

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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.2025.144669.

Data availability

Data will be made available on request.

References

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., … Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties, 20*(8), 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

Ashaolu, T. J., Saibandith, B., Yupanqui, C. T., & Wichienchot, S. (2019). Human colonic microbiota modulation and branched chain fatty acids production affected by soy protein hydrolysate. *International Journal of Food Science & Technology, 54*(1), 141–148. https://doi.org/10.1111/ijfs.13916

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 bioaccessibility 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

Bazzocco, S., Mattila, I., Guyot, S., Renard, C. M., & Aura, A. M. (2008). Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *European Journal of Nutrition, 47*(8), 442–452. https://doi.org/10.1007/s00394-008-0747-2

Blaak, E. E., Canfora, E. E., Theis, S., Frost, G., Groen, A. K., Mithieux, G., … Verbeke, K. (2020). Short chain fatty acids in human gut and metabolic health. *Beneficial Microbes, 11*(5), 411–455. https://doi.org/10.3920/BM2020.0057

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Brown, E. M., Nitecki, S., Pereira-Caro, G., McDougall, G., Stewart, D., Rowland, I., & Gill, C. I. R. (2015). Comparison of in vivo and in vitro digestion of polyphenol composition in lingonberries: Potential impact on colonic health. *Proceedings of the Nutrition Society, 74*(OCE1). https://doi.org/10.1017/s0029665115000282

Burgos-Edwards, A., Fernández-Romero, A., Carmona, M., Thuissard-Vasallo, I., Schmeda-Hirschmann, G., & Larrosa, M. (2020). Effects of gastrointestinal digested polyphenolic enriched extracts of Chilean currants (*Ribes magellanicum* and *Ribes punctatum*) on in vitro fecal microbiota. *Food Research International, 129*, Article 108848. https://doi.org/10.1016/j.foodres.2019.108848

Campos-Vega, R., García-Gasca, T., Guevara-Gonzalez, R., Ramos-Gomez, M., Oomah, B. D., & Loarca-Piña, G. (2012). Human gut flora-fermented nondigestible fraction from cooked bean (*Phaseolus vulgaris* L.) modifies protein expression associated with apoptosis, cell cycle arrest, and proliferation in human adenocarcinoma colon cancer cells. *Journal of Agricultural and Food Chemistry, 60*(51), 12443–12450. https://doi.org/10.1021/jf303940r

Campos-Vega, R., Reynoso-Camacho, R., Pedraza-Aboytes, G., Acosta-Gallegos, J. A., Guzman-Maldonado, S. H., Paredes-Lopez, O., … Loarca-Piña, G. (2009). Chemical composition and in vitro polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.). *Journal of Food Science, 74*(7), T59–T65. https://doi.org/10.1111/j.1750-3841.2009.01292.x

Canfora, E. E., Jocken, J. W., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews Endocrinology, 11*(10), 577–591. https://doi.org/10.1038/nrendo.2015.128

Chen, P. X., Dupuis, J. H., Marcone, M. F., Pauls, P. K., Liu, R., Liu, Q., … Tsao, R. (2015). Physicochemical properties and in vitro digestibility of cooked regular and nondarkening cranberry beans (*Phaseolus vulgaris* L.) and their effects on bioaccessibility, phenolic composition, and antioxidant activity. *Journal of Agricultural and Food Chemistry, 63*(48), 10448–10458. https://doi.org/10.1021/acs.jafc.5b04005

Chen, W., Liu, R., Zhu, X., Lu, Q., Yang, H., & Liu, R. (2024). Microbial phenolic metabolites 3-(3′, 4′-dihydroxyphenyl) propanoic acid and 3′, 4′-dihydroxyphenylacetic acid prevent obesity in mice fed with high-fat diet. *Food*

