Food Research International 173 (2023) 113348

Contents lists available at ScienceDirect

Food Research International

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

Butyrogenic, bifidogenic and slight anti-inflammatory effects of a green kiwifruit powder (Kiwi FFG®) in a human gastrointestinal model simulating mild constipation

Elizabeth Goya-Jorge [a], Pauline Bondue [a,b], Irma Gonza [a], Fanny Laforêt [a], Céline Antoine [a], Samiha Boutaleb [c], Caroline Douny [c], Marie-Louise Scippo [c], Jeoffrey Christyn de Ribaucourt [b], Fabienne Crahay [b], Véronique Delcenserie [a,*]

[a] Laboratory of Food Quality Management, Department of Food Sciences, FARAH - Veterinary Public Health, University of Liège, B43b, 4000 Liège, Belgium
[b] ORTIS S.A., Hinter der Heck 46, 4750 Elsenborn, Belgium
[c] Laboratory of Food Analysis, Department of Food Sciences, FARAH - Veterinary Public Health, University of Liège, B43b, 4000 Liège, Belgium

ARTICLE INFO

Keywords:
Kiwifruit
Microbiota
In vitro simulator
Fermentation
Nutritional supplement
Colon health
Intestinal bacteria
SHIME system

ABSTRACT

Green kiwi (*Actinidia deliciosa* var. Hayward) is a fruit with important nutritional attributes and traditional use as a laxative. In this work, we studied *in vitro* the colonic fermentation of a standardized green kiwifruit powder (Kiwi FFG®) using representative intestinal microbial content of mildly constipated women. Static (batch) and dynamic configurations of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) were used to estimate the impact of Kiwi FFG® in the human gut. Analysis of metabolites revealed a significant butyrogenic effect of the kiwifruit powder and, consistently, butyrate-producing bacterial populations (*i.e.*, *Faecalibacterium prausnitzii*, Cluster IV, *Roseburia* spp.) were greatly increased in the dynamic gastrointestinal model. *Bifidobacterium* spp. was also found boosted in the microflora of ascending and transverse colon sections, and a significant rise of *Akkermansia muciniphila* was identified in the transverse colon. Reporter gene assays using human intestinal cells (HT-29) showed that kiwifruit fermentation metabolites activate the aryl hydrocarbon receptor (AhR) transcriptional pathway, which is an important regulator of intestinal homeostasis and immunity. Moreover, modulation in the production of human interleukins (IL-6 and IL-10) in Caco-2 cells suggested a potential mild anti-inflammatory effect of the kiwifruit powder and its gut microbiota-derived metabolites. Our results suggested a potential health benefit of Kiwi FFG® in the gut microbiota, particularly in the context of constipated people.

1. Introduction

The kiwifruit was first described in the twelfth-century Chinese Song Dynasty. The plant was introduced to the western world at the end of the nineteenth century and owns its name to the iconic native bird of New Zealand where there is the first and still a major kiwi plant industry (Ward & Courtney, 2013).

Constipation is one of the most common gastrointestinal conditions estimated to affect about 15 % of adults worldwide (Bharucha & Lacy, 2020). Green kiwifruit (*Actinidia deliciosa* var. Hayward) is considered a natural and well-tolerated treatment alternative to alleviate constipation symptoms (Bayer et al., 2018; Turck et al., 2021). In fact, green kiwi has been shown to increase the frequency and consistency of stool, decrease transit time, and reduce abdominal discomfort in healthy subjects (Caballero et al., 2020). Scientific evidence associates kiwi's content of vitamins, proteins, carbohydrates, and dietary fibers, as well as a high-water retention capacity with its constipation-related health benefits (Haytowitz et al., 2019; Mishra & Monro, 2012). Furthermore,

Abbreviations: AC, ascending colon; AhR, aryl hydrocarbon receptor; BCFA, branched chain fatty acids; DC, descending colon; ddH_2O, double-distilled water; DMEM, Dulbecco's Modified Eagle Medium; IL-6, interleukin-6; IL-10, interleukin-10; IBD, inflammatory bowel disease; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ns, non-significant; OD, optical density; RLU, relative light units; SCFA, short-chain fatty acid; SHIME®, Simulator of the Human Intestinal Microbial Ecosystem; TC, transverse colon..

* Corresponding author.
E-mail address: veronique.delcenserie@uliege.be (V. Delcenserie).

https://doi.org/10.1016/j.foodres.2023.113348
Received 12 April 2023; Received in revised form 1 August 2023; Accepted 3 August 2023
Available online 20 August 2023

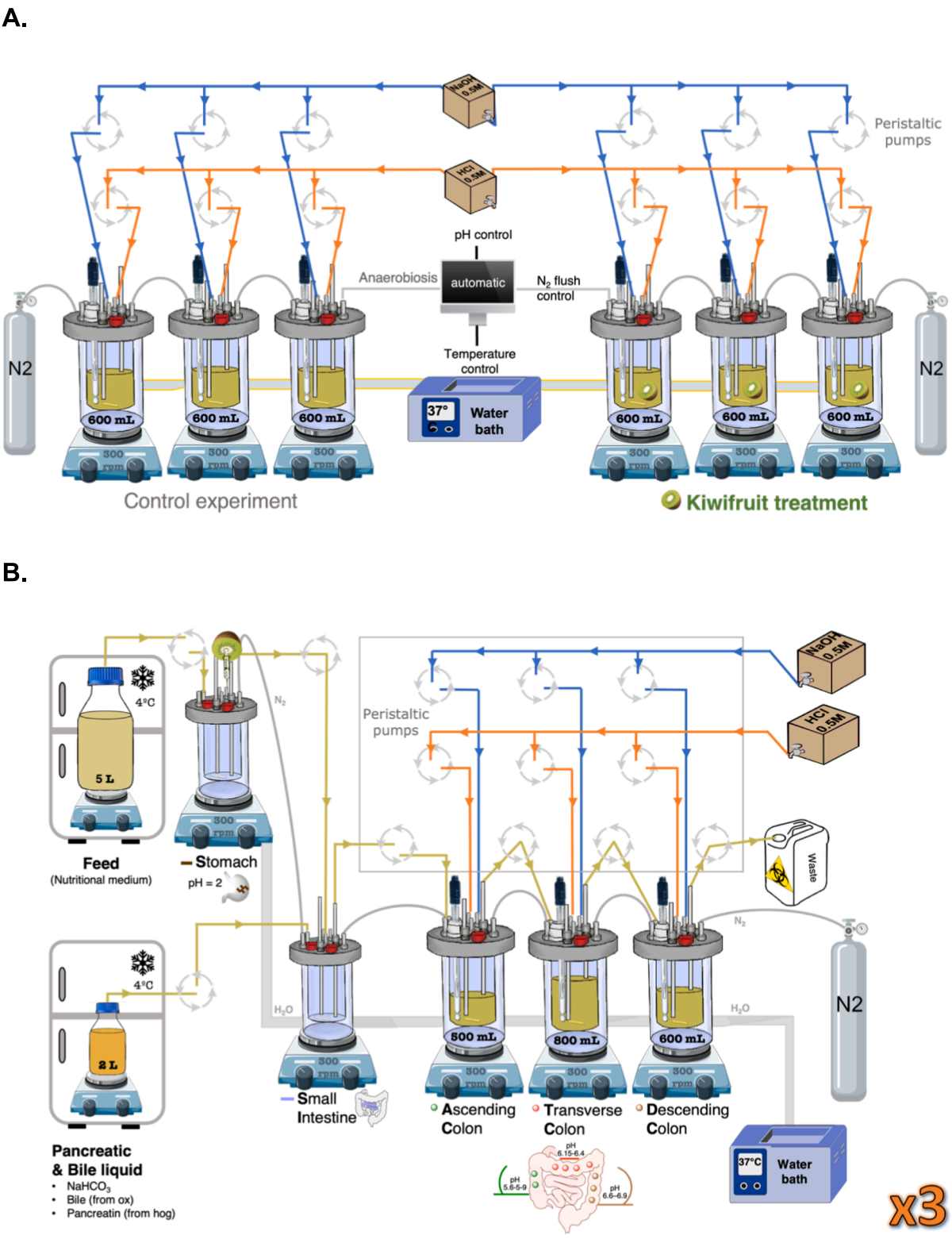

Fig. 1. Experimental design of the two variants of the model used for the Simulation of the Human Intestinal Microbial Ecosystem (SHIME®) to evaluate green kiwifruit powder Kiwi FFG®. **A.** Static (kiwi-Batch) experiment: vessels (6) were inoculated with nutritional media and a three-donors pool of feces (5 %) to complete 600 mL. Three fermenters were treated with the green kiwifruit powder Kiwi FFG® and three were used as parallel control (triplicate independent repetitions). The maintenance of the anaerobiosis, the pH control (6.6-6.9), and the human basal temperature (37 °C) are automatically regulated. **B.** Dynamic (kiwi-SHIME®) experiment (independently repeated- 3X). Five vessels are connected in a dynamic flow representing the stomach, small intestine, and ascending, transverse, and descending colon sections, respectively. Three times/day the system is fed with 140 mL of nutritional medium and 60 mL of pancreatic-bile juice. Anaerobic conditions (checked with N_2 flush), specific pH intervals, basal temperature and volume are computer-controlled automatically by the SHIME® machine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kiwifruits have antioxidant (Collins et al., 2001; Latocha et al., 2015) and anti-inflammatory (Bentley-Hewitt et al., 2020) effects that further support their nutritional importance in human's daily diet (Richardson et al., 2018). However, only a few studies have addressed the fate of kiwifruit on the human gastrointestinal tract (Carnachan et al., 2012; Chen et al., 2018; Rosendale et al., 2012), or studied the metabolic and gut microbial impacts of kiwifruit fermentation (Blatchford et al., 2015; Wang et al., 2022).

The study of the human intestinal microbiota remains a challenge whether *in vitro* or *in vivo* experiments are used. On one hand, controlled environments are accomplished with animal models (*e.g*, mouse, rat, and piglet). However, in many cases the gut microbiota of these animals has a low physiological resemblance with human gut microbiota metabolic activity and, consequently, their translation to humans has limitations (Vikrant Berde et al., 2021). In fact, the use of simple animal models such as zebrafish (*Danio rerio*) and the nematode *Caenorhabditis elegans* has gained popularity in the study of microbiome-host interactions since they constitute an effective engine for fundamental discovery (Douglas, 2019). Meanwhile, *in vitro* fermentation experiments provide rapid, reproducible, economically, and ethically advantageous alternatives (Biagini et al., 2023; Roupar et al., 2021) and are highly encouraged by regulatory entities to analyze the human gut microbiota.

As the colon is the most important compartment to study gut transit (Kim & Rhee, 2012), a simulation of several human colon sections was designed in this work. Thus, we aimed to provide a thorough *in vitro* analysis of the impact of a green kiwifruit powder (Kiwi FFG®) on the human colon microbiota. Using the representative intestinal microbial content of mildly constipated women, we simulated the evolution of the microbial ecosystem in the presence of Kiwi FFG®. For the first time a comparison between a static/short-term colonic fermentation *versus* a dynamic/long-term gut model, both testing a standardized green kiwifruit powder, is provided.

2. Materials and methods

2.1. Chemical reagents and consumable materials

All chemicals and reagents were obtained from Sigma Aldrich, Merck KGaA (Darmstadt, Germany) unless otherwise stated. Cell culture materials were obtained from Greiner Bio-One BioScience (Vilvoorde, Belgium) VWR International BV (Leuven, Belgium), or Thermofisher Scientific (Waltham, USA). The nutritional medium ("feed") used for simulation of the adult human gastrointestinal environment contained per liter of distilled water: arabinogalactan (1.2 g), pectin (2.0 g), xylan (0.5 g), glucose (0.4 g), yeast extract (3.0 g), special peptone (1.0 g), mucin from porcine stomach lining (3.0 g), L-cystein-HCl (0.5 g) and starch (4.0 g). The prepared feed was autoclaved prior to use and adjusted to acidic pH (pH = 2) for dynamic simulations. The pancreatic-bile liquid contained per liter of previously sterilized water: 12.5 g of $NaHCO_3$ from Chem-Lab (Zedelgem, Belgium), 6.0 g of Difco™ Oxgall (ox bile) from BD (Sparks, US), and 0.9 g of a mixture of amylase, trypsin, lipase, ribonuclease, and protease digestive enzymes produced by the exocrine cells of the porcine pancreas (Pancreatin from hog - Sigma Aldrich). All consumable materials and nutritional media used in the SHIME® experiments were obtained through ProDigest company (Ghent, Belgium).

