In vitro fermentability of a broad range of natural ingredients by fecal microbiota from lean and obese individuals: potential health benefits

Left running head: A. GUAL-GRAU ET AL.

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Andreu Gual-Grau[a] Maria Guirro[b] Anna Crescenti[c] Noemí Boqué[c] Lluís Arola[a] AQ1

[a]Department of Biochemistry and Biotechnology, Nutrigenomics Research Group, Universitat Rovira i Virgili, Tarragona, Spain;

[b]Eurecat, Centre Tecnològic de Catalunya, Centre for Omic Sciences (Joint Unit Eurecat-Universitat Rovira i Virgili), Unique Scientific and Technical Infrastructures (ICTS), Reus, Spain;

[c]Eurecat, Centre Tecnològic de Catalunya, Technological Unit of Nutrition and Health, Reus, Spain;

CONTACT Noemí Boquénoemi.boque@eurecat.org Eurecat, Centre Tecnològic de Catalunya, Technological Unit of Nutrition and Health, Avinguda Universitat 1., Reus 43204, Spain

Abstract

The prevalence of obesity and related complications is continuously increasing while the gut microbiota might have a significant role to address this challenge. In this context, the food industry generates large amounts of residues that could be likely revalorised as functional ingredients. Hence, we evaluated the fermentability of food skins, husks, shells, trimming residues, mosses and mushrooms, which were subjected to *in vitro* fermentation with faecal microbiota from lean and obese adults. We demonstrated for the first time that pumpkin skin is highly fermented by human faecal microbiota showing pH-lowering effects and promoting gas and SCFA production. Furthermore, brewers' spent grain generated an inulin-like SCFA profile after microbial fermentation, whereas Irish moss, plum skin, quinoa husk and mushrooms, including *Armillaria mellea* and *Boletus edulis*, showed high fermentation rates. Remarkably, although propionate production was significantly higher in obese individuals, the fermentability of the ingredients was similar between lean and obese conditions.

Keywords: Natural ingredients; food by-products; human faecal microbiota; obesity; *in vitro* fermentation

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Introduction

The microbial communities living to mammals' gastrointestinal tract provide a wide range of essential metabolites that hosts are not able to synthetise on their own (Cani et al. 2019). Our microbiome takes advantage of the dietary components that reach the large intestine, such as indigestible plant polysaccharides, which are primarily transformed into short chain fatty acids (SCFA) (Henningsson et al. 2001; Makki et al. 2018). These bacterial products have been related to multiple beneficial effects on mammalian energy metabolism, mainly through the signalling of satiety and the enhancement of energy expenditure, whereas a healthy gut microbiota composition is a key determinant to its balanced production (Freeland et al. 2010; Daud et al. 2014). Of note, apart from fibre-derived SCFAs, microbiota fermentation leads to the production of a broad range of other molecules such as polyphenol-derived compounds that, in turn, might impact on the gut microbial communities and its interaction with the host (Rowland et al. 2018). In this scenario, both microbes and host co-live in a mutually beneficial symbiosis. However, exogenous events, such as stress, drugs and diet, can alter the phylogenetic structure and function of gut microbiota (Graf et al. 2015; Karl et al. 2018), leading to the impairment of host homeostasis.

In recent years, the gut microbiota has been identified as a key element in the aetiology of obesity (Kobyliak et al. 2016). Accordingly, microbiota actively contributes to host metabolic efficiency by enhancing energy harvest from the diet (Wichmann et al. 2013). In this sense, the lack of gut microbiota prevents the development of diet-induced obesity in rodents (Bäckhed et al. 2007), whereas obesity-resistant germ-free mice become obese after receiving a microbiota transplant (Turnbaugh et al. 2006). Although human studies have shown controversial results concerning the contributory role of specific phyla to the obese phenotype, changes in the prevalence of certain species and the diversity of bacterial communities between lean and obese individuals have been widely described (Turnbaugh et al. 2009; Million et al. 2012; Ignacio et al. 2016), highlighting the importance of the intestinal microbiome in obesity

progression. Thus, the dietary modulation of the gut microbiome has become one of the major targets in the prevention of metabolic diseases (Okubo et al. 2018). In this regard, the prebiotic potential of some dietary fibres, which are the main substrates for gut microbiota, has been reported by several researchers (Flint et al., 2012; Rossi et al. 2005). For instance, inulin-type fructans favour the growth of beneficial bacteria, SCFA production, fat oxidation and reduced food intake during overweight and obesity (Vandeputte et al. 2017; van der Beek et al. 2018; Hiel et al. 2019).

Every year, the food industry generates large amounts of food wastes or by-products that negatively

impact the biosphere because of their poor oxidative and biological stability (Thompson and Darwish 2019), representing one major concern around the world. Currently, the circular economy is emerging as a new sustainable approach to confront the substantial economic losses for manufacturing industries related to waste production (Zuin and Ramin 2018). In this model, the demand to optimise the impact of industrial by-products on society is addressed through the management and revalorisation of food wastes by benefitting from their nutritional properties. In this sense, some food by-products such as husks, skins, seeds, shells and trimming residues are mostly composed by highly valuable components, such as polysaccharides, proteins, fibres, phytochemicals and bioactive compounds.

In vitro methods for screening untapped prebiotic candidates enable a simple and rapid evaluation of its impact on the ecosystem and the microbial community response. Several publications have obtained encouraging results in evaluating the potential prebiotic effects of different industrial by-products, such as coffee wastes (López-Barrera et al. 2016), fruit peels (Tejada-Ortigoza et al. 2019), soybean Okara (Pérez-López et al. 2016) and Aloe Vera mucilage (Gullón et al. 2015), using *in vitro* fermentation procedures. In this study, we aimed to evaluate the fermentability of a wide range of industrial by-products in both normoweight and obese conditions using an *in vitro* fermentation procedure with human faecal samples. To this end, several industrial residues including husks, shells, skins, grains and branches, as well as other potentially miscellaneous candidates, were tested.

Materials and methods

Sample preparation

The industrial by-products used in this screening study included nutshell (mixed varieties including Alcalde, Onteniente and Baldo II, Carcagente, Cerdá, Escribá and Villena, Tarragonès, Tarragona, Spain), rice husk (Montsianell variety, *Oryza sativa*, El Montsià, Tarragona, Spain), brewers' spent grain (BSG) (white IPA-derived residues, Alcover, Tarragona, Spain), pumpkin skin (*Curcubita maxima*, La Pobla de Montornès, Tarragona, Spain), olive press cake (OPC) (Arbequina variety, *Oleo europea*, Tarragonès, Tarragona, Spain), stevia branches (*Stevia rebaudiana bertoni*, Balaguer, Lleida, Spain), quinoa husk (*Quenopodium quinoa*, Almenar, Lleida, Spain), and plum skin (*Prunus domestica*, Lleida, Spain). We also tested the fermentability of some miscellaneous ingredients including fungus (*Boletus edulis*, Ripollès, Girona, Spain; *Armillaria mellea*, Solsonès, Lleida, Spain) and Irish moss (*Chondrus crispus*; A Coruña, Galicia, Spain). All products were accurately selected according to these criteria: (1) presenting a valuable nutrient composition, essentially pointing up the fibre content shown in the literature; (2) limited previous scientific evidences of the potential fermentability of the selected products by gut microbiota; (3) currently unexploited residues from the food industry; (4) high or moderate generation during industrial processes, thus ensuring a sustainable and worthwhile revalorisation; and (5) market availability of the products, essentially obtained from local manufacturers as detailed above. Inulin (Orafti® HP, BENEO GmbH, Germany) and cellulose

(C6288, Merck, Spain) were used as positive and negative controls, respectively. All samples were stored at −80 °C upon arrival until they were manually grounded, crushed with a grinder and lyophilised. Dry samples were stored at room temperature, properly protected from light and preserved from moisture in a desiccator.

In vitro digestion

Prior to the fermentation procedure, an *in vitro* gastrointestinal digestion was conducted to simulate the mammalian conditions after food intake, which was performed as previously described (Minekus et al. 2014) with slight modifications. Briefly, lyophilised samples were diluted with pH 7.0-controlled 1X phosphate buffer saline (PBS) at a 3:50 (weight-to-volume) ratio and subsequently heated to 37 °C for 10 min. During the entire procedure, samples were maintained under magnetic stirring and temperature-controlled conditions (37 °C). The oral phase was simulated by adding 50 µL of α-amylase from human saliva (A1031, Merck, Spain) and incubating for 15 min. To reproduce the gastric phase, the pH was set to 2.0 ± 0.1

with HCl, 250 µL of 1 mg/mL pepsin from porcine gastric mucosa (P7012, Merck, Spain) was added and the solution was incubated for 30 min. During the intestinal phase, pH was readjusted to 6.9 ± 0.1 with NaOH before adding 1 mL of 50 mg/mL pancreatin from porcine pancreas (P7545, Merck, Spain) and incubated for 3 h. Samples were transferred into dialysis membranes with a 1000 Da cut off (Spectra/Por 1000 Da MWCO Dialysis Tubing, Spectrum Laboratories Inc., UK) as previously described (Noack et al. 2013). The dialysis procedure was performed to reflect the absorption of low-molecular-weight compounds before reaching the large intestine during digestion. After 24 h under constant water circulation, the digestion residues were lyophilised and kept into a desiccator at room temperature until they were used for *in vitro* fermentation.

