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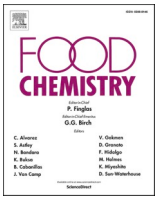

Distinct effects of fiber and colon segment on microbiota-derived indoles and short-chain fatty acids

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ABSTRACT

Effects of pectin, inulin, and their combination on the production of microbiota-derived indoles and short-chain fatty acids (SCFAs) from different colon segments were investigated in a batch system inoculated with microbiota from proximal colon (PC) and distal colon (DC) compartments of the Simulator of Human Intestinal Microbial Ecosystem. Bacteria from DC compartment had a higher abundance of *Firmicutes* and a stronger capacity to produce indoles and SCFAs than bacteria from PC compartment. Fiber supplementation significantly increased the production of SCFAs, indole-3-propionic acid, and indole-3-lactic acid, but decreased the production of oxindole, tryptamine, and serotonin. Pectin specifically promoted the production of indole-3-acetic acid and indole-3-aldehyde. Interestingly, supplementation of pectin or inulin increased the relative abundance of *Bacteroidetes* whereas supplementation of a mixture of two fibers decreased it. Overall, these results suggest that fiber supplementation and colon segment affect the composition of gut microbiota and the microbial catabolism of tryptophan.

1. Introduction

Dietary fiber is a group of non-digestible carbohydrates plus lignin passing to the colon (EFSA, 2010). It is mainly present in nuts, legumes, cereals, fruits, and vegetables and is broadly categorized into soluble and insoluble fiber. Most insoluble fiber is not fermented or only poorly fermented in the colon and has a fecal bulking effect (Makki, Deehan, Walter, & Bäckhed, 2018). Conversely, soluble fiber is fermentable by gut microbiota (Makki et al., 2018), producing short-chain-fatty acids (SCFAs) as metabolic by-products which play an important role in intestinal homeostasis and affect the tissues and organs beyond the gut (van der Hee & Wells, 2021). Meta-analyses of prospective studies and randomized trials provide evidence for the important role of dietary fiber in the prevention of cardiovascular related mortality, type 2 diabetes, and colorectal cancer (Cui et al., 2019; Reynolds et al., 2019). Dietary fiber is also able to promote the growth of beneficial bacteria in the gut (Kuo, 2013). Therefore, dietary fiber, mostly soluble fiber, is widely used to modulate the composition and metabolic profile of the gut microbial community.

SCFAs are the primary end products of fiber fermentation. It is well established that dietary fiber can promote the production of SCFAs in the gut. Recently, there is increasing interest in several indole derivatives originating from bacterial catabolism of tryptophan (Trp), such as indole-3-aldehyde (I3A), indole-3-propionic acid (IPA), and indole-3-acetic acid (IAA), due to their activation of the Aryl hydrocarbon Receptor (AhR), a cytosolic ligand-activated transcription factor that regulates intestinal immune homeostasis (Lamas, Natividad, & Sokol, 2018). Moreover, IPA can regulate the intestinal barrier function by acting as a ligand of the Pregnane X Receptor (PXR), an effect highly potentiated in the presence of indole (Venkatesh et al., 2014). A recent human cohort study indicated that dietary fiber intake was positively associated with the circulating level of microbial produced IPA (Qi et al., 2022), but there are no studies on the impact of fiber or combinations of fibers on the bacterial Trp catabolism for the production of indole derivatives in the gut.

The functional capacity of gut microbiota varies along the colon with proximal colon (PC) being the major location for saccharolytic fermentation, as most microbiota preferentially ferment carbohydrates

Abbreviations: 5HT, Serotonin; AhR, Aryl hydrocarbon Receptor; BCFA, Branched-chain fatty acid; DC, Distal colon; I3A, Indole-3-aldehyde; IAA, Indole-3-acetic acid; ILA, Indole-3-lactic acid; Ind, Indole; IPA, Indole-3-propionic acid; Kyn, Kynurenine; Oxi, Oxindole; PC, Proximal colon; PXR, Pregnane X Receptor; SCFA, Short-chain fatty acid; SHIME®, Simulator of Human Intestinal Microbial Ecosystem; TA, Tryptamine; Trp, Tryptophan.

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(Oliphant & Allen-Vercoe, 2019). When carbohydrates reaching the colon are depleted, bacteria tend to utilize other energy sources, such as peptides or amino acids, and this typically occurs in the distal colon (DC) (Windey, De Preter, & Verbeke, 2012). Interestingly, both PC and DC microbiota are dominated by *Bacteroidetes* and *Firmicutes* phyla (Marzorati et al., 2017), but *Bacteroidetes* is more active in a close to neutral pH environment in the DC compared to mildly acidic conditions in the PC (Duncan, Iyer, & Russell, 2021). The nature of the substrates available to the microbial community and environmental conditions in the PC and DC would lead to a difference in the composition and metabolic activity of the microbiota. However, whether this leads to a different utilization pattern of dietary fiber or a different tryptophan catabolism remains unknown.

In this study, we investigated the effect of pectin, inulin, and their combination on the microbiota composition and the production of SCFAs and indole derivatives by *ex vivo* human gut microbiota from proximal and distal colon compartments of the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) model. We hypothesized that different fibers would have different effects on the composition and metabolic activity of gut microbiota and that this would also be influenced by the colon segment (proximal or distal) and the resident microbiota adapted to this niche.