2.2. Volunteer donors and fecal sampling

The Ethical Committee of the Liège University - Hospital (file number 2020/293) approved the proposed study where volunteer donors should comply with the stated criteria. Thus, three healthy female adults (A, B, C) of 44, 38 and 33 years old, respectively, were selected. They all had a typical western diet (not vegan nor vegetarian) and a normal body mass index (BMI < 30). All donors declared not to have consumed antibiotics, prebiotics, or probiotics treatments for at least 6 months before sampling. During at least few weeks prior to sampling, they had not consumed kiwifruit. After answering a survey, the three donors were identified to have mild constipation (Type 2) according to the Bristol stool form scale (Lewis & Heaton, 1997) and the frequency declared.

Fecal samples were collected in anaerobic jars and anaerobiosis was ensured using Anaerogen™ bags (Oxoid, Basingstoke, UK). A phosphate buffer solution containing per liter: 8.8 g of K_2HPO_4, 6.8 g of KH_2PO_4 and 0.1 g of sodium thioglycolate was used to prepare a fecal homogenate of 20 % (w/v). Mechanical homogenization was achieved in a Stomacher VWR® Star-Blender LB400. Double-coated sterile stomacher bags (300 × 190 mm) were used to collect and filter the fecal suspensions. For long-term storage (-80 °C), glycerol 20 % (v/v) was added to the feces as a cryoprotectant agent. For the static short-term fermentation experiments, a pool of feces was prepared by mixing equal proportions of samples from the three donors. A selected donor (B) provided the fecal material for the dynamic long-term fermentation experiments.

2.3. SHIME® system experiments

Two different configurations of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) were settled as represented in Fig. 1. The first one (Fig. 1A) was a static "batch" model where pH (6.6-6.9), temperature (37 °C), and anaerobiosis were controlled in a microbial system that remained closed and in contact with the treatment for 72 h. In this static-SHIME® system, the tested substance (Kiwi FFG®) was inoculated along with the fecal homogenate (5 %) and the nutritional medium. In parallel, a control system without any treatment was run for comparison purposes.

The second configuration (Fig. 1B) was a dynamic-SHIME® system where 5 fermenters representing different sections of the gastrointestinal tract were connected consecutively and airtight. The basal human body temperature (37 °C) was maintained through warm water circulation and automatic control of different pH intervals was achieved through peristaltic pumps connected to acid (HCl, 0.5 M) and base (NaOH, 0.5 M) solutions. Three times per day the stomach vessel was fed with nutritional medium (140 mL) that was later mixed with pancreatic-bile liquid (60 mL) in the small intestine vessel to gradually migrate from one section of the colon to the next through transfer tubes. The volume of ascending colon (AC, 500 mL), transverse colon (TC, 800 mL) and descending colon (DC, 600 mL) sections containing the representative microbial ecosystem obtained from human feces remained constant by discharging the surplus volume in a waste bottle.

2.4. Kiwi FFG® treatments

Kiwi FFG®, a green kiwifruit (*Actinidia deliciosa*) powder, was obtained from Ortis S.A. that certified that the product was characterized and standardized for specific enzymatic profile combined with defined amounts of soluble and insoluble fibers and polyphenols. Treatment doses of Kiwi FFG® and the timelines of static and dynamic SHIME® experiments are represented in Fig. 2A and Fig. 2B, respectively.

As shown in Fig. 2A, in the static-SHIME® the dose was 2.4 g of Kiwi FFG®. Under constant stirring (300 rpm), the powder was poured slowly until complete homogenization with the fecal material and the medium (final volume of 600 mL). Fermentation samples were taken every 24 h for three days. Triplicate parallel repetitions of the experiment (with and without adding Kiwi FFG®) were done (see Fig. 1A).

In the timeline of the dynamic-SHIME® experiment designed (Fig. 2B) is shown that the system was kept for two weeks allowing the stabilization of the intestinal microbiota before starting the treatment. Then, Kiwi FFG® mixed with the first meal of the day was added for two weeks in increasing doses as follows: 0.6 g (5 days), 1.2 g (5 days), and 2.4 g (5 days). Finally, the system was kept running for two more weeks without treatment (washout period). Fermentation samples were taken three times per week and selected time points corresponding to pre-

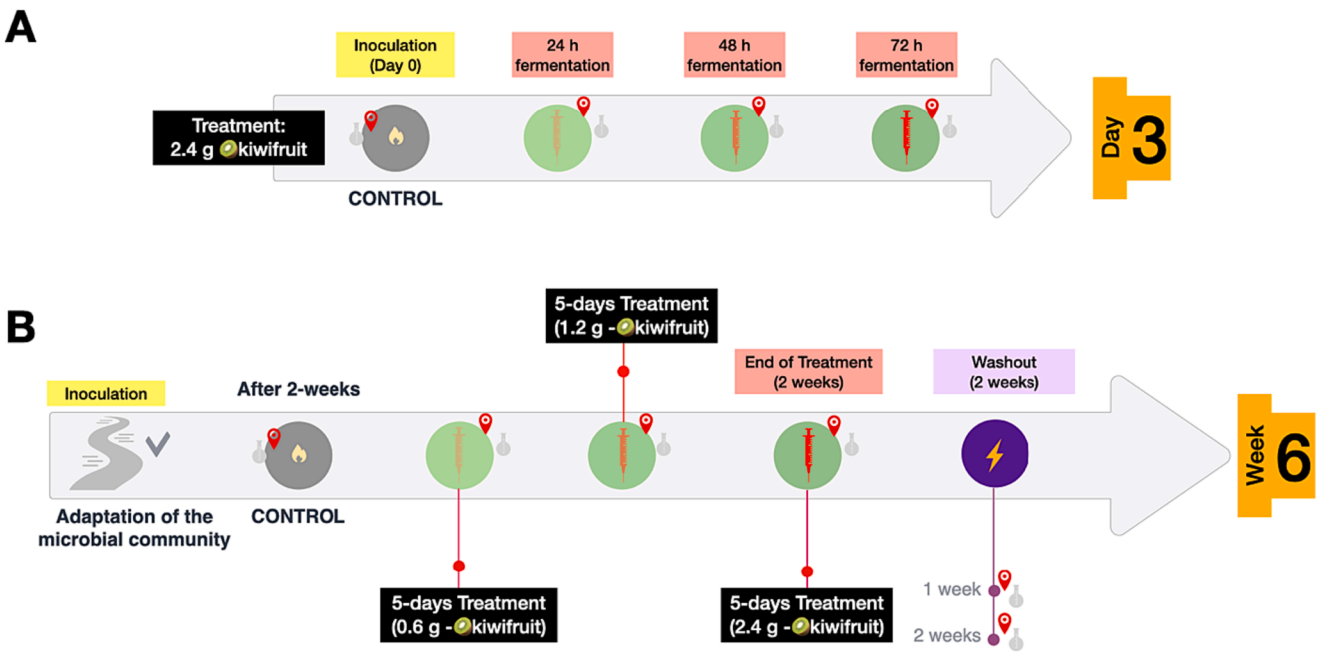

Fig. 2. Timeline and dosage of the human gastrointestinal simulation experiments of the standardized green kiwifruit powder Kiwi FFG®. Samples were taken at crucial time points (red pins). **A.** Static (kiwi-Batch) experiment: At day 0 the system was inoculated with the fecal material and the treatment (or not- for control). The treatment dose (2.4 g of FFG® kiwifruit powder) was slowly added until total dispersion. **B.** Dynamic (kiwi-SHIME®) experiment: After feces inoculation, the system was maintained for two weeks to reach microbial stabilization, and pre-treatment control samples were taken. Then, increase doses of FFG® kiwifruit powder were daily inoculated into the stomach vessel for 2 weeks as follows: 5 days (0.6 g) + 5 days (1.2 g) + 5 days (2.4 g). The system was maintained for two more weeks (washout period) to analyze potential residual effects. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatment controls, end of treatments, and washout periods were analyzed. The six weeks long dynamic experiment was independently repeated three times.

2.5. SCFA quantification

A previously validated method of solid-phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry (GC–MS) (Douny et al., 2019) was used to quantify simultaneously the SCFAs produced during the SHIME®-experiments. The analytical technique has the following lower and upper limits of quantification (LOQ), respectively: acetic acid (C2): 2.40 – 119.90 mM, propionic acid (C3): 1.09 – 54.67 mM, butyric acid (C4): 0.79 - 39.72 mM, isobutyric acid (iC4): 0.18 - 9.08 mM, isovaleric acid (iC5): 0.14 - 6.85 mM, valeric acid (C5): 0.47 - 23.50 mM and caproic acid (C6): 0.02 - 0.86 mM.

2.6. D/L-lactic acids quantification

D-lactic acid and L-lactic acid produced during the fermentation in the dynamic-SHIME® were determined using an enzymatic kit from Biosentec (Portet-sur-Garonne, France). The preparation of fermentation supernatants was validated in four steps as follows: two-fold dilution in 0.1 M of triethanolamine buffer (pH practically 10) followed by a 5 min centrifugation (3900 rpm), protein precipitation by adding 10 % trichloroacetic acid (6.1 N) and a 20 min centrifugation (3900 rpm, 4 °C). Lactic acids were measured in the supernatant following kit supplier instructions with a prior validation of the method using a standard solution of D/L-lactic acids (detection limits between 0.33 and 2.22 mM). Optical density (OD) was measured at 340 nM in a Genesys10UV spectrophotometer from Thermofisher Scientific.

2.7. Ammonia quantification

An enzymatic kit from Biosentec was used to determine the production of ammonia during fermentation experiments. The pH of the samples was first adjusted to 8 and then, the protein precipitation using trichloroacetic acid and the OD measurements (340 nM) were conducted as for D/L-lactic acids detailed above. The method was validated with a standard solution of ammonia and the detection limits are between 0.59 and 5.0 mM.

2.8. Cell culture conditions

Two human colorectal adenocarcinoma cell lines (Caco-2 and AhR_HT29-Lucia) were used. Cells were maintained in 75 cm^2 and 175 cm^2 culture flasks, at 37 °C in 5 % CO_2 and used for bioassays upon reaching 80 % - 90 % confluency. Caco-2 cells were obtained from ATCC (Manassas, USA) and cultured in DMEM supplemented with FBS (10%), NEAA 1X and 0.05 mg/mL of Gentamicin. Meanwhile, AhR_HT29-Lucia cells were obtained from Invivogen (Toulouse, France) and cultured according to providers instruction in DMEM supplemented with FBS (10%), PEEN (100 U/mL Penicillin G + 100 µg/mL Streptomycin) and the specific antibiotics Normocin™ and Zeocin™ (100 µg/mL each). For all assays, the used media was DMEM with FBS (10%) and antibiotic free.

2.9. Cytotoxicity experiments

Kiwi FFG® was diluted in sterile double-distilled water (ddH_2O) (free of DNA, RNase, and DNase contamination) for cell treatments and the stock concentration was 200 mg/mL. Treatments for exposure were prepared in pre-warmed assay medium.