Donors and fecal sample collection

Faecal samples from male adult volunteers between 30 and 50 years-old were collected for *in vitro* fermentation. In order to ensure that microbial communities obtained from both normoweight and obese volunteers were representative of both lean and obese conditions, respectively, the subjects were excluded if they met any of the following criteria: smoking, recent use of antibiotics or nutraceutics, chronic alcohol intake, presence of gastrointestinal pathologies, presence of obesity-related disorders such as chronic inflammation or diabetes, and presence of recent and sudden body weight changes (>2 kg change in the last three months). All procedures were approved by the Ethics Committee on Clinic Investigation from the Hospital Sant Joan, Reus, Spain (CEIM 035/2018). All the volunteers provided a signed informed consent. Six adult volunteers were included in the study, including three normoweight (BMI = 20–25 kg/m^2) and three obese (BMI = 30–35 kg/m^2) individuals.

Donors were previously instructed to defaecate directly into a sterile recipient and quickly placed into an anaerobic bag system (Bio-Bag type C, BD, Germany) including an anaerobic sachet to ensure the maintenance of a CO_2 atmosphere. The samples were immediately refrigerated and brought to the laboratory within the next 12 h. Once obtained, faecal samples were pooled according to groups (normoweight or obese) as previously reported (Nordlund et al. 2012), and the inoculum was freshly prepared for *in vitro* fermentation as described below.

In vitro fermentation

The *in vitro* fermentation experiment was performed under strict sterile conditions at 37 °C and mostly maintained an anaerobic atmosphere during the entire procedure. The *in vitro* fermentation protocol was performed as previously described (Sánchez-Moya et al. 2017) with slight modifications. Briefly, the fermentation media was composed of 2 g peptone water (CM0009, Oxoid, United Kingdom), 2 g yeast extract (Y4250, Merck, Spain), 100 mg sodium chloride (S5886, Merck, Spain), 40 mg potassium phosphate dibasic (121,512, PanReac AppliChem, Germany), 40 mg potassium phosphate monobasic (131,509, PanReac AppliChem, Germany), 10 mg magnesium sulphate heptahydrate (230,391, Merck, Spain), 10 mg calcium chloride dehydrate, 2 g sodium bicarbonate (S5761, Merck, Spain), 2 mL Tween 80 (P4780, Merck, Spain), 0.5 g bile salt (B8631, Merck, Spain) and 50 mg haemin (H9039, Merck, Spain). The pH was set to 7.0 ± 0.1 before adding 4 mL of 0.025% resazurin (R7017, Merck, Spain), which acts as an indicator of anaerobic conditions through colour change. The fermentation media was autoclaved, and 10 µL of vitamin K1 (v3501, Merck, Spain) was added prior to starting the experimental procedure. Fifty milligrams of each digestion-derived product were placed into sterile Hungate tubes (2047-16125, Bellco Glass Inc., USA) with rubber and screw caps. Then, 4 mL of media along with 100 µL of oxyrase (30620059-1, bioWORLD, USA), which removes the oxygen from the environment, was added at each fermentation tube and kept in an anaerobic atmosphere for 30 min at 37 °C for its hydration.

To prepare the inoculums, pooled faecal samples from each group (obese or normoweight) were separately diluted into PBS 0.1 M at 10% (w/v), maintained at 37 °C under a flux of CO_2 gas to ensure anaerobic conditions, and finally filtered through a sterile 1 mm nylon mesh (FMNY 250, Filter Lab, Spain). Once the residues were properly hydrated, 1 mL of faecal inoculum from normoweight or obese individuals was added their corresponding tubes using a syringe, going through its rubber cap. Intra-day triplicates for each condition were maintained in a shaking water bath at 37 °C for 0 h, 4 h or 24 h until 200 µL of copper sulphate (209,198, Merck, Spain) at 2.75 mg/mL was added to inhibit further fermentation. Of note, triplicates for the *in vitro* fermentation of our positive and negative controls (inulin and cellulose, respectively), were performed intra- and inter-day for each condition. Early (4 h) and late (24 h) fermentation time points were selected to discriminate the capacity

of microbial communities to utilise the selected ingredients in a short or long-term basis, respectively, while the basal point (0 h) corresponded to the initial composition of the samples.

In situ determinations

Total gas volume was measured by inserting an empty graduated syringe through the rubber cap and measuring the plunger displacement after 24 h of fermentation. At the corresponding time point, all reactions were inhibited as described above and 400 µL per tube were aliquoted into 1.5 mL sterile tubes and frozen at −80 °C for further metabolomic analysis. Finally, pH was measured with a pH Metre (PHS-3D, Shanghai San-Xin Instrumentation, Inc., China) previously calibrated.

After *in vitro* fermentation, the supernatants obtained were subjected to metabolomic analysis using nuclear magnetic resonance 1H (1H NMR). Three hundred µL of the fermented solution was centrifuged to eliminate particulates (21,000 g, 25 min, 4 °C). Then, 400 µL of 0.05 M PBS buffer in D2O (pH 7.2, TSP 0.7 mM) was added, vigorously vortexed, sonicated until complete homogenisation and centrifuged (14,000 g, 5 min, 25 °C). For NMR measurement, 600 µL of the upper phase was placed into a 5 mm O.D. NMR tube. 1H NMR spectra were recorded at 300 K on an Avance III 600 spectrometer (Bruker, Germany) operating at a proton frequency of 600.20 MHz using a 5 mm PBBO gradient probe. One-dimensional (1 D) 1H pulse experiments were carried out using the nuclear Overhauser effect spectroscopy (NOESY- Noesypr1d pulse programme in Bruker). Solvent presaturation with an irradiation power of 160 uW was applied during recycling delay (5 s) and mixing time (100 ms). The 90° pulse length was calibrated for each sample and varied from 10.31 to 11.12 ms. The spectral width was 9.6 kHz (16 ppm), and a total of 256 transients were collected into 64 k data points for each 1H spectrum. The exponential line broadening applied before Fourier transformation was 0.3 Hz. The frequency domain spectra were manually phased and baseline-corrected using Topspin software (v. 3.2, Bruker). Metabolite identification and quantification were performed using HMDB, AMIX 3.9 (Bruker) and Chenomx ® software spectra databases.

Data analysis

All results are expressed as the mean ± standard error of the mean (SEM). Because the fermentation procedure was not performed on all samples at the same time, the negative control was properly used to correct the pH measurements and SCFA results, which were expressed as the relative variation within each time point. To assess significant differences regarding the relative variation of the measurements between ingredients and our positive control (inulin), statistical analyses were based on repeated measures ANOVA within each condition (normoweight or obese individuals). In the case of a significant ingredient × time interaction, the main effects within each time point were studied through one-way ANOVA followed by Sidak post hoc test. A probability of $p < .05$ was considered to be statistically significant. Statistical analyses were performed using SPSS 22 software (SPSS, Inc.,

