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The microbiome modulating potential of superheated steam (SHS) treatment of dietary fibres

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ABSTRACT

In this study, wheat and oat bran fibres were used to investigate the potential beneficial prebiotic-enhancing effects of superheated steam (SHS) treatment. Following SHS treatment of fibres, *in-vitro* simulated gastric and intestinal digestion followed by batch culture fermentation was performed. 16S rRNA gene sequencing and short chain fatty acid analysis were conducted, which demonstrated that SHS treatment was associated with significant differences in beta diversity and the relative abundances of several microbial taxa. Principal Coordinate Analysis (PCoA) revealed significant separation between SHS-treated oat and non-treated oat ($p = 0.013$), and between SHS-treated wheat and non-treated wheat following *in vitro* batch fermentation ($p = 0.042$). Abundances of *Ruminococcus* were found to be significantly higher by 1.09-log in SHS-treated oat when compared with non-treated oat while abundances of *Escherichia_Shigella,* and *Alistipes* were significantly lower by 1.47-log and 0.39-log, respectively in the SHS-treated oat samples than the non-treated oat samples ($p = 6.85E-06$, $p = 0.002037$, $p = 0.002458$; respectively). These data demonstrate that SHS treatment of dietary fibre enhanced its effectiveness to modulate the gut microbiome.

Industrial Relevance: Superheated steam (SHS) treatment has emerged as an effective method to improve the shelf-life of grains by reducing lipid hydrolysis and oxidation. Our results revealed that SHS treatment of wheat and oat brans led to increased physiological and chemical properties including higher water-binding capacity, water-extractable arabinoxylan, and total phenolics as well as improvement in human gut microbiota. Our results suggest that this method could be implemented in the food industry in order to improve beneficial properties of oat and wheat brans.

1. Introduction

Dietary fibres are a complex mixture of carbohydrates that resist digestion in the small intestine and can be fermented by the microorganisms in the colon (Jones, 2014). Dietary fibre intake is associated with a variety of health effects including overall metabolic function (Barber, Kabisch, Pfeiffer, & Weickert, 2020; Cronin, Joyce, O'Toole, & O'Connor, 2021). According to European Food Safety Authority (EFSA) guidelines, an intake of 25 g/day or more of dietary fibre is associated with health benefits (Scientific Opinion on Dietary Reference Values for Carbohydrates and Dietary Fibre, 2010). However, the daily intake of dietary fibre has been decreasing as countries adopt a westernized diet (Oliver et al., 2021), characterized by a high fat, high cholesterol, and low fibre intake (Statovci, Aguilera, MacSharry, & Melgar, 2017). The westernized diet has been associated with several diseases such as irritable bowel syndrome (IBS) (Uranga, Lopez-Miranda, Lombo, & Abalo, 2016), cardiovascular disease (Zampelas & Magriplis, 2020), asthma (Leiria, Martins, & Saad, 2015), obesity, and type 2 diabetes mellitus (Flint, Duncan, Scott, & Louis, 2015; Mayerhofer et al., 2020).

Many of the beneficial effects of dietary fibre have been attributed to its influence on the gut microbiome and the promotion of the production of several beneficial metabolites including short chain fatty acids (SCFAs) (Shah, Li, Al Sabbah, Xu, & Mraz, 2020). Recently, a three-week high fibre intervention study in humans resulted in a 1.4-fold increased

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abundance of *Bifidobacterium* between pre and post-intervention periods (Oliver et al., 2021). This gut modulating effect may be attributed to the production of SCFAs at the end of the fermentation process. SCFAs reduce colonic pH and can inhibit the growth of pathogenic microorganisms including *Enterobacteraciae* (den Besten et al., 2013; Parada Venegas et al., 2019). One of the major SCFAs produced in the colon is acetate which has been shown to inhibit colorectal cell proliferation, induce apoptosis, and promote lysosomal membrane permeabilization (Ferro et al., 2016; Marques et al., 2013; Matthews, Howarth, & Butler, 2012). Propionate and butyrate are the other major SCFAs produced in the colon (Parada Venegas et al., 2019) which play significant roles in the intestinal lumen including suppressing tumour formation and inflammatory pathways through the inhibition of histone deacetylase activity (Zhang & Davies, 2016). Butyrate is also used by colonocytes as an energy source and is considered protective against colorectal disease (Encarnação, Abrantes, Pires, & Botelho, 2015).

Oat bran and wheat bran are examples of fibres that exert health benefits and have been shown to have a prebiotic effect (Koç, Mills, Strain, Ross, & Stanton, 2020). A dietary prebiotic is defined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Gibson et al., 2017). Oat is rich in β-glucan, a soluble fibre associated with several health benefits including cholesterol-lowering effects and improving blood glucose levels in patients with diabetes (Francelino Andrade et al., 2014). Wheat is one of the most important and most widely consumed crops in the world with many reported health benefits including reducing the risk of cardiovascular disease, cancer, and gastrointestinal disease (Hu, Wang, & Li, 2018; Reynolds et al., 2019). These health-promoting effects can be mainly attributed to the bran layer which contains antioxidants, fibre, vitamins, and minerals (Prasadi & Joye, 2020). However, lipid oxidation in wheat bran can result in reduced shelf life, functionality, and palatability. As a result, the development of methods to inactivate or stabilize enzymatic hydrolysis and extend shelf life is of great importance (Hu et al., 2018).

Several methods have been used to improve the shelf life of wheat bran including hot air heating, the addition of metal salts, and superheated steam (SHS) treatment. SHS treatment has emerged as a promising method and has been shown to be effective at increasing shelf life as well as increasing antioxidant activity of brans. SHS treatment creates an oxygen free environment that can reduce oxidation loss (Hu et al., 2018). A recent study investigated the effect of SHS treatment on the lipid stability of noodles during storage and found that SHS treatment significantly reduced lipid hydrolysis and oxidation compared with non-treatment during 12-weeks storage period (Jia, Yang, Guo, & Zhu, 2021). The stabilization effect of SHS was also found to be effective for oat bran (Head, Cenkowski, Arntfield, & Henderson, 2010).

Physical pre-treatment of bran material may increase the extractability of soluble fibres and bioactives from the cellulosic material, and further enhances the bioavailability of bioactives by improving the fermentability of the bran (Liu, Li, Liu, Guan, & Bian, 2021; Merali et al., 2015). The use of SHS processing as a novel clean process route has been successfully tested to increase the extraction of arabinoxylan from cereal by-products (Ma, Zhang, Xu, Jin, & Xu, 2021) and is comparable or better than using existing industrial processes alone (Phillips & Williams, 2009). The increased extraction of soluble bran compounds by SHS treatment appears to be related to structural and morphological changes in bran structure. These structural changes alter the physical properties of bran, by increasing the water holding capacity as well as the capacity to bind (simulated) gastric fluid (Diaz, Hübner, Noort, Slaghek, & Essers, 2015). Therefore, these physical and structural changes may promote the fermentation process directly (binding and/or hosting microorganisms) or indirectly (improved accessibility of compounds).

To our knowledge, no previous research has investigated the effects of SHS-treated bran on the gut microbiome. This study aimed to investigate the prebiotic potential of SHS-treated wheat and SHS-treated oat. An *in vitro* batch fermentation system was used to mimic colonic fermentation following *in vitro* simulated oral, gastric, and intestinal digestion of fibres. Samples were analysed using 16S rRNA gene sequencing and SCFA analysis to examine potential microbiome and metabolome modulation effects.

2. Materials and methods

2.1. Raw materials

Wheat and oat brans were provided by industry partners from the JPI LONGLIFE project, inulin and cellulose were sourced from Sigma Aldrich (Ireland). Wheat bran (15.4% protein, 17% starch, 0.5% sugar, 45% dietary fibre and 4.3% fat) was provided by Ernst Böcker GmbH (Germany) and oat bran (18% protein, 31.5% starch, 1.5% sugar, 25% dietary fibre, of which 14% is β-glucan, and 9% fat) was provided by Barilla (Italy). The soluble dietary fibres were 3.6% and 14% for wheat and oat bran, respectively, based on AOAC 991.43. Both were subjected to SHS treatment at Wageningen Food and Biobased Research (WFBR, Netherlands) (Section 2.2 SHS Treatment Method). The soluble dietary fibre content of the SHS treated wheat and SHS treated oat bran were 5.9% and 15%, respectively. Brans were stored at −20 °C prior to INFOGEST processing.

2.2. Effect of SHS treatment on physicochemical properties of wheat and oat bran

The ability of SHS treatment to enhance the release of soluble material and bioactive compounds from wheat and oat brans was screened in order to select the treatment conditions for the *in-vitro* digestion and fermentation studies. SHS treatments were performed in a pilot apparatus designed and manufactured by WFBR, The Netherlands. Samples were prepared by hydrating the bran in water at 30% (*w/v*) concentration (on a dry matter basis). This condition was chosen based on previous experience in treating lignocellulosic materials. The wetted material was loaded into the SHS chamber and treated at temperatures of 120 °C, 140 °C and 160 °C. The treatment times were 10 min and 40 min for temperatures below 160 °C and a treatment time of 10 min was applied at 160 °C. For all treatments, steam flow was set at 150 kg/h. The pressure in the SHS chamber was adjusted to obtain a water activity (a_w) of 0.7 for all samples. Samples were coded following on the treatment temperature and time combinations, *e.g.* W-120-10 for wheat bran at 120 °C for 10 min, and O-120-10 for oat bran at 120 °C for 10 min. Additionally, the effect of saturated steam ($a_w = 1$) was also studied for the conditions of 120 °C and 40 min treatment as compared to an a_w of 0.7. These samples are coded as W-120-40-1 and O-120-40-1. After treatment, SHS treated samples were frozen at −30 °C and freeze-dried. Following freeze-drying, the bran particles were loosened using a laboratory blender from Waring commercial (Stamford, CT, USA) for 15 s at high speed.