Science and Human Wellness, 13(1), 327–338. https://doi.org/10.26599/FSHW.2022.9250027
Chen, Y., Chang, S. K., Zhang, Y., Hsu, C. Y., & Nannapaneni, R. (2020). Gut microbiota and short chain fatty acid composition as affected by legume type and processing methods as assessed by simulated in vitro digestion assays. *Food Chemistry, 312*, Article 126040. https://doi.org/10.1016/j.foodchem.2019.126040
Chen, Y., McGee, R., Vandemark, G., Brick, M., & Thompson, H. J. (2016). Dietary fiber analysis of four pulses using AOAC 2011.25: Implications for human health. *Nutrients, 8*(12), 829. https://doi.org/10.3390/nu8120829
Creedon, A. C., Hung, E. S., Berry, S. E., & Whelan, K. (2020). Nuts and their effect on gut microbiota, gut function and symptoms in adults: A systematic review and meta-analysis of randomised controlled trials. *Nutrients, 12*(8), 2347. https://doi.org/10.3390/nu12082347
Dai, X., & Karring, H. (2014). A determination and comparison of urease activity in feces and fresh manure from pig and cattle in relation to ammonia production and pH changes. *PLoS One, 9*(11), Article e110402. https://doi.org/10.1371/journal.pone.0110402
De Carvalho, N. M., Oliveira, D. L., Dib Saleh, M. A., Pintado, M., & Madureira, A. R. (2021). Preservation of human gut microbiota inoculums for in vitro fermentations studies. *Fermentation, 7*(1), 14. https://doi.org/10.3390/fermentation7010014
De Martino, P., & Cockburn, D. W. (2020). Resistant starch: Impact on the gut microbiome and health. *Current Opinion in Biotechnology, 61*, 66–71. https://doi.org/10.1016/j.copbio.2019.10.008
Di Iorio, B. R., Rocchetti, M. T., De Angelis, M., Cosola, C., Marzocco, S., Di Micco, L., … Gesualdo, L. (2019). Nutritional therapy modulates intestinal microbiota and reduces serum levels of total and free indoxyl sulfate and p-cresyl sulfate in chronic kidney disease (Medika study). *Journal of Clinical Medicine, 8*(9), 1424. https://doi.org/10.3390/jcm8091424
Dima, C., Assadpour, E., Dima, S., & Jafari, S. M. (2020). Bioavailability and bioaccessibility of food bioactive compounds; overview and assessment by in vitro methods. *Comprehensive Reviews in Food Science and Food Safety, 19*(6), 2862–2884. https://doi.org/10.1111/1541-4337.12623
Dore, J., Ehrlich, S. D., Levenez, F., Pelletier, E., Alberti, A., Bertrand, L., Bork, P., Costea, P. I., Sunagawa, S., Guarner, F., Manichanh, C., Santiago, A., Zhao, L., Shen, J., Zhang, C., Versalovic, J., Luna, R. A., Petrosino, J., Yang, H., … IHMS Consortium. (2015). *IHMS_SOP 07 V1: Standard operating procedure for fecal samples DNA extraction*. International Human Microbiome Standards: Protocol H. http://www.microbiome-standards.org.
Dou, Y., Yu, X., Luo, Y., Chen, B., Ma, D., & Zhu, J. (2022). Effect of fructooligosaccharides supplementation on the gut microbiota in human: A systematic review and meta-analysis. *Nutrients, 14*(16), 3298. https://doi.org/10.3390/nu14163298
EFSA (European Food Safety Authority). (2017). Dietary reference values for nutrients: Summary report. *EFSA Supporting publication*. https://doi.org/10.2903/sp.efsa.2017.e1512. e15121.92 pp.
Eisenstein, M. (2020). The hunt for a healthy microbiome. *Nature, 577*(7792), S6–S8. https://doi.org/10.1038/d41586-020-00193-3
Feng, Y., Wang, Y., Wang, P., Huang, Y., & Wang, F. (2018). Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy. *Cellular Physiology and Biochemistry, 49*(1), 190–205. https://doi.org/10.1159/000492853
Fernández, J., Redondo-Blanco, S., Gutiérrez-del-Río, I., Miguélez, E. M., Villar, C. J., & Lombó, F. (2016). Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. *Journal of Functional Foods, 25*, 511–522. https://doi.org/10.1016/j.jff.2016.06.032
Ferreira, H., Duarte, D., Carneiro, T. J., Costa, C., Barbosa, J. C., Rodrigues, J. E., … Gil, A. M. (2024). Impact of a legumes diet on the human gut microbiome articulated with fecal and plasma metabolomes: A pilot study. *Clinical nutrition ESPEN, 63*, 332–345. https://doi.org/10.1016/j.clnesp.2024.06.051
