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Research Article

In Vitro Gastrointestinal Digestion of *Polyonatum cyrtonema* Polysaccharides and Impact on Intestinal Flora

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Polyonatum cyrtonema polysaccharides (PCPs) have been illustrated to have pharmacological and biological activity. However, the impact of PCPs on gut microorganisms and their metabolic pathways has been scarcely addressed. In order to investigate the antidigestion potential of PCPs, an *in vitro* simulation of digestion was developed. Moreover, an *in vitro* fermentation model was used to explore the influence of PCPs on the microbial community. The findings revealed that the concentration of the remaining carbohydrate and reducing end of the polysaccharide chain grew slightly, while the molecular weight was unaltered after being digested. Notably, the gut microbiota degraded the PCPs during the subsequent fermentation, resulting in the production of short-chain fatty acids and a significant decrease in the pH level ($p < 0.05$). Compared with the control group, the result of 16S rRNA sequencing revealed that PCPs could affect the diversity of the community and the composition of the gut microbes significantly ($p < 0.05$), having similar regulatory effects to inulin on the structure of the microbial community. The PCPs group notably promoted the relative abundance of acetic-acid bacteria, such as *Bifidobacterium*. In addition, PICRUSt2 analysis demonstrated that PCPs mainly enhanced phenotypic KEGG pathways such as amino acid metabolism and carbohydrate metabolism. Overall, PCPs may have prebiotic properties that support human health by controlling the make-up and metabolic activity of intestinal flora.

1. Introduction

Polyonatum cyrtonema, a herb with homology of medicine and food, was first recorded in the Song Dynasty of China [1], including various bioactive compounds such as *Polyonatum cyrtonema* polysaccharides (PCPs), saponins, flavones, alkaloids, and so on [2]. Therein, PCPs were the most abundant component in *Polyonatum cyrtonema*, which was considered a new source of nutrients [3]. PCPs have been reported to have many pharmacological applications and biological activities [4]. Research indicated that PCPs could enhance immunoregulatory activity by stimulating the production of immune factors [5]. It has been shown that PCPs have an antifatigue effect on mice in the swimming exhaustion model [6]. In addition, PCPs could lower blood sugar levels and improve insulin resistance to have an antidiabetic effect [7]. Interestingly, the increasing studies have demonstrated that these biological activities are closely associated with the action of gut flora [8]. Recent evidence confirmed that the increased relative abundance of beneficial bacteria and the increased amount of short-chain fatty acids (SCFAs) expression in the gut could enhance immune regulation [9]. Also, there was a high correlation between fatigue syndrome and changes in gut microbiome

composition [10]. Previous literature found that there was a significant difference in intestinal microflora between diabetics and normal populations, which demonstrated that intestinal flora was closely related to diabetics [11]. Obviously, the diversity, composition, and distribution of the intestinal flora played an important role in the health of the human body [12]. The dynamic balance of intestinal flora had distinct effects on the immune system and participated in the important physiological functions of various human systems [13]. Dysregulation of intestinal flora would affect and lead to various diseases [14]. Therefore, PCPs might exert a series of pharmacological and biological activities by regulating intestinal flora.

