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Complementary foods in infants: an *in vitro* study of the faecal microbial composition and organic acid production†

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The transition from breastmilk to complementary foods is critical for maturing the colonic microbiota of infants. Dietary choices at weaning can lead to long-lasting microbial changes, potentially influencing health later in life. However, the weaning phase remains underexplored in colonic microbiome research, and the current understanding of how complementary foods impact the infant's colonic microbiota is limited. To address this knowledge gap, this study assessed the influence of 13 food ingredients on the *in vitro* microbial composition and production of organic acids by the faecal microbiota in New Zealand infants aged 5 to 11 months. To better represent real feeding practices, ingredients were combined with infant formula, other complementary foods, or both infant formula and other foods. Among the individual food ingredients, fermentation with peeled kūmara (sweet potato) increased the production of lactate and the relative abundance of the genus *Enterococcus*. Fermentation with blackcurrants, strawberries, or raspberries enhanced acetate and propionate production. Additionally, fermentation with blackcurrants increased the relative abundance of the genus *Parabacteroides*, while raspberry fermentation increased the relative abundance of the genera *Parabacteroides* and *Eubacterium*. When combined with infant formula or with blackcurrants, fermenting black beans increased butyrate production and stimulated the relative abundance of *Clostridium sensu stricto 1*. These foods are promising candidates for future clinical trials.

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1. Introduction

The large intestine harbours a diverse microbial community that relies on dietary compounds unabsorbed by the host. Numerous studies have highlighted the crucial role of the colonic microbiota in digestion and have demonstrated the impact of microbial metabolites produced in the colon on host health and well-being.[1–3] The relationship between colonic commensals and the host is dynamic and mutual, influenced by multiple factors, with diet playing a major role. Notably, disruptions in faecal microbial composition and concentration of organic acids are often observed in individuals experiencing negative health outcomes, ranging from gastrointestinal diseases to neurological disorders, compared to healthy controls.[4–6]

Dysbiosis in the colonic microbiota (an imbalance in the microbiota) is frequently marked by reduced faecal concentration of short-chain fatty acids (SCFAs).[4,7] SCFAs are organic acids produced by the microbial metabolism of complex carbohydrates and benefit the host by supporting intestinal barrier integrity, supplying energy, and regulating metabolic functions, among other benefits.[8–11] Given the relationship between the colonic microbiota and host physiology, understanding how diet shapes colonic microbes to promote health has attracted great interest recently. However, research in this area often neglects a critical period for the development of the colonic microbiota: infancy.

In early life, breastmilk is the gold standard for nourishing beneficial colonic commensals.[12,13] However, little is known about how complementary foods influence the colonic microbiota when infants start consuming solids (weaning). Longitudinal observations demonstrated that the faecal microbiota, as a proxy of the colonic microbiota, is particularly

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adaptable during weaning, with dietary-induced changes potentially lasting into later life and affecting long-term health.[14,15] At this stage, the gastrointestinal tract is still developing, allowing macronutrients from complementary foods to reach the colon and promote the growth of new commensal microbes.[16,17] Therefore, a deeper understanding of how foods impact the microbiota of weaning infants is essential for fostering the adequate development of the colonic microbiota from an early age.

Clinical trials allow for assessing dietary interventions on the faecal microbiota and tracking related health outcomes. However, trials involving vulnerable populations, such as infants, can be particularly time-consuming, costly, and ethically complex. *In vitro* experimental models, while unable to capture the full complexity of host-microbiota interactions, offer a cheaper and less invasive alternative that addresses some of the ethical and logistical challenges associated with clinical trials.[18] Among these methods, static *in vitro* protocols for food digestion and subsequent faecal fermentation of food remnants provide a useful screening approach to evaluate how dietary compounds influence faecal microbes.[19,20]

This study investigated the effects of complementary foods on the microbial composition and organic acid production of the faecal microbiota in weaning infants after 24 hours of fermentation. Uniquely, food ingredients were combined with infant formula, other foods, or both to better replicate real-life infant feeding patterns. This research aimed to identify *in vitro* foods that support adequate development of the faecal microbiota in New Zealand weaning infants.

2. Materials and methods

2.1. Food ingredients

A total of 13 food ingredients were used in this study (Table 1). These included vegetables (pumpkin), legumes (black beans, chickpeas, soybeans, and yellow peas), starchy foods (kūmara and couscous), meat (pork), seafood (prawn), berries (blackcurrants, raspberries, strawberries), and infant formula. These foods were identified through an *in silico* analysis as candidates for promoting changes in the production of SCFAs by the faecal microbiota of New Zealand weaning infants.[21] Foods were purchased from local stores and prepared under various conditions. Fruits and infant formula were obtained as dried powders and used as purchased, while the other ingredients were brought fresh, sous-*vide* cooked, freeze-dried, and ground using a standardised method (see ESI Table 1† for conditions). Potato starch (Sigma-Aldrich, St Louis, MO, USA) was used as a positive control due to its resistant starch content, which serves as a fermentable substrate for faecal microbes. The moisture content of food ingredient powders was determined by the weight difference before and after 48 hours of incubation at 105 °C. Additionally, compositional analyses of freeze-dried and ground food ingredients were performed at the Massey University Nutrition Laboratory. Analyses were conducted in duplicates for carbohydrates, sugar, total dietary fibre, protein, fat, saturated fat, and energy content (ESI Table 2†).

2.2 Simulated infant digestion of the food ingredients

Food ingredients were digested *in vitro* either alone, combined with other foods (1 : 1 food-food ratio), combined with infant formula (1 : 4 food-formula ratio), or combined with other foods and infant formula (1 : 1 : 8 food-food-formula ratio). These ratios were selected to reflect the high intake of infant formula by formula-fed infants at 6 months of age, which accounts for approximately 80% of their caloric intake.[22] A total of 53 samples, each with three replicates, were randomised into batches and independently digested using a protocol adapted to mimic the digestion of a 6-month-old infant. Simulated digestive fluids were prepared as described in the adult INFOGEST protocol,[20,23] with enzyme concentrations modified according to a dynamic model for infant digestion[24] and a static model for newborn digestion.[25]

To simulate oral digestion, 1.5 g of food ingredients were homogenised with 5 mL of deionised water and 5 mL of simulated salivary fluids. No mastication was assumed due to the liquid nature of the resulting mixture. The mixture was incubated for 2 minutes at pH 7.0 and 37 °C with 75 U mL^{-1} of α-amylase under agitation at 150 rpm. The reaction was

Table 1 List of food ingredients used in this study

Ingredient	Description	Source
Black beans	Dried grains of turtle black beans	Davis Food Ingredients, Palmerston North, New Zealand
Blackcurrants	Freeze-dried New Zealand-grown blackcurrants	Fresh As, Auckland, New Zealand
Chickpeas	Dried grains of chickpeas (garbanzo beans)	Davis Food Ingredients, Palmerston North, New Zealand
Couscous	Medium size grains of dried couscous (Durum wheat)	DARI, Salé, Morocco
Infant formula	Nestlé NAN SUPREMEpro 2	Nestlé New Zealand Limited, Auckland, New Zealand
Kūmara	Fresh red kūmara	Countdown, Palmerston North, New Zealand
Pork	Fresh lean pork fillet (tenderloin)	Online meats, Ōtāhuhu, New Zealand
Prawn	Fresh Australian prawn	Solander Seafood & Fishing, Nelson, New Zealand
Pumpkin	Fresh crown pumpkin	Countdown, Palmerston North, New Zealand
Raspberries	Freeze-dried New Zealand-grown raspberries	Fresh As, Auckland, New Zealand
Soybeans	Dehulled grains of soybeans	Jia Hua Asian Mart, Palmerston North, New Zealand
Strawberries	Freeze-dried New Zealand-grown strawberries	Fresh As, Auckland, New Zealand
Yellow peas	Dried grains of yellow peas	Davis Food Ingredients, Palmerston North, New Zealand

stopped with concentrated hydrochloric acid, and simulated gastric fluid was added to bring the volume to 20 mL. The mixture was then incubated for 2 hours at pH 3.0 and 37 °C with 500 U mL^{-1} of porcine pepsin under agitation at 150 rpm. The reaction was stopped with concentrated sodium hydroxide, and simulated intestinal fluid was added to bring the final volume to 40 mL. Intestinal digestion was simulated by incubating the mixture for 2 hours at pH 7.0 and 37 °C with 100 U mL^{-1} of protease activity of pancreatin, 200 U mL^{-1} of pancreatic lipase, 100 U mL^{-1} of amyloglucosidase, and 10 mmol L^{-1} of bile salts under agitation at 150 rpm. All chemicals and enzymes were purchased from Sigma-Aldrich (St Louis, MO, USA).