2. Experimental section

2.1. Materials and chemicals

Pectin from apple (product No. 93854) and inulin from chicory (product No. I2255) were purchased from Sigma Aldrich (St. Louis MO, USA), as well as other chemicals used in this study, unless stated otherwise.

2.2. Preparation of faecal inocula

Inocula for batch fermentation were collected from SHIME® (ProDigest, Belgium), a dynamic *in vitro* human gastrointestinal model to simulate the *in vivo* conditions (Van de Wiele, Van den Abbeele, Ossieur, Possemiers, & Marzorati, 2015). In this study, SHIME® was used to mimic the proximal and distal colon and it was set up according to a previous study (Koper et al., 2019). In brief, fresh fecal samples from two healthy donors who had no history of antibiotic usages in the last 6 months prior to donation were inoculated into the PC and DC compartments of SHIME®. As host genetics, sex, age, and diet affect the gut microbiota (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Org et al., 2016), the two selected donors are Dutch female with similar ages, body mass index, and self-reported dietary habits, so as to reduce inter-individual differences in microbial functionality. The *ex vivo* human gut microbiota were stabilized for two weeks in SHIME® to produce a stable human microbial community by continuously feeding with the adult SHIME® growth medium (PD-NM001B, ProDigest). The pH of PC compartment was kept between 5.6 and 5.9 and DC compartment was kept between pH 6.6 and 6.9. Bacteria adapted to PC compartment was referred to as PC bacteria and adapted to DC compartment as DC bacteria. Stabilized bacteria from each donor and each colon segment were subsequently used in the batch fermentation.

2.3. In vitro batch fermentation

The batch fermentation was performed in sterilized penicillin bottles containing 43 mL of autoclaved basal medium and 20 mL of autoclaved water (Control) or water containing 2 g/L pectin (P) or 2 g/L inulin (I) or a combination of 1 g/L pectin and 1 g/L inulin (P + I). The basal medium consisted of 2.0 g/L $NaHCO_3$, 2.0 g/L yeast extract, 2.0 g/L special peptone, 1.0 g/L mucin, 0.5 g/L L-cysteine HCl, and 2.0 mL/L Tween 80. In addition, 2.11 g/L K_2HPO_4 and 18.77 g/L KH_2PO_4 were used in the fermentation with PC bacteria (PC fermentation) to create a pH between 5.6 and 5.9 and 12.34 g/L K_2HPO_4 and 10.88 g/L KH_2PO_4 were used in the fermentation with DC bacteria (DC fermentation) for a pH between 6.6 and 6.9. An additional 0.14 g/L of Trp was added to the basal medium for investigating the bacterial catabolism of Trp. Bottles were flushed with N_2/CO_2 (80/20, v/v) gases to create an anaerobic environment before adding 7 mL of the SHIME® inoculum from each donor and each colon segment, then incubated at 37 °C with gentle shaking. The batch fermentation was carried out in duplicate. After 24 h fermentation, 5 mL of fermented samples were immediately centrifuged at 12500 rpm for 5 min. The supernatants were filtered by a 0.20 μm regenerated cellulose filter and then frozen at −20 °C before quantification of SCFAs and indole derivatives. The pellets were frozen at −20 °C prior to the isolation of bacterial DNA.

2.4. Quantification of SCFAs

SCFAs were measured as previously described (Huang, Schoones, Wells, Fogliano, & Capuano, 2021). In brief, the frozen supernatants were defrosted at 4 °C and then mixed with the internal standard (0.45 mg/mL 2-ethylbutyric acid in 0.3 mol/L HCl and 0.9 mol/L oxalic acid) at 2:1 (v/v) ratio for SCFA quantification via a Shimadzu GC-2014 (Kyoto, Japan) equipped with a flame-ionization detector and a capillary fatty acid-free Stabil wax-DA column (1 μm × 0.32 mm × 30 m) (Restek, Bellefonte, PA, USA). The injection volume was 0.5 μL and the carrier gas was nitrogen. The initial oven temperature was 100 °C for 0.35 min, and increased to 172 °C by 10.8 °C/min, then to 200 °C by 50 °C/min, and held for 1 min. The temperature of the injector and detector was 100 °C and 250 °C, respectively. Standard solutions of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate were prepared and used for identification and quantification. The results were processed using Chromeleon 7.2.10 (Thermo Fisher Scientific Inc., San Jose, CA).