2.3. Chemical and physical characterization of native and SHS-treated wheat and oat bran

2.3.1. Total analysable carbohydrate content

Total analysable carbohydrate content of brans was performed according to a previously described protocol as follows: (i) free sugars from the water extract, and (ii) after hydrolysis with 2 M trifluoracetic acid (TFA) at 100 °C for 1 h (arabinoxylan) of water extract (Renzetti, Theunissen, & Horrevorts, 2021). For the water extracts, 1 g of sample was weighed in a 50 mL Greiner tube, and deionized water was added to reach 25 mL in volume. The solution was then kept under stirring at room temperature for 2 h. After stirring, the solution was centrifuged at 16000*g* for 10 min, and the supernatant was recovered. For the water-extractable arabinoxylan (WEAX) content, 1 mL of 2 M TFA was

added to 1 mL of water extract. The obtained monosaccharides were determined by high-performance anion exchange chromatography (HPAEC) using an ICS-3000 Ion Chromatography HPLC system equipped with a CarboPac PA-1 column (250 × 2 mm) in combination with a CarboPac PA guard column (25 × 2 mm) and a pulsed electrochemical detector in pulsed amperometric detection mode (Dionex, Sunnyvale, CA, USA) at 20 °C, according to a previously described protocol (Gilbert-López et al., 2015). Analyses were performed in duplicate. Arabinoxylan content was determined as the sum of the arabinose and xylose multiplied by the factor of 0.88 (after correcting free arabinose and xylose in the water extract before hydrolysis). The content of galactose and glucose generated from soluble polysaccharides (*i.e.* polymeric galactose and polymeric glucose) was determined from the TFA hydrolysis after correction before hydrolysis.

2.3.2. β-Glucan content in water extract

The water extract was obtained similarly as described for the total analysable carbohydrate content (Renzetti et al., 2021). The β-glucan content from the supernatant of the oat bran was measured using a mixed-linkages assay kit (AOAC Method 995.16, Megazyme, Bray, Ireland). Briefly, 100–120 mg of freeze-dried supernatant was treated sequentially with lichenase and β-glucosidase before determination of released glucose by means of the GOPOD reagent. Quantification of glucose was carried out on Varian Cary 50 Scan UV Visible Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2.3.3. Soluble proteins

Protein content in the water extract obtained from the total analysable carbohydrate analysis was determined using a DC Protein assay kit in duplicate (Bio-Rad Laboratories Inc., USA).

2.3.4. Total phenolic content

Extraction of phenolic content was performed using 80% (*v*/v) methanol at 70 °C for 15 min at 1/10 (*w*/*v*) solid material to solvent ratio. After cooling, the supernatant was decanted, the residue was re-extracted twice, and supernatants were combined. The content of total phenolic compounds in the extracts was investigated using Folin and Ciocalteu's phenol reagent. (+)-Catechin was used as a standard. The results were expressed as mg catechin equivalent per g of dry bran.

2.3.5. Water-binding capacity and soluble solids

The water-binding capacity (WBC) of the bran fractions was determined in duplicate according to a modified version of the protocol (Zanoletti et al., 2017). The bran was soaked in demi-water with a bran/water weight ratio of 1/30. After mixing in a vortex, the samples were left to shake at room temperature for 2 h on a Multi Reax Vortex (Heidolph, Schwabach, Germany). Then, the samples were centrifuged at 5000*g* for 60 min using an Avanti J-26XP High Speed Centrifuge from Beckman Coulter (Indianapolis, IN, USA). The supernatant was collected and dried to determine the soluble solids per g of bran on dry matter basis. The pellet was drained for 15 min at an angle of 45° and then weighed. WBC was expressed as g water taken up per g bran on dry matter basis (dm).

2.3.6. Colour measurements

The colour of the brans was measured with a Minolta CR310 Colour Meter (L*, a*, b* colour space). Three measurements per variation were performed.

2.4. In vitro simulated gastric digestion, dialysis, and freeze drying

Samples were processed using the harmonized INFOGEST method to mimic oral, gastric, and intestinal digestion as previously described (Egger et al., 2016; Minekus et al., 2014). Fifty millilitre aliquots of the INFOGEST end products were placed in sterile 110 × 110 mm petri dishes and freeze-dried for 24 h using a previously described protocol (Patterson et al., 2019) prior to *in vitro* faecal fermentation.

2.5. Preparation of faecal fermentation medium

The faecal fermentation medium (FFM) was prepared according to the Fooks and Gibson's protocol (Fooks & Gibson, 2003) with the following composition: Tryptone water (2 mg/L), yeast extract (2 mg/L), cysteine HCl (1 mg/L), bile salts (0.5 mg/L), Tween 80 (2 mL/L), hemin (0.05 mg/L; dissolved in 3 drops of 1 M NaOH), vitamin K_1 (10 μL/L), NaCl (100 mg/L), KH_2PO_4 (40 mg/L), K_2HPO_4 (40 mg/L), $CaCl_2.6H_2O$ (40 mg/L), $MgSO_4.7H_2O$ (10 mg/L), $NaHCO_3$ (2 mg/L). The medium was autoclaved at 121 °C for 15 min and stored at 4 °C until use.

2.6. Preparation of faecal slurry

Stool samples from six healthy individuals (no antibiotic use in 6 months prior to sample donation) were collected, homogenized, dispensed into equal parts in an anaerobic chamber and frozen at −80 °C until use for batch fermentation (O'Donnell et al., 2016). Participant recruitment and collection of stool samples were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (review ref. no: ECM 4 (gg) 11/02/20 & ECM (iiii) 22/02/2022).

Faecal samples were processed in an anaerobic chamber (Don Whitley anaerobic workstation, West Yorkshire, UK) within 2–3 h of sample collection. Approximately 60 g sample was placed into a large stomacher bag with a 70-μM filter insert (Sparks lab supplies, Rathcoole, Co. Dublin). Four hundred millilitres of 50 mM phosphate buffer supplemented with 0.05% (*w*/*v*) L-cysteine hydrochloride (Sigma Aldrich-Ireland) was added into stomacher bag and the faecal sample was homogenized. The filtered slurry was centrifuged at 4000*g* for 25 min at 4 °C in a Sorvall SLA-3000 centrifuge (Thermofisher, MA, USA). The pellet was re-suspended in 400 mL 50 mM phosphate buffer solution containing 25% glycerol (*v*/v). The faecal slurry was divided into even sets of aliquots (30 mL for each aliquot) and kept at −80 °C until use for batch fermentation experiment.

2.7. In vitro faecal fermentation

The faecal fermentation was performed using the MicroMatrix™ (Applikon Biotechnology, Heertjeslaan 2, 2629 JG Delft, The Netherlands) 24-well cassette system. For each well, 1 mL of thawed faecal slurry was inoculated into 4 mL FFM in the anaerobic chamber and mixed with 0.25 g pre-digested fibre. Running parameters included nitrogen gas at 40%, orbiter at 250 rpm, temperature at 37 °C, dissolved oxygen (dO_2) at 0%, and pH of 6.8. NaOH (4 M) and CO_2 gas were used to adjust the pH. The fermentation was performed for 12 h with eight replicates per fibre. Samples were collected from each well into sterile 2 mL screw-cap tubes and centrifuged at 15000 *g* for 15 min at 4 °C. Supernatant was collected for SCFA analysis and pellet was kept for DNA extraction. Both supernatant and pellet were frozen at −80 °C for further analysis.

2.8. SCFA analysis

SCFAs were analysed from supernatant according to a previously described protocol (Lynch et al., 2021). Briefly, thawed samples were mixed with acidified water (pH = 2–3), 1:1 (*v*/v) and syringe filtered (0.22 μM, Corning). A 270 μL sample was mixed with 30 μL 10 mM internal standard (IS) in duplicate. The samples were vortex mixed and centrifuged at 16000 *g* for 10 min at 4 °C. Following centrifugation, 250 μL supernatant was transferred in glass inserts (Agilent, California, USA) and placed in amber glass 2 mL GC vials (Agilent, California, USA). A standard curve (acetate, propionate, butyrate, and valerate (0.1 mM–10 mM); isobutyrate and isovalerate (0.01-1 mM)) was created for quantification. All standards were purchased from Sigma- Merck (Missouri, USA).

The analysis of standards and samples was carried out by gas chromatography flame ionisation detection (GC-FID) using a Varian 3800 GC system DB-FFAP column (30 m -length, 0.32 mm -inner diameter, 0.25 μM -film thickness; Agilent, California, USA) and a flame ionisation detector. Standards and samples (0.2 μL splitless injection) were loaded with a CP-8400 autosampler (Varian, California, USA). Helium was used as carrier gas at an initial flow rate of 1.3 mL/min. The initial oven temperature was 50 °C, and was maintained for 0.5 min, ramped up to 140 °C at 10 °C/min and held for 0.5 min. The temperature was then increased to 240 °C at 20 °C/min and held for 5.0 min. The total run time was 20 min. The detector and oven temperatures were set at 300 °C and 240 °C, respectively. Peaks were integrated with Varian Star Chromatography Workstation Version 6.0.