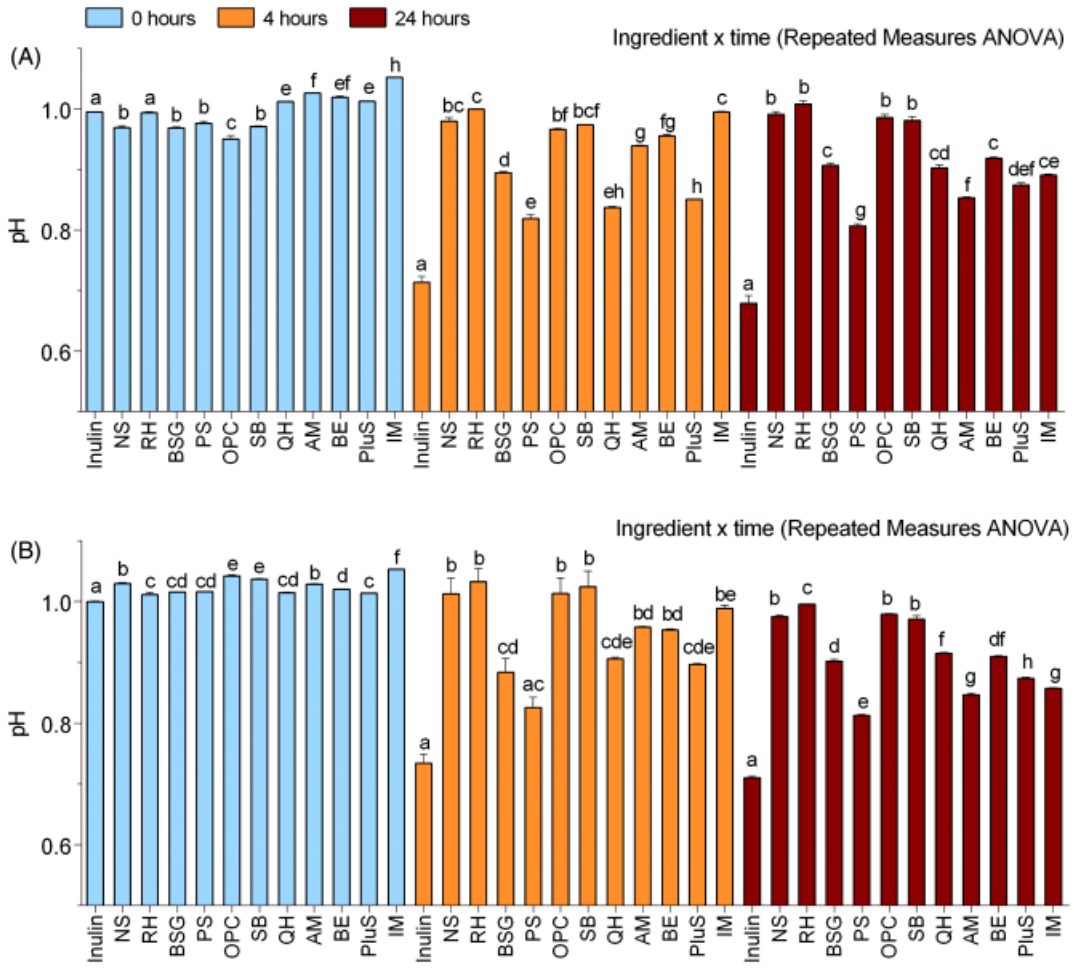
Chicago, IL, USA). Principal component analysis (PCA) was performed through the multivariate modelling of metabolomics data in MATLAB (MathWorks) using in-house scripts as previously described (Massot-Cladera et al. 2017).

Results

pH changes and total gas production

Overall, the pH changes were closely related to those observed in gas production. Repeated measures ANOVA revealed a significant ingredient × time interaction ($p < .01$) explaining the pH variation within both normoweight and obese conditions (Figure 1). After 4 h of fermentation and focussing on normoweight samples, pumpkin skin induced the highest pH decrease (18%), followed by quinoa husk, plum skin and BSG (16%, 15% and 11%, respectively). In this instance, other substrates such as *Armillaria mellea* and *Boletus edulis* caused a slight drop in pH that was significantly higher than that promoted by nutshell, rice husk and Irish moss, which showed the largest pH values. However, these values were all significantly different from those obtained with inulin, which induced a decrease in pH of 29%. Importantly, pumpkin skin triggered the highest pH decrease (17%) in obese faecal samples after 4 hours of fermentation, showing no significant differences when compared to inulin (23%). In addition, substantial pH-lowering effects were also attributed to BSG (12%), plum skin (10%) and quinoa husk (9%).

Figure 1. Normalised pH values of the faecal slurries after 0 h, 4 h and 24 h of *in vitro* fermentation using faecal inoculums from lean (A) and obese (B) individuals. The pH measurements obtained from ingredient fermentation were properly corrected by negative controls within the same time point and expressed as the relative pH variation. Data are expressed as the mean ± SEM (*n* = 3). Ingredient × time interactions were assessed using repeated measures ANOVA. The main effects were evaluated and different letters represent significant differences among ingredients within each time point (one-way ANOVA followed by Sidak post hoc test). AM: *Armillaria mellea*; BE: *Boletus edulis*; BSG: brewers' spent grain; IM: Irish moss (*Chondrus crispus*); NS: nutshell, OPC: olive press cake; PluS: plum skin; PS: pumpkin skin; QH: quinoa husk; RH: rice husk; SB: stevia branches.



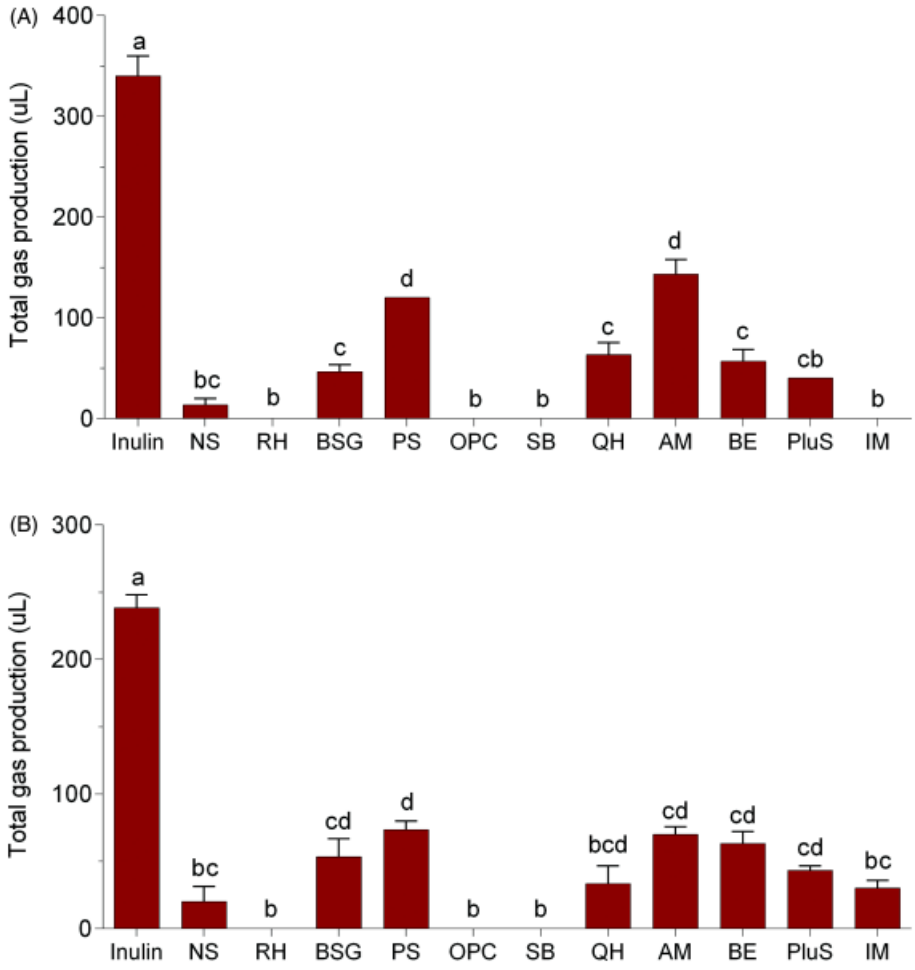
After 24 h of fermentation, the pH decreased up to 32% and 29% when inulin was included as a fermentable substrate in normoweight and obese conditions, respectively (Figure 1). Overall, the pH-lowering effects observed in normoweight faecal samples at 4 h were maintained after 24 h by

pumpkin skin, plum

skin and BSG ingredients. In addition, other ingredients showing marginal pH-lowering effects at 4 h drove an important decrease in pH at the end time point, such as *Armillaria mellea*, *Boletus edulis* and Irish moss, within both normoweight and obese conditions. However, nutshell, rice husk, OPC and stevia branches did not induce changes in pH during the entire fermentation period within either normoweight or obese individuals.

In vitro fermentation of faecal samples from both normoweight and obese individuals induced gas production at the end of the incubation period (24 h), which acted as an indicator of particular fermentation rates depending on the tested substrates, as shown in Figure 2. Prominently, *Armillaria mellea* and pumpkin skin showed the highest fermentation rate within normoweight conditions, reaching 42.2% and 34.7% of the total amount of gas produced by the positive control and significantly differing from the rest of the ingredients. Moreover, quinoa husk, *Boletus edulis* and BSG produced 18.6%, 16.7% and 13.4% of the total gas volume generated by inulin, respectively, showing no significant differences between them. In contrast, nutshell generated significantly lower amounts of gas than the other substrates, whereas rice husk, OPC, stevia branches and Irish moss did not promote gas production after 24 h of fermentation. Similarly, the highest gas production within the obese condition was observed for pumpkin skin (33.1%), *Armillaria mellea* (27.9%), *Boletus edulis* (25.3%), BSG (23.9%), plum skin (17.3%) and quinoa husk (13.6%) after 24 h (Figure 2). Finally, Irish moss and nutshell were identified as the ingredients inducing the lowest gas production, while no gas generation was detected when rice husk, OPC or stevia branches were used as fermentable substrates, as previously described within normoweight conditions.