2.5. Quantification of Trp and indole derivatives

Trp and indole derivatives were measured via a Shimadzu Nexera XR LC-20ADxr UPLC system coupled with a Shimadzu LCMS-8050 mass spectrometer (Kyoto, Japan). Chromatographic separation was accomplished on a Phenomenex Kinetex 1.7 μm EVO C18 100 Å LC column (100 × 2.1 mm). The mobile phase and elution program were described elsewhere (Huang et al., 2021). Indoles were identified by comparing the transitions (*m/z*) and retention time (RT) with reference standards including Trp (*m/z* 204.9 → 188.1; RT 3.08 min) and indole derivatives: serotonin (5HT; *m/z* 177.0 → 160.1; RT 1.66 min), kynurenine (Kyn; *m/z* 209.0 → 192.1; RT 1.92 min), tryptamine (TA; *m/z* 161.1 → 144.0; RT 2.11 min), oxindole (Oxi; *m/z* 134.0 → 77.1; RT 8.55 min), indole-3-lactic acid (ILA; *m/z* 205.9 → 118.1; RT 9.02 min), I3A (*m/z* 146.0 → 118.1; RT 9.26 min), IAA (*m/z* 176.0 → 130.1; RT 9.30 min), indole (Ind; *m/z* 118.2 → 91.1; RT 9.59 min), and IPA (*m/z* 190.1 → 130.0; RT 9.78 min). Data analysis was performed on LabSolutions LCMS 5.6 (Shimadzu Corporation, Japan).

2.6. Microbiota profiling

Bacterial DNA was extracted from the pellets according to the manufacturer instruction of DNeasy® PowerSoil® Kit (12888-50, Qiagen). The purified DNA was quantified by Qubit™ dsDNA BR Assay Kit (Q32853, Invitrogen) using Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., USA) and then kept at −80 °C. The sequencing procedures were carried out by Novogene Europe (Cambridge, United Kingdom). In brief, PCR amplification of 16S rRNA gene (V3-V4 regions) was performed by using 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) primers connecting with barcodes. PCR products with 450–550 bp were selected by 2 % agarose gel electrophoresis. Same amounts of PCR products from each sample were pooled, end-repaired, A-tailed and further ligated with Illumina

adapters. Libraries were sequenced on a paired-end Illumina platform (NovaSeq 6000) to generate 250 bp paired-end raw reads. After receiving raw sequencing data, the primers were trimmed with cutadapt 2.3 (Martin, 2011). We created amplicon sequence variants (ASVs) with DADA2 (Callahan et al., 2016) and used the SILVA database v138 for taxonomic assignment (Quast et al., 2012). We excluded ASVs with taxonomic assignment as eukaryote, mitochondria, and chloroplast.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by IBM SPSS Statistics 25 (SPSS Inc, Chicago, USA). Principal coordinate analysis (PCoA) was performed by Canoco 5.12 based on Bray-Curtis distance of microbial relative abundance at the genus level and default settings for the analysis type "Specialized analysis: Principal-coordinates". Shannon diversity was calculated in Python from the microbial relative abundance at the genus level. Differences in the concentrations of microbial metabolites were tested by one-way ANOVA followed by a Tukey post-hoc test, with a $p < 0.05$ considered as statistical significance.

3. Results

3.1. Microbial community

Alpha diversity describes the species richness in the ecosystem. After 24 h fermentation, PC bacteria had a lower alpha diversity than DC bacteria (Fig. 1A). The inclusion of pectin and inulin slightly increased the alpha diversity of bacteria compared to the control (Fig. 1A). In the PCoA plot (Fig. 1B), the first principal coordinate (PC1, 39.7 %) explains the intragroup variation due to the colon segment and the second principal coordinate (PC2, 28.4 %) explains the intergroup variation due to the fiber supplementation, in which the total amount of fibers was kept the same in the P, I, and P + I groups. The four groups were well-separated in the PC1 and PC2 (Fig. 1B), suggesting a different composition of gut microbiota between PC and DC, and an effect of fibers on the microbiota of each donor.

At phylum level, the microbial community was dominated by *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* in both colon segments (Fig. 2). PC and DC bacteria had slight differences in microbial composition, in which a higher level of *Proteobacteria* and a lower level of *Firmicutes* was in PC bacteria than in DC bacteria. The P and I groups had a higher percentage of *Bacteroidetes* and a lower percentage of *Proteobacteria* compared to the control group, although this effect was small for DC bacteria of donor 2. Interestingly, an opposite outcome was observed in the P + I group.

At genus level, *Bacteroides* was overall the most abundant, followed by *Lachnoclostridium* and *Suttonella* (Fig. 3). The relative abundance of *Citrobacter* and *Enterococcus* showed large variations between two donors, especially in PC bacteria. The relative abundance of *Lachnospiraceae UCG-010* and *Pyramidobacter* was lower in PC bacteria than in DC bacteria. Addition of pectin or inulin resulted in a small increase in the relative abundance of *Bacteroides*, while addition of a mixture of the two fibers appeared to substantially lower the relative abundance of *Bacteroides* compared to the control group. The effect of fiber on *Citrobacter*, *Suttonella*, and *Enterococcus* was different for each donor, hence repeat studies are needed to determine whether this variability is due to the donor variation in gut microbiome.

3.2. SCFAs

Fig. 4 shows changes in SCFA concentration after 24 h fermentation. Acetate, propionate, and butyrate were the three major produced SCFAs with acetate being the most abundant as previously reported (Sánchez-Patán et al., 2015). For all measured SCFA except valerate, which was only found in fermentations with DC bacteria, higher concentrations were observed in samples fermented with DC bacteria than with PC bacteria. Fiber supplementation significantly increased the production of acetate, propionate, and butyrate compared to the control group, but no significant differences were observed for valerate, isobutyrate, and isovalerate. Addition of a mixture of the two fibers had a similar effect on SCFA production as the addition of either pectin or inulin alone. Compared to pectin, inulin was more effective in increasing SCFA production, leading to significantly higher concentrations of propionate in the PC fermentation and acetate in the DC fermentation.