Intestinal digestion was stopped by heat treatment (3 minutes at 95 °C). After digestion, nutrient absorption in the large intestine was simulated by placing digested samples into Spectra/Por® cellulose membrane dialysis tubing (Thermo Fisher Scientific, Waltham, MA, USA) for 24 hours, with at least 2 changes of room-temperature deionised water. Post-dialysis samples were stored at −20 °C until fermentation.

2.3. Faecal fermentation of the digested food ingredients

This research was approved by the Massey University Human Ethics Committee Southern A (Application 22/48). A total of six healthy New Zealand infants at weaning age (5–11 months) were recruited for this study after written consent for their participation was obtained from their primary caregivers. The recruited infants were born at over 32 gestational weeks and weighed more than 2.5 kg (ESI Table 3†). None had received antibiotics in the last three weeks and were not consuming prebiotics or probiotics. All infants had already been exposed to complementary foods and had no known medical conditions.

Participants donated multiple faecal samples and were provided with scooping-lid plastic containers and written instructions on collecting and storing the stool samples. Samples were preferentially collected fresh after defaecation and transported refrigerated to the laboratory. Alternatively, samples could be stored in the participant's freezer until transportation. Upon arrival at the laboratory, samples were diluted with 50 mM potassium phosphate buffer pH 6.8 to a concentration of 32% (w/v). The resulting faecal slurry was filtered using a filter bag and stored at −80 °C.

Faecal fermentation followed a standard batch protocol with slight modifications.[19] Before fermentation, aliquots from different donors were defrosted and pooled in equal proportions to create an inoculum representative of the faecal microbiota of New Zealand weaning infants. Digested food samples were randomised into independent fermentation batches, and 6 mL of each sample was mixed with 2 mL of 0.15M potassium phosphate buffer pH 7.4 in two 16 × 125 mm Hungate tubes. The potassium phosphate buffer was also used as a negative control. The mixture was degassed with nitrogen, and the headspace of the tubes was filled with carbon dioxide. To ensure the absence of oxygen, 100 μL of 3% (w/v) L-cysteine was added to the tubes. Finally, 2 mL of faecal inoculum was added to the tubes, resulting in a total volume of 10.1 mL. Half of the tubes were immediately incubated on ice (time zero), while the remaining tubes were incubated for 24 hours at 37 °C.

2.4. Gas pressure and pH

After 24 hours of fermentation, the gas pressure of the Hungate tubes was measured (in kPa) using the Go Direct® Gas Pressure Sensor and the software Vernier Graphical Analysis (Vernier Science Education, Beaverton, OR, USA). The pH of samples at the start and end of fermentation was measured using the PL-700AL bench meter (Pacific Sensor Technologies, Rowville, VIC, Australia). Results were expressed as a decrease in pH after 24 hours. Additionally, 1 mL aliquots were collected and centrifuged at 13 000*g* for 1 min using the Minispin Plus mini centrifuge (Eppendorf, Hamburg, Germany). The supernatants and pellets were recovered and stored at −80 °C for subsequent analysis of organic acids and microbial composition, respectively.

2.5. Organic acids analysis

Organic acids were extracted and derivatised following a published protocol,[26] with slight modifications. Extractions were performed by mixing 450 μL of fermentation supernatant with 50 μL of the internal standard 50 mM 2-ethyl butyric acid (Sigma-Aldrich, St Louis, MO, USA). Then, 1250 μL of diethyl ether and 250 μL of hydrochloric acid (37%) were added to the mixture. Samples were vortexed, and 100 μL of the diethyl ether phase was transferred to a glass vial containing 20 μL of the derivatising agent *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (Sigma-Aldrich, St Louis, MO, USA). Derivatisation occurred by incubating the mixture for 20 minutes at 80 °C, followed by 48 hours at room temperature.

Standard solutions of the organic acids formate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, hexanoate, heptanoate, lactate, and succinate, containing 5 mM 2-ethyl butyric acid were prepared alongside the samples. The standard solutions at varying concentrations (0.15, 0.25, 0.50, 1, 2.50, 5, 10, and 20 mM) were used to generate a calibration curve for determining the concentration of the organic acids in the samples. The supernatants from samples at the end of fermentation were diluted with 0.15 M potassium phosphate buffer pH 7.4 to ensure that the concentrations fell within the range of the calibration curve. Organic acid production was calculated as the difference between the concentrations at time zero and 24 hours, expressed in mmol g^{-1} (dry weight) to account for the theoretical dry mass of the fermented sample.

Organic acids were detected using the GC-2010 gas chromatograph system coupled with a flame ionisation detector (Shimadzu, Kyoto, Japan) and fitted with an HP-1 column (30 m × 0.25 mm ID × 0.25 μm) (Agilent Technologies, Santa Clara, CA, USA). Helium was used as carrier gas with a flow rate of 21.2 mL min^{-1}, a pressure of 131.2 kPa, and a split ratio of 5 : 1. The temperature programme began at 70 °C, increasing to 115 °C at a rate of 6 °C min^{-1}, followed by a final increase to 300 °C at 60 °C min^{-1}, holding for 3 minutes. The detector temperature was 310 °C. Data were acquired and pro-

cessed using the LabSolutions software (version 5.98) (Shimadzu, Kyoto, Japan).

2.6. Microbial compositional analysis

The DNA from fermentation pellets was extracted using the NucleoSpin DNA Soil kit (Macherey-Nagel, Dueren, Germany) according to the manufacturer's instructions. The quantity of extracted DNA was measured using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and its quality was assessed through gel electrophoresis using a 1% agarose gel and the lambda HindIII DNA marker (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA was stored at −80 °C before sequencing. The V3–V4 regions of the 16S rRNA were amplified using the 341 forward (5′-CCTACGGGAGGCAGCAG-3′) and the 806 reverse (5′-GGACTACHVGGGTWTCTAAT-3′) primers with custom barcodes. All samples from the 24 hours of fermentation were sequenced, while only five randomly selected samples from time zero were sequenced due to resource limitations.

PCR amplification, amplicon quantification, purification, and sequencing using a MiSeq platform (Illumina, San Diego, CA, USA) with 2 × 250 bp paired-end reads were performed at Magigene Biotechnology Co. Ltd (Guangzhou, China). Raw data was processed using the New Zealand eScience Infrastructure (NeSI) high-performance computing facilities. In short, primers were removed from raw demultiplexed reads using Cutadapt[27] (version 2.3), followed by Trimmomatic.[28] The DADA2 pipeline (version 1.32)[29] was employed for denoising, truncating reads (to 214 bp for forward reads and 195 bp for reverse reads), chimera removal, and inferring amplicon sequence variants (ASVs) in R (version 4.4).[30] Taxonomy assignment was performed using the SILVA database (version 138.1).[31]

Inconsistencies and missing classifications in the ASV data were addressed using the microbiome package (version 1.26)[32] by collapsing taxa into higher taxonomic ranks. Microbial alpha diversity analyses were conducted on unfiltered and unrarefied ASVs using the package phyloseq (version 1.48)[33] to measure the Chao1 richness estimator, Shannon index, and Simpson index. For microbial beta diversity analysis, samples were rarified to 49 433 reads, and dissimilarities in microbial abundances were assessed using the Bray–Curtis index. Data were ordinated using principal coordinate analysis (PCoA) based on the Bray–Curtis index employing phyloseq. Microbial relative abundance was visualised using the microViz package (version 0.12.4)[34] after filtering taxa that were present in at least 10% of samples and had a relative abundance greater than 0.01%.