Giusti, F., Capuano, E., Sagratini, G., & Pellegrini, N. (2019). A comprehensive investigation of the behaviour of phenolic compounds in legumes during domestic cooking and in vitro digestion. *Food Chemistry, 285*, 458–467. https://doi.org/10.1016/j.foodchem.2019.01.148
Gutiérrez-Sarmiento, W., Sáyago-Ayerdi, S. G., Goñi, I., Gutiérrez-Miceli, F. A., Abud-Archila, M., Rejón-Orantes, J. D. C., … Ruíz-Valdiviezo, V. M. (2020). Changes in intestinal microbiota and predicted metabolic pathways during colonic fermentation of mango (Mangifera indica L.)-based bar indigestible fraction. *Nutrients, 12*(3), 683. https://doi.org/10.3390/nu12030683
He, B., Bai, Y., Jiang, L., Wang, W., Li, T., Liu, P., Tao, S., Zhao, J., Han, D., & Wang, J. (2018). Effects of oat bran on nutrient digestibility, intestinal microbiota, and inflammatory responses in the hindgut of growing pigs. *International Journal of Molecular Sciences, 19*(8), 2407. https://doi.org/10.3390/ijms19082407
He, C., Jiang, J., Liu, J., Zhou, L., Ge, Y., & Yang, Z. (2024). *Pseudostellaria heterophylla* polysaccharide mitigates Alzheimer's-like pathology via regulating the microbiota-gut-brain axis in 5× FAD mice. *International Journal of Biological Macromolecules, 270*, Article 132372. https://doi.org/10.1016/j.ijbiomac.2024.132372
Hernández-Salazar, M., Osorio-Diaz, P., Loarca-Piña, G., Reynoso-Camacho, R., Tovar, J., & Bello-Pérez, L. A. (2010). In vitro fermentability and antioxidant capacity of the indigestible fraction of cooked black beans (*Phaseolus vulgaris* L.), lentils (*Lens culinaris* L.) and chickpeas (*Cicer arietinum* L.). *Journal of the Science of Food and Agriculture, 90*(9), 1417–1422. https://doi.org/10.1002/jsfa.3954
Jakobek, L., & Matić, P. (2019). Non-covalent dietary fiber-polyphenol interactions and their influence on polyphenol bioaccessibility. *Trends in Food Science & Technology, 83*, 235–247. https://doi.org/10.1016/j.tifs.2018.11.024
Jarett, J. K., Carlson, A., Serao, M. R., Strickland, J., Serfilippi, L., & Ganz, H. H. (2019). Diets with and without edible cricket support a similar level of diversity in the gut microbiome of dogs. *PeerJ, 7*, Article e7661. https://doi.org/10.7717/peerj.7661
Kageyama, A., & Benno, Y. (2000). *Catenibacterium mitsuokai* gen. Nov., sp. nov., a gram-positive anaerobic bacterium isolated from human faeces. *International Journal of Systematic and Evolutionary Microbiology, 50*(4), 1595–1599. https://doi.org/10.1099/00207713-50-4-1595
Ketnawa, S., Reginio, F. C., Jr., Thuengtung, S., & Ogawa, Y. (2022). Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition, 62*(17), 4684–4705. https://doi.org/10.1080/10408398.2021.1878100
Kim, Y. J., Jung, D. H., & Park, C. S. (2024). Important roles of Ruminococcaceae in the human intestine for resistant starch utilization. *Food Science and Biotechnology, 33*(9), 2009–2019. https://doi.org/10.1007/s10068-024-01621-0
Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), Article e1. https://doi.org/10.1093/nar/gks808
Kutschera, M., Engst, W., Blaut, M., & Braune, A. (2011). Isolation of catechin-converting human intestinal bacteria. Journal of Applied Microbiology, 111(1), 165–175. doi: https://doi.org/10.1111/j.1365-2672.2011.05025.x.
Li, D., Wang, P., Wang, P., Hu, X., & Chen, F. (2016). The gut microbiota: A treasure for human health. *Biotechnology Advances, 34*(7), 1210–1224. https://doi.org/10.1016/j.biotechadv.2016.08.003
Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications, 11*(1), 3514. https://doi.org/10.1038/s41467-020-17041-7
Liu, H., Zhang, M., Ma, Q., Tian, B., Nie, C., Chen, Z., & Li, J. (2020). Health beneficial effects of resistant starch on diabetes and obesity via regulation of gut microbiota: A review. *Food & Function, 11*(7), 5749–5767. https://doi.org/10.1039/D0FO00855A