2.9. DNA extraction

DNA was extracted from each pellet (from a 2 mL sample tube) using the repeated bead-beating DNA extraction method according to Yu and Morrison (Yu & Morrison, 2004). All chemicals were purchased from Sigma-Merck (Missouri, USA) unless stated otherwise. Pellets were suspended using 1 mL lysis buffer (500 mM NaCl, 50 mM Tris-HCl, pH: 8.0, 50 mM EDTA, and 4% sodium dodecyl sulphate (SDS)). The suspension was transferred to a 2 mL screw-capped microtube (Sarstedt, Nümbrecht, Germany) containing different size and amounts of Zirconia/Silica beads (0.125 g of 0.1 mm, 0.125 g of 1.5 mm, a single 2.5 mm; Biospec, Oklahoma, USA). The mixture was homogenized in a bead beater (Biospec, Oklahoma, USA) at 4000 rpm for 3 min. Following homogenization, the tubes were incubated at 70 °C for 15 min and centrifuged at 16000 *g* for 5 min at 4 °C. The supernatant was transferred in a sterile 2 mL Eppendorf tube and 300 μL lysis buffer was added to the pellet. Homogenization, incubation, and centrifugation steps were repeated. Supernatants were collected in sterile 2 mL Eppendorf tubes. A 260 μL of 7.5 M cold ammonium acetate solution was then added and incubated on ice for 5 min. Following incubation, tubes were centrifuged at 16000 *g* for 10 min at 4 °C, then 650 μL supernatant was transferred into two 1.5 mL tubes, 650 μL isopropanol was added, and incubated at −20 °C overnight. Samples were centrifuged at 16000 *g* for 15 min at 4 °C. The supernatant was discarded and the pellet was washed with 200 μL of 70% ethanol. The pellet was then resuspended in 100 μL Tris-EDTA buffer and 2 μL RNase (10 mg/mL; Thermofisher, MA, USA) was added and incubated at 37 °C for 15 min. Following incubation, 15 μL proteinase K (QIAmp DNA Stool Mini Kit, Qiagen, Germany) and 200 μL AL buffer (QIAmp DNA Stool Mini Kit, Qiagen, Germany) were added to samples and incubated at 70 °C for 10 min. 200 μL 100% ethanol was added to samples for precipitation. Samples were transferred to QIAmp mini columns (Qiagen, Germany) and washing and elution procedures were performed according to the QIAmp kit protocol. DNA was stored at −20 °C until 16S rRNA library preparation.

2.10. 16S rRNA Sequencing Library Preparation

DNA extracts were prepared according to Illumina 16S Metagenomics Sequencing Library Preparation guidelines. All PCR reactions were conducted in a 2720 thermal cycler (Life Technologies, MA, USA) using KAPA HiFi Hot Start Ready-mix Kapa Biosystems (MA, USA). The variable V3 and V4 regions of the 16S rRNA gene were amplified using 16S Amplicon PCR Forward Primer = 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Amplicon PCR Reverse Primer = 5' GTCTCGTGGGCTCGGAGATGT GTATAAGAGACAGGACTACHVGGGTATCTAATCC. The amplicon PCR program was; 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by 5 min 72 °C for the elongation step. For index PCR, the Illumina Index primer set was used according to the manufacturer's protocol. All PCR amplicons were visualised on 1.5% agarose gel (Sigma-Merck) stained with SYBR safe (Thermofisher, MA, USA). PCR products were cleaned up using AMPure XP bead solution (Beckman Coulter, CA, USA) according to Illumina's guidelines. Before pooling, concentrations of final products were measured with Qubit dsDNA high-sensitivity kit (Thermofisher, MA, USA) and Qubit 3.0 fluorimeter (Thermofisher, MA, USA). Samples were sequenced at the Teagasc Next Generation DNA Sequencing Facility using the Illumina MiSeq platform with a MiSeq Reagent Kit v3 (Illumina, Inc. San Diego, USA).

2.11. Bioinformatics and statistics

Sequence reads were filtered using DADA2, version 1.16 using standard filtering parameters; maxN = 0, truncQ = 2, rm.phix = TRUE, and maxEE = 2. Primers were trimmed using trimLeft = c (17, 21). Filtered reads were then de-replicated and de-noised using DADA2 default parameters. An amplicon sequence variant (ASV) table was constructed and chimeric sequences are identified and removed. Taxonomy was assigned to the sequence variants using the IdTaxa taxonomic classification method *via* the DECIPHER Bioconductor package and the SILVA SSU r138 database.

MicrobiomeAnalyst was used to analyse the ASV table for alpha diversity, beta diversity, and differential abundance measures (Chong, Liu, Zhou, & Xia, 2020). The alpha-diversity analysis function was performed on unfiltered data using Shannon and Simpson diversity measures. Data were subsequently filtered for abundance for those ASVs that had a minimum total count of 4, or less than 20% total abundance in all samples. The ASVs in the lowest 5% for variance based on the inner-quartile range were then removed. Lastly, a centred log-ratio (CLR) transformation was performed. Abundance profiles were visualised at phylum and genus levels using GraphPad Prism version 9.1.0. A Bray-Curtis dissimilarity matrix was calculated and visualised using Principal Coordinate analysis (PCoA). The PERMANOVA statistical test was used to determine if microbial communities of sample groups are significantly different. Differential analyses were used to assess potential differentially abundant taxa between negative control (cellulose) and test group (for each fibre) using DESeq2 on untransformed data (Love, Huber, & Anders, 2014). DESeq2 uses a negative binomial generalized linear model with a variance stabilizing transformation on abundances. Linear discriminant analysis effect size (LEfSe) was used to identify the most effective genus in the groups. False detection rates (FDR) were reported to limit false positive results (Dong, Li, Chen, Kusalik, & Xu, 2020). FDR adjusted p value (FDR = 0.05) was used to determine significance level of relative abundance. Statistical analysis of SCFAs, physical, and chemical properties of bran fibres were performed using IBM SPSS version 27.0. Differences among groups were determined by a one-way Anova test with Tukey's honestly significant difference (HSD) test at a 5% level of significance.

3. Results

3.1. Physical and chemical properties of native and shs-treated brans

SHS treatments were performed at different temperature and time combinations in order to assess their effects on the chemical and physical properties of the brans. In particular, the solubilisation of bran components was studied as an indicator of opening up of the bran matrix and increased accessibility of compounds. A PCA analysis was performed on wheat and oat bran separately, in order to distinguish the effect of the SHS treatments on each bran source. The sample variations (score plot) and the chemical and physical properties (loading plot) are shown for wheat (Fig. 1A and 1B, respectively) and oat brans (Fig. 2A and 2B, respectively). The first two principal components accounted for 88.7% and 91.3% of the variance for wheat and oat bran samples, respectively. Therefore, PC_1–PC_2 plane can be considered as indicative of the differences between the samples.

For both wheat and oat brans, all the SHS-treated samples were clearly separated from the native brans along the PC_1 axis (Fig. 1A and

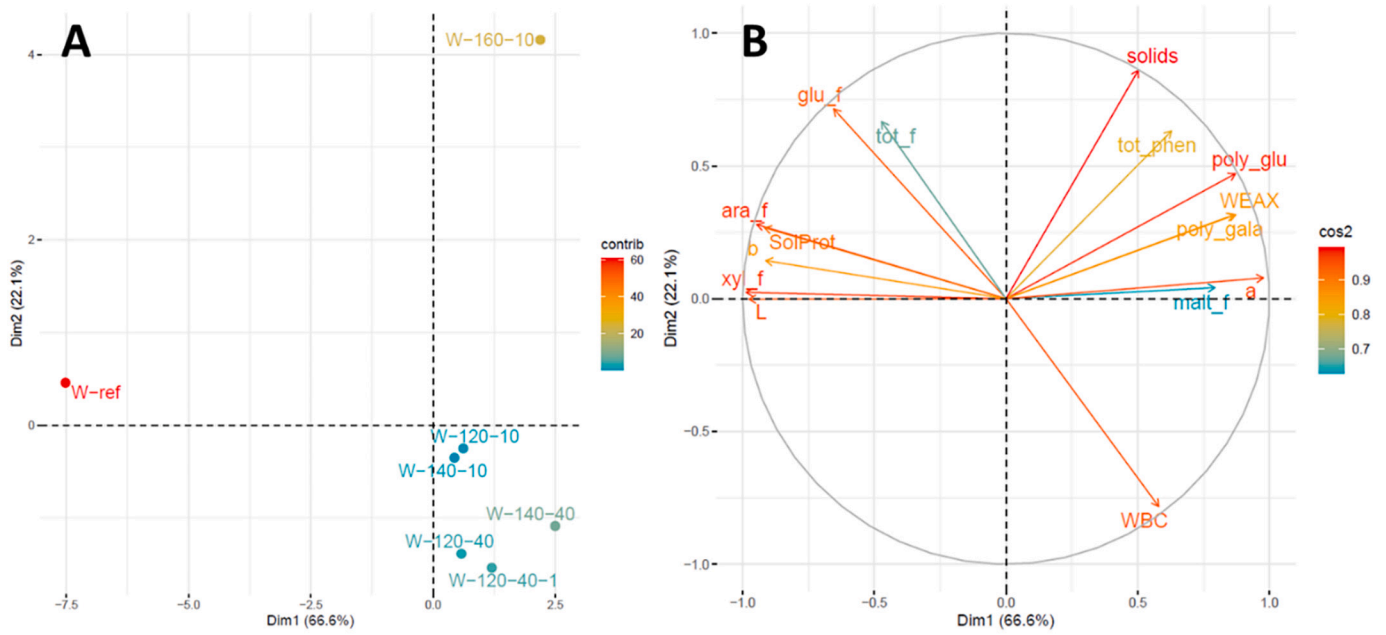

Fig. 1. PCA (Principal component analysis) of wheat brans with samples score plot (A) and loading plot of compositional and physical properties (B). For the compositional and physical characteristics: ara_f = free arabinose, glu_f = free glucose, xyl_f = free xylose, malt_f = free maltose, tot_f = total free sugars, WEAX = water extractable arabinoxylans, ply_glu = polymeric glucose, poly_gala = polymeric galactose, tot_phen = total detectable phenolics, solids = soluble solids, WBC = water-binding capacity, L = lightness, a = a* in the CIELAB colour space, b = b* * in the CIELAB.