2.7. Statistical analysis

All statistical analyses were performed individually for each subset of samples, which were grouped according to their composition: food ingredients alone, foods combined with infant formula, foods combined with other foods, and foods combined with both infant formula and other foods. A one-way analysis of variance (ANOVA) was used to assess the influence of the substrate (food ingredient or food combination) on pH, gas pressure, and organic acid production after 24 hours of fermentation. Differences in absolute pH changes, gas pressure, and organic acid production between samples were determined using the Tukey Honestly Significant Difference (HSD) test with a 95% confidence level to account for multiple comparisons. Results were plotted using the ggplot2 package (version 3.5.1).[35]

The effect of the substrate on the microbial alpha diversity of samples was assessed using the Kruskal–Wallis test. For diversity indices with significant differences, subsequent pairwise comparisons were performed using Dunn's test *via* the FSA package (version 0.9.5).[36] The Benjamini–Hochberg adjustment was employed to control for false discovery rates. Differences in beta diversity between samples were evaluated through a pairwise permutational multivariate analysis of variance (PERMANOVA), with p-values adjusted using the Benjamini–Hochberg method. Analyses were conducted using the adonis2 function from the vegan package with 9999 permutations (version 2.6–6).[37]

Differential abundance testing was performed for taxa present in more than 5% of the samples using the ANCOM-BC2 package (version 2.6).[38] The ANCOM-BC2 global test served as a preliminary approach to identify taxa varying between at least two samples, while sensitivity analyses assessed the reliability of the results. For taxa identified through the global test, abundance log-fold changes (LFC) between samples were evaluated through multiple pairwise comparisons using a Dunnett's type of test, with *p*-values adjusted using the Holm-Bonferroni method.

Two-sided Spearman's rank correlation tests were performed to assess the strength of the associations between the following pairs: the nutritional composition of food samples and organic acids produced after 24 hours of fermentation; the nutritional composition of food samples and the relative abundance of microbial genera after 24 hours of fermentation; and produced organic acids and the relative abundance of microbial genera at the end of the fermentation. Only genera with more than 0.05% relative abundance were included in the analyses. The Benjamini–Hochberg method was used to control for false discovery rates. Significant correlations (at a false discovery rate-adjusted $p < 0.05$) were displayed as heatmaps using the corrplot package (version 0.95).[39]

3. Results

3.1. Changes in pH and gas pressure

Changes in pH and gas pressure between fermented substrates were only observed for fermentations with food ingredients alone (ANOVA, $p < 0.001$). Fermentations with kūmara with skin and couscous resulted in the greatest decreases in pH and gas pressures. Fermentation with peeled kūmara also exhibited one of the highest decreases in pH but moderate gas pressure. In contrast, fermenting pork, prawn, raspberries, and blackcurrants promoted the least decreases in pH and the lowest gas

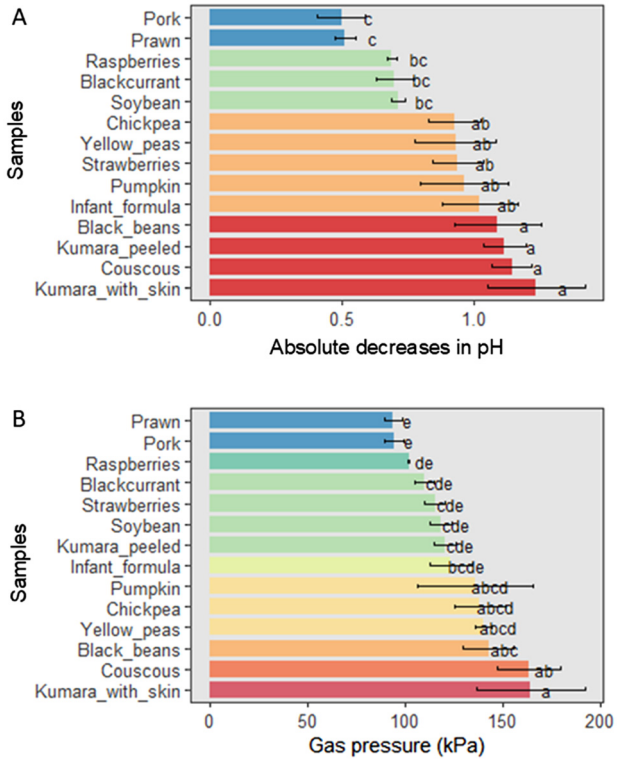

Fig. 1 Absolute decreases in pH and gas pressure after 24 hours of fermentation for food ingredients alone. Bars are coloured according to intensity and statistical significance, with higher values in red and lower values in blue. Samples with the same colour and same letters belong to the same group according to the Tukey HSD test with a 95% confidence interval. Decreases in pH (A) and gas pressure (B) are displayed.

pressures (Fig. 1). Fermentation with strawberries showed one of the lowest gas pressures but a moderate decrease in pH. Additionally, combining foods with infant formula resulted in greater pH decreases and increased gas pressures compared to food ingredients alone (ESI Fig. 1 and 2†).

3.2. Produced organic acids

After 24 hours of fermentation, fermented food samples produced formate, acetate, propionate, butyrate, isovalerate, lactate, and succinate. Isobutyrate, valerate, hexanoate, and heptanoate were undetected (see ESI Table 4† for detection limits). Individually, fermenting blackcurrants and strawberries resulted in the highest production of organic acids. The same was observed for the fermentation of these berries when combined with infant formula, with each other, or in combination with each other and formula (ESI Fig. 3†).

The type of food ingredient significantly influenced the production of formate, acetate, propionate, butyrate, isovalerate, lactate, and succinate, as well as total SCFAs (sum of acetate, propionate, and butyrate) (ANOVA one-way, $p < 0.05$). Fermentations with blackcurrants, strawberries, and, to a lesser extent, raspberries increased the production of acetate, propionate, and total SCFAs compared to other foods (Tukey HSD, adjusted $p < 0.05$). The fermentation of kūmara, either peeled or with skin, primarily produced lactate (Fig. 2) (ESI Table 5†).

Fermenting food ingredients combined with infant formula or other foods resulted in fewer differences in organic acid production across samples (ESI Tables 6 and 7†). The type of fermented food-formula combination only influenced butyrate production after adjusting for multiple comparisons, which was highest in fermentation with black beans combined with infant formula (Tukey HSD, adjusted $p < 0.005$). Similarly, differences between fermented food-food combination samples were only noted for butyrate, with fermentation with black beans combined with blackcurrants yielding the highest production (Tukey HSD, adjusted $p < 0.005$). No differences in organic acid production were observed between fermentations of food-food-formula combinations after adjusting for multiple comparisons (ESI Table 8†).

3.3. Microbial diversity

Samples at fermentation time zero exhibited higher alpha diversity indices (Chao1, Shannon, and Simpson) compared to those at the end (Kruskal test, $p < 0.001$) (ESI Fig. 4†). There were no differences in microbial alpha diversity scores between samples at the end of the fermentation after adjusting for multiple comparisons (Dunn's test, adjusted $p > 0.05$). Samples at fermentation time zero had distinct beta diversity from samples at 24 hours of fermentation, as measured by the Bray–Curtis dissimilarity index (PERMANOVA, $p < 0.001$) (ESI Fig. 5†). No differences in the Bray–Curtis dissimilarity index were observed between samples at the end of the fermentation after adjusting for multiple comparisons.

3.4. Microbial relative abundance

The major phyla present in samples at fermentation time zero were Actinobacteriota, Firmicutes (or Bacillota), Proteobacteria (or Pseudomonadota), and Bacteroidota, with respective relative abundances of 35%, 32%, 20%, and 10%. The most abundant families included *Bifidobacteriaceae*, *Enterobacteriaceae*, *Lachnospiraceae*, and *Bacteroidaceae*, while the predominant genera were *Bifidobacterium*, *Escherichia-Shigella*, *Bacteroides*, and *Veillonella*. After 24 hours of fermentation, a shift in the dominant microbial taxa was observed (ESI Tables 9, 10, and 11†). Bacteroidota, followed by Proteobacteria, became the most abundant phyla. The predominant families were *Bacteroidaceae*, *Enterobacteriaceae*, *Bifidobacteriaceae*, and *Enterococcaceae*, while *Bacteroides*, *Escherichia-Shigella*, *Bifidobacterium*, and *Enterococcus* became the dominant genera (ESI Fig. 6†).