Figure 2. Absolute gas production measured after 24 h of *in vitro* fermentation using faecal inoculums from lean (A) and obese (B) individuals. Data are expressed as the mean ± SEM (n = 3). Different letters represent significant differences among ingredients (one-way ANOVA followed by Sidak post hoc test). AM: *Armillaria mellea*; BE: *Boletus edulis*; BSG: brewers' spent grain; IM: Irish moss (*Chondrus crispus*); NS: nutshell, OPC: olive press cake; PluS: plum skin; PS: pumpkin skin; QH: quinoa husk; RH: rice husk; SB: stevia branches.



SCFA production

The relative amounts of SCFAs produced after 0 h, 4 h and 24 h are presented in Table 1. Overall, we found ingredient-dependent significant variations in the SCFA butyrate, propionate and acetate production over time in both normoweight and obese conditions ($p < .001$, repeated measures ANOVA).

Table 1. SCFAs production after 0 h, 4 h and 24 h of *in vitro* fermentation with the faecal microbiota from normoweight individuals.

	Inulin	Nutshell	Rice husk	BSG	Pumpkin skin	OPC	Stevia branches	Quinoa husk	*Armillaria Mellea*	*Boletus Edulis*	Plum skin	Irish moss
Butyrate												
0h	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.3 ± 0.0*	0.9 ± 0.1	1.2 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.7 ± 0.0*	1.0 ± 0.0
4 h	2.1 ± 0.1	1.1 ± 0.0*	1.1 ± 0.0*	2.9 ± 0.1*	3.3 ± 0.1*	1.1 ± 0.2*	1.7 ± 0.0	1.3 ± 0.1*	1.2 ± 0.0*	1.1 ± 0.1*	1.2 ± 0.1*	1.4 ± 0.1*
24 h	3.5 ± 0.2	1.1 ± 0.0*	1.0 ± 0.0*	2.5 ± 0.1*	3.2 ± 0.1	1.1 ± 0.1*	1.4 ± 0.1*	1.6 ± 0.0*	1.6 ± 0.0*	1.3 ± 0.0*	1.3 ± 0.0*	1.2 ± 0.0*
Propionate												
0h	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.5 ± 0.0*	1.0 ± 0.0	1.1 ± 0.0
4 h	0.8 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	1.2 ± 0.0*	1.2 ± 0.0*	1.1 ± 0.2	1.3 ± 0.0*	0.8 ± 0.0	1.0 ± 0.1	1.3 ± 0.1*	0.9 ± 0.1	1.3 ± 0.1*
24 h	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0.0*	1.0 ± 0.0*	0.9 ± 0.0	1.1 ± 0.1*	1.0 ± 0.0	1.1 ± 0.0*	1.5 ± 0.0*	1.5 ± 0.0*	1.1 ± 0.0*	1.6 ± 0.0*
Acetate												
0h	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4 h	3.3 ± 0.1	1.2 ± 0.1*	1.1 ± 0.1*	2.2 ± 0.0*	2.8 ± 0.0	1.2 ± 0.2*	1.6 ± 0.1*	1.5 ± 0.1*	1.2 ± 0.1*	1.3 ± 0.1*	1.5 ± 0.1*	1.3 ± 0.1*
24 h	2.5 ± 0.0	1.1 ± 0.0*	1.0 ± 0.0*	1.5 ± 0.0*	1.6 ± 0.1*	1.0 ± 0.0*	1.2 ± 0.1*	1.6 ± 0.0*	1.6 ± 0.1*	1.6 ± 0.0*	1.6 ± 0.0*	1.6 ± 0.1*
Total SCFA												
0h	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	0.9 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4 h	1.8 ± 0.0	1.1 ± 0.0*	1.1 ± 0.1*	1.8 ± 0.0	2.0 ± 0.0	1.2 ± 0.2*	1.4 ± 0.0	1.2 ± 0.1*	1.2 ± 0.1*	1.2 ± 0.1*	1.3 ± 0.1*	1.3 ± 0.1*
24 h	1.9 ± 0.0	1.0 ± 0.0*	1.1 ± 0.0*	1.5 ± 0.0*	1.5 ± 0.1*	1.0 ± 0.0*	1.1 ± 0.1*	1.4 ± 0.0*	1.5 ± 0.0*	1.4 ± 0.0*	1.3 ± 0.0*	1.5 ± 0.0*

Data is presented as the mean ± SEM, and normalised by the negative control (cellulose) at the same time point ($n = 3$ per group). Repeated Measures ANOVA revealed ingredient × time interaction in all cases. Main effects within each time were examined using one-way ANOVA followed by Sidak post-hoc test. *Significant differences compared to inulin at each time point ($p < .05$). BSG: brewers' spent grain; OPC: olive press cake.

Focussing on normoweight samples (Table 1) AQ2, pairwise comparisons revealed that pumpkin skin, BSG and stevia branches promoted a similar generation of total SCFAs than inulin at 4 h, although it was not maintained at the end time point (24 h). Surprisingly, pumpkin skin and BSG induced a

greater increase in butyrate production than inulin after 4 h, denoting significant differences. In addition, while BSG fermentation resulted in a lower butyrate concentration after 24 h, pumpkin skin maintained an elevated butyrate formation emerging as the only ingredient showing no significant differences when compared to inulin. On the other hand, stevia branches were the unique ingredient presenting an increment of butyrate similar to that induced by our positive control after 4 h of fermentation.

Inulin fermentation did not yield propionate production after 4 or 24 h. Most of the ingredients also showed a lack of propionate formation during the first 4 h of fermentation, whereas nutshell, stevia branches and pumpkin skin did not differ from inulin at the end of the study. Other ingredients including *Armillaria mellea*, *Boletus edulis* and Irish moss, significantly differed from the inulin control because of an increase in propionate generation at 24 h. Furthermore, the concentration of acetate followed an inulin-like pattern only when pumpkin skin was used as a fermentable substrate, showing acetate overproduction within the first 4 h of fermentation. However, this effect was not maintained after 24 h,

where acetate production induced by all of the ingredients significantly differed from that promoted by inulin.

In obese faecal samples, BSG, pumpkin and plum skins, quinoa husk and mushrooms increased the total SCFA production after 4 h of fermentation, following an inulin-like pattern. After 24 h, *Armillaria mellea*, *Boletus edulis* and Irish moss maintained an elevated generation of SCFAs, showing no significant differences with respect to inulin, as presented in Table 2. At 4 h, five ingredients, including BSG, pumpkin skin, Irish moss, rice husk and stevia branches followed an inulin-like butyrate generation. Although the rise of butyrate levels after 24 h in the inulin group was not reproduced by any ingredient, a numerically greater butyrate production was observed with pumpkin skin, BSG, *Armillaria mellea*, *Boletus edulis* and plum skin compared to less effective ingredients such as nutshell, rice husk, OPC and stevia branches at 24 h.

Table 2. SCFAs production after 0 h, 4 h and 24 h of *in vitro* fermentation with the faecal microbiota from obese individuals.

	Inulin	Nutshell	Rice husk	BSG	Pumpkin skin	OPC	Stevia branches	Quinoa husk	*Armillaria Mellea*	*Boletus Edulis*	Plum skin	Irish moss
Butyrate												
0h	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.1	1.2 ± 0.0*	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	0.8 ± 0.0*	1.0 ± 0.0

	Inulin	Nutshell	Rice husk	BSG	Pumpkin skin	OPC	Stevia branches	Quinoa husk	*Armillaria Mellea*	*Boletus Edulis*	Plum skin	Irish moss
4 h	1.2 ± 0.2	1.0 ± 0.1*	1.1 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	1.0 ± 0.2*	1.1 ± 0.1	1.8 ± 0.0*	1.5 ± 0.0*	1.5 ± 0.0*	1.7 ± 0.0*	1.4 ± 0.0
24 h	2.3 ± 0.0	1.0 ± 0.1*	1.1 ± 0.0*	1.6 ± 0.0*	1.8 ± 0.0*	1.2 ± 0.0*	1.2 ± 0.0*	1.7 ± 0.1*	1.7 ± 0.0*	1.6 ± 0.0*	1.5 ± 0.0*	1.4 ± 0.0*
Propionate												
0h	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.4 ± 0.1*	1.1 ± 0.0	1.1 ± 0.1
4 h	1.4 ± 0.0	1.0 ± 0.0*	1.1 ± 0.0*	1.2 ± 0.0*	1.1 ± 0.0*	1.1 ± 0.0*	1.1 ± 0.0*	1.2 ± 0.0	1.2 ± 0.0*	1.3 ± 0.1	1.3 ± 0.0	1.1 ± 0.1*
24 h	1.5 ± 0.1	1.0 ± 0.1*	1.1 ± 0.0*	1.3 ± 0.0	1.3 ± 0.0	1.1 ± 0.0*	1.2 ± 0.0*	1.3 ± 0.1	1.8 ± 0.0*	1.8 ± 0.0*	1.4 ± 0.0	1.9 ± 0.0*
Acetate												
0h	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0*	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0*	1.0 ± 0.0	1.0 ± 0.0
4 h	1.4 ± 0.0	1.0 ± 0.0*	1.1 ± 0.0*	1.4 ± 0.0	1.4 ± 0.1	1.1 ± 0.0*	1.1 ± 0.0*	1.4 ± 0.0	1.1 ± 0.0*	1.2 ± 0.0*	1.4 ± 0.0	1.0 ± 0.0*
24 h	1.4 ± 0.1	1.1 ± 0.0*	1.1 ± 0.0*	1.3 ± 0.0	1.3 ± 0.0	1.1 ± 0.0*	1.3 ± 0.0	1.5 ± 0.1	1.7 ± 0.0*	1.7 ± 0.0*	1.6 ± 0.1	1.6 ± 0.0
Total SCFA												
0h	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0*	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4 h	1.4 ± 0.0	1.0 ± 0.0*	1.1 ± 0.0*	1.3 ± 0.0	1.3 ± 0.0	1.1 ± 0.0*	1.1 ± 0.0*	1.4 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.1 ± 0.0*
24 h	1.7 ± 0.1	1.0 ± 0.1*	1.1 ± 0.0*	1.4 ± 0.0*	1.5 ± 0.0*	1.1 ± 0.0*	1.2 ± 0.0*	1.5 ± 0.1*	1.7 ± 0.0	1.6 ± 0.0	1.5 ± 0.0*	1.6 ± 0.0