Liu, T., Zhen, X., Lei, H., Li, J., Wang, Y., Gou, D., & Zhao, J. (2024). Investigating the physicochemical characteristics and importance of insoluble dietary fiber extracted from legumes: An in-depth study on its biological functions. *Food Chemistry: X, 22*, Article 101424. https://doi.org/10.1016/j.fochx.2024.101424
Los, F. G. B., Zielinski, A. A. F., Wojeicchowski, J. P., Nogueira, A., & Demiate, I. M. (2018). Beans (*Phaseolus vulgaris* L.): Whole seeds with complex chemical composition. *Current Opinion in Food Science, 19*, 63–71. https://doi.org/10.1016/j.cofs.2018.01.010
Ma, J., Wang, J., Jin, X., Liu, S., Tang, S., Zhang, Z., Long, S., & Piao, X. (2023). Effect of dietary supplemented with mulberry leaf powder on growth performance, serum metabolites, antioxidant property and intestinal health of weaned piglets. *Antioxidants, 12*(2), 307. https://doi.org/10.3390/antiox12020307
Ma, Y., Zhu, L., Ma, Z., Gao, Z., Wei, Y., Shen, Y., Li, L., Liu, X., & Ren, M. (2021). Distinguishing feature of gut microbiota in Tibetan highland coronary artery disease patients and its link with diet. *Scientific Reports, 11*(1), 18486. https://doi.org/10.1038/s41598-021-98075-9
Makarewicz, M., Drożdż, I., Tarko, T., & Duda-Chodak, A. (2021). The interactions between polyphenols and microorganisms, especially gut microbiota. *Antioxidants, 10*(2), 188. https://doi.org/10.3390/antiox10020188
Mao, Y., Yang, Q., Liu, J., Fu, Y., Zhou, S., Liu, J., Ying, L., & Li, Y. (2024). Quercetin increases growth performance and decreases incidence of diarrhea and mechanism of action in weaned piglets. *Oxidative Medicine and Cellular Longevity, 2024*(1), 5632260. https://doi.org/10.1155/2024/5632260
Márquez, K., Arriagada, O., Pérez-Díaz, R., Cabeza, R. A., Plaza, A., Arévalo, B., … Carrasco, B. (2024). Nutritional characterization of Chilean landraces of common bean. *Plants, 13*(6), 817. https://doi.org/10.3390/plants13060817
Mena, P., Bresciani, L., Brindani, N., Ludwig, I. A., Pereira-Caro, G., Angelino, D., … Del Rio, D. (2019). Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: Synthesis, analysis, bioavailability, and bioactivity. *Natural Product Reports, 36*(5), 714–752. https://doi.org/10.1039/c8np00062j
Nina, N., Theoduloz, C., Paillán, H., Jiménez-Aspee, F., Márquez, K., Schuster, K., … Schmeda-Hirschmann, G. (2023). Chemical profile and bioactivity of Chilean bean landraces (*Phaseolus vulgaris* L.). *Journal of Functional Foods, 104*, Article 105513. https://doi.org/10.1016/j.jff.2023.105513
Nogal, A., Valdes, A. M., & Menni, C. (2021). The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes, 13*(1), 1897212. https://doi.org/10.1080/19490976.2021.1897212
O'Donnell, M. M., Rea, M. C., O'Sullivan, Ó., Flynn, C., Jones, B., McQuaid, A., … Ross, R. P. (2016). Preparation of a standardized fecal slurry for ex-vivo microbiota studies which reduces inter-individual donor bias. *Journal of Microbiological Methods, 129*, 109–116. https://doi.org/10.1016/j.mimet.2016.08.002
Ortega, R. M., López-Sobaler, A. M., Andrés, P., Requejo, A. M., Aparicio, A., & Molinero, L. M. (2021). *Programa DIAL para valoración de dietas y cálculos de alimentación. Versión 3.15.1.0. Dpto. de Nutrición (UCM) y Alce Ingeniería, S.L. Madrid, España. Disponible en https://www.alceingenieria.net/nutricion/descarga.htm Último acceso: 06/01/2025*.
Ou, K., Sarnoski, P., Schneider, K. R., Song, K., Khoo, C., & Gu, L. (2014). Microbial catabolism of procyanidins by human gut microbiota. *Molecular Nutrition & Food Research, 58*(11), 2196–2205. https://doi.org/10.1002/mnfr.201400243
Parkar, S. G., Trower, T. M., & Stevenson, D. E. (2013). Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe, 23*, 12–19. https://doi.org/10.1016/j.anaerobe.2013.07.009
Paukkonen, I., Törrönen, E. N., Lok, J., Schwab, U., & El-Nezami, H. (2024). The impact of intermittent fasting on gut microbiota: A systematic review of human studies. *Frontiers in Nutrition, 11*, 1342787. https://doi.org/10.3389/fnut.2024.1342787