Indeed, a growing body of literature indicates that polysaccharides extracted from herbs have a regulatory effect on the intestinal microenvironment [15, 16]. It was reported that polysaccharides derived from *Lycium barbarum* and *Panax ginseng* could promote the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactococcus*, respectively [17, 18]. Moreover, polysaccharides in herbs could be utilized by colon microbes to increase the levels of SCFAs which have many roles in promoting gut health [19]. For example, *Astragalus membranaceus* and *Codonopsis pilosula* polysaccharides were able to reduce intestinal mucosal damage and rebuild immune balance by upregulating the amount of isovaleric and butyrate acids and significantly adjusting corresponding acid-producing bacteria, such as *Bacteroides* and *Clostridia*, in mice with ulcerative colitis [20]. Whereas, different fermentation characteristics of herbal polysaccharides may lead to various changes in the gut microbiota [21]. Thus, it is extremely important to explore suitable herbal polysaccharides from medicine and food homology materials to improve host health. As far as the authors know, there are few studies on the impact of PCPs on the complex microbial environment and their metabolic pathways in the gut, in spite of several previous studies on the effect of PCPs on a single bacterial community [22]. As previous literature confirmed, polysaccharides had the potential to regulate the intestinal flora and their metabolites only if they were not fully digested by the saliva and gastrointestinal digestive system [23]. For instance, the INU from *Cichorium intybus* could reach the gut instead of degrading during the oral and gastrointestinal digestive systems [24]. However, other studies indicated that due to digestive enzymes, salt solution, and pH, polysaccharides might be hydrolyzed in models of gastric and small intestine digestion [16, 25]. For example, intestinal fluids might break down *Nelumbo nucifera* polysaccharides into smaller bits [26]. Nevertheless, whether PCPs will be affected by digested saliva and gastrointestinal juice has been unclear yet.

In order to assess the digestion properties of PCPs in saliva and gastrointestinal settings, an *in vitro* digestion method was used in the current study. In addition, an *in vitro* fermentation method was carried out to investigate how PCPs affect the makeup of the population gut microorganisms and the production of SCFAs. Moreover, PICRUSt2 analysis was first used to determine the phenotypic metabolic pathways in the PCPs group and predict the metabolic function of the gut microbiota. The current effort will contribute to revealing the digestion and fermentation properties of PCPs and will offer empirical evidence to promote their development and use in functional foods.

2. Materials and Methods

2.1. Materials and Reagents. The samples of *Polyonatum cyrtonema* were provided and named as such by the Fujian Sanming Academy of Agricultural Sciences (China). For chromatographic analysis, all compounds were either HPLC grade or reagent grade. The supplier of anhydrous ethanol was Titan Technology Co., Ltd in Shanghai, China. D-galactose (Gal), D-mannose (Man), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), D-glucose (Glc), D-galacturonic acid (GalA), sulfuric acid (95–98%, A.C.S. grade), reagent of Folin-phenol, trifluoroacetic acid (Reagent Plus), sodium tetraborate, sulfamic acid (99.3%), potassium sulfamate, gallic acid, and α-Amylase, pepsin, gastric lipase, and pancreatic enzyme were purchased from Sigma Aldrich (St. Louis, MO, USA). Sinopsin Group Chemical Reagent Co., Ltd. (Shanghai, China) provided the medium reagents. The supplier of bile salt is Aladdin Reagent Co., Ltd. in Shanghai, China.

2.2. Preparation of PCPs. PCPs were prepared from *Polyonatum cyrtonema* according to the previous method with slight modifications [27]. Briefly, *Polyonatum cyrtonema* was treated with ethanol solution (70°C) to remove the polyphenols, pigments, and others. After drying in the drying oven (50°C) for 2 h, *Polyonatum cyrtonema* was extracted thrice with the 25-fold volume at 80°C for 2 h. The sample was combined with a 4-fold volume of ethanol at 4°C for a duration of 24 hours to induce precipitation of the polysaccharides. After freeze-drying, PCPs were finally obtained.

2.3. Physicochemical Characterization of PCPs. The methods were used to measure the total phenol, protein, and carbohydrate contents of PCPs, respectively [28, 29]. With certain adjustments, the monosaccharide composition of PCPs was analyzed [30]. Briefly, PCPs were hydrolyzed in trifluoroacetic acid (2M) at 110°C for 4 hours. Monosaccharides in hydrolyzed samples were identified using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) employing the Dionex CarboPac PA20 analytical column (3150 mm). The high-performance size exclusion chromatography system, incorporating multiangle laser light scattering and a refractive index detector (HPSEC-MALLS-RID Shimadzu, Kyoto, Japan), was utilized for measuring the molecular weight (Mw) of PCPs using the modified approach previously reported [17]. Moreover, PCPs (2 mg) were evenly ground and compressed into a transparent round sheet and were recorded on a Fourier transform infrared spectrophotometer with the infrared spectrum of the vibration region 400–4000 cm^{-1}.