Differential abundance testing for fermentations with food ingredients alone revealed significant changes between samples at the phylum, family, and genus levels (ANCOM-BC2 global test, adjusted $p < 0.05$) (ESI Tables 9, 10, and 11†). Fermentation with pork, followed by fermentation with raspberries, had the highest relative abundances of the phylum Bacteroidota, the family *Bacteroidaceae*, and the genus *Bacteroides* (41% and 40%, respectively). In contrast, fermenta-

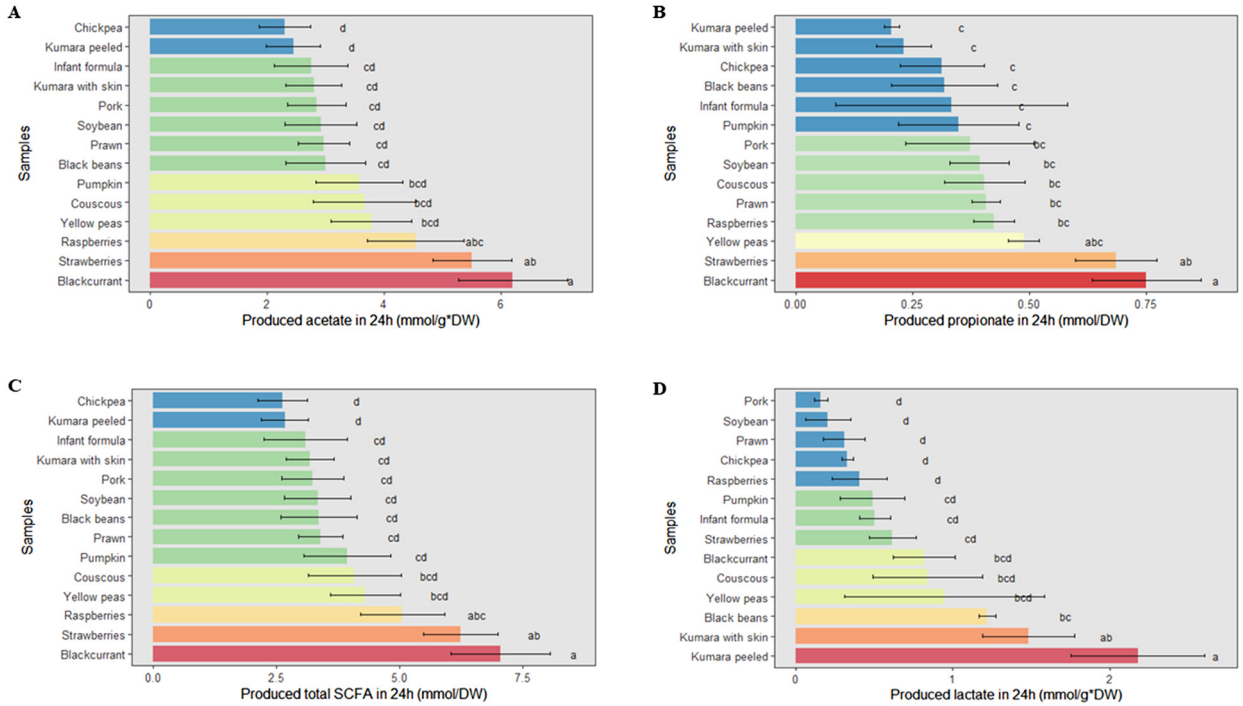

Fig. 2 Production of organic acids after 24 hours of fermentation of individual food ingredients. Only key results are presented, as follows: acetate (A), propionate (B), total major SCFAs (C), and lactate (D). Bars are coloured according to intensity and statistical significance, with higher values in red and lower values in blue. Samples with the same colour and letters belong to the same group according to the Tukey HSD test with a 95% confidence interval.

tion with kūmara with skin exhibited the lowest abundances of these taxa, with *Bacteroides* accounting for 32%. The family *Tannerellaceae* and the genus *Parabacteroides* reached their highest abundances in fermentations with pork and blackcurrants (1% and 0.8%, respectively) and their lowest in fermentations with kūmara both peeled and with skin (0.08%).

The relative abundances of the phylum Actinobacteriota, the family *Bifidobacteriaceae*, and the genus *Bifidobacterium* were highest in fermentations with peeled kūmara and prawn (20%) and lowest in fermentations with blackcurrants and strawberries (15%). Fermentations with peeled kūmara, blackcurrants, and raspberries promoted the highest abundances of the phylum Firmicutes, the family *Enterococcaceae*, and the genus *Enterococcus* (18%, 17%, and 17%, respectively). In contrast, fermentations with soybeans and chickpeas had the lowest abundances of these taxa, with *Enterococcus* accounting for 5% of each food. The family *Streptococcaceae* and the genus *Streptococcus* were least abundant in fermentations with blackcurrants and strawberries (0.9% each) but showed their highest abundances in fermentation with prawns (2.1%). Additionally, fermentations with kūmara peeled and with skin had the highest abundances of the family *Lactobacillaceae* and the genus *Lacticaseibacillus*. Fermentations with raspberries and blackcurrants exhibited the highest abundances of the family *Eubacteriaceae* and the genus *Eubacterium*.

Multiple pairwise comparisons against a reference group (ANCOM-BC2 Dunnett-type test) demonstrated that fermentations with blackcurrants and raspberries significantly increased the log-fold change (LFC) in the abundance of the family *Tannerellaceae* and the genus *Parabacteroides* and, to a lesser extent *Enterococcus*, compared to fermentations with other food ingredients (adjusted $p < 0.05$) (Fig. 3) (ESI Fig. 7†). Importantly, LFC values represent differences in bias-correct abundances between groups and do not directly reflect the relative abundance of taxa. Fermentation with raspberries also exhibited higher LCF values for the phylum Firmicutes, the families *Eubacteriaceae* and *Enterococcaceae*, and the genera *Sellimonas* and *Eubacterium* compared to fermentations with other foods (ESI Fig. 8†). In contrast, fermentations with kūmara peeled or with skin decreased the LFC in the abundance of the genus *Parabacteroides* compared to fermentations with other foods (Fig. 3).

Significant differences in taxa relative abundance between fermentations with food-food combinations were at the phylum, family, and genus levels (ANCOM-BC2 global test, p adjusted < 0.05). Fermentation with the couscous-pork combination promoted the highest abundances of the phylum Bacteroidota, the family *Bacteroidaceae*, and the genus *Bacteroides* (43%), while fermentation with couscous-pumpkin exhibited the lowest abundance of these taxa (35%). The family *Tannerellaceae* and the genus *Parabacteroides* showed the highest relative abundances in the fermentation with pork-raspberries (1.2%) and the lowest in the fermentation with couscous-pork (0.2%).