Peng, Y., Yan, Y., Wan, P., Dong, W., Huang, K., Ran, L., Mi, J., Lu, L., Zeng, X., & Cao, Y. (2020). Effects of long-term intake of anthocyanins from *Lycium ruthenicum* Murray on the organism health and gut microbiota in vivo. *Food Research International, 130*, Article 108952. https://doi.org/10.1016/j.foodres.2019.108952

Pérez-Burillo, S., Molino, S., Navajas-Porras, B., Valverde-Moya, Á. J., Hinojosa-Nogueira, D., López-Maldonado, A., … Rufián-Henares, J.Á. (2021). An in vitro batch fermentation protocol for studying the contribution of food to gut microbiota composition and functionality. *Nature Protocols, 16*(7), 3186–3209. https://doi.org/10.1038/s41596-021-00537-x

Rocchetti, G., Gregorio, R. P., Lorenzo, J. M., Barba, F. J., Oliveira, P. G., Prieto, M. A., … Lucini, L. (2022). Functional implications of bound phenolic compounds and phenolics–food interaction: A review. *Comprehensive Reviews in Food Science and Food Safety, 21*(2), 811–842. https://doi.org/10.1111/1541-4337.1292

Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants: An essential physiological function. *Journal of Agricultural and Food Chemistry, 59*(1), 43–49. https://doi.org/10.1021/jf1036596

Sáyago-Ayerdi, S. G., Zamora-Gasga, V. M., & Venema, K. (2020). Changes in gut microbiota in predigested *Hibiscus sabdariffa* L calyces and Agave (*Agave tequilana* weber) fructans assessed in a dynamic in vitro model (TIM-2) of the human colon. *Food Research International, 132*, Article 109036. https://doi.org/10.1016/j.foodres.2020.109036

Serra, A., Macià, A., Romero, M. P., Reguant, J., Ortega, N., & Motilva, M. J. (2012). Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food Chemistry, 130*(2), 383–393. https://doi.org/10.1016/j.foodchem.2011.07.055

Shi, Z., Zhu, Y., Teng, C., Yao, Y., Ren, G., & Richel, A. (2020). Anti-obesity effects of α-amylase inhibitor enriched-extract from white common beans (Phaseolus vulgaris L.) associated with the modulation of gut microbiota composition in high-fat diet-induced obese rats. *Food & Function, 11*(2), 1624–1634. https://doi.org/10.1039/C9FO01813A

Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: A review. *Trends in Food Science & Technology, 21*(4), 168–180. https://doi.org/10.1016/j.tifs.2009.12.001

Stoupi, S., Williamson, G., Drynan, J. W., Barron, D., & Clifford, M. N. (2010). A comparison of the in vitro biotransformation of (−)-epicatechin and procyanidin B2 by human faecal microbiota. *Molecular Nutrition & Food Research, 54*(6), 747–759. https://doi.org/10.1002/mnfr.200900123

Sun, C., Song, R., Zhou, J., Jia, Y., & Lu, J. (2023). Fermented bamboo fiber improves productive performance by regulating gut microbiota and inhibiting chronic inflammation of sows and piglets during late gestation and lactation. *Microbiology Spectrum, 11*(3), e04084–e04122. https://doi.org/10.1128/spectrum.04084-22

Tabone, M., García-Merino, J. A., Bressa, C., Rocha Guzman, N. E., Herrera Rocha, K., Chu Van, E., … Larrosa, M. (2022). Chronic consumption of cocoa rich in procyanidins has a marginal impact on gut microbiota and on serum and fecal metabolomes in male endurance athletes. *Journal of Agricultural and Food Chemistry, 70*(6), 1878–1889. https://doi.org/10.1021/acs.jafc.1c07547

Teixeira-Guedes, C., Sánchez-Moya, T., Pereira-Wilson, C., Ros-Berruezo, G., & López-Nicolás, R. (2020). In vitro modulation of gut microbiota and metabolism by cooked cowpea and black bean. *Foods, 9*(7), 861. https://doi.org/10.3390/foods9070861

Tomás-Barberán, F. A., González-Sarrías, A., García-Villalba, R., Núñez-Sánchez, M. A., Selma, M. V., García-Conesa, M. T., & Espín, J. C. (2017). Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. *Molecular Nutrition & Food Research, 61*(1), 1500901. https://doi.org/10.1002/mnfr.201500901

Tzounis, X., Vulevic, J., Kuhnle, G. G., George, T., Leonczak, J., Gibson, G. R., … Spencer, J. P. (2008). Flavanol monomer-induced changes to the human faecal microflora. *British Journal of Nutrition, 99*(4), 782–792. https://doi.org/10.1017/S0007114507853384