2.4. Simulated Oral-Gastric-Small Intestinal Digestion. A simulated *in vitro* digestion method was employed with minor modifications [31]. First, 0.025 mL of $CaCl_2$ solution (0.3M) and 0.5 mL of α-Amylase solution (75 U/mL) were mixed as simulated salivary fluid (SSF). Ultrapure water was added to reach a total volume of 5 mL, while 5 mL of PCPs solution (10 mg/mL) was then mixed with SSF. Moreover, the PCPs solution was substituted with ultrapure water and an INU solution (10 mg/mL) to serve as the control group (CON) and positive control group (INU), respectively. All groups were placed in an oscillating bath at a temperature of 37°C for a duration of 5 minutes. The pH level of the solution was subsequently modified to 3.0 by the addition of hydrochloric acid (6M). Following this, simulated gastric fluid (SGF) underwent preheating in a water bath set at 37°C. 10 mL of the previously prepared simulated digestive fluid, 0.01 ml of $CaCl_2$ solution (0.3M), 1 mL of pepsin solution (2000U/mL), and ultrapure water were added into 8 mL SGF to achieve a volume of 20 mL. Subsequently, the pH was modified to 7.0 using sodium hydroxide (6M). Subsequent to this, simulated intestinal fluid (SIF) was subjected to preheating at 37°C. To this, 2.5 mL of bile salt solution (10 mM), 0.04 mL of $CaCl_2$ solution (0.3M), 0.5g of pancreatic enzyme solution (100U/mL), and ultrapure water were added to 12 mL of previously prepared SIF. The volume was then replenished to 40 mL. Samples were collected at 0, 1, 2, and 4-hour intervals into the digestion process. Samples were procured for further examination concurrent with the previously delineated time intervals.

2.5. Simulated Fermentation by Gut Microbiota. The *in vitro* fermentation experiment was conducted with slight modifications as outlined in the literature [32]. Briefly, fecal samples were freshly procured from three male and three female donors, aged 22 to 30 years, who adhered to normal dietary patterns and had abstained from probiotics or antibiotics for a minimum of three months. The fecal samples underwent processing by mixing with 0.1M phosphate-buffered saline (pH 7.0) to prepare fecal microbiota samples. Add 1 mL of fecal suspension to 9 mL of basic nutrient medium containing 10 mg/mL INU or PCPs (the INU, PCPs group), while the CON group replaces the sample with 9 mL of basic nutrient medium without carbon source. All groups were incubated at 37°C and subsequent analysis was conducted every 6, 12, and 24 hours during the fermentation process.

2.6. The Characterization of Changes during In Vitro Digestion and Fermentation. The Mw of PCPs was measured by Waters e2695 HPLC (Waters Technology Co. Ltd., USA) equipped with G4000PWXL (7.8 mm × 300 mm) and G6000PWXL (7.8 mm × 300 mm) through High-Performance Gel Permeation Chromatography (HPGPC). The concentrations of reducing sugar and residual carbohydrates were determined using the dinitro salicylic acid (DNS) and phenol-sulphuric acid procedures, respectively.