3.3. Trp and indole derivatives

Trp was catabolized by gut microbiota to produce indole derivatives. After 24 h fermentation, the concentration of residual Trp in samples fermented with PC bacteria was about 40-fold higher than in samples fermented with DC bacteria (Fig. 5), suggesting a stronger capacity of DC bacteria to utilize Trp. Fiber supplementation suppressed the bacterial catabolism of Trp by PC bacteria as P, I, and P + I groups had significant higher concentrations of residual Trp and thus lower concentrations of indole derivatives than the control group after 24 h fermentation. However, there were no significant differences in the concentration of indole derivatives produced by DC fermentation.

When looking at specific indole derivatives (Fig. 6), Ind was produced in the greatest amount likely due to the fact that many *Citrobacter* spp. and *Bacteroides* spp. presented in PC and DC bacteria express tryptophanase enzyme that converts Trp into Ind (Lee & Lee, 2010). PC and DC bacteria showed different capacities to produce indole derivatives, giving high concentrations of ILA, TA, and 5HT in PC fermentation and high concentrations of Ind, Oxi, and IPA in DC fermentation. Fiber supplementation significantly decreased the

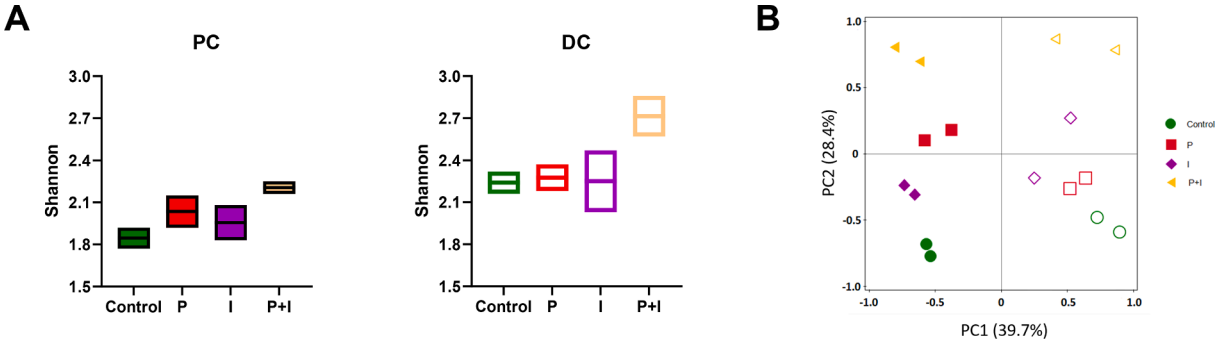

Fig. 1. Floating bars (min to max, line at mean) of alpha diversity (A) and PCoA plot with Bray-Curtis dissimilarity metrics (B) of microbial relative abundance at the genus level after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin. Closed symbols are PC samples and open symbols are DC samples.

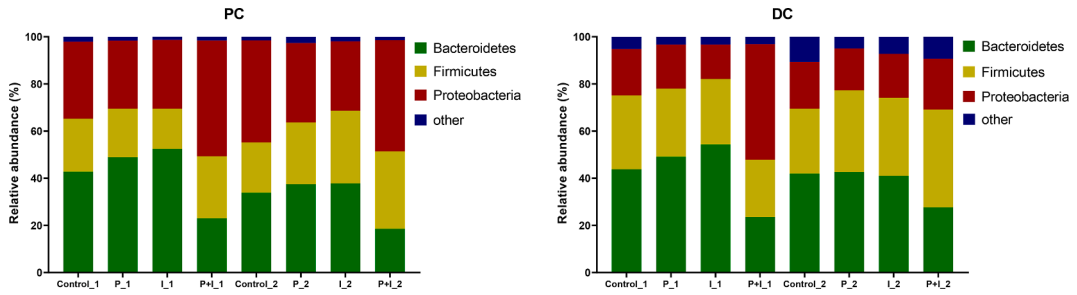

Fig. 2. Relative abundance (%) of microbiota at the phylum level after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin; _1: donor 1; _2: donor 2.