MTT cell viability assay. A range of concentrations of Kiwi FFG®

(0.25 - 5.0 mg/mL) was tested in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay using Caco-2 cells. Cells were counted using the Scepter™ 3.0 Cell Counter from Merck KGaA (Darmstadt, Germany) and densities between 2.0×10^5 and 5.0×10^5 cells/mL were seeded in CellStart® 24-well plates. When reached proper confluency, cells were exposed to the treatments for 24 h, 48 h and 72 h. On the day of the assay the culture media was removed from the wells and cells were gently washed with warm (37 °C) and sterile phosphate-buffered saline solution (PBS, 1X, pH 7.4). Then, 400 μL/well of MTT dye prepared in PBS (0.5 mg/mL) were added and plates were incubated for 3 h at 37 °C to allow the mitochondrial reduction of the yellow water-soluble tetrazolium salt and to yield the water-insoluble purple formazan crystals. In principle, the capacity of metabolically active cells to reduce the MTT reagent indicate the effects caused by Kiwi FFG® treatments in the proportion of live, healthy cells within a population (*i.e.*, cell viability) (Mosmann, 1983). Once MTT supernatant was removed, formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and softly shaken (140 rpm) for 10 min in a Heidolph Unimax 2010 platform shaker (Schwabach, Germany) before measuring the absorbance at 550 nm in a ELx800™ microplate reader spectrophotometer from Agilent BioTek Inc. (Winooski, USA).

LDH cytotoxicity assay. The LDH-Cytox™ Assay Kit from BioLegend Inc (San Diego, USA) was used to determine the cytotoxic potential of kiwifruit (0.25 - 5.0 mg/mL) in Caco-2 cells. This test measures the presence of lactate dehydrogenase (LDH)- a cytoplasmic enzyme- in the cell culture medium, indicating damage in the plasma cell membrane (Decker & Lohmann-Matthes, 1988). Briefly, cells were seeded in CellStart® 96-well plates overnight, then exposed to Kiwi FFG® dilutions for 24 h. Preparation steps from the kit suppliers were followed and the absorbance was measured at 490 nm in the ELx800™. Results are presented as cytotoxicity percentage relative to the total LDH release when adding a lysis buffer and considering the spontaneous LDH release.

2.10. Metabolic output induction of AhR transactivity

Supernatant samples from the SHIME® experiments were sterilized by filtration using Whatman™ syringe filters (0.2 μm) from Cytiva, (Marlborough, USA). AhR_HT29 Lucia cells were seeded at 3.0×10^5 cells/mL in CellStart® 96-well plates, incubated overnight and exposed to 20 μL/well of the SHIME® fermentation samples for 24 h, as described elsewhere (Goya-Jorge et al., 2022). Then, 20 μL/well of cells supernatant were transferred to Nunc™-white 96-well plates. Finally, 50 μL/well of Quanti-Luc™ assay reagent from Invivogen were added for luminescence reading in an ORION-II Berthold Detection System (Pforzheim, Germany). Signals were detected as relative light units (RLUs) and presented as fold response relative to cells treated with the growth medium from the SHIME® system. Simultaneously, MTT cell viability assays were conducted to rule out cytotoxic effects caused by the treatments in the AhR_HT29 Lucia cells (following the same protocol detailed above).

2.11. Measurement of IL-6 and IL-10

Caco-2 cells were seeded and incubated overnight in CellStart® 96-well plates at 10^5 cells/mL. Then, cells were exposed for 24 h to Kiwi FFG® dilutions (0.25 - 5.0 mg/mL) or to sterile-filtered fermentation supernatant from the SHIME® experiments (20 μL/well). Triplicated samples from the SHIME® experiments (n = 6) and from Kiwi FFG® dilutions (n = 3) were exposed to Caco-2 cells. Then, the sample transfer protocol of IL-6 and IL-10 Lumit™ kits from Promega (Madison, USA) was followed. Briefly, after the 24 h treatment, 50 μL of cell medium was transferred from each well to the corresponding wells of two separate white assay plates to measure IL-6 and IL-10, indistinctly. An antibody mixture was added to the cell's supernatant, plates were shaken (10 min) and incubated (1 h) at 37 °C. The luminescence (as RLU) was read upon adding 25 μL/well of Lumit™ detection reagent. The luminescence of standard controls of IL-6 and IL-10 (18.2 - 25 000 pg/mL) prepared in the assay medium was registered for calculations. Thus, the

Table 1
Primer sequences and annealing temperatures used for each taxon-specific qPCR experiment.

Tann [a]	Taxon	Primer sequences	Ref. [b]
	Total bacteria	F: AAA-CTC-AAA-KGA-ATT-GAC-GG R: CTC-ACR-RCA-CGA-GCT-GAC	(Bacchetti De Gregoris et al., 2011)
61.5 °C	Bacteroidota	F: CRA-ACA-GGA-TTA-GAT-ACC-CT R: GGT-AAG-GTT-CCT-CGC-GTA-T	
	Bacillota	F: TGA-AAC-TYA-AAG-GAA-TTG-ACG R: ACC-ATG-CAC-CAC-CTG-TC	
	Actinomycetota	F: TAC-GGC-CGC-AAG-GCT-A R: TCR-TCC-CCA-CCT-TCC-TCC-G	
	Christensenellaceae	F: GYT-CGC-GTC-CCA-TTA-GVT-AGT-TGG R: CAC-GTA-GTT-AGC-CGG-RGC-TTC-CTC	(Kurina et al., 2020)
	Cluster XIVa	F: AAA-TGA-CGG-TAC-CTG-ACT-AA R: CTT-TGA-GTT-TCA-TTC-TTG-CGA-A	(Matsuki et al., 2004)
	Cluster IV	F: GCA-CAA-GCA-GTG-GAG-T R: CTT-CCT-CCG-TTT-TGT-CAA	
	Roseburia spp.	F: GCG-GTR-CGG-CAA-GTC-TGA R: CCT-CCG-ACA-CTC-TAG-TMC-GAC	(Jiang et al., 2016; Ramirez-Farias et al., 2009)
	Enterococcus spp.	F: CCC-TTA-TTG-TTA-GTT-GCC-ATC-ATT R: ACT-CGT-TGT-ACT-TCC-CAT-TGT	(Rinttilä et al., 2004)
55 °C	*Oscillospira* spp.	F: AAG-GAG-TTT-TCG-GAC-AAC-GG R: ATT-CAA-GGG-GTA-CCG-TCT-TC	(Mackie et al., 2003)
	Bacteroides-Prevotella spp.	F: GAG-AGG-AAG-GTC-CCC-CAC R: CGC-TAC-TTG-GCT-GGT-TCA-G	(Morel et al., 2015)
54 °C	*Bifidobacterium* spp.	F: GGG-TGG-TAA-TGC-CGG-ATG R: CCA-CCG-TTA-CAC-CGG-GAA	(Amit-Romach et al., 2004)
	A. muciniphila	F: CAG-CAC-GTG-AAG-GTG-GGG-AC R: CCT-TGC-GGT-TGG-CTT-CAG-AT	(Everard et al., 2013)
	F. prausnitzii	F: AGA-TGG-CCT-CGC-GTC-CG R: CCG-AAG-ACC-TTC-TTC-CTC-C	(Morel et al., 2015; Wang et al., 1996)

[a] Annealing temperature (Tann) validated in this study following a cycling protocol that includes denaturation at 95 °C (5 min), 35 cycles (95 °C for 15 sec, Tann for 15 sec, and 72 °C for 30 sec), and final elongation at 72 °C (5 min) (Bacchetti De Gregoris et al., 2011).
[b] References of the primer sequences.

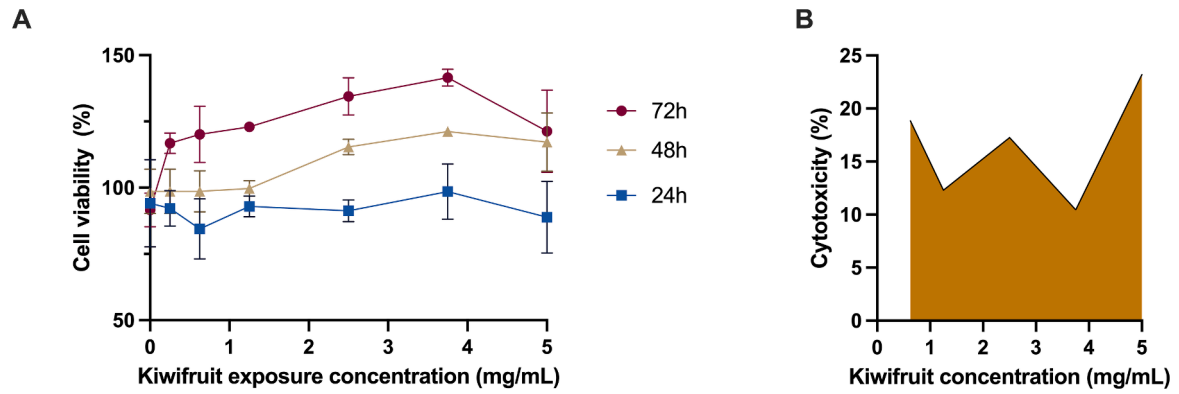

Fig. 3. Results of FFG® green kiwifruit powder cytotoxicity experiments in Caco-2 cells (n = 9). **A.** Cell viability percentage (mean ± SD) obtained in the MTT assay after exposing cells to different kiwifruit powder dilutions (mg/mL) during 24 h, 48 h and 72 h. **B.** Cytotoxicity percentage in the LDH assay after 24 h of exposure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration of IL-6 and IL-10 in the cell medium supernatant was determined from the interpolation into the calibration curves of each standard interleukin.

2.12. Gut bacteria DNA extraction

Bacterial DNA from the SHIME® samples was extracted using the PSP® Spin Stool DNA Kit from Invitek Molecular GmbH (Berlin, Germany). Briefly, fermentation samples were centrifuged (13 000 rpm, 5 min) when harvested and supernatants were removed to recover pellets of approximately 200 mg that were stored at -20 °C until use. On the day of the extraction, 1.2 mL of stool stabilizer solution was added to each pellet, followed by a 10 min incubation at 95 °C under shaking (900 rpm) on a thermomixer from Eppendorf (Aarschot, Belgium). Then, several purification steps were done, including an incubation for 10 min with proteinase K at 70 °C for 10 min to eliminate endogenous nucleases. Mixed with a binding buffer, the nucleic acids from the sample were bound to a column and washed in several steps to be finally eluted using a warm (70 °C) buffer solution. DNA concentrations were measured in a Nanodrop 2000 spectrophotometer from ThermoFisher Scientific. To evaluate the quality and purity of the extracted DNA, 260/280 nm and 230/260 nm ratios were used. All samples were normalized (4 ng/μL of DNA) for qPCR experiments using sterile ddH_2O.

2.13. Evolution of gut microbial populations

Selected bacterial populations of interest were analyzed by qPCR using the Takyon™ No ROX SYBR MasterMix from Eurogentec S.A. (Seraing, Belgium). The C1000™ Touch Thermal Cycler from Bio-Rad (Hercules, USA) was used for detection and a single validated qPCR protocol was applied. The only parameter in the qPCR protocol that varies between taxon targets tested is the annealing temperature (see Table 1). Melt curves (65 °C - 95 °C) were analyzed to guarantee the specificity of amplified products. Primer efficiencies (90 % - 105 %) and regression coefficients (>0.98) were determined in all cases as recommended by MIQE guidelines (Bustin et al., 2010). Results are expressed by comparative quantification via the $2^{-\Delta\Delta Cq}$ method (Livak & Schmittgen, 2001). The calibration samples for the static-SHIME® were the ones from inoculation (day 0), while for the dynamic-SHIME®, samples from the pre-treatment control were used. Normalization was done using universal bacteria sequences.

2.14. Statistical Analysis

All statistical analyses were done using GraphPad Prism version 9.5.1 for macOS (San Diego, California, USA). In the static experiment, comparisons with the parallel control were done using Unpaired *t*-test or Mann-Whitney test. For the dynamic experiment, comparisons relative to the pre-treatment time point were done using Repeated-Measures One-way ANOVA or Friedman test. In all cases, the normality of the data was previously analyzed using Shapiro-Wilk test, which allowed to select the parametric or the non-parametric method, as convenient.