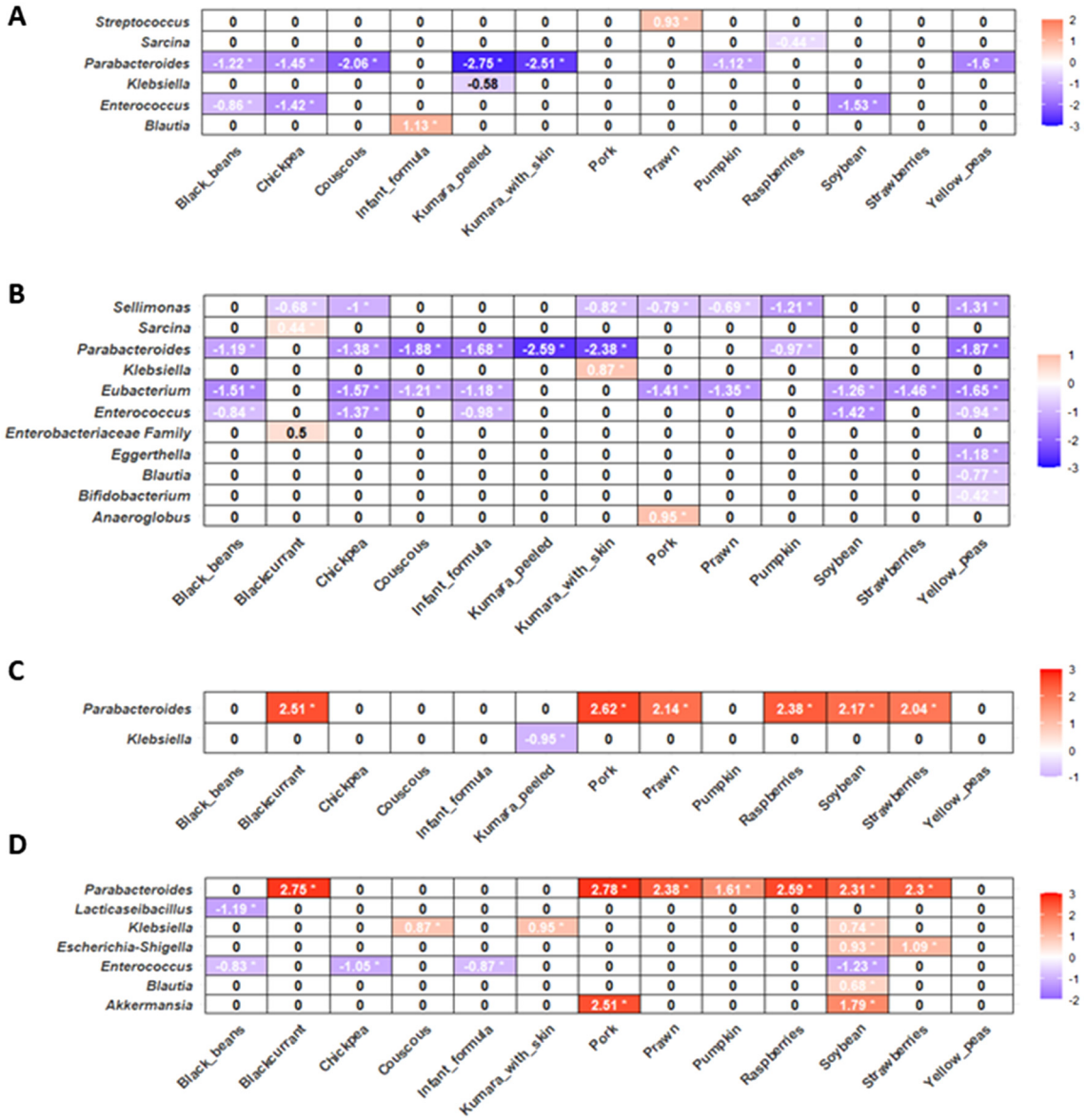

Fig. 3 Heatmap of log-fold changes (LFC) in the abundance of bacterial genera after 24 hours of fermentation with selected food ingredients. LFC values are presented in comparison to other foods. Fermentation with blackcurrant, compared to fermentation with other foods, is at the top (A), followed by raspberries (B), kūmara with skin (C), and peeled kūmara (D) compared to other food ingredients. Cells are coloured according to intensity, with higher values in red and lower values in blue. Significant changes in LFC (adjusted $p < 0.05$) that passed sensitivity analyses are marked with an asterisk (*).

The phylum Proteobacteria and the family *Enterobacteriaceae* had the highest abundances in the fermentation with the blackcurrants-strawberries combination (30%) and the lowest in the fermentation with blackcurrants-kūmara with skin (20%). In contrast, the phylum Actinobacteria, the family *Bifidobacteriaceae*, and the genus *Bifidobacterium* exhibited the highest abundances in the fermentation with black beans-blackcurrants (19%) and the lowest in the fermentation with blackcurrants-strawberries (14%). Fermentation with blackcurrants-pork had the highest relative abundances of the phylum Verrucomicrobiota, the family *Akkermansiaceae*, and the genus *Akkermansia* (1.4%).

The relative abundances of the phylum Firmicutes, the family *Enterococcaceae*, and the genus *Enterococcus* were highest in fermentations with blackcurrants combined with kūmara peeled or kūmara with skin (19%). In contrast, the fermentation of the combination blackcurrants-soybean exhibited the lowest abundance of these taxa, with *Enterococcus* accounting for 6%. The families *Eubacteriaceae* and *Clostridiaceae* and the genera *Eubacterium* and *Clostridium sensu stricto 1* had their highest relative abundances in fermentation with black beans-blackcurrants (0.2% each).

Multiple pairwise comparisons of taxa LFC demonstrated that fermentations with the combination of black beans-black-

currants had increased LFC values in the abundance of the genera *Eubacterium* and *Clostridium sensu stricto 1* compared to fermentations with other food-food combinations (Fig. 4). Fermentation with black beans-blackcurrant also had higher LFC for the families *Eubacteriaceae* and *Clostridiaceae* (ANCOM-BC 2 Dunnett's test, adjusted $p < 0.05$), while no significant differences were observed at the phylum level (ESI Fig. 9†).

Combining infant formula with food ingredients or food-food combinations reduced the observed differences in microbial relative abundance between fermented samples. No differences in the abundance of bacterial phyla, families, or genera detected between fermentations with food-formula combinations by the ANCOM-BC2 global test passed the sensitivity analyses. This suggests that the variations between fermented samples were likely due to model parameters or assumptions rather than biological differences. Multiple pairwise comparisons, using fermentation with black beans-formula as a reference group due to its increased butyrate produced after 24 hours of fermentation, showed increased LFC in the abundance of the family *Clostridiaceae* and the genus *Clostridium sensu stricto 1* (adjusted $p < 0.05$), compared to fermentations with other food-formula combinations (Fig. 4).

Significant differences in taxa abundance between fermentations with food-food-formula combinations were observed at the family and genus levels (ANCOM-BC global test, adjusted $p < 0.05$). The family Bacteroidaceae and the genus *Bacteroides* had the highest abundances in fermentation with chickpea-yellow peas-formula (43%) and the lowest in fermentation with blackcurrants-kūmara with skin-formula (32%). Additionally, fermentation with chickpea-yellow peas-formula combination exhibited the lowest abundances of the families *Streptococcaceae* and *Eubacteriaceae* and the *genera Streptococcus* and *Eubacterium* (1.2% and 0.06%, respectively). In contrast, fermentation with couscous-pork-formula promoted the highest abundances of these taxa (1.7% for *Streptococcus* and 0.1% for *Eubacterium*). No significant changes in bacterial taxa LFC values between fermentations with food-food-formula combinations were observed after multiple pairwise comparisons using the fermentation with blackcurrants-strawberries-formula combination as a reference group.

3.5. Correlations between food composition, organic acids and microbiota composition

The produced major and total SCFAs exhibited weak positive correlations with the total dietary fibre content across all