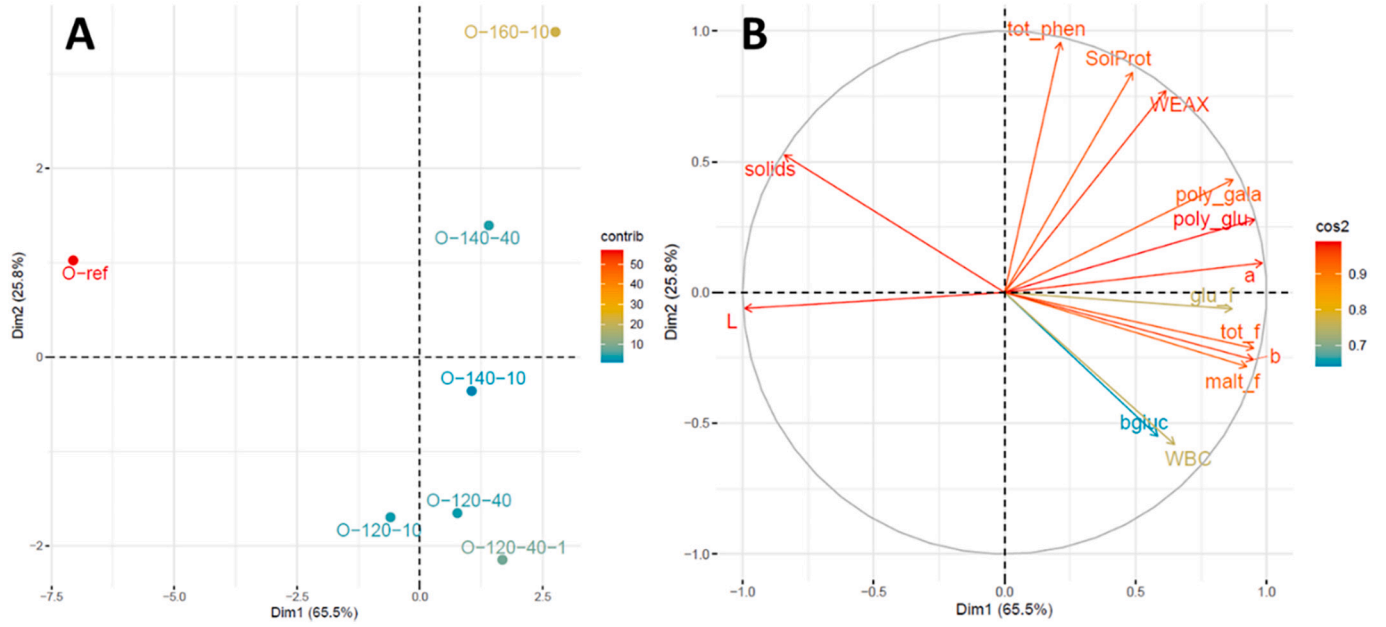

Fig. 2. PCA of oat brans with samples score plot (A) and loading plot of compositional and physical properties (B). For the compositional and physical characteristics: ara_f = free arabinose, glu_f = free glucose, xyl_f = free xylose, malt_f = free maltose, tot_f = total free sugars, WEAX = water extractable arabinoxylans, ply_glu = polymeric glucose, poly_gala = polymeric galactose, tot_phen = total detectable phenolics, solids = soluble solids, WBC = water-binding capacity, L = lightness, a = a* in the CIELAB colour space, b = b* * in the CIELAB.

Fig. 2A, respectively). SHS-treated wheat brans showed considerably higher amounts of WEAX, polymeric glucose, polymeric galactose, and total phenolics compared to the native wheat bran (Fig. 1B and Supplementary Table 1). On the contrary, free sugars (*i.e.* glucose, arabinose, and xylose) and soluble proteins were lower in the SHS-treated wheat samples compared to the native wheat bran. The SHS-treated samples were also darker and with an enhancement in the red colour space (*i.e.* a*), suggesting that the decrease in soluble proteins and free (reducing) sugars may be the result of Maillard reaction taking place. Overall, the measured soluble solids were correlated with the sum of individual compounds detected in the compositional analysis (R^2 = 0.882, $p < 0.00$).

Among the SHS-treated wheat brans, sample W-160-10 could be clearly distinguished as the one with highest release of WEAX, polymeric glucose, and galactose, total phenolics and overall soluble solids (Supplementary Table 1).

For the SHS-treated oat bran, an increase in total free sugars, WEAX, polymeric glucose, total phenolics and soluble proteins was observed compared to the native oat bran (Fig. 2B and Supplementary Table 2). Contrary to the wheat bran, a significant reduction in soluble solids was detected for the SHS-treated oat samples compared to the native oat bran. Concomitantly, an increase in WBC was observed. It seemed likely that the soluble solids might have been retained in the gel layered formed in the pellet during centrifugation. This hypothesis is supported by the fact that no correlation was observed between the measured soluble solids and the sum of individual compounds detected in the compositional analysis of Supplementary Table 2. Also for the oat brans, the treatment at 160 °C, *i.e.* sample O-160-10, showed the largest effects in enhancing the extraction of compounds, *i.e.* polymeric glucose, WEAX, and soluble proteins (Supplementary Table 2).

Overall, it can be suggested that the 160 °C-10 min SHS treatment resulted in wheat and oat brans with the highest accessibility of compounds. Therefore, samples W-160-10 and O-160-10 were selected for further investigation of the effect of the SHS treatment on *in vitro* digestion and gut microbiota modulation.

Several chemical and physical properties of SHS-treated oat (160 °C–10 min) and native oat differed significantly (Supplementary Table 2). Increased levels of maltose and total free sugars were found in SHS-treated oat compared with native oat ($p < 0.05$). Polymeric glucose, WEAX levels and WBC were higher in SHS-treated oat than native oat ($p < 0.05$). Soluble solids were found to be lower in SHS-treated oat than native oat ($p < 0.05$). Colour measurements were significantly different between SHS-treated oat and native oat ($p < 0.05$, Supplementary Table 2).

3.2. Alpha and Beta diversity

Following *in vitro* oral, gastric, and upper intestinal digestion of SHS-treated wheat and oat brans and their native (non-SHS-treated) counterparts, *in vitro* faecal fermentation was performed to mimic colonic fermentation of the pre-digested fibres. The controls included inulin (positive control) and cellulose (negative control). At the endpoint of fermentation (T12), 16S rRNA gene sequencing was performed on samples. Shannon and Simpson diversity indices were used to assess the alpha diversity of samples. Both indices revealed a number of differences in diversity following fermentation in the presence of cellulose, resulting in the highest level of diversity among samples (Fig. 3).

The Shannon index was significantly lower following fermentation in the presence of oat, SHS-treated oat, and SHS-treated wheat samples compared with cellulose ($p = 0.0379$, $p = 0.0147$, $p = 0.0498$; respectively). The Simpson index was significantly lower following fermentation in the presence of SHS-treated oat samples compared to cellulose ($p = 0.0147$) (Fig. 3).

Principal Coordinate analysis (PCoA) using Bray-Curtis distance was used to examine community compositional differences following fermentation in the presence of different fibre samples. The community composition following fermentation in presence of all fibre samples (with the exception of inulin) separated significantly from the negative control (cellulose) using permutational multivariate analysis of variance (PERMANOVA) (Fig. 4). Moreover, a significant separation was observed in community composition between SHS-treated oat and non-treated oat ($p = 0.013$) as well as between SHS-treated wheat and non-treated wheat ($p = 0.042$) (Supplementary Table 3).

3.3. Taxonomic composition

At the phylum level, six phyla were detected in all groups following fermentation. As expected, Firmicutes and Bacteroidetes were the predominant phyla in all samples. DESeq2 was used to evaluate the differential abundance of bacteria at phylum and genus levels. Firmicutes was the dominant phylum following fermentation in the presence of SHS-treated oat where it had significantly higher relative abundance compared with the cellulose group ($p = 2.00E\text{-}0.7$, FDR = 1.40E-06; Supplementary Table 6) and oat samples ($p = 1.65E\text{-}07$, FDR = 1.15E-06; Supplementary Table 12).

Similarly, the relative abundances of Firmicutes following fermentation in the presence of wheat ($p = 0.000878$, FDR = 0.003073; Supplementary Table 8) and SHS-treated wheat ($p = 1.49E\text{-}8$, FDR = 1.04E-07; Supplementary Table 10) were significantly higher than in the presence of cellulose (Fig. 5). The Bacteroidetes phylum had significantly higher relative abundance following fermentation in the presence of oat than in presence of cellulose (Fig. 5) ($p = 0.000595$, FDR = 0.002427; Supplementary Table 4). Actinobacteria were detected at a significantly higher relative abundance following fermentation in presence of all fibres (with the exception of inulin) than in cellulose (Fig. 5; Supplementary Table 4, 6, 8, and 10). Verrucomicrobiota was found at significantly lower relative abundance following fermentation of oat ($p = 0.007589$, FDR = 0.017708; Supplementary Table 4), SHS-treated oat ($p = 0.003443$, FDR = 0.008034; Supplementary Table 6), and wheat ($p = 0.00435$, FDR = 0.010149; Supplementary Table 8) compared with cellulose (Fig. 5). However, it was significantly higher in the SHS-treated wheat group compared with cellulose ($p = 0.010316$, FDR = 0.018052; Supplementary Table 10). Proteobacteria was also found at significantly lower relative abundance in SHS-treated oat ($p = 0.006064$, FDR = 0.010613; Supplementary Table 6), wheat ($p = 0.008122$, FDR = 0.014214; Supplementary Table 8), and SHS-treated wheat ($p = 0.002747$, FDR = 0.00641; Supplementary Table 10) following fermentation than in cellulose (Fig. 5). Moreover, Desulfobacterota was found at lower relative abundance following fermentation in both SHS-treated oat ($p = 0.034987$, FDR = 0.048982; Supplementary Table 6) and SHS-treated wheat ($p = 0.014161$, FDR = 0.019825; Supplementary Table 10) compared with cellulose (Fig. 5).