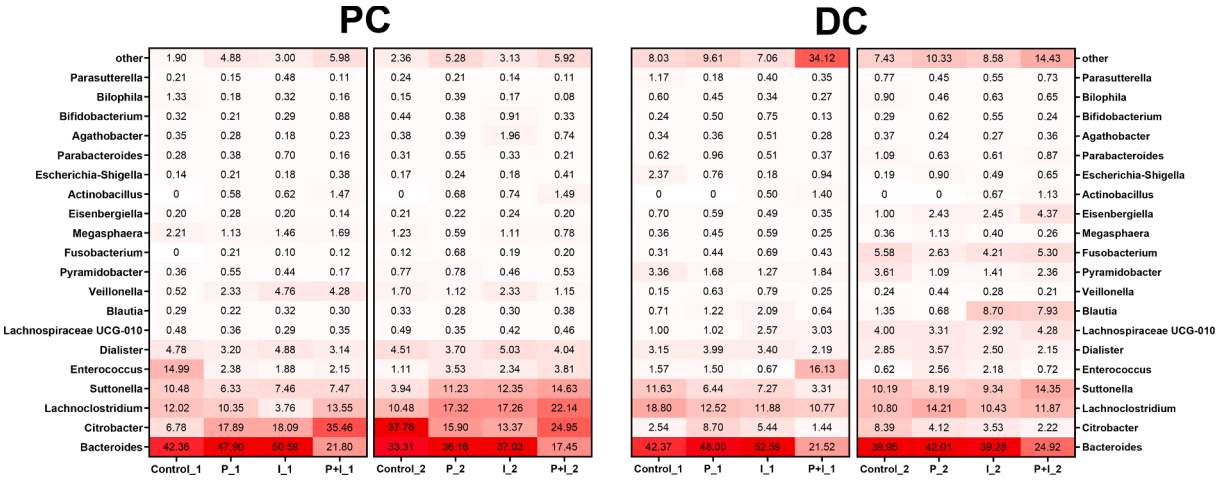

Fig. 3. Heatmap of relative abundances (%) of microbiota at the genus level after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin. _1: donor 1; _2: donor 2.

production of Oxi, TA, and 5HT, but increased the production of IPA, IAA, and ILA by PC and DC bacteria, especially in the fermentation containing a mixture of the two fibers. Interestingly, fiber supplementation suppressed the production of Ind in the PC fermentation, but not in the DC fermentation. Fermentation with pectin yielded significant higher concentrations of IAA and I3A than fermentation with inulin, while fermentation with inulin promoted the production of Kyn and 5HT compared to fermentation with pectin.

4. Discussion

Dietary fiber is recognized as an important component in a health-promoting diet, which can modulate the gut microbial community and promote the production of SCFAs in the colon so as to reduce the risks of several diseases, such as inflammatory bowel disease, obesity, diabetes, and intestinal cancers (Cui et al., 2019; Makki et al., 2018). Emerging data shows that indole derivatives produced from bacterial catabolism of Trp are also positively involved in the host-microbiota crosstalk in health and disease (Agus, Planchais, & Sokol, 2018; Roager & Licht, 2018), but how the production of indole derivatives is modulated by dietary fiber remains largely unknown. In this study, we used an *in vitro* experimental framework to investigate the effect of pectin and inulin on the production of SCFAs and indole derivatives by gut microbiota from PC and DC compartments of the SHIME® model.

We found that pectin and inulin induced a striking impact on gut microbiota after 24 h fermentation compared to the control. *Bacteroides* was enriched in the fermentation with pectin in line with the ability of species in this genus to utilize pectin as a carbon source (Luis et al., 2018). In the PC fermentation, inulin also favored the growth of *Bacteroides* which can digest and metabolize inulin-type fructans (Bai et al., 2021). An increased relative abundance of a specific taxon, genus or species depends on primary utilization of a specific carbohydrate or cross-feeding of products from primary degraders. As hypothesized, fiber supplementation increased the production of SCFAs in the *in vitro* fermentation system, thereby significantly decreasing the pH values (Fig. S1). Acetate was the major produced SCFA in agreement with the abundance of *Bacteroidetes*, which contains many bacterial groups capable of producing acetate in the intestine (Cummings, Pomare, Branch, Naylor, & Macfarlane, 1987). Propionate is produced by a few dominant genera, including *Akkermansia muciniphila*, *Coprococcus catus*, and certain species of *Bacteroidetes* (Louis & Flint, 2017). The increased relative abundance of *Bacteroidetes* is consistent with the increased production of acetate and propionate in the fermentation with pectin and inulin, especially with inulin. Isobutyrate and isovalerate are branched-chain fatty acids (BCFAs), which are exclusive products of fermentation of branched-chain amino acids. In our study, fermentation with fibers had no noticeable effects on the production of BCFAs. When the bacterial degradation of Trp is considered, supplementation of pectin and inulin markedly decreased the production of indole derivatives by PC bacteria. This could be explained by the microbial preference of carbohydrates over proteins as energy source (Oliphant & Allen-Vercoe, 2019). However, this was not evident in DC fermentation, possibly due to the functional capacities of bacteria in the PC and DC. The PC bacteria hosts mainly saccharolytic specialists, while DC bacteria hosts more proteolytic specialists. Fermentation with pectin and inulin differently altered the bacterial catabolism of Trp, resulting in different