3. Results and discussion

Human health is determined by the microbial communities that colonize the intestine and these, in turn, by diets and nutritional habits (Flint et al., 2012). Indeed, dysbiosis on the gut microbiota is a clinical sign of all kinds of human diseases (*i.e.*, infectious, physiological, deficiency, hereditary) (Shreiner et al., 2015). The consumption of green kiwifruits is highly recommended to alleviate abdominal discomfort and constipation (Gearry et al., 2023). As signature composition of *Actinidia* genus, kiwifruits are known to be a rich source of vitamin C, vitamin E, potassium, folate, actinidin, fiber, and polyphenols (Abdul Hamid et al., 2017). These components attribute to the fruit a high nutritional score (Drummond, 2013) and multiple benefits in digestive, immune, and metabolic health (Richardson et al., 2018) as well as a great interest in its antioxidant potential, particularly related to its vitamin C and polyphenols contents (Du et al., 2009).

In this work, we aim to contribute to the understanding of the effects caused by green kiwis in the evolution and metabolism of the bacterial population in the human large intestine. As recommended (Louis et al., 2007), a combination of several approaches to analyze the microbial ecosystem is used.

3.1. Kiwi FFG® is not cytotoxic in intestinal cell culture experiments

Results from MTT and LDH assays indicated that below 5 mg/mL dose of Kiwi FFG®, there was no apparent cytotoxicity in Caco-2 cells (See Fig. 3). Thus, the MTT assay (Fig. 3A) revealed high cell viability (> 90%), while low cytotoxicity (< 25%) was estimated from the LDH assay (Fig. 3B) for all tested concentrations. At 4 mg/mL of exposure dose, maximum cell viability (> 95%) and minimal cytotoxicity (<10%) were obtained. Therefore, this dosage was selected for subsequent experiments.

3.2. Treatment with Kiwi FFG® influences the metabolic production of the human colonic microbiota

The impact caused by Kiwi FFG® on the metabolic production of the intestinal microbiota was evaluated using two approaches. First, analyses of gut health biomarkers (*i.e.*, SCFA, lactate, ammonia) were conducted. Then, a cell-based reporter gene analysis allowed to assess the influence of kiwi's colonic fermentation output on AhR signaling

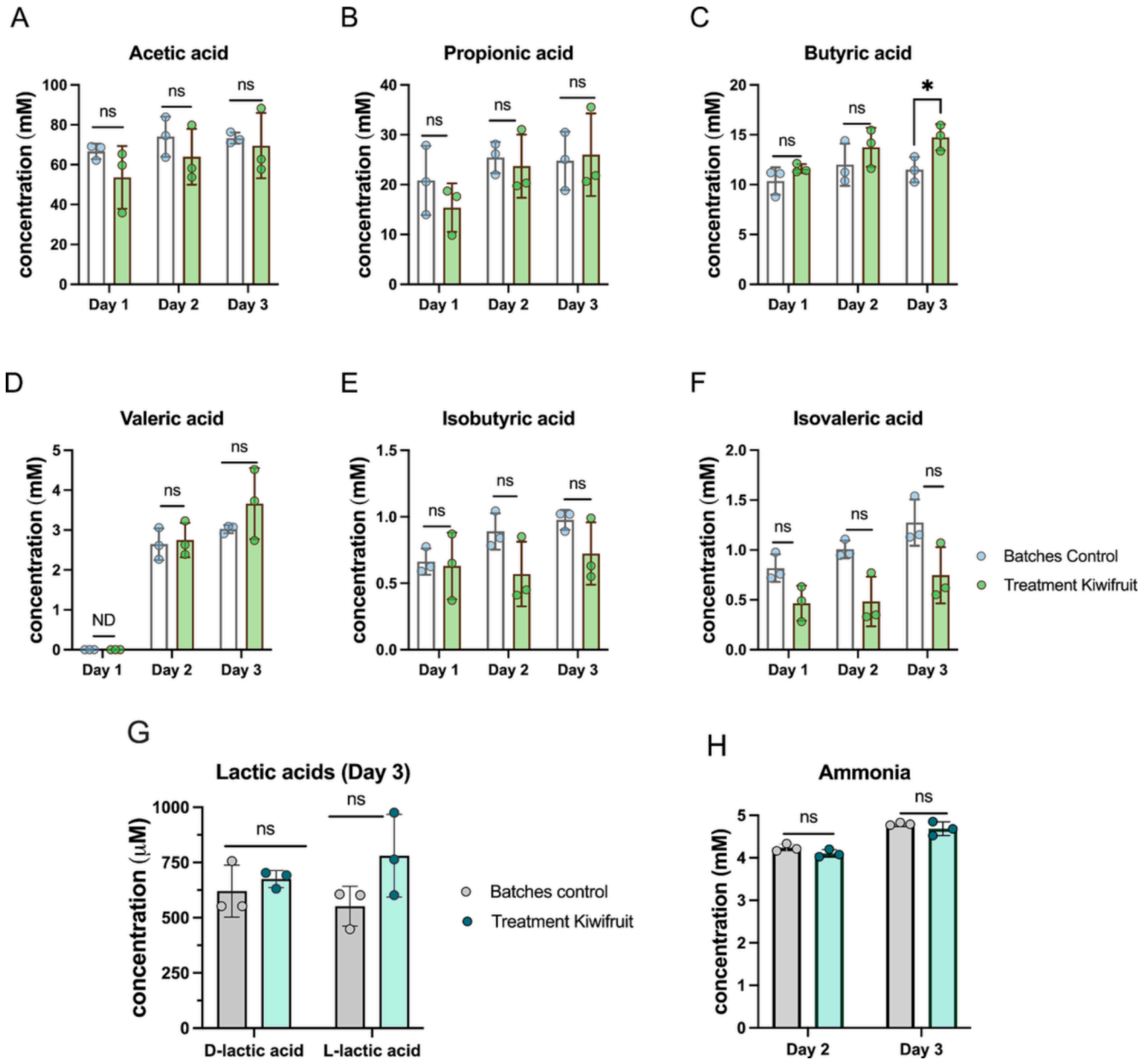

Fig. 4. Microbial production of SCFA (**A-D**), BCFA (**E**, **F**), D/L-lactic acids (**G**), and ammonia (**H**) induced by green kiwifruit powder Kiwi FFG® treatment (2.4 g) in the static human gastrointestinal simulation (3 days). Data are shown as molar concentration (μM or mM) and error bars represent standard deviation. Comparisons with the parallel control experiment were done using Unpaired *t*-test or Mann-Whitney test (depending on data normality), non-significant (ns) ($P > 0.05$), * ($P \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathways.

SCFAs are major end-products of bacterial anaerobic breakdown of non-digestible carbohydrates in the human gut. In human intestines, potassium, calcium, magnesium, and ammonia represent the major cations and SCFAs are the dominant anions (Cummings & Macfarlane, 1991). The proper balance of these metabolites is fundamental for colon motility and therefore crucial in the context of constipation (Q. Zhao et al., 2021). In Fig. 4 and Fig. 5 are shown the results of the quantification of SCFA, lactate and ammonia during the simulated fermentation of the standardized powder Kiwi FFG® in the static- and dynamic-SHIME® systems, respectively.

The treatment with Kiwi FFG® caused increased production of butyrate in the intestinal ecosystem. In the case of the static-SHIME®, this effect was seen by the end of the fermentation (day 3) as shown in Fig. 4C. The microbial productions of other SCFA, lactate and ammonia were not significantly changed by kiwifruit treatment in the static colon simulation.

In the dynamic gastrointestinal simulation, butyric acid was significantly increased in TC and DC compartments after 15 days. Such increase returned to the initial (control) levels in TC once the treatment stopped (after the washout period), as shown in Fig. 5C. Raised levels of butyrate in the colon are considered a positive health biomarker. This short-chain fatty acid reinforces the epithelial defense barrier and has a positive influence on mucosal inflammation and the oxidative status of the gastrointestinal tract (Canani et al., 2011). Moreover, high concentrations of butyrate can stimulate water and electrolyte absorption which can reduce stool volume (Zhao & Yu, 2016), particularly important in constipated subjects (Pituch et al., 2013). Indeed, butyrate supplementation in mice has shown important benefits in the context of slow-transit constipation (Ge et al., 2017). Our *in vitro* results suggested that the standardized green kiwifruit powder Kiwi FFG® is a promoter of butyrate secretion in the colon, which is in line with reports

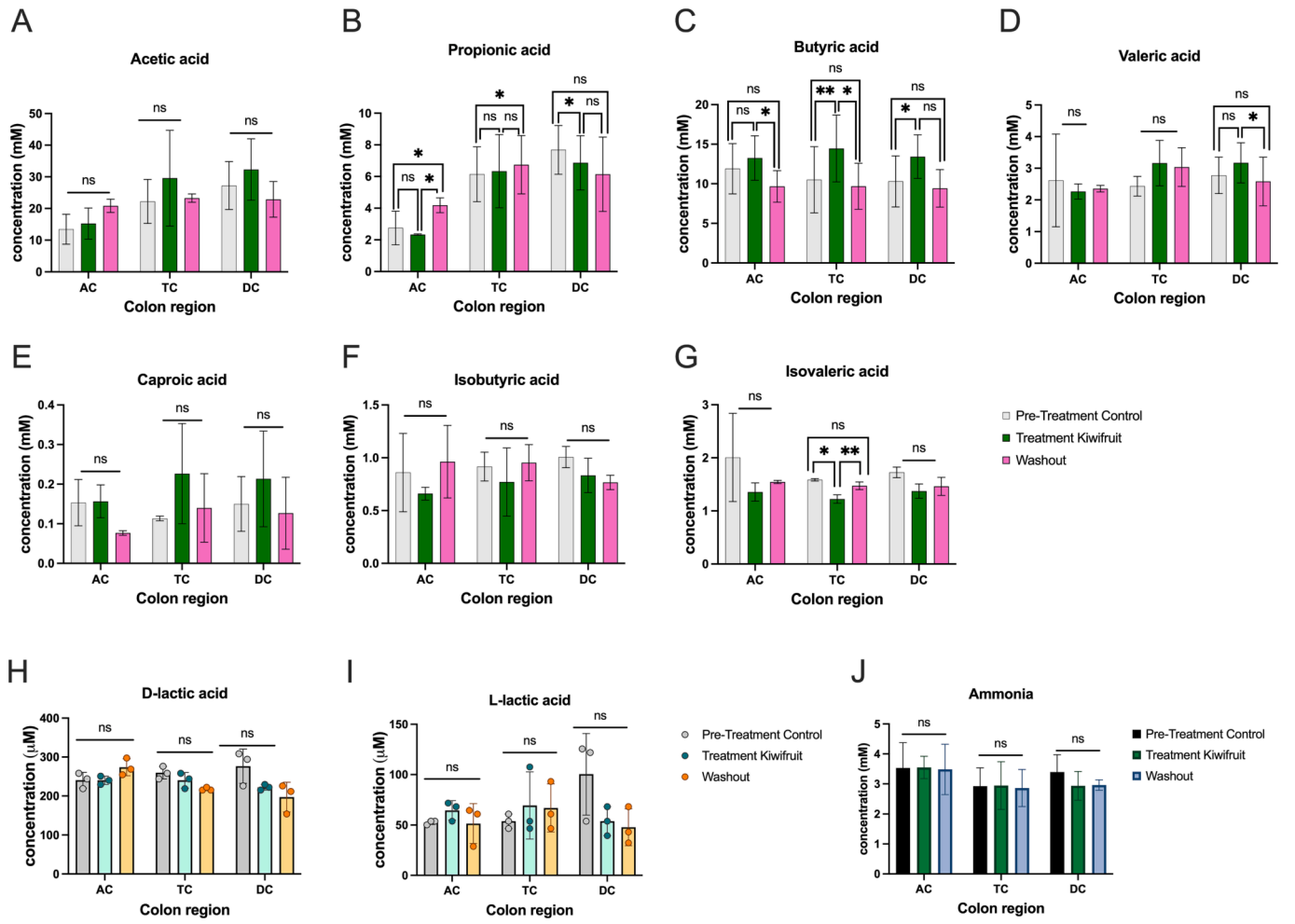

Fig. 5. Microbial production of SCFA (**A**-**E**), BCFA (**F**, **G**), D/L-lactic acids (**H**, **I**), and ammonia (**J**) induced by FFG® green kiwifruit powder treatment in AC, TC, DC regions of the dynamic human gastrointestinal simulation. Mean concentration (μM or mM) are shown and error bars represent standard deviation. Comparisons relative to the pre-treatment control were done depending on data normality using Repeated-Measures One-way ANOVA or Friedman test. non-significant (ns), ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on the usefulness of the fruit in constipation symptoms alleviation. However, the mechanisms determining the laxative effects of kiwifruits are multifactorial and still to be closer investigated (Eltorki et al., 2022).