A number of significant differences were revealed in community composition at genus level following fermentation in the presence of the

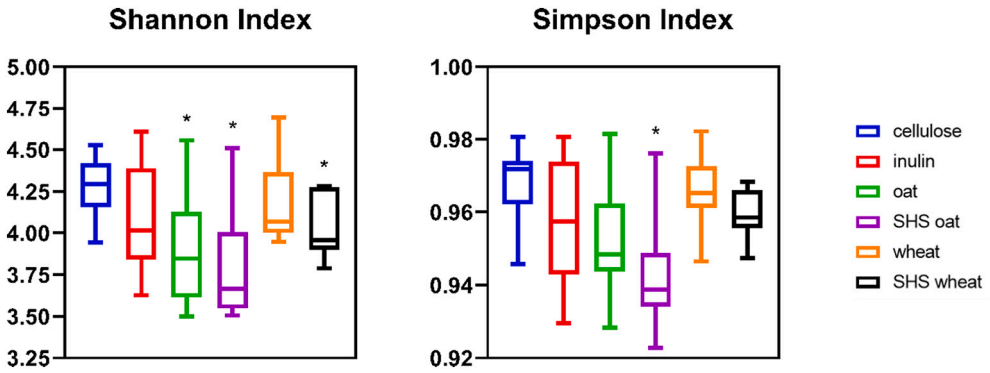

Fig. 3. Box plots showing the Shannon and Simpson indices following 12 h fermentation. Mann-Whitney *U* test was used to compare groups to the negative control (*$p < 0.05$).

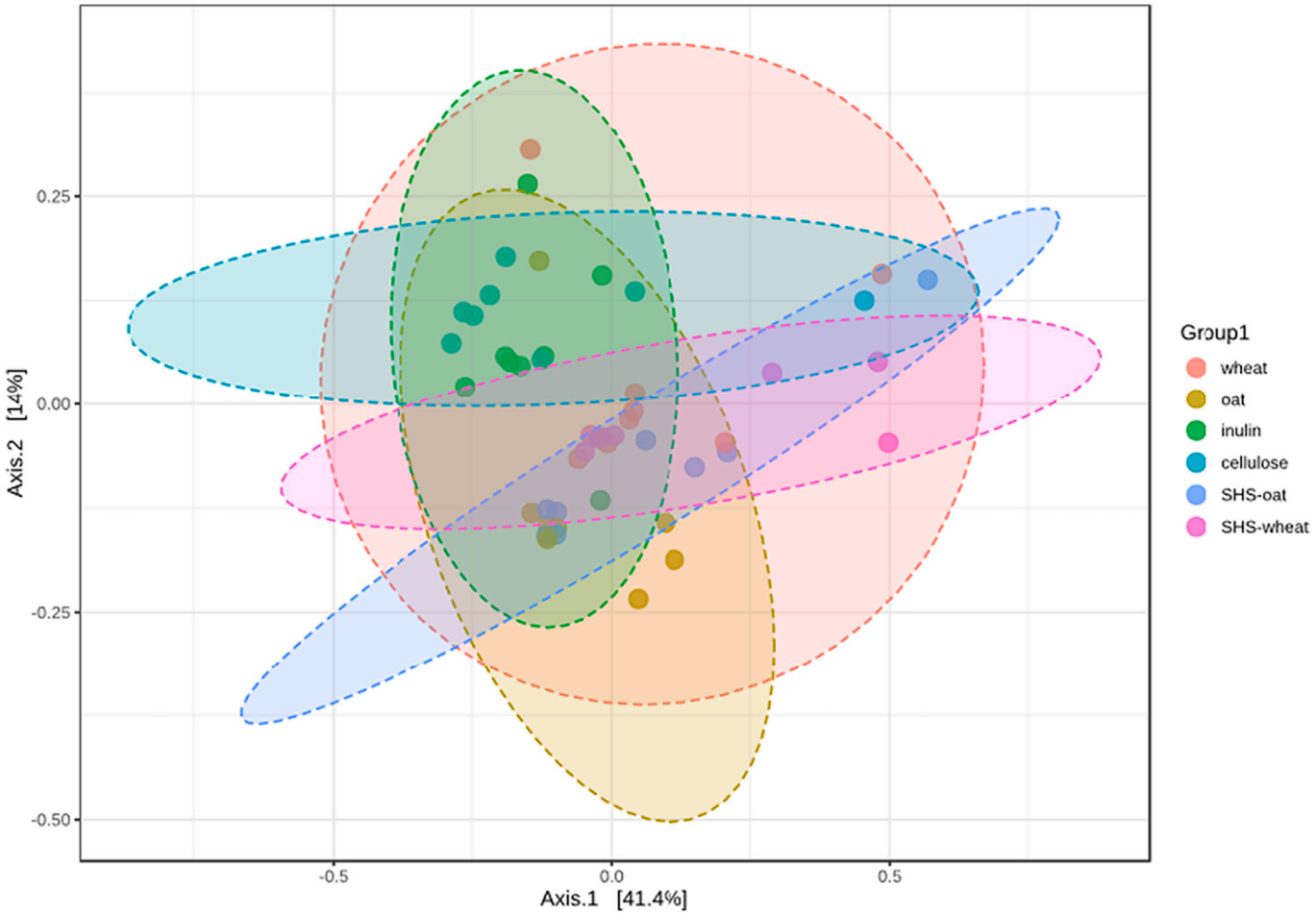

Fig. 4. Principal Coordinate analysis (PCoA) using Bray-Curtis dissimilarity shows separation between groups. PERMANOVA reveals significant differences ($p < 0.05$) among groups. Eclipses indicate 95% confidence intervals around samples from each group.

different fibres. In total, 21 genera were found to be significantly different between oat and cellulose samples (Supplementary Table 5). Fig. 6 shows that abundances of *Bifidobacterium* and *Lactobacillus* were 1.94-fold ($p = 7.06E-05$, FDR = 0.0000261) and 3.55-fold ($p = 0.00019$, FDR = 0.003175) higher following fermentation in the presence of oat samples than in presence of cellulose, respectively.

Similarly, relative abundances of *Faecalibacterium* were 1.04-fold ($p = 0.001564$, FDR = 0.009434), *Coprobacter* 3.85-fold ($p = 0.00169$, FDR = 0.009434), and *Pediococcus* 3.85-fold ($p = 0.002228$, FDR = 0.009951) significantly increased in the oat samples compared with cellulose. *Megamonas* was 2.88-fold ($p = 0.000248$, FDR = 0.003322) and *Escherichia_Shigella* was 1.47-fold ($p = 0.024393$, FDR = 0.077825) significantly lower following fermentation in presence of oat than in presence of cellulose.

Abundances of nineteen genera were significantly altered following fermentation in the presence of SHS-treated oat and cellulose (Supplementary Table 7). Following fermentation in SHS-treated oat, *Escherichia-Shigella* was found to be 2.99-fold ($p = 5.93E-05$, FDR = 0.000519) significantly lower than in cellulose group while *Lactobacillus* was 3.81-fold ($p = 3.24E-05$, FDR = 0.000378) significantly higher in SHS-treated oat group compared with cellulose group (Fig. 6). *Pediococcus* was significantly higher by 4.2-fold ($p = 5.85E-05$, FDR = 0.000519), *Bifidobacterium* by 1.87-fold ($p = 9.85E-07$, FDR = 3.95E-05), and *Faecalibacterium* by 1.35-fold ($p = 3.11E-05$, FDR = 0.000378) following fermentation in presence of SHS-treated oat than in presence of cellulose, respectively. In the presence of SHS-treated oat, significant decrease in relative abundance of *Megamonas* was observed by 3.03-fold when compared with cellulose ($p = 0.000182$, FDR = 0.001275).

The fermentation of wheat yielded significant differences of 21 genera compared to cellulose (Supplementary Table 9). The relative abundances of *Bifidobacterium* were significantly higher by 1.24-fold ($p = 0.002073$, FDR = 0.017042), *Lactobacillus* by 3.13-fold ($p = 0.00101$, FDR = 0.010675), and *Pediococcus* by 3.36-fold ($p = 0.009684$, FDR = 0.055126) following fermentation in presence of wheat than in presence of cellulose, respectively (Fig. 6). Fermentation of wheat resulted in 3.33-fold reduction in the relative abundance of *Megamonas* and a 2.72-fold reduction of *Escherichia_Shigella* compared to cellulose ($p = 2.69E-05$, FDR = 0.0019875; $p = 0.000226$, FDR = 0.0048262; respectively).

Comparison of relative abundances at the genus level also revealed that 26 genera were significantly different following fermentation in presence of SHS-treated wheat and cellulose (Supplementary Table 11). In presence of the SHS-treated wheat, *Bifidobacterium* was significantly higher by 1.83-fold ($p = 4.55E-08$, FDR = 1.66E-06), *Lactobacillus* by 3.54-fold ($p = 2.78E-05$, FDR = 0.000406), and *Pediococcus* by 3.41-fold ($p = 0.000713$, FDR = 0.004003) than in presence of cellulose. *Escherichia_Shigella* was significantly lower by 3.21-fold ($p = 9.71E-07$, FDR = 1.77E-05), *Sutterella* by 2.07-fold ($p = 1.15E-12$, FDR = 8.40E-11), and *Megamonas* by 3.73-fold ($p = 5.36E07$, FDR = 1.30E-05) in SHS-treated wheat group when compared to cellulose, respectively.