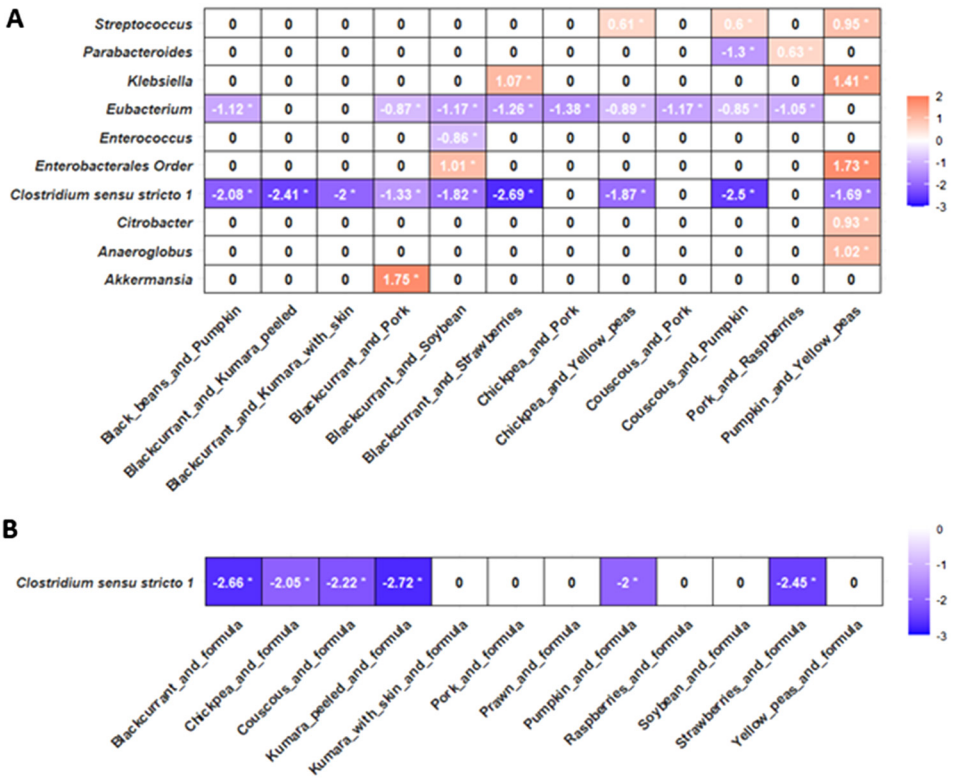

Fig. 4 Heatmap of log-fold changes (LFC) in the abundance of bacterial genera after 24 hours of fermentation with food-formula and food-food combinations. LFC values are presented in comparison to other food combinations. Fermentation with black beans combined with blackcurrant, *versus* fermentation with other food-food combinations, is at the top (A), while black beans combined with infant formula, compared to other food-formula combinations, is at the bottom (B). Cells are coloured according to intensity, with higher values in red and lower values in blue. Significant changes in LFC (adjusted $p < 0.05$) that passed sensitivity analyses are marked with an asterisk (*).

fermented food samples (Spearman's rank correlation, adjusted $p < 0.05$). In contrast, fat, energy, protein, and sugar content correlated negatively with the production of these organic acids (Fig. 5). Notably, acetate production had a strong negative correlation with fat and energy content (Spearman's rank correlation coefficient r_s values of −0.66 and −0.72, respectively). Lactate production also showed negative correlations with energy, fat, and protein content but had a weak positive correlation with carbohydrate content. Additionally, when analysing individual food ingredients and food-food combinations, lactate production had a strong positive correlation with carbohydrate content and a strong negative correlation with fat content (ESI Fig. 10†).

The relative abundances of the genera *Veillonella* and *Enterococcus* were positively correlated with the total fibre content in all samples. Trends indicating a weak positive correlation between fibre content and the relative abundance of the genera *Parabacteroides* and *Lacticaseibacillus* were also observed (Spearman's rank correlation, adjusted $p < 0.1$). Furthermore, the relative abundance of *Lacticaseibacillus* demonstrated a moderate positive correlation with carbohydrate content when analysing only food ingredients (r_s = 0.52). In contrast, energy, fat, and sugar content negatively correlated with the relative abundance of *Enterococcus* and *Lacticaseibacillus* (r_s ranging from −0.23 to −0.52), while they positively correlated with the relative abundance of the genera *Streptococcus* and *Blautia* (r_s ranging from 0.22 to 0.60). Protein content exhibited weak positive correlations with the relative abundance of the genera *Akkermansia*, *Anaeroglobus*, *Clostridium sensu stricto 1*, and *Streptococcus*, also showing a trend toward a positive correlation with the abundance of *Bacteroides* (Fig. 5). A moderate positive correlation was also observed between *Clostridium sensu stricto 1* abundance and protein content in food-food combinations (r_s = 0.49) (ESI Fig. 10†).

When considering the entire set of samples, the production of acetate, propionate, and total SCFAs positively correlated with the relative abundance of *Parabacteroides*, *Lacticaseibacillus*, and *Enterococcus*, among other genera (Fig. 5). Notably, there were strong correlations between the abundance of *Parabacteroides* and propionate (r_s = 0.50) and between *Enterococcus* and acetate (r_s = 0.43). *Enterococcus* abundance also showed positive correlations with lactate production, alongside *Lacticaseibacillus*, as well as with butyrate production in conjunction with *Clostridium sensu stricto 1*. Additionally, the relative abundance of *Lacticaseibacillus* demonstrated a moderate positive correlation with lactate production from food ingredients, while *Clostridium sensu stricto 1* exhibited a similar correlation with butyrate production from food-formula combinations (ESI Fig. 10†). In contrast, the abundances of *Streptococcus* and *Blautia* exhibited negative correlations with the production of major and total SCFAs (r_s ranging from −0.57 to −0.24).

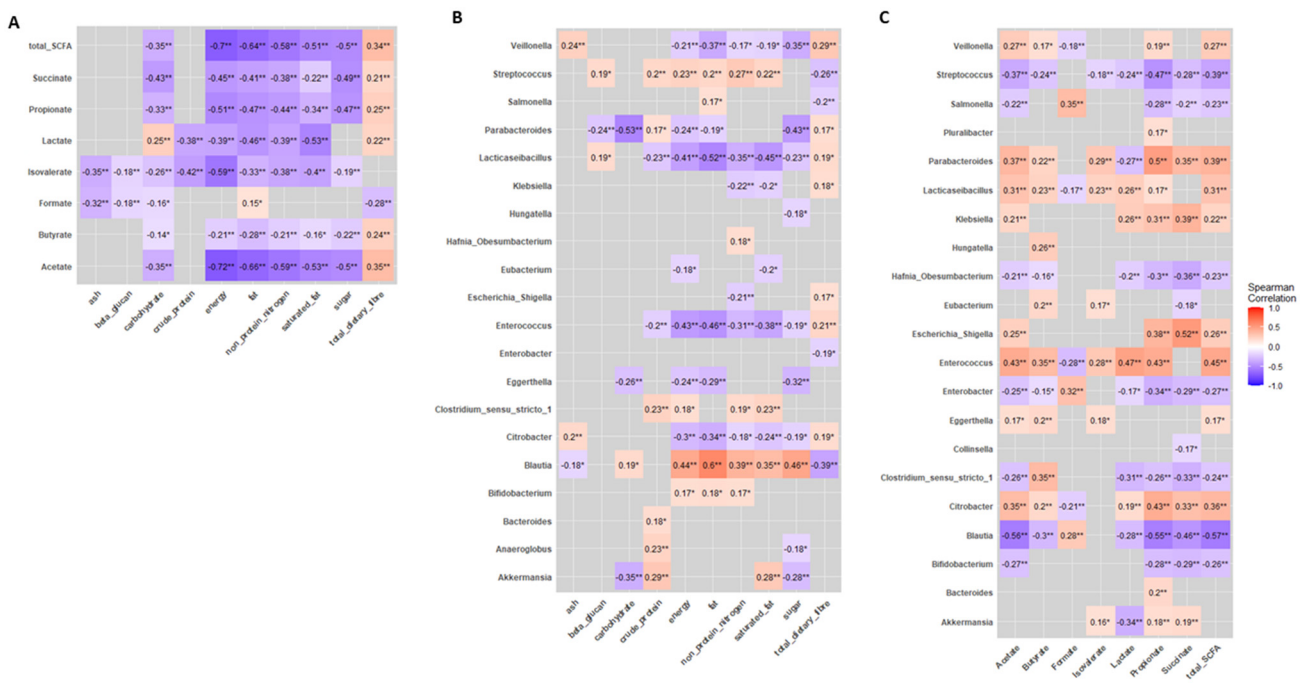

Fig. 5 Spearman's rank correlation heatmaps. (A) Correlations between food composition and organic acid production. (B) Correlations between food composition and microbiota composition. (C) Correlations between organic acid production and microbiota composition. Only significant correlations are shown. Significant relationships (adjusted $p < 0.05$) are marked with a double asterisk (**), while trends (adjusted $p < 0.1$) are marked with a single asterisk (*). Positive correlations are shown in red and negative in blue.