During the treatment with Kiwi FFG® in the dynamic-SHIME® model, propionic acid (Fig. 5B) remained relatively invariable in AC and TC, but a decrease in DC section was identified. After the washout period, a tendency to increase propionate production was observed in AC and TC sections. Thus, Kiwi FFG® supplementation did not influence the production of propionate. A recent study of the role of SCFA in constipation suggested that acetic acid and butyric acid, but not propionic acid could relieve constipation (Wang et al., 2020). However, the tendency to increase the acetate concentration in the dynamic-SHIME® experiment during the treatment was not significant either in any of the colon regions.

Meanwhile, Kiwi FFG® treatment induced a significant decrease in the production of isovaleric acid (Fig. 5G) in TC region. High fecal levels of the branched-chain fatty acids (BCFA) isovaleric and isobutyric have been associated with aging (Rios-Covian et al., 2020). Furthermore, isovalerate in human stool is correlated with a positive diagnosis of depression and elevated cortisol levels (Szczesniak et al., 2016). Therefore, the herein observed decrease of isovaleric acid with Kiwi FFG® treatment can be considered a beneficial outcome.

As a key mediator of host-microbiota interplay (Dong & Perdew, 2020), the AhR transcriptional activation induced by the intestinal metabolic output of the kiwifruit fermentation in the SHIME® systems was analyzed and results are presented in Fig. 6. None of the fermentation samples caused a decrease in cell viability greater than 10% in the MTT assays simultaneously conducted (data not shown).

In the static system (Fig. 6A), no significant differences were observed compared to the control experiment after 24 h and 48 h of fermentation. However, at the end (72 h), a decrease in AhR transactivity was identified compared to the control. On the opposite, a significant increase in AhR transcription was induced during the fermentation of Kiwi FFG® in the dynamic-SHIME® system after 15 days of administration of the fruit.

Intestinal AhR activity is directly correlated with the abundance of microbiota-derived tryptophan catabolites such as indole and kynurenic acid (Dong et al., 2020). Bacterial tryptophan metabolites are fundamental to maintain intestinal and systemic homeostasis and are considered important signaling molecules in host-microbial crosstalk (Roager & Licht, 2018). Kiwi FFG® caused a significant increase in AhR transcription in the TC (Fig. 6C) and, to a lesser extent, in the DC region (Fig. 6D) after 15 days of treatment. The microbial production of metabolites causing AhR transcriptional activation was consistently maintained in the dynamic-SHIME® even two weeks after stopping the treatment. These results were in line with the recent finding of a synergistic effect of SCFAs, particularly butyrate, with gut microbiota derived AhR ligands (Modoux et al., 2022). Thus, the increase of butyrate concentration in TC and DC regions (see Fig. 5) correlates with the AhR transactivity displayed by the metabolic pool in human intestinal

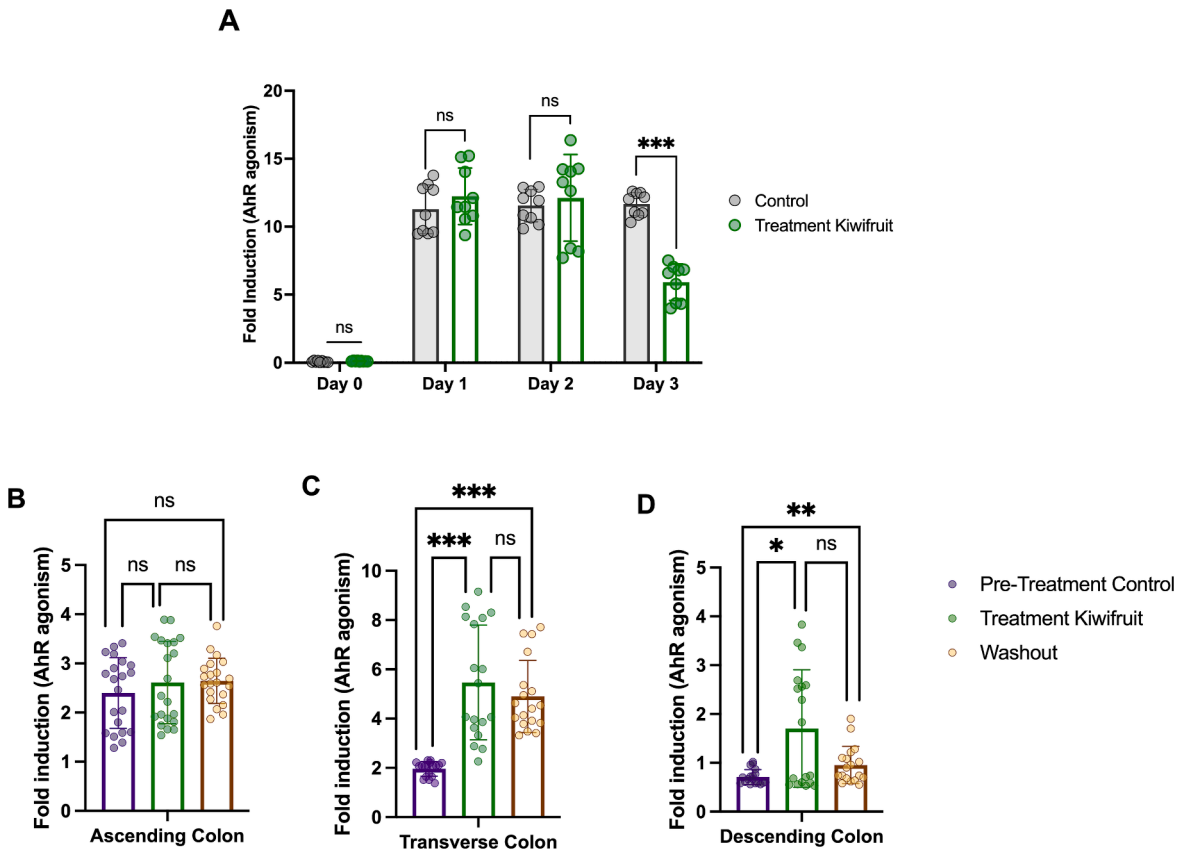

Fig. 6. AhR fold induction in HT29_Lucia cells of the metabolic output from the static-SHIME® experiment (**A**) and the ascending (**B**), transverse (**C**) and descending (**D**) colon sections of the dynamic-SHIME® experiment. **A.** Short-term fermentation (3 days) on the presence of 2.4 g of the green kiwifruit powder Kiwi FFG® (treatment) or in the absence (control). Technical replicates of the triplicate batches are shown (n = 9). Unpaired *t*-test or Mann-Whitney test (depending on data normality) were applied for comparisons, non-significant (ns) ($P > 0.05$), *** ($P \leq 0.001$). **B, C, D.** Three time points of the dynamic-SHIME® experiment were selected corresponding to the pre-treatment control, the end of the 2-weeks treatment, and the end of the washout period. Technical replicates of the triplicate SHIME® are shown (n > 9) and error bars represent standard deviation. Repeated-Measures One-way ANOVA were applied for comparisons, ns ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cells. However, in the static system, the increase of butyrate observed by the end of the fermentation was not extrapolated to an AhR activation. This suggests that the synergistic effects of butyrate on AhR activation are probably less linear when multiple interferences are involved, as expected in a short-term static fermentation where metabolites accumulate (van de Wiele et al., 2015).

3.3. Kiwi FFG® seems to have a mild anti-inflammatory activity in Caco-2 cells

Bioluminescent immunoassays (Lumit™ Technology) were used for detecting the proinflammatory human interleukin-6 (IL-6) and the anti-inflammatory human interleukin-10 (IL-10). Caco-2 cells were exposed to increasing doses of Kiwi FFG® and to the supernatants of Kiwi FFG® fermentation in the SHIME® systems. Results of the quantification of these interleukins upon 24 h exposures to Caco-2 cells are shown in Fig. 7.

All doses of Kiwi FFG® caused a reduction of IL-6 production (Fig. 7A), which was significant in some of the exposure doses tested (*i.e.*, 1 mg/mL, 4 mg/mL, 5 mg/mL). As shown in Fig. 7B, at the maximum exposure concentration of Kiwi FFG® (5 mg/mL), a significant increase of IL-10 was identified. For the rest of the exposure doses, the production of IL-10 remained relatively invariable.

On the other hand, the metabolic pool at the end of the fermentation (72 h) of the static-SHIME® caused a significant decrease in IL-6 production (Fig. 7C) while no significant change in IL-10 concentration was identified when compared with the parallel control experiment (Fig. 7D).

In line with the results of the static-SHIME®, the fermentation supernatants from AC and TC regions of the dynamic-SHIME® experiment caused a significant reduction of the IL-6 produced by Caco-2 cells when compared with the pre-treatment control time point (Fig. 7E). Therefore, a mild anti-inflammatory effect can be attributed to Kiwi FFG® as both, the direct exposure, and the microbiota-derived metabolic output reduced the IL-6 produced by Caco-2 cells. Abundant bioactive compounds in kiwifruit such as polyphenols and galactolipids are known to reduce inflammation (Bentley-Hewitt et al., 2020). Besides, kiwifruit protein molecules such as the peptide kissper have displayed anti-inflammatory and antioxidant potential in *ex vivo* and *in vitro* human intestinal models (Ciacci et al., 2014). Recently, another human gastrointestinal tract simulation study reported the impact of a total gut restoration system (*i.e.*, five probiotic *Bacillus* strains, a prebiotic mixture, an immunoglobulin concentrate, amino acids and prebiotic flavonoids) on the production of IL-6 and IL-10 in a Caco-2/THP1 coculture, which benefited the gut microbiome in inflammatory bowel disease (IBD) (Marzorati et al., 2022).