Data is presented as the mean ± SEM, and normalised by the negative control (cellulose) at the same time point ($n = 3$ per group). Repeated Measures ANOVA revealed ingredient × time interaction in all cases. Main effects within each time were examined using one-way ANOVA followed by Sidak post-hoc test. *Significant differences compared to inulin at each time point ($p < .05$). BSG: brewers' spent grain; OPC: olive press cake.

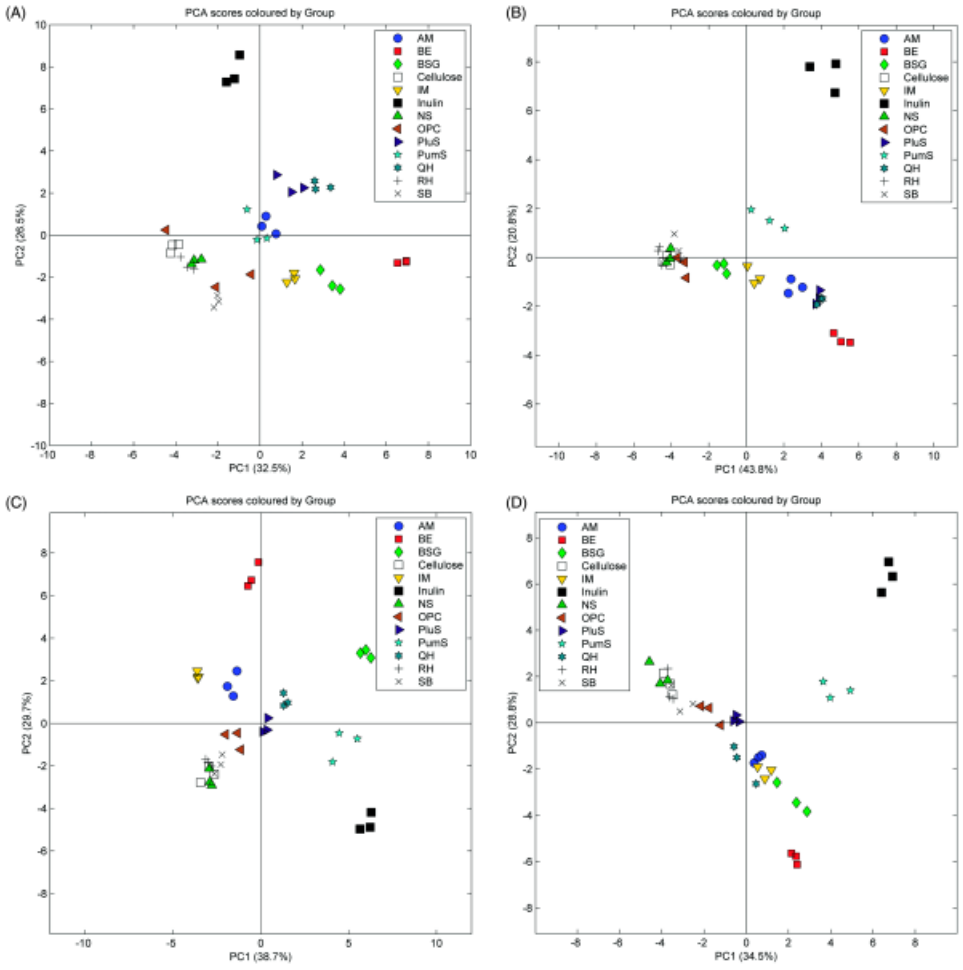
In contrast to that observed in faecal samples from normoweight individuals, inulin fermentation led to a raised production of propionate in obese conditions. Moreover, BSG, pumpkin skin, quinoa husk and plum skin promoted the generation of propionate paralleled to inulin after 24 h. The same ingredients also induced the production of acetate after 4 and 24 h, presenting no differences with inulin.

Clustering analysis

Metabolomic techniques allowed for the wide-range identification and quantification of metabolites

resulting from *in vitro* fermentation in a comprehensive approach, thereby revealing particular metabolomic profiles. PCAs constructed from metabolomic profiles were useful to identify clusters within each condition after 4 and 24 hours of *in vitro* fermentation (Figure 3). These analyses revealed that inulin generated the most dissimilar metabolic pattern at every time point within both conditions. Concerning normoweight faecal samples, PCA explained up to 59% of the overall variation (PC1: 32.5%; PC2: 26.5%) after 4 hours of fermentation as shown in Figure 3(A). Among all the ingredients tested, quinoa husk, plum and pumpkin skins, *Armillaria mellea* and BSG shaped the closest clusters to positive control samples. Consistently, ingredients showing no relevant impact on pH or gas production such as nutshell, rice husk, OPC and stevia branches were clustered together nearby to cellulose. Indeed, the clustering patterns after 24 h of fermentation, where 43.8% and 20.8% of the total variation were explained by PC1 and PC2, respectively, revealed that pumpkin skin exhibited the most inulin-like displacement along the PC2 axis (Figure 3(B)).

Figure 3. Principal component analysis constructed from the faecal slurries metabolic profiles. Clustering analyses included all the ingredients tested and both positive (inulin) and negative (cellulose) controls. *In vitro* fermentations with faecal inoculum from lean individuals at 4 h (A) and at 24 h (B), as well as from obese individuals at 4 h (C) and at 24 h (D) are included. AM: *Armillaria mellea*; BE: *Boletus edulis*; BSG: brewers' spent grain; IM: Irish moss (*Chondrus crispus*); NS: nutshell, OPC: olive press cake; PluS: plum skin; PC: principal component (axis); PumS: pumpkin skin; QH: quinoa husk; RH: rice husk; SB: stevia branches.



Fermentation of obese faecal samples revealed several clusters after 4 h, as presented in Figure 3(C), where PC1 and PC2 explained up to 38.7% and 29.7% of the overall dissimilarities, respectively. According to the clustering obtained within the normoweight individuals, pumpkin skin and inulin closely clustered at this time point, revealing a highly similar metabolic pattern after fermentation. Moreover, BSG was also associated with both pumpkin skin and inulin along the PC1, while *Boletus edulis* clustered separately throughout the PC2. As also observed within normoweight samples, such

ingredients as nutshell, stevia branches, rice husk and OPC, which showed no

significant variation in fermentation rates compared to cellulose, clustered alongside the negative control. After 24 h of fermentation, the positive control clearly differed from the rest of the ingredients, showing only close distances with the metabolic profiles generated after using the pumpkin skin as a fermentable source (Figure 3(D)). In this case, 34.5% of the overall variation explained by PC1 defined substantial differences regarding the fermentable susceptibility of ingredients, where the presence of mostly non-fermentable substrates was intensified on the left side. Finally, 28.8% of the total dissimilarities explained by PC2 elucidated that the metabolic profiles generated by *Boletus edulis* strongly differed from the positive control.

Discussion

Using a validated procedure for the *in vitro* fermentation of human faecal samples, we investigated the pH changes, gas production and metabolomic profiles generated by distinct ingredients. Gut microbial fermentation of indigestible plant polysaccharides produces a wide range of acidic end metabolites, such as SCFAs and lactate. Then, changes in pH can provide relevant information regarding the fermentation rate occurring in a system, while gas production is concomitant with fermentative processes acting as the main marker of complex carbohydrate utilisation by gut microbiota. Moreover, metabolomic techniques revealed the specific contributory role of each ingredient on the resulting metabolic profiles after being exposed to faecal microbiota. Despite that several compounds contained in our ingredients could be interacting with microbial communities during faecal fermentation, we have paid special attention on the microbial capacity to utilise plant indigestible polysaccharides.