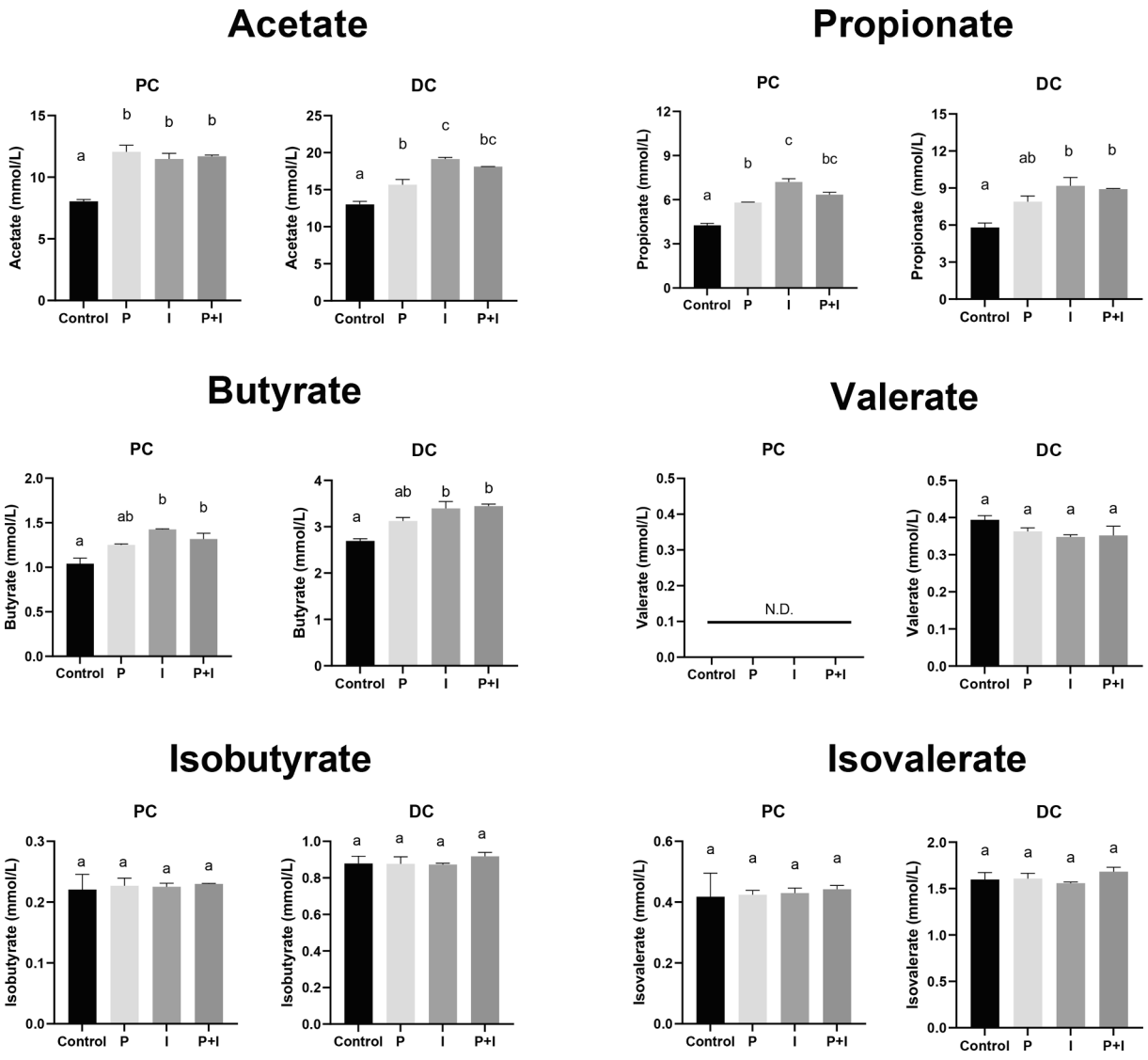

Fig. 4. Concentrations of SCFAs after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin. Bars not sharing any letters indicate treatments that are significantly different ($p < 0.05$, one-way ANOVA followed by a Tukey post-hoc test). N.D.: not detected.

profiles of indole derivatives. An increased level of some specific catabolites, like IPA, IAA, and ILA, were observed in the fermentation with fibers. Although the conversion of indoles by gut microbiota is not well understood, several *Bacteroides* and *Bifidobacterium* species have been reported to be able to convert Trp into IAA and ILA (Roager & Licht, 2018). Intake of fiber-rich foods was found to be positively associated with the serum level of microbial produced IPA (Qi et al., 2022). Our study provided direct evidence that fermentation with pectin and inulin increased the production of IPA by gut microbiota. Together, these results confirmed the general observation that the type of fiber influences the microbiota composition and the microbial metabolites produced.

We observed that gut microbiota were differently modulated depending on whether fiber was added alone or in combination. To rule out an effect of the total amount of fibers, this was kept the same for all conditions. Compared to fermentation with pectin or inulin alone, the relative abundance of *Bacteroidetes* which contains the largest repertoire of carbohydrate-active enzymes (Korpela, 2018) was markedly reduced in the fermentation with a mixture of the two fibers. Additionally, the combination of pectin and inulin greatly promoted the production of IPA and ILA, and decreased the production of Oxi and TA, which suggests a synergistic or antagonistic effect of fiber combination on indoles production. These results highlight the importance to study the combination of different fibers rather than one specific fiber in the understanding of the health effects of fiber-rich foods where a complex mixture of fibers is always present.