The analysis of metabolites herein presented suggests a positive impact of Kiwi FFG® in the colonic ecosystem of mild constipated women. On one side, the large intestinal environment and the absorption process from the lumen could benefit from the observed increase in microbially produced SCFA, the decrease of the BCFA isovalerate and even the maintenance of ammonia and D/L-lactic acids levels (Havenaar, 2011; Russell et al., 2013). On the other hand, the activation of AhR signaling caused by kiwifruit fermentation could be related to the

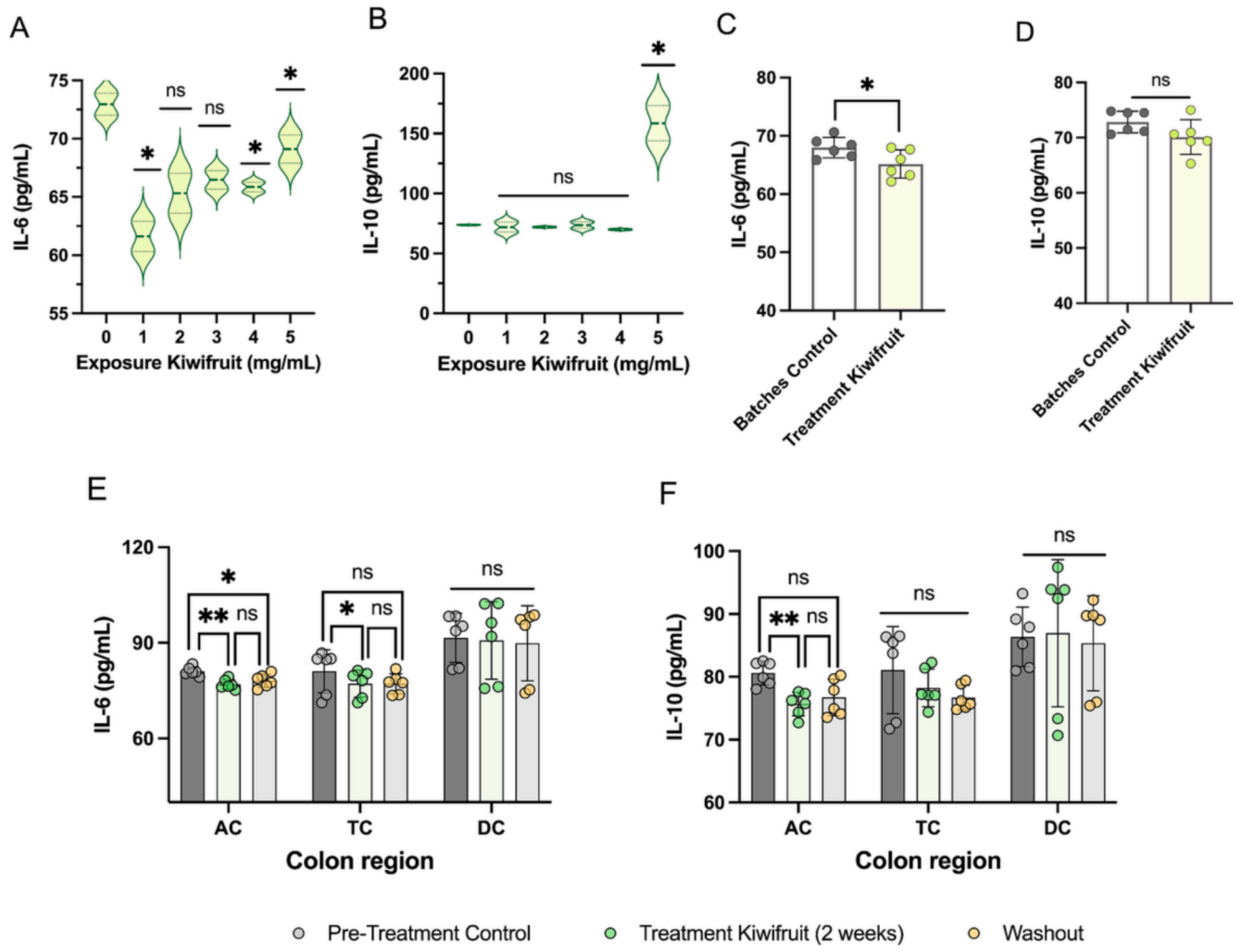

Fig. 7. Lumit™ detection of IL-6 and IL-10 production in Caco-2 cells exposed to the green kiwifruit powder Kiwi FFG® (**A**, **B**), and to fermentation samples at the end (day 3) of the static-SHIME® (**C, D**), and on the three colon regions (AC, TC, DC) of the dynamic SHIME® experiment (**E, F**). Results are shown as concentrations (pg/mL) of IL-6 and IL-10 estimated from the interpolation in their respective calibration curves. Error bars represent standard deviation. Unpaired t-tests were used for comparisons of kiwifruit powder serial dilutions *versus* no kiwi (0 mg/mL- water control) and for comparisons with the parallel control in the static-SHIME® experiment. Repeated-Measures One-way ANOVA was used for comparing samples from the pre-treatment control in the dynamic-SHIME® *versus* the end of the treatment or the end of the washout period, non-significant (ns) ($P > 0.05$), * ($P \leq 0.05$), ** ($P \leq 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

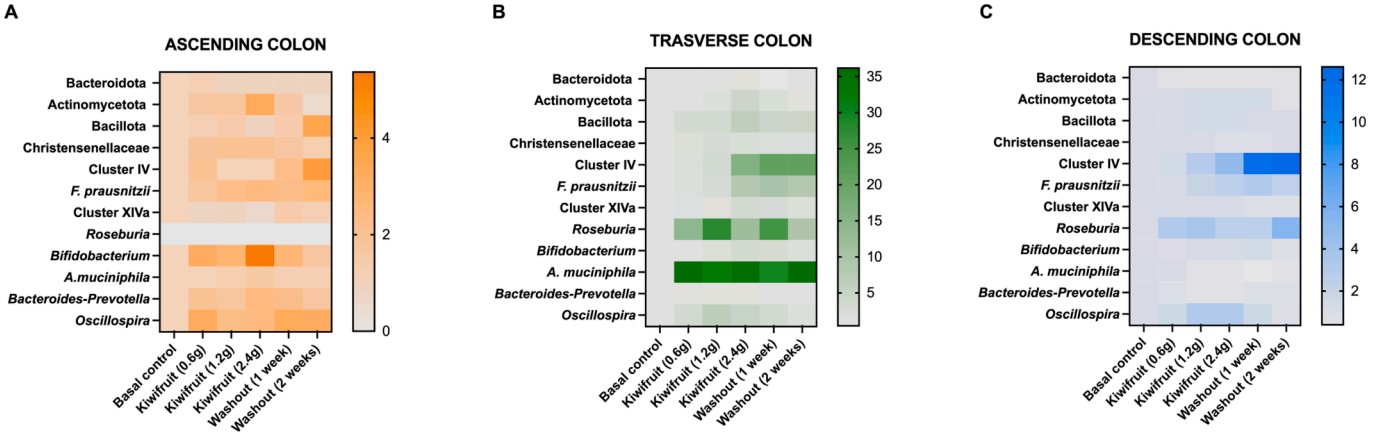

Fig. 8. Evolution of target bacterial populations measured by qPCR in the ascending (A), transverse (B), and descending (C) colon regions of the dynamic -SHIME® experiment testing the green kiwifruit powder Kiwi FFG®. Mean values represented in the heat maps correspond to a relative quantification using the $2^{-\Delta\Delta Cq}$ method. Detailed graphical representations including error measurements and statistical comparisons are provided as Supplementary Material (Table S2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulation of immune and inflammatory responses of colonic epithelial cells (Lamas et al., 2018; Yoshimatsu et al., 2022). Consistently, microbiota-derived metabolites from kiwifruit fermentation caused a mild decreased in IL-6 production. This pleiotropic pro-inflammatory cytokine is associated with colorectal cancer angiogenesis and IBD pathogenesis (Atreya & Neurath, 2005; Nagasaki et al., 2014).

3.4. Long-term colonic fermentation identifies a positive impact of Kiwi FFG® on gut microbial communities

For the analysis of the evolution of microbial populations during the fermentation experiments, target populations known to have a direct association with health in the intestinal ecosystem were measured. Results for all target taxon and statistical comparisons with the control experiment or pre-treatment control for the static and dynamic SHIME® experiment, respectively, are provided as Supplementary Material (Tables S1 and S2).

In the Table S1 is shown that Kiwi FFG® treatment did not cause a great impact in any of the target bacterial communities evaluated during the 3-days fermentation of the static-SHIME® experiment. In fact, some butyrate producing populations such as Cluster IV, Cluster XIV and *Roseburia* genus decreased by the end of the fermentation where, according to the metabolites analysis, an increase of butyrate was identified. *A. muciniphila* was reduced on the first and second day of fermentation and *Bacteroides-Prevotella* genera decreased compared to the control on the second day. *Bifidobacterium* genus was found unchanged by Kiwi FFG® treatment. These results could be a consequence of the high substrate depletion rate in the static-SHIME®. Although the pH regulation used herein may somehow ameliorate the weak microbiological control typical of static fermentations, the accumulation of toxic products and the growth arrest of bacterial populations are well-known limitations of batch fermentation systems (Payne et al., 2012). Furthermore, other studies have also not identified alterations in some of these bacterial populations or in the microbial abundance during simulated green kiwifruit gastrointestinal digestion compared to the parallel control experiment (Katsirma et al., 2021; Parkar et al., 2012).

On the other hand, detailed analysis of thirteen microbial populations in the ascending, transverse, and descending colons of the dynamic-SHIME® experiment revealed an overall positive and dose-dependent influence of Kiwi FFG® treatment in the gastrointestinal microbiota (Fig. 8). Individual values and statistical comparisons are provided in Table S2.

As summarized in Fig. 8A, in the ascending colon region, health-related populations such as *Bacteroides-Prevotella* and *Bifidobacterium* genera were significantly increased upon Kiwi FFG® treatment. These results are consistent with previous studies using *in vitro* batch fermentation models (48 h) of gastric-ileal digestion and fecal fermentation of gold and green kiwifruit (Blatchford et al., 2015; Parkar et al., 2012). The herein observed bifidogenic effect was dose-dependent, as a maximum increase of the genus was reached by the end of the second week of treatment and gradually decreased during the washout period.

The induction of butyrogenic effects on the intestinal microbiota was corroborated based on specific microbial colonization. Indeed, butyrate producing bacteria such as *F. prausnitzii* species and Cluster IV group were found to increase in the three colon regions due to Kiwi FFG® supplementation. Similarly, human clinical trials involving gold kiwifruit (Livaux™) formulations have caused an increase of commensal *F. prausnitzii* which was claimed as a biomarker for mitigation of functional constipation symptoms and for reducing gut inflammatory conditions (Blatchford et al., 2017). Furthermore, in our dynamic-SHIME® experiment, the major butyrate producing *Roseburia* genus was boosted in a no-dose dependent manner in TC and DC, as shown in Fig. 8B and Fig. 8C, respectively. As a microbial community with important roles in metabolic and anti-inflammatory gastrointestinal processes (Kumari et al., 2013; la Rosa et al., 2019), *Roseburia*'s increase was considered a very positive outcome of kiwifruit treatment.

Another positive impact that we attributed to Kiwi FFG® in the simulated microbial ecosystem, was the promotion of a significant increase (in TC) in the mucin-degrading bacterium *A. muciniphila* (Fig. 8B). Likewise, other authors have reported an increase in the *Akkermansia* genus upon gold-fleshed kiwifruit treatment (Parkar et al., 2018). *A. muciniphila* species is an intestinal symbiont suggested to be included as part of the new generation of therapeutic probiotics (Zhang et al., 2019). Among its functions is the regulation of mucus thickness and gut barrier integrity (Ottman et al., 2017). Furthermore, *A. muciniphila* presence in the gut microbiota is a bioindicator of human cardiometabolic health (Lakshmanan et al., 2022).

CRediT authorship contribution statement

Elizabeth Goya-Jorge: Conceptualization, Methodology, Validation, Investigation, Visualization, Formal analysis, Data Curation, Writing - Original Draft, Writing – Review & Editing. **Pauline Bondue:** Validation, Investigation, Resources, Funding acquisition, Project administration, Writing – Review & Editing. **Irma Gonza:** Validation, Investigation, Writing – Review & Editing. **Fanny Laforêt:** Validation, Investigation, Funding acquisition, Project administration, Writing – Review & Editing. **Céline Antoine:** Visualization, Writing – Review & Editing. **Samiha Boutaleb:** Validation, Investigation, Writing – Review & Editing. **Caroline Douny:** Validation, Investigation, Writing – Review & Editing. **Marie-Louise Scippo:** Validation, Investigation, Supervision, Writing – Review & Editing. **Jeoffrey Christyn de Ribaucourt:** Resources, Funding acquisition, Project administration, Writing – Review & Editing. **Fabienne Crahay:** Resources, Writing – Review & Editing. **Véronique Delcenserie:** Conceptualization, Methodology, Supervision, Funding acquisition, Project administration, Writing – Review & Editing.