According to the literature (Holscher 2017), inulin induced an important drop in pH and a high gas production after being fermented with microbiota from both normoweight and obese individuals, whereas cellulose is known to be poorly fermented by the gut microbiota. In humans, a lower colonic pH has been suggested to play a determinant influence on the microbiota composition and function by favouring the occurrence of butyrate-producing bacteria such as *Roseburia spp.* (Walker et al. 2005). In fact, this genus was found to be increased in subjects consuming high amounts of indigestible polysaccharides (Duncan et al. 2007). Otherwise, an increment in the populations of *Bacteroides spp.*, which is robustly adapted to low-fibre diets (Koropatkin et al. 2012) and previously found increased in diet-induced obese rodents (Gual-Grau et al. 2019), was reported when the pH increased from 5.5 to 6.5, highly correlating with a an increment of propionate production (Musso et al. 2011). Thus, the pH-lowering effect, which is linked to the SCFA production as a result of microbial fermentation of dietary compounds, contributes to the overall microbiota composition and, in turn, is prevents the overgrowth of pathogenic bacteria, such as *Enterobacteriaceae* and *Clostridia* (den Besten et al. 2013). In this regard, pumpkin skin, which caused the largest drop in pH and promoted butyrate

production, is suggested to be preferably utilised by the favoured butyrogenic bacteria. Concerning this ingredient, high mucilage content was previously assessed in pumpkin skin peel extract (Bahramsoltani et al. 2017). In parallel, mucilage-containing plants have been shown to improve the glycaemic and lipid control in diabetic

patients (Ziai et al. 2004). The prebiotic properties of different mucilage sources have been elucidated in bacterial cultures by favouring the growth of beneficial bacteria and promoting SCFA production (Guevara-Arauza et al. 2012; Gullón et al. 2015; Mueller et al. 2017).

On the other hand, we hypothesised that insoluble fibres, which are highly present in pumpkin skin (Butke et al. 2018), could partially mediate the fermentability and SCFA production described for this ingredient. For many years, insoluble fibres have been considered inert bulking compounds unable to be utilised by gut microorganisms (Wong et al. 2006). However, it has been stated that insoluble fibres should not be considered to be non-fermentable carbohydrates (Brotherton 2015), whereas a current

revision concluded that “insoluble” and “soluble” fibre classification may not be sufficient to explain fermentation performances (Wang et al. 2019). In this sense, insoluble fibres more effectively suppressed high-fat diet-induced obesity than soluble fibres in mice, which was accompanied by an active modulation of gut microbiota (Li et al. 2016), thereby supporting our hypothesis. Furthermore, although pumpkin oils have been widely studied, showing beneficial effects on dyslipemia in mice (Morrison et al. 2015), and pumpkin seed mixtures showed anti-atherogenic, hypolipidaemic and immunomodulatory effects in rats (Barakat and Mahmoud 2011), the potential benefits of pumpkin skin has not been previously evaluated. In this sense, the demonstrated acetogenic effect of pumpkin skin, together with a significant rise in butyrate and propionate production in normoweight and obese conditions, respectively, reveals for the first time its potential health outcomes in both lean and obese subjects.

Among the rest of the ingredients, the mushrooms *Armillaria mellea* and *Boletus edulis* showed noticeable pH lowering effects together with high gas and propionate production under lean and obese conditions, which are suggested to be promising ingredients with health benefits. In this sense, many species of mushrooms have been previously reported to be a potential source of dietary fibre with high beta-glucan content (Sari et al. 2017), which were linked to several metabolic and physiological benefits, such as cholesterol lowering effects (Bai et al. 2019). The resulting SCFA production from the fermentation of beta-glucans in the large intestine, particularly propionate, was suggested to directly mediate its described hypocholesterolaemic effects (Hughes et al. 2008). Interestingly, BSG, a source of beta-glucans, and pumpkin skin fermentation also led to the generation of propionate within obese conditions. Propionate production is postulated to be a microbiota-derived response due to its

anti-obesity properties (Ruijschop et al. 2008; Al-Lahham et al. 2010). In this sense, propionate can induce intestinal gluconeogenesis, which is related to the suppression of the appetite through signalling pathways (De Vadder et al. 2014), conferring protection to the host from diet-induced obesity as previously shown (Chambers et al. 2015). Moreover, BSG also contains noteworthy amounts of arabinoxylans (Lynch et al. 2016), which have been shown to impact the gut microbial ecosystems and stimulate the production of SCFAs (Neyrinck et al. 2011). In this instance, the fermentability of BSG was also explained by a high butyric acid production, although it was lower than inulin. Consistently, previous studies have shown an association between the consumption of arabinoxylans and the stimulation of butyrate-producing bacteria (Teixeira et al. 2018). Clinical interventions have demonstrated that the dietary intake of fermentable carbohydrates is linked to changes in gut microbial ecosystems, thus enhancing the production of butyrate and reporting beneficial consequences to the host (Majid et al. 2011; Venkataraman et al. 2016; Baxter et al. 2019). For instance, butyrate has been widely shown to combat distal inflammatory bowel diseases by the inhibition of proinflammatory cytokines (Ogawa et al. 2004) and to promote the apoptosis of colon carcinogenic cells (Yu et al. 2010). In this sense, recent literature has attributed prebiotic properties to BSG, such as an increased caecal and faecal bacterial diversity (Maukonen et al. 2017) and an active modulation of microbiota composition towards a higher butyric acid formation (Teixeira et al. 2018). Altogether, our observations were validated by the clustering obtained from metabolomic profiles, which are in line with recent studies attributing prebiotic capacities to BSG (Sajib et al. 2018; Teixeira et al. 2018) and introducing pumpkin skin as a promising candidate.

On the other hand, the diminution of pH induced by Irish moss (*Chondrus crispus*) in the obese condition, which was accompanied by gas and SCFA production, suggests a significant fermentability of this ingredient. In this regard, chronic supplementation with *C. crispus* triggered an increase in the beneficial bacteria *Bifidobacterium* accompanied by high concentrations of faecal SCFAs in normoweight rats (Liu et al. 2015). Likewise, the *C. crispus*-enriched diet also increased the production of SCFAs in layer hens (Kulshreshtha et al. 2014). Overall, whereas previous studies support our results, we demonstrated that *C. crispus* was able to mediate SCFA production in microbiota samples from obese individuals. In contrast, the absence of pH or gas production during the fermentation period observed in nutshell, rice husk, OPC and stevia branches incubations suggested either a depressed bioavailability or a low concentration of fermentable compounds contained in these ingredients. Other ingredients tested in this study, such as quinoa husk and plum skin, slightly modulated the production of the main SCFAs, despite the significant differences found when compared to inulin. These observations were accompanied by a moderate decrease in pH and a favoured gas production within both obese and normoweight conditions. Thus, although no clear evidence was found concerning the

fermentability of these ingredients, further research on these substrates would be optimal to validate

our results.

The gut microbiota from lean and obese subjects have been previously shown to differentially contribute to the fermentation of certain fibres (Aguirre et al. 2016), suggesting that the utilisation rate of dietary fibres strongly depends on the phylogenetic structure of the microbiome. Our study revealed that the highest gas production was observed within normoweight conditions, suggesting that microbiota from lean individuals predominantly favoured the fermentation of our tested substrates. In this respect, microbiota from obese subjects has been associated with enhanced energy extraction from the diet (Bäckhed et al. 2004; Turnbaugh et al. 2006), whereas a previous study showed no differences in gas production induced by inulin between lean and obese human subjects (Sarbini et al. 2013). Additionally, the pH-lowering effects induced by inulin under both normoweight and obese conditions suggested that fermentation was similarly occurring. Therefore, caution must be taken when interpreting total gas production as an absolute indicator of fermentation in this study. The ingredients promoted a similar pattern of gas production and pH-lowering effects within both lean and obese conditions, indicating that fermentation is mostly substrate-dependent.