4. Discussion

Our study evaluated the effects of complementary foods from various sources, including meat, seafood, starchy foods, and fruits, on the composition and function of the faecal microbiota of New Zealand weaning infants. The transition from breastmilk to solid foods is a critical period for the development of the colonic microbiota of infants, yet it has been traditionally neglected in microbiome research.[40] To address this knowledge gap in infant nutrition, this study assessed the fermentation of food ingredients combined with infant formula, other foods, or both other foods and infant formula. This unique aspect of our study aims to better replicate how complementary foods are introduced to infants in real life while also considering the impact of interactions between different dietary compounds on the relative abundance and organic acid production of colonic microbes. Another strength of our study was using standardised protocols and the latest methods for *in vitro* digestion, faecal fermentation, DNA sequencing, and bioinformatics.

Among the food ingredients, fermentation with kūmara (sweet potato) or couscous produced the most gas and promoted the greatest pH changes. In turn, fermentation with pork, followed by fermentation with prawn, blackcurrants, or raspberries, had the lowest values for both measurements. Another *in vitro* study using faecal inoculum from weaning infants assessed the fermentation of plant-based foods, reporting that fermenting oats, sweetcorn, and carrot produced more gas than apple, blackcurrants, and kiwifruit.[41] These findings indicate that the infant microbiota has adapted to fermenting complex carbohydrates rather than sugar or animal protein.

In particular, the soluble fibre content may be a major factor influencing pH and gas production during fermentation. Evidence from swine faecal fermentation of different ratios of soluble to insoluble fibre indicates that a higher proportion of soluble fibre increases total gas production while decreasing pH.[42] Additionally, soluble fibre content was associated with higher production of lactate and acetate, whereas insoluble fibres were associated with propionate and butyrate yield.[42] However, the study used simple substrates derived from mixes of inulin with non-starch polysaccharides, which did not reflect the complexity of foods. In addition to carbohydrates, foods contain various other components, such as fats and protein, which influence the digestion of other nutrients and impact colonic microbes (see reviews[43,44]). Furthermore, phytochemicals found in plant-based foods can be metabolised by colonic microbes, generating more absorbable and bioactive molecules,[45] or exhibit antimicrobial or prebiotic properties, selectively promoting the growth of certain microbes in the colon.[46,47]

In our study, fermentation with kūmara produced more lactate than fermentations with other food ingredients. This result is likely an artifact of the *in vitro* static fermentation, as lactate accumulation is often observed in faecal fermentations due to an excess of fermentable substrates.[48] Kūmara is rich in complex carbohydrates, primarily in the form of starch, and contains pectin as a soluble fibre and cellulose, hemicellulose, and lignin as an insoluble fibre.[49] Consistent with the increased lactate production, fermentation with peeled kūmara promoted the highest relative abundances of lactic acid bacteria from the genera *Bifidobacterium*, *Enterococcus*, and *Lacticaseibacillus*. Additionally, fermentation with peeled kūmara had higher LFC values for the abundance of the genus *Enterococcus* compared to other food ingredients. Correlation analyses demonstrated that the carbohydrate content in food ingredients was positively associated with lactate production and the relative abundance of *Lacticaseibacillus*. Furthermore, *Lacticaseibacillus* and *Enterococcus* abundances were positively linked to lactate production and total dietary fibre content.

These bacteria belong to a group of potentially beneficial microbes that produce lactate as their major fermentation product, ultimately contributing to SCFAs produced in the colon through cross-feeding with other microbes.[50,51] For instance, the *Bifidobacterium* genus breaks down carbohydrates, particularly human milk oligosaccharides, through the fructose 6-phosphate pathway to produce acetate and lactate. Most members of the *Lacticaseibacillus* genus (previously classified under *Lactobacillus*) are homofermentative, mainly converting carbohydrates into lactate, although some strains are heterofermentative and produce acetate.[52]

The highest lactate yield in the fermentation with kūmara also explains the greatest pH drop, as lactic acid is a stronger acid than the other major SCFAs. In the colon, lactate can be oxidised to pyruvate and subsequently converted into acetyl-CoA, contributing to the pool of acetate and butyrate,[53,54] while propionate can be generated from lactate *via* methylmalonyl-CoA or acrylyl-CoA pathways.[55] Notably, the microbial conversion of lactate into other major SCFAs is sensitive to pH. Evidence *in vitro* demonstrated that lactate is efficiently transformed into propionate and butyrate at around pH 6.5, whereas at pH 5.5 or lower, its conversion is inhibited, resulting in lactate accumulation and overabundance of *Bifidobacteria*.[56,57] However, it is important to acknowledge that such low pH conditions do not accurately reflect the physiology of the human colon.

In line with those findings, we observed that the fermentation with kūmara peeled or with skin produced some of the lowest amounts of acetate and propionate, also having reduced abundance of the genus *Parabacteroides*. This suggests that lactate was not efficiently converted into SCFAs and accumulated during fermentation. Our study used a static fermentation protocol, which does not reflect the dynamic inflow and outflow of substances in the human colon. Under more realistic conditions, lactate produced from kūmara fermentation may contribute to greater production of SCFAs, ultimately conferring host benefits.

Research in rats showed that dietary fibre from sweet potatoes stimulated the growth of *Bifidobacterium* and *Lactobacillus* during *in vitro* faecal fermentation.[58] Additionally, it increased faecal propionate and butyrate levels in rats that received sweet potato fibre supplementation for four weeks.[58] Similarly, faecal fermentation studies using adult inoculum reported

that whole sweet potato and its extracted fibre promoted the production of major SCFAs and the abundance of bifidobacteria.[59–61] Currently, there is no published research on the effect of kūmara on the colonic microbiota of weaning infants. However, two ongoing clinical trials are evaluating this topic, and their results could provide valuable insights into the field of infant nutrition.[62,63]

Fermentations of blackcurrants, strawberries and raspberries led to the highest production of acetate and propionate. Consistently, fermentation with raspberries, followed by fermentation with blackcurrants, exhibited the highest abundances of the genus *Eubacterium.* Additionally, LFC values in the abundance of the genus *Parabacteroides* were greater in fermentations with raspberries or with blackcurrants compared to other food ingredients. These genera encode carbohydrate-active enzymes (CAZy), allowing them to degrade complex carbohydrates to produce SCFAs.[64,65] In contrast, genera colonising the colon in early life, such as *Streptococcus* and *Bifidobacterium*, had the lowest abundances in fermentations with blackcurrants or strawberries, suggesting a transition from infant to adult microbiota.

Blackcurrants, strawberries, and raspberries are sources of dietary fibre, particularly insoluble fibre (mostly cellulose), and contain high amounts of polyphenols, mainly anthocyanins, flavonols, ellagitannins, and ellagic acid.[66–68] Dietary fibre is fermented by colonic bacteria, primarily producing gases and SCFAs.[8,69] In addition, evidence suggests that polyphenols and dietary fibre synergistically affect the colonic microbiota by changing the carbohydrate metabolism of colonic commensals. For instance, cranberry proanthocyanidins enhanced the fermentation of xyloglucans, a type of soluble fibre, by lactic acid bacteria *in vitro*, leading to increased acetate production.[70] Whole-fruit cranberry powder, rather than its fibrous fraction alone, was more efficient in restoring colonic dysbiosis and reducing body weight in obese mice.[71]

Consistently, the production of major and total SCFAs positively correlated with the dietary fibre content of fermented foods here. The production of acetate, propionate and total SCFAs were also positively associated with a higher relative abundance of *Parabacteroides* and lower abundances of *Streptococcus* and *Bifidobacterium* genera. In agreement with our findings, the faecal fermentation of raspberry using adult inoculum mainly produced acetate and propionate.[72] Additionally, the same study demonstrated that polyphenols contributed more to the production of these SCFAs than dietary fibres.[72] A four-week raspberry intervention in prediabetic adults reported no changes in faecal microbial alpha and beta diversity compared to baseline values, but an increase in the relative abundance of *Eubacterium eligens* and *Clostridium orbiscindens*, as well as reduced plasma total and low-density lipoprotein cholesterol.[73]

In contrast to our results, another faecal fermentation study using inoculum from weaning infants found no changes in the production of acetate, propionate, and butyrate between blackcurrant and control fermentations.[74] However, clinical studies assessing the effect of blackcurrant intervention observed an increase in the faecal abundance of the *Ruminococcus* genus in postmenopausal women after six months;[75] as well as an increase in the faecal abundance of the genera *Lactobacillus* and *Bifidobacterium*, alongside a decrease in the abundance of *Clostridium* and *Bacteroides* genera after two weeks in healthy adults.[76]

Little is known about the impact of strawberries on the human colonic microbiota. A study using mice with colitis reported that strawberry supplementation increased the faecal abundance of the genera *Bifidobacterium* and *Lactobacillus*, as well as the caecal content of SCFAs.[77] Additionally, strawberry supplementation increased the colonic abundance of *Bifidobacterium* in diabetic mice.[78] A four-week trial involving healthy adults who consumed strawberries observed increased faecal abundance of the genera *Akkermansia*, *Bacteroides*, and *Bifidobacterium* but no changes in faecal SCFA levels.[79] The evidence above suggests that blackcurrants, strawberries, and raspberries are promising complementary foods for increasing the abundance of SCFA-producing bacteria in the colonic microbiota of infants.