Declaration of Competing Interest

This research work was conducted in the context of an Academic-Industry collaboration financially supported by the Walloon Region (Belgium) to investigate innovative nutritional ingredients to stimulate transit and improve intestinal health. The industrial partner Ortis S.A. coordinated the project. The University of Liège declare no conflict of interest in the results herein presented. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research work was financially supported by GutTransit Project, Wagralim, Wallonia, Belgium (Grant number: 8225). The authors would like to acknowledge the contributions of Dr. Ambre Riba in the design of the study. The Graphical Abstract was created with BioRender.com.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113348.

References

Abdul Hamid, N. A., Mediani, A., Maulidiani, M., Abas, F., Park, Y. S., Leontowicz, H., Leontowicz, M., Namiesnik, J., & Gorinstein, S. (2017). Characterization of metabolites in different kiwifruit varieties by NMR and fluorescence spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis, 138*, 80–91. https://doi.org/10.1016/j.jpba.2017.01.046

Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora Ecology of the Chicken Intestine Using 16S Ribosomal DNA Primers. *Poultry Science, 83*(7), 1093–1098. https://doi.org/10.1093/ps/83.7.1093

Atreya, R., & Neurath, M. F. (2005). Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. *Clinical Reviews in Allergy & Immunology, 28*(3), 187–196. https://doi.org/10.1385/CRIAI:28:3:187

Bacchetti De Gregoris, T., Aldred, N., Clare, A. S., & Burgess, J. G. (2011). Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. *Journal of Microbiological Methods, 86*(3), 351–356. https://doi.org/10.1016/j.mimet.2011.06.010

Bayer, S. B., Gearry, R. B., & Drummond, L. N. (2018). Putative mechanisms of kiwifruit on maintenance of normal gastrointestinal function. *Critical Reviews in Food Science and Nutrition, 58*(14), 2432–2452. https://doi.org/10.1080/10408398.2017.1327841

Bentley-Hewitt, K. L., Perrott, M., Butts, C. A., Hedderley, D. I., Stoklosinski, H. M., & Parkar, S. G. (2020). Influence of kiwifruit on gastric and duodenal inflammation-related gene expression in aspirin-induced gastric mucosal damage in rats. *Scientific Reports, 10*(1), 13055. https://doi.org/10.1038/s41598-020-70006-0

Bharucha, A. E., & Lacy, B. E. (2020). Mechanisms, Evaluation, and Management of Chronic Constipation. *Gastroenterology, 158*(5), 1232–1249.e3. https://doi.org/10.1053/j.gastro.2019.12.034

Biagini, F., Daddi, C., Calvigioni, M., De Maria, C., Zhang, Y. S., Ghelardi, E., & Vozzi, G. (2023). Designs and methodologies to recreate in vitro human gut microbiota models. *Bio-Design and Manufacturing, 6*(3), 298–318. https://doi.org/10.1007/s42242-022-00210-6

Blatchford, P., Stoklosinski, H., Eady, S., Wallace, A., Butts, C., Gearry, R., Gibson, G., & Ansell, J. (2017). Consumption of kiwifruit capsules increases Faecalibacterium prausnitzii abundance in functionally constipated individuals: A randomised controlled human trial. *Journal of Nutritional Science, 6*, e52.

Blatchford, P., Stoklosinski, H., Walton, G., Swann, J., Gibson, G., Gearry, R., & Ansell, J. (2015). Kiwifruit fermentation drives positive gut microbial and metabolic changes irrespective of initial microbiota composition. *Bioactive Carbohydrates and Dietary Fibre, 6*(1), 37–45. https://doi.org/10.1016/j.bcdf.2015.07.001

Bustin, S. A., Beaulieu, J.-F., Huggett, J., Jaggi, R., Kibenge, F. S. B., Olsvik, P. A., Penning, L. C., & Toegel, S. (2010). MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Molecular Biology, 11*(1), 74. https://doi.org/10.1186/1471-2199-11-74

Caballero, N., Benslaiman, B., Ansell, J., & Serra, J. (2020). The effect of green kiwifruit on gas transit and tolerance in healthy humans. *Neurogastroenterology & Motility, 32*(9), e13874.

Canani, R. B., Costanzo, M. D., Leone, L., Pedata, M., Meli, R., & Calignano, A. (2011). Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World Journal of Gastroenterology, 17*(12), 1519–1528. https://doi.org/10.3748/wjg.v17.i12.1519

Carnachan, S. M., Bootten, T. J., Mishra, S., Monro, J. A., & Sims, I. M. (2012). Effects of simulated digestion in vitro on cell wall polysaccharides from kiwifruit (Actinidia spp.). *Food Chemistry, 133*(1), 132–139. https://doi.org/10.1016/j.foodchem.2011.12.084

Chen, A. G. Y., Offereins, M. S. L., Mulder, C. J., Frampton, C. M., & Gearry, R. B. (2018). A Pilot Study of the Effect of Green Kiwifruit on Human Intestinal Fermentation Measured by Hydrogen and Methane Breath Testing. *Journal of Medicinal Food, 21*(12), 1295–1298. https://doi.org/10.1089/jmf.2018.4179

Ciacci, C., Russo, I., Bucci, C., Iovino, P., Pellegrini, L., Giangrieco, I., Tamburrini, M., & Ciardiello, M. A. (2014). The kiwi fruit peptide kissper displays anti-inflammatory and anti-oxidant effects in in-vitro and ex-vivo human intestinal models. *Clinical and Experimental Immunology, 175*(3), 476–484. https://doi.org/10.1111/cei.12229

Collins, B. H., Horská, A., Hotten, P. M., Riddoch, C., & Collins, A. R. (2001). Kiwifruit Protects Against Oxidative DNA Damage in Human Cells and In Vitro. *Nutrition and Cancer, 39*(1), 148–153. https://doi.org/10.1207/S15327914nc391_20

Cummings, J. H., & Macfarlane, G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *Journal of Applied Bacteriology, 70*(6), 443–459. https://doi.org/10.1111/j.1365-2672.1991.tb02739.x

Decker, T., & Lohmann-Matthes, M. L. (1988). A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *Journal of Immunological Methods, 115*(1), 61–69. https://doi.org/10.1016/0022-1759(88)90310-9

Dong, F., Hao, F., Murray, I. A., Smith, P. B., Koo, I., Tindall, A. M., Kris-Etherton, P. M., Gowda, K., Amin, S. G., Patterson, A. D., & Perdew, G. H. (2020). Intestinal microbiota-derived tryptophan metabolites are predictive of Ah receptor activity. *Gut Microbes, 12*(1), 1788899. https://doi.org/10.1080/19490976.2020.1788899

Dong, F., & Perdew, G. H. (2020). The aryl hydrocarbon receptor as a mediator of host-microbiota interplay. *Gut Microbes, 12*(1), 1859812. https://doi.org/10.1080/19490976.2020.1859812

Douglas, A. E. (2019). Simple animal models for microbiome research. *Nature Reviews Microbiology, 17*(12), 764–775. https://doi.org/10.1038/s41579-019-0242-1

Douny, C., Dufourny, S., Brose, F., Verachtert, P., Rondia, P., Lebrun, S., Marzorati, M., Everaert, N., Delcenserie, V., & Scippo, M.-L. (2019). Development of an analytical method to detect short-chain fatty acids by SPME-GC-MS in samples coming from an in vitro gastrointestinal model. *Journal of Chromatography B, 1124*, 188–196. https://doi.org/10.1016/j.jchromb.2019.06.013

Drummond, L. (2013). The Composition and Nutritional Value of Kiwifruit. In M. Boland & P. J. B. T.-A. in F. and N. R. Moughan (Eds.), *Nutritional Benefits of Kiwifruit* (Vol. 68, pp. 33–57). Academic Press. https://doi.org/10.1016/B978-0-12-394294-4.00003-1.

Du, G., Li, M., Ma, F., & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. *Food Chemistry, 113*(2), 557–562. https://doi.org/10.1016/j.foodchem.2008.08.025

Eltorki, M., Leong, R., & Ratcliffe, E. M. (2022). Kiwifruit and Kiwifruit Extracts for Treatment of Constipation: A Systematic Review and Meta-Analysis. *Canadian Journal of Gastroenterology and Hepatology, 2022*, 7596920. https://doi.org/10.1155/2022/7596920

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., Guiot, Y., Derrien, M., Muccioli, G. G., Delzenne, N. M., de Vos, W. M., & Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences, 110*(22), 9066–9071. https://doi.org/10.1073/pnas.1219451110

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology, 9*(10), 577–589. https://doi.org/10.1038/nrgastro.2012.156

Ge, X., Zhao, W., Ding, C., Tian, H., Xu, L., Wang, H., Ni, L., Jiang, J., Gong, J., Zhu, W., Zhu, M., & Li, N. (2017). Potential role of fecal microbiota from patients with slow transit constipation in the regulation of gastrointestinal motility. *Scientific Reports, 7*(1), 441. https://doi.org/10.1038/s41598-017-00612-y

Gearry, R., Fukudo, S., Barbara, G., Kuhn-Sherlock, B., Ansell, J., Blatchford, P., Eady, S., Wallace, A., Butts, C., Cremon, C., Barbaro, M. R., Pagano, I., Okawa, Y., Muratubaki, T., Okamoto, T., Fuda, M., Endo, Y., Kano, M., Kanazawa, M., … Drummond, L. (2023). Consumption of 2 Green Kiwifruits Daily Improves Constipation and Abdominal Comfort-Results of an International Multicenter Randomized Controlled Trial. In *The American Journal of Gastroenterology*. https://doi.org/10.14309/ajg.0000000000002124.

Goya-Jorge, E., Gonza, I., Bondue, P., Douny, C., Taminiau, B., Daube, G., Scippo, M.-L., & Delcenserie, V. (2022). Human Adult Microbiota in a Static Colon Model: AhR Transcriptional Activity at the Crossroads of Host-Microbe Interaction. *Foods, 11*(13), 1946. https://doi.org/10.3390/foods11131946

Havenaar, R. (2011). Intestinal health functions of colonic microbial metabolites: A review. *Beneficial Microbes, 2*(2), 103–114. https://doi.org/10.3920/BM2011.0003

Haytowitz, D. B., Ahuja, J. K. C., Wu, X., Somanchi, M., Nickle, M., Nguyen, Q. A., Roseland, J. M., Williams, J. R., Patterson, K. Y., Li, Y., & Pehrsson, P. R. (2019). *USDA National Nutrient Database for Standard Reference, Legacy Release.* Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. https://data.nal.usda.gov/dataset/usda-national-nutrient-database-standard-reference-legacy-release.

Jiang, S., Xie, S., Lv, D., Zhang, Y., Deng, J., Zeng, L., & Chen, Y. (2016). A reduction in the butyrate producing species Roseburia spp. and Faecalibacterium prausnitzii is associated with chronic kidney disease progression. *Antonie van Leeuwenhoek, 109*(10), 1389–1396. https://doi.org/10.1007/s10482-016-0737-y

Katsirma, Z., Dimidi, E., Rodriguez-Mateos, A., & Whelan, K. (2021). Fruits and their impact on the gut microbiota, gut motility and constipation. *Food & Function, 12*(19), 8850–8866. https://doi.org/10.1039/D1FO01125A

Kim, E. R., & Rhee, P.-L. (2012). How to interpret a functional or motility test - colon transit study. *Journal of Neurogastroenterology and Motility, 18*(1), 94–99. https://doi.org/10.5056/jnm.2012.18.1.94

Kumari, R., Ahuja, V., & Paul, J. (2013). Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India. *World Journal of Gastroenterology, 19*(22), 3404–3414. https://doi.org/10.3748/wjg.v19.i22.3404

Kurina, I., Popenko, A., Klimenko, N., Koshechkin, S., Chuprikova, L., Filipenko, M., Tyakht, A., & Alexeev, D. (2020). Development of qPCR platform with probes for quantifying prevalent and biomedically relevant human gut microbial taxa. *Molecular and Cellular Probes, 52*, Article 101570. https://doi.org/10.1016/j.mcp.2020.101570

la Rosa, S. L., Leth, M. L., Michalak, L., Hansen, M. E., Pudlo, N. A., Glowacki, R., Pereira, G., Workman, C. T., Arntzen, M.Ø., Pope, P. B., Martens, E. C., Hachem, M. A., & Westereng, B. (2019). The human gut Firmicute Roseburia intestinalis is a primary degrader of dietary β-mannans. *Nature Communications, 10*(1), 905. https://doi.org/10.1038/s41467-019-08812-y

Lakshmanan, A. P., Murugesan, S., al Khodor, S., & Terranegra, A. (2022). The potential impact of a probiotic: Akkermansia muciniphila in the regulation of blood pressure—the current facts and evidence. *Journal of Translational Medicine, 20*(1), 430. https://doi.org/10.1186/s12967-022-03631-0.