Abundances of three genera were found to be significantly altered following fermentation in presence of SHS-treated oat and native oat (Supplementary Table 13). The abundance of *Escherichia_Shigella* was significantly lower by 1.47-fold ($p = 0.002037$, FDR = 0.056525) and *Alistipes* by 0.39-fold ($p = 0.002458$, FDR = 0.056525) in SHS-treated oat than non-treated oat groups, respectively. On the contrary, *Ruminococcus* was found to be 1.09-fold higher following fermentation in the

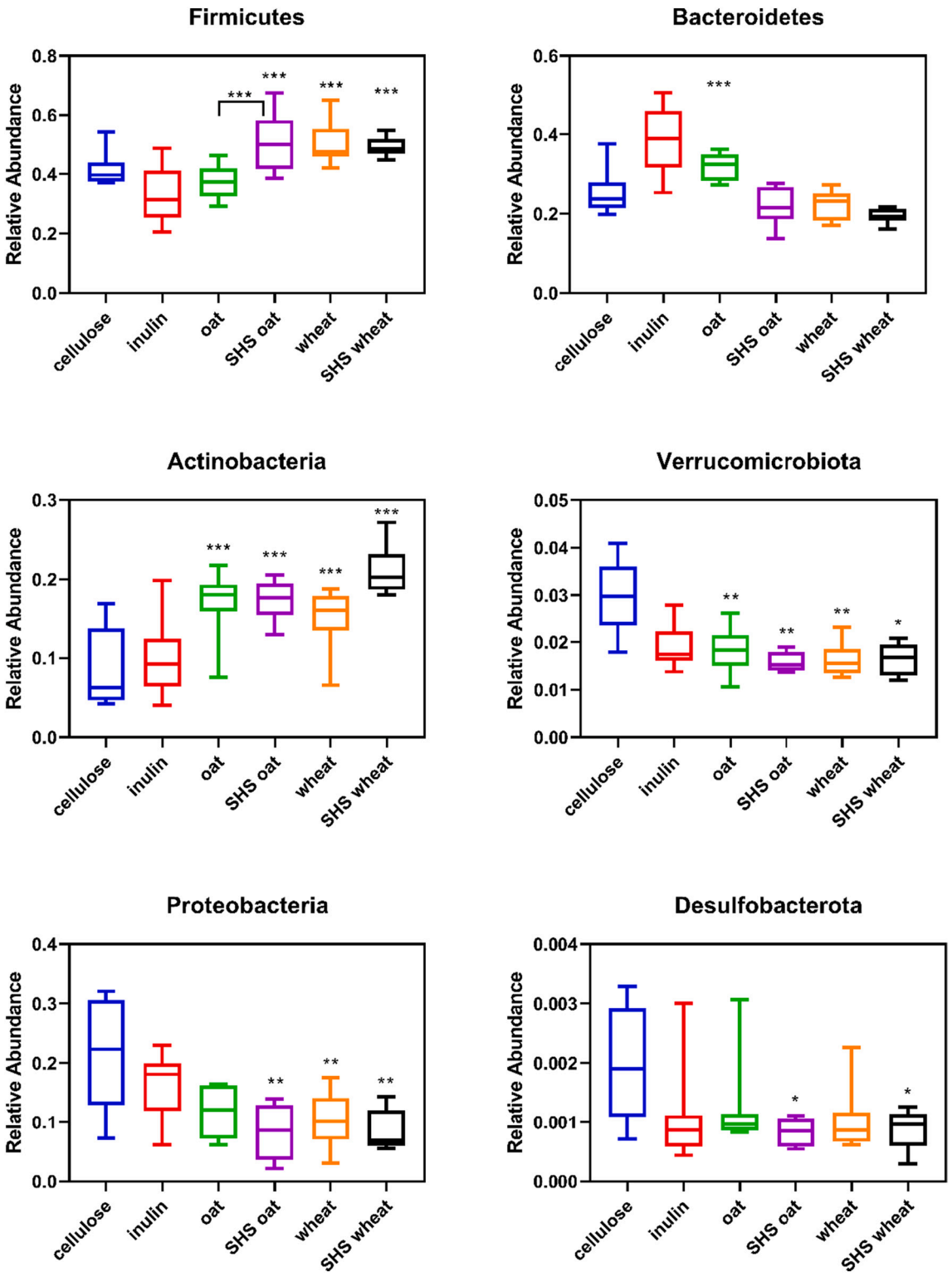

Fig. 5. Relative abundance of Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobiota, Proteobacteria, and Desulfobacterota among groups. DESeq2 was used to evaluate differential abundance of bacterial phyla (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

presence of SHS-treated oat compared with non-treated oat ($p = 6.65E-06$, FDR = 0.00047276).

Fermentation of both SHS-treated oat and wheat resulted in significantly higher relative abundance of *Lachnospiraceae NC2004* group than cellulose by 2.8-fold and 2.25-fold, respectively ($p = 0.009989$, FDR = 0.038844; $p = 0.005652$, FDR = 0.025789).

LEfSe analysis was used to determine the genera most likely to explain differences among groups (Fig. 7). The most differentially abundant bacterial taxa in cellulose were *Escherichia_Shigella* and *Sutterella*. SHS-treated oat, oat and SHS-treated wheat were characterized by a preponderance of *Lactobacillus*.

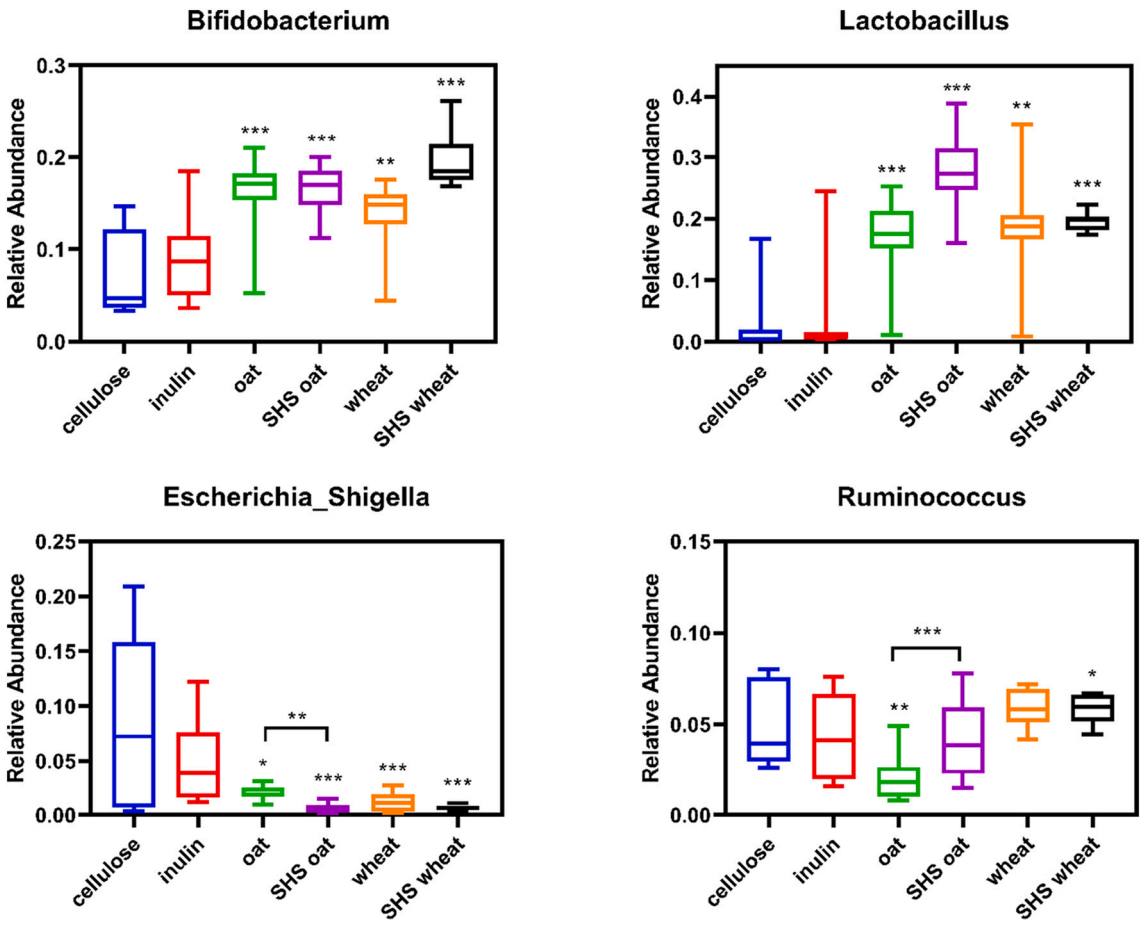

Fig. 6. Relative abundances of *Bifidobacterium*, *Lactobacillus*, *Escherichia_Shigella,* and *Ruminococcus* among groups. DESeq2 was used to evaluate differential abundance of bacterial phyla (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

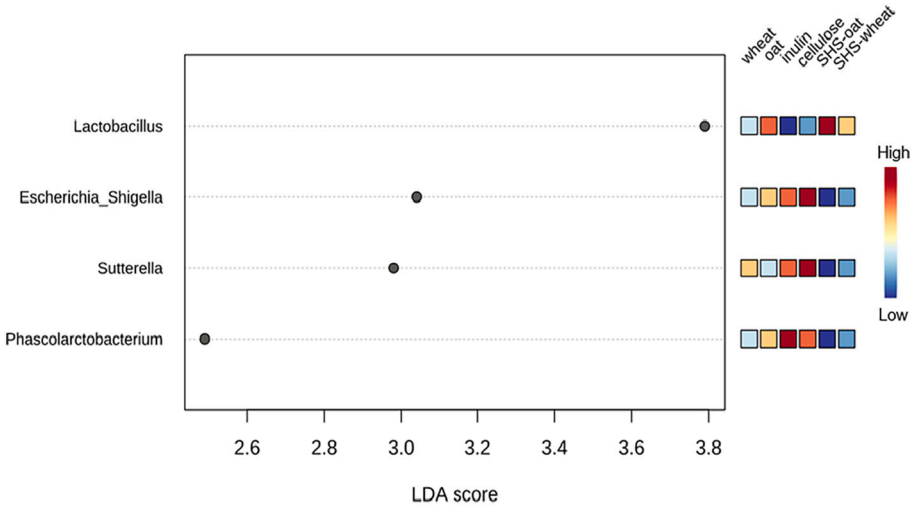

Fig. 7. LEfSe analysis demonstrates the signature genera in each fibre group.