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., & De Angelis, M. (2020). The controversial role of human gut lachnospiraceae. *Microorganisms, 8*(4), 573. https://doi.org/10.3390/microorganisms8040573

Verbeke, K. A., Boobis, A. R., Chiodini, A., Edwards, C. A., Franck, A., Kleerebezem, M., … Tuohy, K. M. (2015). Towards microbial fermentation metabolites as markers for health benefits of prebiotics. *Nutrition Research Reviews, 28*(1), 42–66. https://doi.org/10.1017/S0954422415000037

Vita, A. A., Roberts, K. M., Gundersen, A., Farris, Y., Zwickey, H., Bradley, R., & Weir, T. L. (2024). Relationships between habitual polyphenol consumption and gut microbiota in the INCLD health cohort. *Nutrients, 16*(6), 773. https://doi.org/10.3390/nu16060773

Wang, H. Y., Wang, C., Guo, L. X., Zheng, Y. F., Hu, W. H., Dong, T. T. X., … Tsim, K. W. K. (2019). Simultaneous determination of short-chain fatty acids in human feces by HPLC with ultraviolet detection following chemical derivatization and solid-phase extraction segmental elution. *Journal of Separation Science, 42*(15), 2500–2509. https://doi.org/10.1002/jssc.201900249

Wen, X., Wan, F., Wu, Y., Liu, Y., Zhong, R., Chen, L., & Zhang, H. (2024). Caffeic acid modulates intestinal microbiota, alleviates inflammatory response, and enhances barrier function in a piglet model challenged with lipopolysaccharide. *Journal of Animal Science, skae233*. https://doi.org/10.1093/jas/skae233

Windey, K., De Preter, V., & Verbeke, K. (2012). Relevance of protein fermentation to gut health. *Molecular Nutrition & Food Research, 56*(1), 184–196. https://doi.org/10.1002/mnfr.201100542

Xiao, T., Liang, T., Geng, D. H., Wang, L., Liu, L., Zhou, X., … Tong, L. T. (2021). Dietary proteins alter fermentation characteristics of human gut microbiota in vitro. *Plant Foods for Human Nutrition, 76*, 419–426. https://doi.org/10.1007/s11130-021-00940-5

Xie, J., Sun, N., Huang, H., Xie, J., Chen, Y., Hu, X., Hu, X., Dong, R., & Yu, Q. (2022). Catabolism of polyphenols released from mung bean coat and its effects on gut microbiota during in vitro simulated digestion and colonic fermentation. *Food Chemistry, 396*, Article 133719. https://doi.org/10.1016/j.foodchem.2022.133719

Yamamoto, M., Ogura, H., Kuda, T., Xia, Y., Nakamura, A., Takahashi, H., … Takayanagi, S. (2024). Detection of typical indigenous gut bacteria related to kanpyo *Lagenaria siceraria* var. hispida powder in murine caecum and human faecal cultures. *3. Biotech, 14*(4), 118. https://doi.org/10.1007/s13205-024-03960-5

Yamamura, R., Inoue, K. Y., Nishino, K., & Yamasaki, S. (2023). Intestinal and fecal pH in human health. *Frontiers in Microbiomes, 2, Article 1192316*. https://doi.org/10.3389/frmbi.2023.1192316

Yoo, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., & McSkimming, D. I. (2020). Gut microbiota and immune system interactions. *Microorganisms, 8*(10), 1587. https://doi.org/10.3390/microorganisms8101587

Zeb, F., Naqeeb, H., Osaili, T., Faris, M. E., Ismail, L. C., Obaid, R. S., … Alam, I. (2024). Molecular crosstalk between polyphenols and gut microbiota in cancer prevention. *Nutrition Research, 124*, 21–42. https://doi.org/10.1016/j.nutres.2024.01.012

Zhang, L., Wang, Y., Li, D., Ho, C. T., Li, J., & Wan, X. (2016). The absorption, distribution, metabolism and excretion of procyanidins. *Food & Function, 7*(3), 1273–1281. https://doi.org/10.1039/C5FO01244A

Zhang, S., Deng, M., Zhang, R., Jia, X., Huang, F., Zhao, D., … Zhang, M. (2023). Modulation effect of black rice dietary fiber on the metabolism and fermentation of cyanidin-3-glucoside in an in vitro human colonic model. *Food & Function, 14*(14), 6707–6717. https://doi.org/10.1039/D3FO00955F