Supplementary Methods

In vitro digestion and *in vitro* colonic fermentation

In vitro digestion was performed according to INFOGEST 2.0 method which was described previously [1, 2]. Ten mL of boiled and cooled tarhana soup was used as the starting material. Cellulose and inulin were also subjected to *in vitro* digestion and colonic fermentation as well as tarhana soups. Five grams of starting material was used for both controls. The entire procedure was performed at a constant temperature of 37°C on a rotator (VWR, Radnor, PA, United States). The oral phase of digestion was performed for 2 minutes at 37°C, during which the sample was mixed with simulated saliva solution containing α-amylase (1,500 U mL−1, Sigma, A0521) and the pH was adjusted to 7. Following the oral phase, the mixture underwent gastric digestion for 2 hours. This phase involved the addition of simulated gastric solution and pepsin enzyme (25,000 U mL−1, Sigma, P6887), maintaining the mixture at 37°C while continuously rotating, with the pH adjusted to 3. The final phase was the intestinal digestion, which included gastric chyme, simulated intestinal fluid electrolyte solution, pancreatin (800 U mL−1, Sigma, P7545), and fresh bile (10 mM), the pH was adjusted to 7.0 and samples were incubated in an incubating rocking platform shaker at 37°C for 2 h. Afterward, the post-intestinal phase liquids were transferred to dialysis tubes and immersed in water as the dialysate for 24 h. The retentate was taken from dialysis tubing for MicroMatrixTM *in vitro* colonic fermentation experiment.

Following simulated salivary, gastric and intestinal digestion, samples were used in triplicate for the *in vitro* colonic fermentation experiment. Cellulose and inulin were used as negative and positive controls, respectively, for *in vitro* digestion and *in vitro* colonic fermentation in triplicate. Digested soups were abbreviated as CFB_SD (Chickpea Flour baker's yeast fermented Soup Digested), CFS_SD (Chickpea Flour Sourdough fermented Soup Digested), EFB_SD (Einkorn Flour baker's yeast fermented Soup Digested), EFS_SD (Einkorn Flour

Sourdough fermented Soup Digested), PPB_SD (Purple Potato flour baker's yeast fermented Soup Digested), PPS_SD (Purple Potato flour Sourdough fermented Soup Digested), WFB_SD (Wheat Flour baker's yeast fermented Soup Digested), WFS_SD (Wheat Flour Sourdough fermented Soup Digested).

Subsequent to *in vitro* digestion, *in vitro* colonic fermentation was conducted based on a previously described protocol [3]. Participant recruitment and stool sample collection were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (review reference numbers: ECM 4 (gg) 11/02/20 & ECM 3 (iiii) 22/02/2022). Faecal samples were obtained from nine healthy individuals who had no antibiotic usage for last 3 months, no medicine usage and healthy and pooled in equal amounts for use in a MicroMatrix™ *in vitro* benchtop fermentation system, adhering to a protocol outlined previously [4]. Faecal samples were collected from all individuals in the morning at the Teagasc Moorepark Research Centre. The samples were kept in anaerobic jars in a cold room at 4°C. Within two hours, all samples were transferred to an anaerobic chamber for pooling. Each sample was weighed, and 60 g of each sample was transferred into a stomacher bag and mixed with 400 mL of phosphate buffer. The mixture was then transferred to sterile 50 mL falcon tubes and centrifuged at 4000 g for 25 minutes at 4°C. The supernatant was discarded, and the pellet was washed with phosphate buffer. The falcon tubes were centrifuged again at 4000 g for 25 minutes at 4°C. Following centrifugation, the pellet was dissolved in phosphate buffer containing 25% glycerol. Aliquots were then stored at -80°C until use in MicroMatrix™.

The faecal fermentation medium (FFM) was prepared following the protocol of Fooks and Gibson, 2003 [5] with the following composition: tryptone water (2 g/L), yeast extract (2 g/L), cysteine HCl (1 mg/L), bile salts (0.5 g/L), Tween 80 (2 mL/L), hemin (0.05 mg/L, dissolved in three drops of 1 M NaOH), vitamin K1 (10 μL/L), NaCl (100 mg/L), KH_2PO_4 (40 mg/L), K_2HPO_4 (40 mg/L), $CaCl_2 \cdot 6H_2O$ (40 mg/L), $MgSO_4 \cdot 7H_2O$ (10 mg/L), and $NaHCO_3$ (2 mg/L).

The medium was autoclaved at 121°C for 15 minutes and stored at 4°C until use. For the fermentation process, 2 mL of digesta was added to the fecal fermentation medium and fecal slurry mixture (20%). The fermentation was conducted under anaerobic conditions at 37°C, with the pH maintained at 6.8 [3]. The mixture was continuously agitated at 250 rpm using a cassette to ensure homogeneity. Faecal fermentation was performed for 12 hours and samples were collected at baseline (T0) and at the end of fermentation (T12). All samples were collected in 2 mL sterile screw cap tubes. Subsequently, the tubes containing the samples were centrifuged at 16,000 g for 10 minutes at 4°C. Following centrifugation, the pellet was used for DNA extraction to analyse the microbial composition and the supernatant was processed for short-chain fatty acid (SCFA) and branched-chain fatty acid (BCFA) analysis. Following *in vitro* colonic fermentation, faecal samples were labelled with the suffix 'F'; for example, CFB_SDF represents faecal samples fermented with digested chickpea flour baker's yeast fermented soup.

References

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