2.7. Determination of pH Value and SCFAs. Quantification of short-chain fatty acids (SCFAs) such as acetic, propionic, n-butyric, isovaleric, and n-valeric acids in fermented samples was conducted through gas chromatography-mass spectrometry (GC-MS)-TQ8040 with an SH-Rtx-WAX column (30 m 0.25 mm, 0.25 μm). To facilitate sample preparation, 1 mL of the fermentation samples was combined with 3 mL of an internal standard solution comprising heptanoic acid (5.04 moL/mL). Subsequent to vortex mixing and centrifugation, 300 μL of the resultant supernatant was translocated to a microcentrifuge tube and blended with 10 μL of 1 M phosphoric acid. Following this, the supernatant was subjected to filtration through a 0.20 μm filter and subsequently transferred to a sample injection bottle for further analysis. During analysis using GC-MS, helium was utilized as the carrier gas at a flow rate of 2.0 mL/min. A complete scan mode with a mass-to-charge ratio (m/z) range of 20.0–300.0 was employed. Each sample (1 μL) was initially heated to 140°C for four minutes at a rate of 7.5°C/min before the temperature was ramped up to 200°C at a rate of 60°C/min. The external standard method was used to determine the SCFA concentration, and the related standards were used for calibration.

2.8. Analysis of the Gut Microbiota. Following a 24-hour fermentation period, an investigation into the influence on the gut microbiota was conducted through the utilization of high-throughput bacterial 16S rRNA sequencing. Using the Qiagen QIAamp Fast DNA Stool Mini Kit and following instructions of the manufacturer, each sample from the inulin group (INU group), *Polyonatum cyrtonema* polysaccharides group (PCPs group), and blank control group (CON group) was extracted. All DNA extractions from samples were examined using 1% agarose gel electrophoresis. Subsequent to purification with the AxyPrep DNA gel extraction kit (Axygen Bioscience, California, USA) and quantification with the QuantiFluor™-ST fluorometer (Promega, California, USA), the amplicons were analyzed. The sequencing of the primary PCR products derived from the V3–V4 region of the 16S rRNA gene, which was amplified using the primer pairs 338F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′), was carried out by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China).

2.9. Statistical Analysis. Majorbio Cloud Platform (http://www.majorbio.com) was used to analyze the microbiological data. The method of sequence denoising (DADA2/Deblur) was employed to refine curated data and derive Amplitude Sequence Variants (ASVs) that encapsulate both sequence identity and abundance details. Five times each of the other trials were conducted. The standard deviation (SD) was used to express the data as a mean. SPSS (Version 17.0) was used for the statistical analysis except microbial community analysis.

3. Results and Discussion

3.1. Chemical Compositions of PCPs. Table 1 displays that the total neutral sugar, uronic acid, protein, and polyphenol contents of PCPs were 83.02 0.12%, 8.87 0.13%, 3.63 0.19%, and 4.47 0.24%, respectively. With a molar ratio of 4.15 : 4.57 : 71.21 : 4.47 : 13.21 : 2.39, the monosaccharides in PCPs were primarily glucose and xylose, with minor amounts of fucose, galactose, mannose, and fructose. Fructose, glucose, and mannose were the three main monosaccharides found in *Polyonatum cyrtonema* polysaccharides, which were also typical neutral hetero-polysaccharides, according to earlier research [33]. In addition, the molecular weight of PCPs in deionized water as a mobile phase was 4.20 kDa.

The structural characteristics of PCPs were determined by FT-IR spectroscopy (Figure 1). The prominent absorbance peak at 3305.16 cm^{-1} was attributed to the O–H stretching vibration, and the peak at 2931.37 cm^{-1} was assigned to the C–H stretching vibration [34]. In addition, bands at around 1117.82 and 1009.28 cm^{-1} were assigned to the C–O–C glycosidic bond stretching in the sugar ring, while vibrations at 926.77 and 871.40 cm^{-1} indicated the vibration of the furan ring [35]. Combined with the results of monosaccharide composition and infrared spectroscopy, PCPs might be mainly composed of fructans.