We further clarified the differences in the composition and metabolic activity of gut microbiota from different colon segments (PC and DC). It is reported that the *in vivo* concentration of SCFAs decreases along the length of the colon (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016), possibly due to the decline in the supply of carbohydrates and the continuous absorption by colon epithelium. However, our results show that DC bacteria produced more SCFAs than PC bacteria when they were supplied with the same amount of fibers. This could be due to the higher relative abundance of *Firmicutes* found in DC bacteria compared to PC bacteria, which is active in the degradation of fibers to produce SCFAs, especially butyrate (Berry, 2016). *Blautia* species are well-recognized butyrate-producing bacteria in the gut (Benítez-Páez et al., 2020), and they were relatively more abundant in DC bacteria compared to PC bacteria. The production of SCFAs is affected by the pH of fermentation environment, as some *Bacteroides* species grow well at pH 6.7 but poorly

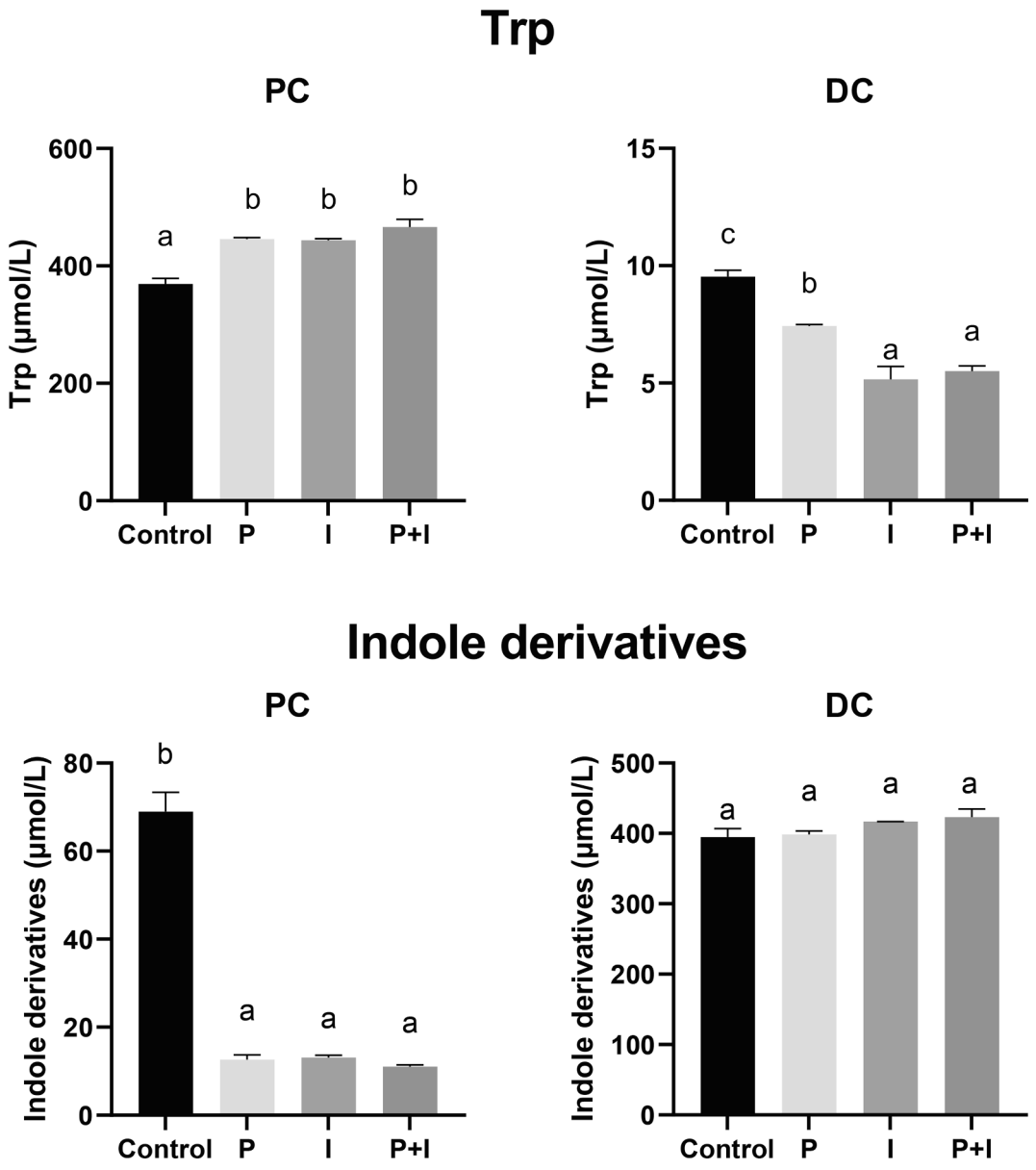

Fig. 5. Concentrations of Trp and indole derivatives after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin. Bars not sharing any letters indicate treatments that are significantly different ($p < 0.05$, one-way ANOVA followed by a Tukey post-hoc test).

at pH 5.5 (Walker, Duncan, Leitch, Child, & Flint, 2005). A pH of 6.5 is able to increase the production of acetate and propionate compared to pH 5.5 (Walker et al., 2005). These results suggest that DC with a pH environment between 6.6 and 6.9 could be a more suitable target segment than PC for promoting SCFA production. Proteolytic fermentation mainly occurs in the DC and this is evident in our study as significant more BCFAs and indole derivatives were produced in the DC fermentation than in the PC fermentation. The functional capacity of PC bacteria to catabolize Trp was relatively low as judged by large amounts of Trp remaining after 24 h fermentation and this capacity was further diminished by fiber supplementation. However, it is interesting to notice that some indole derivatives, like ILA, TA, and 5HT, were found at higher concentrations in the PC fermentation than in the DC fermentation, although less Trp was catabolized by PC bacteria. ILA secreted by *Bifidobacterium* spp. is an agonist of AhR (Ehrlich et al., 2020). TA and 5HT are neurotransmitters with documented effects on gastrointestinal motility (Krautkramer, Fan, & Bäckhed, 2021; Roager & Licht, 2018). These catabolites are considered beneficial to the host health, and therefore, strategies aiming to promote the production of these catabolites should target the PC.