Inulin, BSG and pumpkin skin induced an early increase in total SCFA levels, especially within normoweight faecal samples. Noticeably, relative butyrate amounts induced by these ingredients were higher in lean than obese individuals, which is in accordance with enhanced butyrate production after inulin fermentation by microbiota from lean individuals (Aguirre et al. 2016). Thus, it is suggested that the intrinsic microbiota composition of lean subjects could favour the beneficial effects of highly fermentable substrates. Moreover, although butyrate was the main end-product of inulin fermentation, differential patterns concerning the generation of acetate and propionate were also observed between both lean and obese subjects. Hence, the increase in propionate production found after early inulin fermentation under obese conditions contrasted with the predominant formation of acetate in faecal samples from lean individuals. This finding is in accordance with a favoured propionate production previously described in obese humans (Schwiertz et al. 2010; Aguirre et al. 2016). Furthermore, an increased generation of propionate in obese compared to normoweight faecal inoculums was also previously observed after *in vitro* fermentation of fibres (Yang et al. 2013). While *Firmicutes* are considered butyrate-producers, propionate is the main product of microbial species belonging to *Bacteroidetes* (Walker et al. 2005). Moreover, as discussed above, the growth of propiogenic bacteria is favoured by less acidic pH, which is mainly found in the distal parts of the large intestine, where non-digestible substrates are limited. Taken together, the results of this study suggest that an increased propionate production in obese fermentation is associated with a predominant propiogenic bacterial ecosystem, which, in turn, could be explained by different reasons: a lower consumption of non-digestible dietary compounds, upper pH conditions, and a microbiota-derived response to obesity. Accordingly, increased propionate production was mainly detected in microbial fermentation from obese but not from normoweight individuals. Therefore, while fermentation rates have been proposed to be substrate-dependent, we also suggest that SCFA profiles generated by inulin highly rely on the

intrinsic microbiota composition and function. However, further studies are needed to elucidate the critical role of the microbiota from lean or obese individuals in the fermentation of non-digestible compounds.

Overall, the present *in vitro* fermentation study evaluated a wide range of ingredients that could be potentially utilised by both lean and obese human microbiota. Pumpkin skin revealed an inulin-like fermentation by microbiota from both lean and obese individuals, showing that this by-product could be considered one of the most promising ingredients to be revaluated. Furthermore, we reported a relevant fermentability of BSG mainly based on inulin-like SCFA production by faecal microbiota from obese and normoweight individuals. Other ingredients, such as *Armillaria mellea* and *Boletus edulis*, followed by Irish moss, plum skin and quinoa husk, revealing substantial increments in the fermentation rates and changes in SCFA production, are also proposed to exert microbiota-mediated health benefits. The comparison between fermentations with microbiota from obese and lean individuals suggested that fermentation rates were substrate-dependent, while SCFA profiles strongly relied on microbial origin and action. In this sense, microbiota from obese subjects preferably produced propionate in detriment of butyrate, suggesting a higher butyrogenic activity in the microbiota from lean individuals.

Our *in vitro* fermentation screening study undergoes some limitations such as the use of single faecal slurry for each condition and the absence of gut microbiota characterisation. For this reason, our

results should be further validated using broader sample sizes, while addressing their impact in the microbial communities. Apart from fibre-derived metabolites, gut microbiota also generates a wide range of microbial products as a result of the biotransformation of other plant compounds such as polyphenols, which should be further evaluated in detail. The strengths of our study are (1) the exploration of the potential fermentability of several ingredients in both lean and obese conditions, thus strengthening their potential benefits in different microbial communities across populations; (2) the evaluation of fermentation kinetics by selecting short and a long fermentation time points (0 h, 4 h and 24 h); (3) data analysis using metabolomics techniques (1H-NMR) to characterise a wide range of metabolites after *in vitro* fermentation. In addition, our findings might contribute to the revalorisation of some industrial by-products, thereby alleviating the negative impact of food industry wastes on the ecosystems and countries' economies.

To conclude, we showed the potential fermentability of a broad range of ingredients and by-products from the food industry by both lean and obese microbiota, especially raising pumpkin skin as one of the most promising ingredients to be revaluated. Future *in vitro* studies should focus on the potential role of some of these by-products on shifting microbial profiles from obese into lean ones. Overall, the exploration of the prebiotic capacities and the health benefits linked to these ingredients, especially

with regard to obesity treatment and prevention, are strongly encouraged by our findings.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

References

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Aguirre M, De Souza CB, Venema K. 2016. The gut microbiota from lean and obese subjects contribute differently to the fermentation of arabinogalactan and inulin. PLoS One 11(7):e0159236. ↑

Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. 2010. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochimica et Biophysica Acta – Mol Cell Biol Lipids. 1801(11):1175–1183. ↑

Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA. 101 (44):15718–15723. ↑

Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. 2007. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA. 104(3):979–984. ↑

Bahramsoltani R, Farzaei MH, Abdolghaffari AH, Rahimi R, Samadi N, Heidari M, Esfandyari M, Baeeri M, Hassanzadeh G, Abdollahi M, et al. 2017. Evaluation of phytochemicals, antioxidant and burn wound healing activities of Cucurbita moschata Duchesne fruit peel. Iran J Basic Med Sci 20(7):798–805.

Bai J, Ren Y, Li Y, Fan M, Qian H, Wang L, Wu G, Zhang H, Qi X, Xu M, et al. 2019. Physiological functionalities and mechanisms of β-glucans. Trends Food Sci Technol. 88:57–66.

Barakat LAA, Mahmoud RH. 2011. The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. N Am J Med Sci 3(9):411–417.

Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. 2019. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. mBio. 10(1):e02566-18.

Brotherton CS. 2015. Insoluble fiber and intestinal microbiota metabolism. J Gastroenterol. 50(4):491.

Butke W, et al. 2018. Addition of pumpkin skin flour in pizza changes the physicochemical and sensory acceptability of children. Int J Dev Res. 8:21409–21415.

Cani PD, Van Hul M, Lefort C, Depommier C, Rastelli M, Everard A. 2019. Microbial regulation of organismal energy homeostasis. Nat Metab. 1(1):34–46.

Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, MacDougall K, Preston T, Tedford C, Finlayson GS, et al. 2015. Effects of targeted delivery of propionate to the human colon on appetite

regulation, body weight maintenance and adiposity in overweight adults. Gut. 64(11):1744–1754.

Daud NM, Ismail NA, Thomas EL, Fitzpatrick JA, Bell JD, Swann JR, Costabile A, Childs CE, Pedersen C, Goldstone AP, et al. 2014. The impact of oligofructose on stimulation of gut hormones, appetite regulation and adiposity. Obesity. 22(6):1430–1438.

De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. 2014. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 156(1–2):84–96.

den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J

Lipid Res. 54(9):2325–2340.

Graf D, Di Cagno R, Fåk F, Flint HJ, Nyman M, Saarela M, Watzl B. 2015. Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis. 26:26164.

Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. 2007. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol. 73(4):1073–1078.

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. (2012) Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 3(4):289–306.

Freeland KR, Wilson C, Wolever TMS. 2010. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. Br J Nutr. 103(1):82–90.

Gual-Grau A, Guirro M, Mayneris-Perxachs J, Arola L, Boqué N. 2019. Impact of different hypercaloric diets on obesity features in rats: a metagenomics and metabolomics integrative approach. J Nutr Biochem. 71:122–131.

Guevara-Arauza JC, de Jesús Ornelas-Paz J, Pimentel-González DJ, Rosales Mendoza S, Soria Guerra RE, Paz Maldonado LMT. 2012. Prebiotic effect of mucilage and pectic-derived oligosaccharides from nopal (Opuntia ficus-indica). Food Sci Biotechnol. 21(4):997–1003.

Gullón B, Gullón P, Tavaria F, Alonso JL, Pintado M. 2015. In vitro assessment of the prebiotic potential of Aloe vera mucilage and its impact on the human microbiota. Food Funct. 6(2):525–531.

Henningsson Å, Björck I, Nyman M. 2001. Short-chain fatty acid formation at fermentation of indigestible carbohydrates. Näringsforskning. 45(1):165–168.

Hiel S, Bindels LB, Pachikian BD, Kalala G, Broers V, Zamariola G, Chang BPI, Kambashi B, Rodriguez J, Cani PD, et al. 2019. Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. Am J Clin Nutr. 109(6):1683–1695.

Holscher HD. 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 8(2):172–184.

Hughes SA, Shewry PR, Gibson GR, McCleary BV, Rastall RA. 2008. In vitro fermentation of oat and barley derived beta-glucans by human faecal microbiota . FEMS Microbiol Ecol. 64(3):482–493.

Ignacio A, Fernandes MR, Rodrigues VAA, Groppo FC, Cardoso AL, Avila-Campos MJ, Nakano V. 2016. Correlation between body mass index and faecal microbiota from children. Clin Microbiol Infect . 22(3):258.e1–e8.

Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, Soares JW. 2018. Effects of psychological, environmental and physical stressors on the gut microbiota. Front Microbiol. 9:2013. AQ3

Kobyliak N, Virchenko O, Falalyeyeva T. 2016. Pathophysiological role of host microbiota in the development of obesity. Nutr J. 15(1):43.

Koropatkin NM, Cameron EA, Martens EC. 2012. How glycan metabolism shapes the human gut microbiota. Nat Rev Microbiol. 10(5):323–335.

Kulshreshtha G, Rathgeber B, Stratton G, Thomas N, Evans F, Critchley A, Hafting J, Prithiviraj B. 2014. Feed supplementation with red seaweeds, Chondrus crispus and Sarcodiotheca gaudichaudii, affects performance, egg quality, and gut microbiota of layer hens. Poultr Sci. 93(12):2991–3001.