Lamas, B., Natividad, J. M., & Sokol, H. (2018). Aryl hydrocarbon receptor and intestinal immunity. *Mucosal Immunology, 11*(4), 1024–1038. https://doi.org/10.1038/s41385-018-0019-2

Latocha, P., Łata, B., & Stasiak, A. (2015). Phenolics, ascorbate and the antioxidant potential of kiwiberry vs. common kiwifruit: The effect of cultivar and tissue type. *Journal of Functional Foods, 19*, 155–163. https://doi.org/10.1016/j.jff.2015.09.024

Lewis, S. J., & Heaton, K. W. (1997). Stool Form Scale as a Useful Guide to Intestinal Transit Time. *Scandinavian Journal of Gastroenterology, 32*(9), 920–924. https://doi.org/10.3109/00365529709011203

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Louis, P., Scott, K. P., Duncan, S. H., & Flint, H. J. (2007). Understanding the effects of diet on bacterial metabolism in the large intestine. *Journal of Applied Microbiology, 102*(5), 1197–1208. https://doi.org/10.1111/j.1365-2672.2007.03322.x

Mackie, R. I., Aminov, R. I., Hu, W., Klieve, A. V., Ouwerkerk, D., Sundset, M. A., & Kamagata, Y. (2003). Ecology of uncultivated Oscillospira species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. *Applied and Environmental Microbiology, 69*(11), 6808–6815. https://doi.org/10.1128/AEM.69.11.6808-6815.2003

Marzorati, M., Bubeck, S., Bayne, T., Krishnan, K., & Giusto, M. (2022). Effects of combined prebiotic, probiotic, IgG and amino acid supplementation on the gut microbiome of patients with inflammatory bowel disease. *Future Microbiology, 17*, 1307–1324. https://doi.org/10.2217/fmb-2022-0066

Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T., & Tanaka, R. (2004). Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Applied and Environmental Microbiology, 70* (12), 7220–7228. https://doi.org/10.1128/AEM.70.12.7220-7228.2004

Mishra, S., & Monro, J. (2012). Kiwifruit remnants from digestion in vitro have functional attributes of potential importance to health. *Food Chemistry, 135*(4), 2188–2194. https://doi.org/10.1016/j.foodchem.2012.06.102

Modoux, M., Rolhion, N., Lefevre, J. H., Oeuvray, C., Nádvorník, P., Illes, P., Emond, P., Parc, Y., Mani, S., Dvorak, Z., & Sokol, H. (2022). Butyrate acts through HDAC inhibition to enhance aryl hydrocarbon receptor activation by gut microbiota-derived ligands. *Gut Microbes, 14*(1), 2105637. https://doi.org/10.1080/19490976.2022.2105637

Morel, F. B., Oozeer, R., Piloquet, H., Moyon, T., Pagniez, A., Knol, J., Darmaun, D., & Michel, C. (2015). Preweaning modulation of intestinal microbiota by oligosaccharides or amoxicillin can contribute to programming of adult microbiota in rats. *Nutrition, 31*(3), 515–522. https://doi.org/10.1016/j.nut.2014.09.011

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Nagasaki, T., Hara, M., Nakanishi, H., Takahashi, H., Sato, M., & Takeyama, H. (2014). Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: Anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour–stroma interaction. *British Journal of Cancer, 110*(2), 469–478. https://doi.org/10.1038/bjc.2013.748

Ottman, N., Geerlings, S. Y., Aalvink, S., de Vos, W. M., & Belzer, C. (2017). Action and function of Akkermansia muciniphila in microbiome ecology, health and disease. *Best Practice & Research Clinical Gastroenterology, 31*(6), 637–642. https://doi.org/10.1016/j.bpg.2017.10.001

Parkar, S. G., Rosendale, D., Paturi, G., Herath, T. D., Stoklosinski, H., Phipps, J. E., Hedderley, D., & Ansell, J. (2012). In vitro Utilization of Gold and Green Kiwifruit Oligosaccharides by Human Gut Microbial Populations. *Plant Foods for Human Nutrition, 67*(3), 200–207. https://doi.org/10.1007/s11130-012-0293-1

Parkar, S. G., Simmons, L., Herath, T. D., Phipps, J. E., Trower, T. M., Hedderley, D. I., McGhie, T. K., Blatchford, P., Ansell, J., Sutton, K. H., & Ingram, J. R. (2018). Evaluation of the prebiotic potential of five kiwifruit cultivars after simulated gastrointestinal digestion and fermentation with human faecal bacteria. *International Journal of Food Science & Technology, 53*(5), 1203–1210. https://doi.org/10.1111/ijfs.13697

Payne, A. N., Zihler, A., Chassard, C., & Lacroix, C. (2012). Advances and perspectives in in vitro human gut fermentation modeling. *Trends in Biotechnology, 30*(1), 17–25. https://doi.org/10.1016/j.tibtech.2011.06.011

Pituch, A., Walkowiak, J., & Banaszkiewicz, A. (2013). Butyric acid in functional constipation. *Przeglad Gastroenterologiczny, 8*(5), 295–298. https://doi.org/10.5114/pg.2013.38731

Ramirez-Farias, C., Slezak, K., Fuller, Z., Duncan, A., Holtrop, G., & Louis, P. (2009). Effect of inulin on the human gut microbiota: Stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *The British Journal of Nutrition, 101* (4), 541–550. https://doi.org/10.1017/S0007114508019880

Richardson, D. P., Ansell, J., & Drummond, L. N. (2018). The nutritional and health attributes of kiwifruit: A review. *European Journal of Nutrition, 57*(8), 2659–2676. https://doi.org/10.1007/s00394-018-1627-z

Rinttilä, T., Kassinen, A., Malinen, E., Krogius, L., & Palva, A. (2004). Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology, 97*(6), 1166–1177. https://doi.org/10.1111/j.1365-2672.2004.02409.x

Rios-Covian, D., González, S., Nogacka, A. M., Arboleya, S., Salazar, N., Gueimonde, M., & de los Reyes-Gavilán, C. G. (2020). An Overview on Fecal Branched Short-Chain Fatty Acids Along Human Life and as Related With Body Mass Index: Associated Dietary and Anthropometric Factors. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00973.

Roager, H. M., & Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nature Communications, 9*(1), 3294. https://doi.org/10.1038/s41467-018-05470-4

Rosendale, D. I., Blatchford, P. A., Sims, I. M., Parkar, S. G., Carnachan, S. M., Hedderley, D., & Ansell, J. (2012). Characterizing Kiwifruit Carbohydrate Utilization in vitro and its Consequences for Human Faecal Microbiota. *Journal of Proteome Research, 11*(12), 5863–5875. https://doi.org/10.1021/pr300646m

Roupar, D., Berni, P., Martins, J. T., Caetano, A. C., Teixeira, J. A., & Nobre, C. (2021). Bioengineering approaches to simulate human colon microbiome ecosystem. *Trends in Food Science & Technology, 112*, 808–822. https://doi.org/10.1016/j.tifs.2021.04.035

Russell, W. R., Hoyles, L., Flint, H. J., & Dumas, M.-E. (2013). Colonic bacterial metabolites and human health. *Current Opinion in Microbiology, 16*(3), 246–254. https://doi.org/10.1016/j.mib.2013.07.002

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology, 31*(1), 69–75. https://doi.org/10.1097/MOG.0000000000000139

Szczesniak, O., Hestad, K. A., Hanssen, J. F., & Rudi, K. (2016). Isovaleric acid in stool correlates with human depression. *Nutritional Neuroscience, 19*(7), 279–283. https://doi.org/10.1179/1476830515Y.0000000007

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Knutsen, H. K., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Thies, F., Tsabouri, S., Vinceti, M., Bresson, J.-L., & Siani, A. (2021). Green kiwifruit (lat. Actinidia deliciosa var. Hayward) and maintenance of normal defecation: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006. *EFSA Journal*, *19*(6), e06641. https://doi.org/10.2903/j.efsa.2021.6641.

van de Wiele, T., van den Abbeele, P., Ossieur, W., Possemiers, S., & Marzorati, M. (2015). *The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) BT - The Impact of Food Bioactives on Health: in vitro and ex vivo models* (K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers, Eds.; pp. 305–317). Springer International Publishing. https://doi.org/10.1007/978-3-319-16104-4_27.

Vikrant Berde, C., Salvi Sagar, P., Kajarekar Kunal, V., Joshi Suyoj, A., & Berde Vikrant, B. (2021). Insight into the Animal Models for Microbiome Studies. In P. V. Bramhachari (Ed.), *Microbiome in Human Health and Disease* (pp. 259–273). Springer Singapore. https://doi.org/10.1007/978-981-16-3156-6_13.

Wang, L., Cen, S., Wang, G., Lee, Y., Zhao, J., Zhang, H., & Chen, W. (2020). Acetic acid and butyric acid released in large intestine play different roles in the alleviation of constipation. *Journal of Functional Foods, 69*, Article 103953. https://doi.org/10.1016/j.jff.2020.103953

Wang, R. F., Cao, W. W., & Cerniglia, C. E. (1996). PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Applied and Environmental Microbiology, 62*(4), 1242–1247. https://doi.org/10.1128/aem.62.4.1242-1247.1996

Wang, Z., Feng, Y., Yang, N., Jiang, T., Xu, H., & Lei, H. (2022). Fermentation of kiwifruit juice from two cultivars by probiotic bacteria: Bioactive phenolics, antioxidant activities and flavor volatiles. *Food Chemistry, 373*, Article 131455. https://doi.org/10.1016/j.foodchem.2021.131455

Ward, C., & Courtney, D. (2013). Kiwifruit: Taking its place in the global fruit bowl. *Advances in Food and Nutrition Research, 68*, 1–14. https://doi.org/10.1016/B978-0-12-394294-4.00001-8

Yoshimatsu, Y., Sujino, T., Miyamoto, K., Harada, Y., Tanemoto, S., Ono, K., Umeda, S., Yoshida, K., Teratani, T., Suzuki, T., Mikami, Y., Nakamoto, N., Sasaki, N., Takabayashi, K., Hosoe, N., Ogata, H., Sawada, K., Imamura, T., Yoshimura, A., & Kanai, T. (2022). Aryl hydrocarbon receptor signals in epithelial cells govern the recruitment and location of Helios+ Tregs in the gut. *Cell Reports, 39*(6). https://doi.org/10.1016/j.celrep.2022.110773

Zhang, T., Li, Q., Cheng, L., Buch, H., & Zhang, F. (2019). Akkermansia muciniphila is a promising probiotic. *Microbial Biotechnology, 12*(6), 1109–1125. https://doi.org/10.1111/1751-7915.13410

Zhao, Q., Chen, Y.-Y., Xu, D.-Q., Yue, S.-J., Fu, R.-J., Yang, J., Xing, L.-M., & Tang, Y.-P. (2021). Action Mode of Gut Motility, Fluid and Electrolyte Transport in Chronic Constipation. *Frontiers in Pharmacology, 12*, Article 630249. https://doi.org/10.3389/fphar.2021.630249

Zhao, Y., & Yu, Y.-B. (2016). Intestinal microbiota and chronic constipation. *SpringerPlus, 5*(1), 1130. https://doi.org/10.1186/s40064-016-2821-1