5. Conclusions

In conclusion, our study showed that pectin and inulin had different effects on the composition and metabolic activity of gut microbiota, leading to different profiles of SCFAs and indole derivatives. Our study provides evidence for a novel potential mechanism by which dietary fiber can benefit the host, i.e. via modulating the bacterial catabolism of Trp, in particular promoting the production of IAA, ILA, and IPA which are the ligands of AhR and PXR (Lamas et al., 2018; Venkatesh et al., 2014). Importantly, our study also showed that the proteolytic specialists in DC bacteria possessed a strong capacity to produce SCFAs when they were supplied with a sufficient amount of fiber. The bacterial catabolism of Trp was not limited in the DC as several catabolites, like ILA, TA, and 5HT, were produced in large amounts in the PC fermentation. These findings highlight the impact of microbiota metabolic

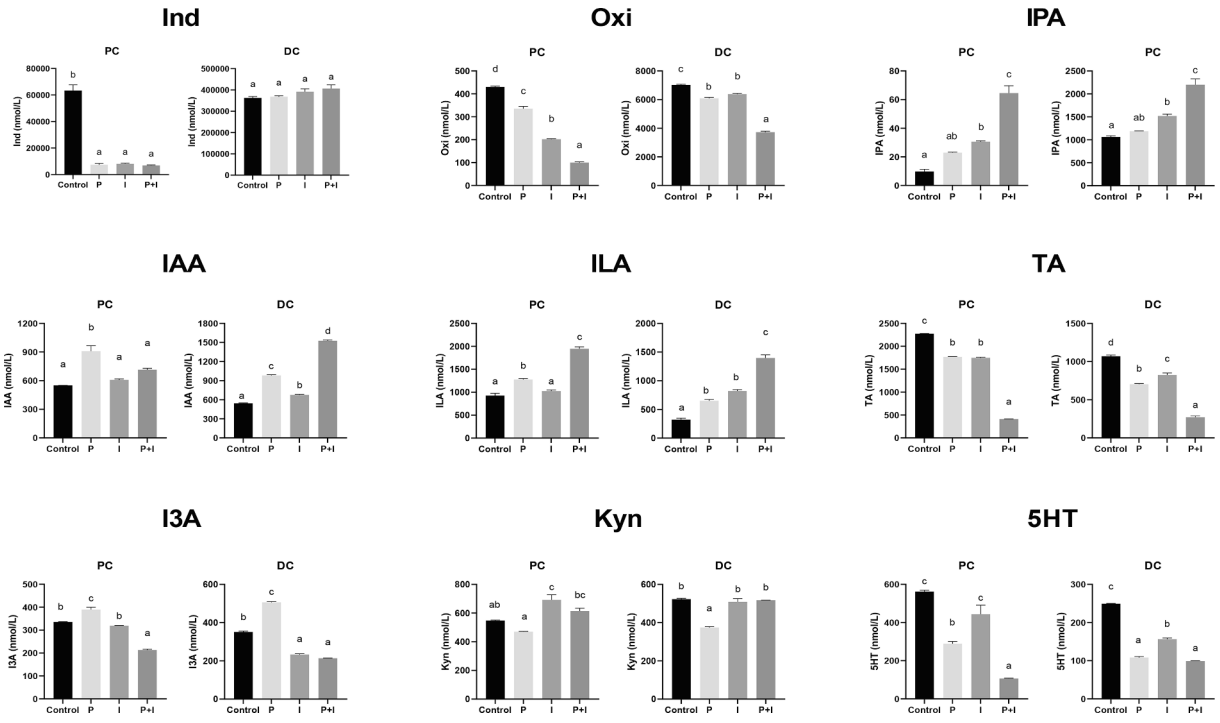

Fig. 6. Concentration of specific indole derivative after 24 h fermentation with inocula from proximal colon (PC) and distal colon (DC) compartments of SHIME® using two donors. Control: fermentation without dietary fibers; P: fermentation with 2 g/L pectin; I: fermentation with 2 g/L inulin; P + I: fermentation with 1 g/L pectin and 1 g/L inulin. Bars not sharing any letters indicate treatments that are significantly different ($p < 0.05$, one-way ANOVA followed by a Tukey post-hoc test).

functions in proximal and distal colon on fermentation of different fibers and production of beneficial metabolites which has implications for the design of health-promoting foods.

CRediT authorship contribution statement

Zhan Huang: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Jos Boekhorst:** Formal analysis, Writing – review & editing. **Vincenzo Fogliano:** Conceptualization, Resources, Writing – review & editing. **Edoardo Capuano:** Conceptualization, Writing – review & editing. **Jerry M. Wells:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133801.

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