Li X, Guo J, Ji K, Zhang P. 2016. Bamboo shoot fiber prevents obesity in mice by modulating the gut microbiota. Sci Rep. 6:32953.

Liu J, Kandasamy S, Zhang J, Kirby CW, Karakach T, Hafting J, Critchley AT, Evans F, Prithiviraj B. 2015. Prebiotic effects of diet supplemented with the cultivated red seaweed Chondrus crispus or with fructo-oligo-saccharide on host immunity, colonic microbiota and gut microbial metabolites. BMC Complement Altern Med. 15:279.

López-Barrera DM, Vázquez-Sánchez K, Loarca-Piña MGF, Campos-Vega R. 2016. Spent coffee grounds, an innovative source of colonic fermentable compounds, inhibit inflammatory mediators *in vitro*. Food Chem. 212:282–290.

Lynch KM, Steffen EJ, Arendt EK. 2016. Brewers' spent grain: a review with an emphasis on food and health. J Inst Brew. 122(4):553–568.

Majid HA, Emery PW, Whelan K. 2011. Faecal microbiota and short-chain fatty acids in patients receiving enteral nutrition with standard or fructo-oligosaccharides and fibre-enriched formulas. J Hum Nutr Diet. 24(3):260–268.

Makki K, Deehan EC, Walter J, Bäckhed F. 2018. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe. 23(6):705–715.

Massot-Cladera M, Mayneris-Perxachs J, Costabile A, Swann JR, Franch À, Pérez-Cano FJ, Castell M. 2017. Association between urinary metabolic profile and the intestinal effects of cocoa in rats. Br J

Nutr. 117(5):623–634.

Maukonen J, Aura A-M, Niemi P, Raza GS, Niemelä K, Walkowiak J, Mattila I, Poutanen K, Buchert J, Herzig K-H, et al. 2017. Interactions of insoluble residue from enzymatic hydrolysis of Brewer's spent grain with intestinal microbiota in mice. J Agric Food Chem. 65(18):3748–3756.

Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, Valero R, Raccah D, Vialettes B, Raoult D, et al. 2012. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium

animalis and Methanobrevibacter smithii. Int J Obes. 36(6):817–825.

Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carrière F, Boutrou R, Corredig M, Dupont D, et al. 2014. A standardised static in vitro digestion method suitable for food – an international consensus . Food Funct. 5(6):1113–1124.

Morrison MC, Mulder P, Stavro PM, Suárez M, Arola-Arnal A, van Duyvenvoorde W, Kooistra T, Wielinga PY, Kleemann R. 2015. Replacement of dietary saturated fat by PUFA-rich pumpkin seed oil attenuates non-alcoholic fatty liver disease and atherosclerosis development, with additional health effects of virgin over refined oil. PLoS One. 10(9):e0139196.

Mueller M, Čavarkapa A, Unger FM, Viernstein H, Praznik W. 2017. Prebiotic potential of neutral oligo- and polysaccharides from seed mucilage of Hyptis suaveolens. Food Chem. 221:508–514.

Musso G, Gambino R, Cassader M. 2011. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 62:361–380.

Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, Larondelle Y, Delzenne NM. 2011. Prebiotic effects of wheat Arabinoxylan related to the increase in bifidobacteria, roseburia and bacteroides/prevotella in diet-induced obese mice. PLoS One. 6(6):e20944.

Noack J, Timm D, Hospattankar A, Slavin J. 2013. Fermentation profiles of wheat dextrin, Inulin and partially hydrolyzed guar gum using an in Vitro digestion pretreatment and *in vitro* batch fermentation system model. Nutrients. 5(5):1500–1510.

Nordlund E, Aura A-M, Mattila I, Kössö T, Rouau X, Poutanen K. 2012. Formation of phenolic microbial metabolites and short-chain fatty acids from rye, wheat, and oat bran and their fractions in the metabolical in vitro colon model. J Agric Food Chem. 60(33):8134–8145.

Ogawa H, Iimura M, Eckmann L, Kagnoff MF. 2004. Regulated production of the chemokine CCL28 in human colon epithelium. Am J Physiol Gastrointest Liver Physiol. 287(5):G1062–G1069.

Okubo H, Nakatsu Y, Kushiyama A, Yamamotoya T, Matsunaga Y, Inoue M-K, Fujishiro M, Sakoda H, Ohno H, Yoneda M, et al. 2018. Gut microbiota as a therapeutic target for metabolic disorders. Curr Med Chem. 25(9):984–1001. ↑

Pérez-López E, Cela D, Costabile A, Mateos-Aparicio I, Rupérez P. 2016. *In vitro* fermentability and prebiotic potential of soyabean Okara by human faecal microbiota. Br J Nutr. 116(6):1116–1124. ↑

Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. 2005. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. Appl Environ Microbiol. 71(10):6150–6158. ↑

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. 2018. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 57(1):1–24. ↑

Ruijschop RMAJ, Boelrijk AEM, Te Giffel MC. 2008. Satiety effects of a dairy beverage fermented with propionic acid bacteria. Int Dairy J. 18(9):945–950. ↑

Sajib M, Falck P, Sardari RRR, Mathew S, Grey C, Karlsson EN, Adlercreutz P. 2018. Valorization of Brewer's spent grain to prebiotic oligosaccharide: production, xylanase catalyzed hydrolysis, in-vitro evaluation with probiotic strains and in a batch human fecal fermentation model . J Biotechnol. 268:61–70. ↑

Sánchez-Moya T, et al. 2017. *In vitro* modulation of gut microbiota by whey protein to preserve intest inal health. Food Funct. 8:3053–3063. ↑

Sarbini SR, Kolida S, Gibson GR, Rastall RA. 2013. In vitro fermentation of commercial α-gluco-oligosaccharide by faecal microbiota from lean and obese human subjects. Br J Nutr. 109(11):1980–1989. ↑

Sari M, Prange A, Lelley JI, Hambitzer R. 2017. Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. Food Chem. 216:45–51. ↑

Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. 2010. Microbiota and SCFA in lean and overweight healthy subjects. Obesity. 18(1):190–195. ↑

Teixeira C, Prykhodko O, Alminger M, Fåk Hållenius F, Nyman M. 2018. Barley products of different fiber composition selectively change microbiota composition in rats. Mol Nutr Food Res 62(19):e1701023. ↑

Tejada-Ortigoza V, Garcia-Amezquita L, Kazem A, Campanella O, Cano M, Hamaker B, Serna-Saldívar S, Welti-Chanes J. 2019. *In vitro* fecal fermentation of high pressure-treated fruit peels used

as dietary fiber sources. Molecules. 24(4):697. ↑

Thompson LA, Darwish WS. 2019. Environmental chemical contaminants in food: review of a global problem. J Toxicol. 2019:1–14. ↑

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444(7122):1027–1031. ↑

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. 2009. A core gut microbiome in obese and lean twins. Nature. 457(7228):480–484. ↑

van der Beek CM, Canfora EE, Kip AM, Gorissen SHM, Olde Damink SWM, van Eijk HM, Holst JJ , Blaak EE, Dejong CHC, Lenaerts K, et al. 2018. The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. Metab: Clin Exp. 87:25–35. ↑

Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. 2017. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut. 66(11):1968–1974. ↑

Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. 2016. Variable responses of human microbiomes to dietary supplementation with resistant starch. Microbiome. 4(1):33. ↑

Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. 2005. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. Appl Environ Microbiol. 71(7):3692–3700. ↑

Wang M, Wichienchot S, He X, Fu X, Huang Q, Zhang B. 2019. In vitro colonic fermentation of dietary fibers: fermentation rate, short-chain fatty acid production and changes in microbiota. Trends Food Sci Technol. 88:1–9. ↑

Wichmann A, Allahyar A, Greiner TU, Plovier H, Lundén GÖ, Larsson T, Drucker DJ, Delzenne NM, Cani PD, Bäckhed F, et al. 2013. Microbial modulation of energy availability in the colon regulates intestinal transit. Cell Host Microbe. 14(5):582–590. ↑

Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJA. 2006. Colonic health: fermentation

and short chain fatty acids. J Clin Gastroenterol. 40(3):235–243.

Yang J, Keshavarzian A, Rose DJ. 2013. Impact of dietary fiber fermentation from cereal grains on metabolite production by the fecal microbiota from normal weight and obese individuals. J Med Food 16(9):862–867.

Yu DCW, Waby JS, Chirakkal H, Staton CA, Corfe BM. 2010. Butyrate suppresses expression of neuropilin I in colorectal cell lines through inhibition of Sp1 transactivation. Mol Cancer. 9:276.

Ziai SA, et al. 2004. Study of psyllium (Plantago ovata L.) effects on diabetes and lipidemia in the Iranian type II diabetic patients. J Med Plants. 4:33–42.

Zuin VG, Ramin LZ. 2018. Green and sustainable separation of natural products from agro-industrial waste: challenges, potentialities, and perspectives on emerging approaches. Top Curr Chem. 376(1):3.

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