Unlike other major SCFAs, butyrate production did not vary between food ingredient fermentations. However, when black beans were fermented with infant formula or blackcurrants, there was an increase in butyrate production compared to other food-formula or food-food combinations. Similarly, combining black beans with infant formula or blackcurrants in fermentation led to the highest relative abundance of *Clostridium sensu stricto 1*, a group of bacteria that metabolise carbohydrates and amino acids, producing butyrate *via* butyryl-CoA and butyrate kinase pathways.[80,81] Correlation analyses supported these findings, showing a relationship between higher protein content and increased relative abundance of *Clostridium sensu stricto 1*, which abundance was also positively associated with butyrate production.

Black beans are a source of protein, dietary fibre, and polyphenols, notably containing high amounts of resistant starch.[82] Additionally, soaking and cooking beans before consumption further increases their resistant starch content.[83] Traditionally, the colonic fermentation of resistant starch produces butyrate through a cross-feeding mechanism involving key resistant starch degraders, such as *Ruminococcus bromii* and *Bifidobacterium adolescentis*, along with butyrate producers from the genera *Faecalibacterium*, *Roseburia*, *Eubacterium*, and *Anaerostipes.*[84,85] However, recent evidence demonstrated that members of *Clostridium sensu stricto 1* can also produce butyrate from resistant starch.[86]

Previous faecal fermentation studies evaluating the effect of black beans on colonic microbes have shown contrasting results. One reported that black beans exhibited a prebiotic effect by increasing the abundance of *Bifidobacterium* and *Lactobacillus* genera during fermentation. This increase was associated with a rise in the production of acetate and propionate but a decrease in butyrate levels compared to the fermentation control.[87] On the other hand, another study observed that the fermentation of the insoluble indigestible fraction of

black beans produced butyrate, as well as acetate and propionate.[88] It is important to note that neither study specified the age of the faecal donors nor evaluated changes in the overall composition of the microbiota, limiting comparison with our findings.

Evidence in murine models suggests that consuming black beans benefits the microbiota by increasing the abundance of key taxa producing SCFAs and subsequently leading to greater production of SCFAs. For instance, healthy mice had increased faecal abundance of *Prevotella* and caecal contents of acetate, propionate, and butyrate after black bean intervention.[89] Similarly, rats fed a high fat and sugar diet supplemented with cooked beans exhibited increased faecal abundance of the *Clostridia* class and the genera *Ruminococcus*, *Coprococcus*, and *Prevotella*, as well as elevated faecal butyrate levels.[90] In contrast, a navy bean intervention did not alter faecal SCFA content in overweight adults, while a common bean intervention in weaning infants showed no changes in faecal microbiota diversity of taxa abundance.[91,92]

Unexpectedly, combining foods with infant formula drastically reduced the variability in organic acid production, taxa abundance, and microbial diversity scores between samples. Since the food-formula combinations consisted of 80% infant formula by mass, this high proportion of formula probably masked the effects of the individual food ingredients on colonic microbes. Similarly, we observed fewer changes in microbiota composition and SCFA production between fermentations with food-food and food-food-formula combinations, suggesting that the impact of specific foods on colonic microbes is less evident when considering the overall dietary pattern. Ultimately, long-term dietary patterns rather than spontaneous consumption of individual foods are more likely to promote notable and lasting changes in colonic commensals.[93,94]

Nevertheless, our study has limitations. During the transition to solid foods, infants often continue to consume breastmilk.[95] However, our study did not evaluate the effect of combining complementary foods with human milk. Instead, breastmilk was replaced with infant formula, which may have influenced the observed effects of complementary foods on the faecal microbiota of weaning infants. This limitation is particularly relevant during the first year of life, as breastfed infants have distinct faecal microbiota and metabolite profiles compared to formula-fed infants.[96]

Faeces were used due to the ease of collection and non-invasive procedure, which are essential when involving vulnerable participants. However, faecal samples mainly represent microbial communities from the distal colon and do not accurately reflect the microbes that adhere to the mucosa or those found in the proximal colon.[97] Due to the screening approach of this study, static protocols were used to simulate infant digestion and subsequent colonic fermentation of foods. These static conditions do not capture the dynamic nature of the gastrointestinal tract of infants. Notably, in static faecal fermentations, microbial metabolites can accumulate, and substrates may become depleted, potentially distorting the microbial community compared to what would be found in a dynamic environment.[98]

The microbial composition was characterised by 16S rRNA sequencing. While this method is accurate, it has limitations, particularly in resolution and cannot resolve taxonomy at the species level.[99] The composition of the identified bacterial taxa was expressed as relative abundances, indicating the proportion of individual microbes within the entire community. Consequently, this may lead to an inaccurate characterisation of the actual microbial community.

Our study evaluated a higher proportion of plant-based foods compared to animal-based foods. This choice was justified by our previous research, which identified complementary foods with the greatest potential for producing SCFAs *in silico*.[21] Although the food ingredients were prepared as similarly as possible to real-life conditions, the preparation likely altered their original structure, ultimately influencing their impact on colonic microbes.[100] For instance, cooking and cooling plant-based foods can increase their resistant starch content.[83] Finally, while correlation analyses could link changes in the production of SCFAs or microbial relative abundance to protein, fat, and fibre content in the evaluated foods, phytochemicals were not analysed, which is warranted in further research.

Finally, as our study focused on the faecal microbiota of New Zealand weaning infants, our findings may not be directly generalisable to infants from other geographic locations. Geographic location is known to influence the composition of the infant faecal microbiota.[101] Furthermore, dietary patterns and eating habits may differ across countries and cultures.[102] Consequently, the complementary foods evaluated in our *in vitro* study may not fully represent those consumed by weaning infants in other parts of the world.

5. Conclusions

This study investigated how various food ingredients and food combinations affect the composition and function of the colonic microbiota in New Zealand weaning infants *in vitro*. Foods promoting the most favourable changes in the infant microbiota were identified. Notably, fermentation with kūmara, a variety of sweet potatoes rich in complex carbohydrates, effectively promoted lactate production by stimulating the growth of the lactic acid bacteria from the genera *Enterococcus* and *Lacticaseibacillus*. Fermentation with blackcurrants, strawberries, and raspberries, notable sources of dietary fibre and polyphenols, increased acetate and propionate production. This increase was linked to a higher relative abundance of *Parabacteroides* and *Eubacterium* genera. Additionally, when black beans were fermented with infant formula or blackcurrants, they produced the highest yields of butyrate and increased the abundance of the group *Clostridium sensu stricto 1*. This is likely due to the high protein and resistant starch content in black beans. Overall, these findings contribute to an under-investigated topic of

colonic microbiome research in infants. Kūmara, berries, and black beans are promising candidates for further clinical trials involving infants.

Author contributions

Vitor Geniselli: conceptualisation, data curation, formal analysis, investigation, visualisation, and writing – original draft. Jane Mullaney: conceptualisation, data curation, formal analysis, supervision, and writing – review & editing. Nicole Roy: conceptualisation, funding acquisition, supervision, and writing – review & editing. Nick Smith: supervision and writing – review & editing. Clare Wall: funding acquisition, supervision, and writing – review & editing. Callum Tatton: investigation. Warren McNabb: conceptualisation, funding acquisition, project administration, supervision, and writing – review & editing.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors have no conflicts to declare.

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