3.4. SCFA analysis

Acetate was significantly higher following fermentation in the presence of all fibre samples ($p < 0.001$) when compared with cellulose (negative control) (Fig. 8). Acetate concentration was significantly higher in oat compared to all other fibre groups ($p < 0.001$).

Propionate was significantly higher following fermentation in the presence of inulin when compared to all other fibre groups ($p < 0.001$), and was significantly higher following fermentation with oat samples when compared to cellulose and SHS-treated wheat ($p = 0.025$, $p = 014$; respectively) (Fig. 8).

Butyrate levels were not significantly altered following fermentation in the presence of wheat compared to cellulose. Inulin, oat, SHS-treated oat, and SHS-treated wheat had reduced butyrate production compared

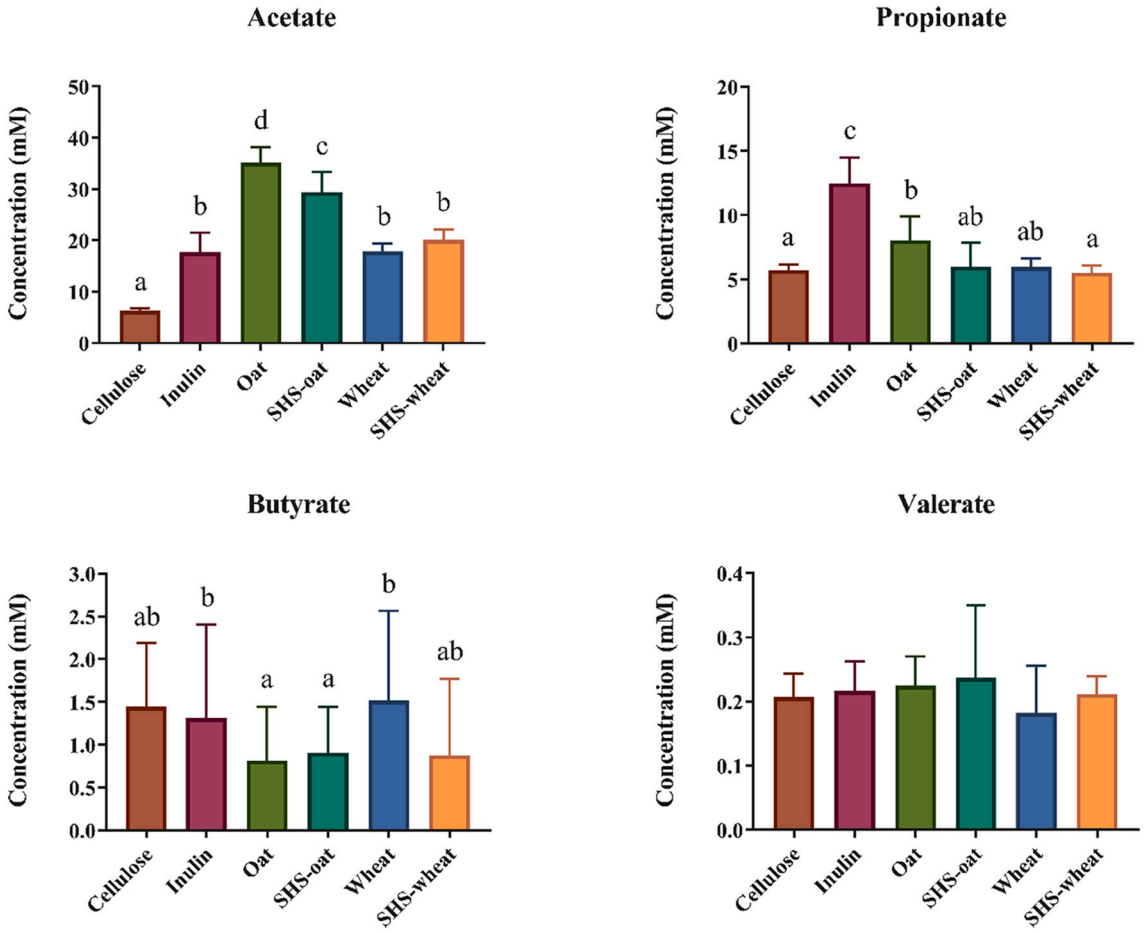

Fig. 8. Concentrations of acetate, propionate, butyrate, and valerate among groups. A one-way ANOVA was performed with Tukey's test. Values with different letters indicate significant differences among the groups.

to cellulose (although differences were not significant). Butyrate levels were significantly higher following fermentation in the presence of inulin compared to SHS-treated oat and native oat ($p = 0.004$, $p = 0.007$; respectively). Wheat had a significantly higher butyrate level than both native and SHS-treated oat ($p = 0.012$, $p = 0.008$; respectively).

Valerate levels were slightly higher following fermentation in the presence of all fibres, with the exception of wheat and SHS-treated wheat, when compared to cellulose (not significant). No significant differences were observed in the concentrations of isobutyrate and isovalerate among the groups (Fig. 9).

4. Discussion

SHS treatment of bran has been reported as a promising method to enhance its nutritional properties and/or make more readily available (Diaz et al., 2015). This study examined the modulating effects of SHS-treated dietary fibres on the human gut microbiota as well as their abilities to promote SCFA production.

Physical and chemical properties of brans showed a number of significant differences between SHS-treated and non-treated brans. SHS treatment of both oat and wheat resulted in increased level of maltose, polymeric glucose, and WEAX (Supplementary Tables 1 and 2). A 2019 study reported that increased total SCFA levels were found following *in vitro* fermentation of wheat and oat bran. It was also shown that during faecal fermentation, the beta glucan level was gradually decreased in both oat and wheat bran samples (Roye et al., 2019).

Analysis of alpha diversity following fermentation in the presence of SHS-treated oat, native (non-treated) oat, and SHS-treated wheat revealed that it was less diverse than following treatment with cellulose, which resulted in the highest diversity, using Shannon index. The Simpson index also revealed that fermentation in the presence of SHS-treated oat led to significantly less diverse microbiota compared with cellulose. PCoA analysis revealed significant separation of each fibre group from cellulose, with the exception of inulin. Of particular note was the observation that SHS-treated oat and SHS-treated wheat separated significantly from native oat and wheat, respectively ($p = 0.013$, $p = 0.042$; respectively). This finding indicates that SHS treatment promotes altered gut microbiota composition compared to the untreated bran.

Fibre supplementation resulted in a significant increase in the relative abundance of Firmicutes, which is not surprising as this phylum is dominated by lactic acid bacteria including butyrate-producing bacteria (Flint et al., 2015; Tanaka & Nakayama, 2017). Similarly, Actinobacteria were found to be significantly higher in all fibre groups when compared with cellulose. A recent study demonstrated that the abundance of Firmicutes and Actinobacteria were significantly higher in young adults than in the elderly population (Li et al., 2021). This increase in abundance of Actinobacteria may be driven by the abundance of the *Bifidobacterium* genus (Li et al., 2021). Another study also showed that the abundance of several butyrate producing bacteria including *Eubacterium hallii* and *Ruminococcus obeum et rel.* were lower in elderly subjects (over 100 years old) than in younger subjects (Biagi et al., 2010). This reduction in Firmicutes phylum throughout ageing might be a result of reduced levels of butyrate producing bacteria.

Following fibre supplementation, significant increases in several potentially probiotic bacterial genera were observed. One of the most well-established probiotic bacteria is *Bifidobacterium* which is associated

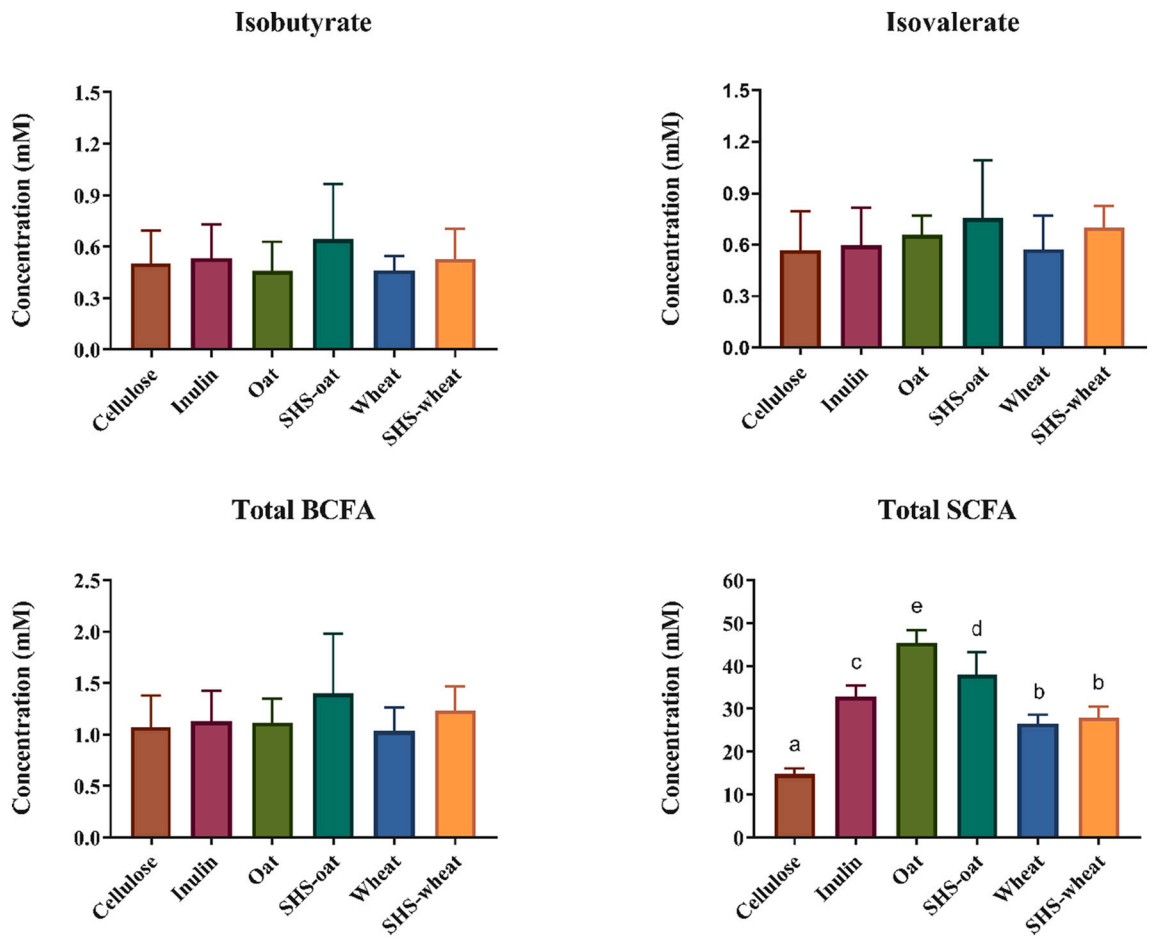

Fig. 9. Concentrations of isobutyrate, isovalerate, total branched chain fatty acid (BCFA), and total short chain fatty acid (SCFA) among groups. A one-way ANOVA was performed with Tukey's test. Values with different letters indicate significant differences among the groups.

with the healthy human gut and is widely used as a probiotic supplement due to its health promoting effects (O'Callaghan & van Sinderen, 2016). Relative abundances of *Bifidobacterium* were significantly higher following fermentation in the presence of oat and wheat and their SHS-treated counterparts compared with cellulose. Studies have shown that both plant and host-derived carbohydrates can stimulate the growth of *Bifidobacterium* (O'Callaghan and van Sinderen, 2016). No significant differences were observed between the oat samples (*i.e.* SHS-treated oat and native oat) and the wheat samples (*i.e.* SHS-treated wheat and native wheat) in terms of relative abundances of *Bifidobacterium*. *Lactobacillus* abundances were also higher following fermentation in the presence of oat, wheat, and their SHS-treated counterpart groups when compared to the negative control (cellulose). LEfSe analysis revealed that the SHS-treated oat group was shaped mostly by *Lactobacillus* which has been shown to have a pathogen-inhibiting effect *via* binding to epithelial cell receptors, producing bile salt hydrolase to survive in the small intestine and producing lactic acid which results in a reduction of pH (Er, Erim, Koç, & Kıvanç, 2019). Bile salts have been reported as toxic to bacterial cells *via* disrupting cell membrane (Succi et al., 2005). Reduction of pH inhibits the growth of pathogenic microorganisms including food-borne pathogens such as *Salmonella enterica* and *Listeria monocytogenes* (Lund et al., 2020).

Our study demonstrated a significant reduction in *Escherichia_Shigella* following fermentation in the presence of SHS-treated brans. LEfSe analysis revealed that *Escherichia_Shigella* was the signature genera of the negative control (cellulose) group. *Escherichia_Shigella* and *Alistipes* were found to be significantly lower following fermentation in the presence of SHS-treated oat compared with non-treated oat. *Escherichia* and *Shigella* are known as enteric pathogens which are associated with several gastric diseases (Devanga Ragupathi, Muthuirulandi Sethuvel, Inbanathan, & Veeraraghavan, 2018). While *Escherichia coli* might be considered as a commensal bacteria in the healthy human gut, some strains have virulence factors (Kaper, Nataro, & Mobley, 2004). A broad range of infections are attributed to different *Escherichia* strains such as urinary tract infection, meningitis/sepsis in children, and diarrhoea (Nataro & Kaper, 1998). Increases in abundance of *Escherichia* are commonly observed in gut microbiota dysbiosis such as small intestinal bacterial overgrowth or inflammatory bowel diseases (Leite et al., 2020; Siniagina et al., 2020).

Alistipes is a relatively new genus found in the human gut microbiota and belongs to the Bacteroidetes phylum. It is anaerobic and generally considered commensal bacteria (Parker, Wearsch, Veloo, & Rodriguez-Palacios, 2020). This genus was first discovered in tissue samples of children diagnosed with appendicitis (Rautio et al., 2003). Some studies have revealed a link between *Alistipes* and a number of diseases including liver fibrosis, colorectal cancer, and cardiovascular disease (Moschen et al., 2016; Rau et al., 2018; Zuo et al., 2019). A recent study conducted in humans revealed a positive correlation between high systolic blood pressure and *Alistipes indistinctus* (Pearson correlation = 0.33, $p = 0.0357$; Spearman correlation = 0.28, $p = 0.0818$) and *Alistipes finegoldii* (Pearson correlation = 0.26, $p = 0.069$; Spearman correlation = 0.39, $p = 0.0125$) (Kim et al., 2018). Moreover, this genus has been implicated in inflammation and bacteraemia in gastrointestinal disorders (Parker et al., 2020).

Following fermentation in the presence of SHS-treated oat and wheat samples, both showed significantly increased relative abundances of

Lachnospiraceae NC2004 group as compared to cellulose. Recently, a reduction of *Lachnospiraceae NC2004* group in Chinese children with autism spectrum disorder was reported (Liu et al., 2019). *Lachnospiraceae* is known as one of the key butyrate producers in the gut (Pryde, Duncan, Hold, Stewart, & Flint, 2002). Another genus that was found at significantly higher relative abundance in all fibre groups (with the exception of inulin) is *Pediococcus* with 4.2-fold increase in SHS-treated oat when compared to cellulose. *Pediococcus* is a member of the lactic acid bacteria group and is mainly used in the production of fermented products such as porridges, cooled gels, alcoholic, and non-alcoholic beverages (Coda, Rizzello, Trani, & Gobbetti, 2011; Nout, 2009). *Pediococcus* is also a pediocin producer, one of the most effective bacteriocins against *Listeria monocytogenes* (Porto, Kuniyoshi, Azevedo, Vitolo, & Oliveira, 2017). Interestingly, we observed that *Megamonas* was significantly lower in all fibre groups compared to cellulose. A 2015 study examined the faecal microbiota composition of patients diagnosed with major depressive disorder. *Megamonas*, *Alistipes*, and *Phascolarctobacterium* were found to be more abundant in patients with major depressive disorder when compared with healthy controls (Jiang et al., 2015). In another study, *Megamonas* was also found to be 8.83-fold greater in subjects with pre-diabetes mellitus than in normal glucose tolerant subjects (Zhang et al., 2013). A number of studies have demonstrated that some strains of the *Megamonas* genus can ferment glucose to acetate and propionate (Chevrot, Carlotti, Sopena, Marchand, & Rosenfeld, 2008; Sakon, Nagai, Morotomi, & Tanaka, 2008).

Faecalibacterium is another genus that contains butyrate-producing species such as *Faecalibacterium prausnitzii* (Zuo et al., 2019). The abundance of *Faecalibacterium* was significantly higher in relative abundance in all fibre groups (with the exception of inulin) compared with cellulose. A 2019 study investigating the heart-gut axis revealed that *Faecalibacterium* was lower in patients with atrial fibrillation than in healthy subjects (Zuo et al., 2019). Similarly, reduced abundances of *Faecalibacterium* have been reported in Crohn's disease patients (Takahashi et al., 2016).

Altogether, our results suggest that SHS treatment is more effective for oat bran than wheat bran. This effect might be attributed to the higher beta glucan content in oat samples. Following *in vitro* batch fermentation with fibre supplementation, a significant increase in relative abundance of Firmicutes and Actinobacteria was found. As the consumption of fibre has decreased over the years, it is important to produce novel foods to improve human health.

5. Conclusions

This study has demonstrated that SHS-treated oat and wheat bran can positively modulate gut microbiota composition generating differential effects when compared with the non-treated counterparts. SHS treatment can be useful in the food industry to increase the functionality of dietary fibre to positively modify the gut microbiome. Even though this *in vitro* study showed the potential health promoting effects of SHS treatment, it is not certain that treated fibres have beneficial effects on human health. Animal studies and human clinical trials should be performed to confirm the beneficial effects *in vivo*.

CRediT authorship contribution statement

Fatma Koc: Methodology, Investigation, Formal analysis, Writing – original draft. **Ivan Sugrue:** Methodology, Investigation, Writing – original draft. **Kiera Murphy:** Formal analysis, Writing – original draft. **Stefano Renzetti:** Methodology, Formal analysis, Resources, Writing – review & editing. **Martijn Noort:** Resources, Writing – review & editing. **R. Paul Ross:** Visualization, Supervision, Writing – review & editing. **Catherine Stanton:** Visualization, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2022.103082.

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