3.2. Variations in the Content of Residual Carbohydrate, Reducing End of Polysaccharide Chain, and Mw of PCPs throughout In Vitro Salivary-Gastrointestinal Digestion. Although the genome from human could not encode carbohydrate-active enzymes (CAZymes) to hydrolyze polysaccharides [36], the amylase in salivary and the extremely acidic pH environment in simulated gastric juices might have an impact on the digestion of polysaccharides [37]. In addition, the chemical composition of polysaccharides might be destroyed during intestine digestion because of the existence of bile acids. Once the polysaccharide is hydrolyzed by the digestive solution, the glycosidic bond of the polysaccharide will be destroyed along with the increase of the reducing end. As shown in Figure 2(a), there was no significant change in the total sugar content of both the INU and PCPs groups. The reducing end of polysaccharide components within PCPs remained unaltered throughout the *in vitro* digestive process (Figure 2(b)), indicating there was no breakdown of glycosidic bonds.

Further research was explored on how the Mw of PCPs changed throughout simulated saliva, gastric, and small intestinal digestion. The Mw of PCPs was not changed significantly ($p > 0.05$) during the digestion (Table 2). Literature has illustrated that the Mw of polysaccharides decreases during *in vitro* digestion due to the disruption of polysaccharide aggregates [38]. Therefore, the breakdown of polymers and the rupture of glycosidic bonds may result in the fluctuation of Mw [39]. The result confirmed that the simulated saliva-gastrointestinal fluid had little impact on the Mw of PCPs, which indicated PCPs reached the colon where the gut flora eventually used it because it could neither be obviously digested nor absorbed.

Table 1: Basic physicochemical properties of PCPs.

Composition (%)	PCPs
Total neutral sugar	83.02 ± 0.12
Uronic acid	8.87 ± 0.13
Protein	3.63 ± 0.19
Polyphenol	4.47 ± 0.24
Monosaccharide composition (%moL)	
(i) Fucose	4.15 ± 0.15
(ii) Galactose	4.57 ± 0.19
(iii) Glucose	71.21 ± 0.18
(iv) Mannose	4.47 ± 0.20
(v) Xylose	13.21 ± 0.09
(vi) Fructose	2.39 ± 0.32
Average molecular weight (kDa)	4.20 ± 0.03

3.3. In Vitro Fermentation Characteristic of PCPs by Gut Microbiota. Due to a deficiency in CAZymes, the polysaccharides from *Polyonatum cyrtonema* cannot be broken down in our upper GI system. The gut bacteria might make use of the indigestible polysaccharides once they reach the colon and boost the reducing end of the polysaccharide chain [40].

The fermentation of polysaccharides would produce SCFAs, which would lower the pH levels in the gut [39]. The pH values of the PCPs group were significantly ($p < 0.05$) lower than that of the CON group during the initial 6-hour stage of closed anaerobic fermentation (Figure 3(a)). Meanwhile, the residual carbohydrates of PCPs decreased continuously (Figure 3(b)). Combined with the significant reduction of reducing end of polysaccharide chain content in Figure 3(c) ($p < 0.05$), it was evident that the gut microbiota broke down PCPs in 6 hours into polysaccharide chains with reducing ends. It can be inferred that intestinal microorganisms utilize PCPs as carbon sources and lead to rapid growth and accumulation of SCFAs. A previous study indicated that a decreased acidity level in the gut could potentially promote the proliferation of beneficial bacteria and inhibit the propagation of pathogenic microorganisms [41]. There was a significant decrease in the molecular weight of PCP during the fermentation, suggesting that PCPs have been utilized and degraded (Table 2).

SCFAs have the potential to increase intestinal peristalsis, safeguard the intestinal barrier, and lower levels of inflammation and colon cancer [42]. Most gut microbes were capable of fermenting indigestible carbohydrates to create organic acids such as acetic, propionic, butyric, and lactic acids [43]. The quantities of acetic acid produced upon the fermentation of PCPs were much greater than those of the INU group, as shown in Table 3 by the amounts of SCFAs produced at various fermentation periods. It was repoted that INU could promote the growth of probiotics such as *Bifidobacterium* and *Lactobacillaceae*, which were connected

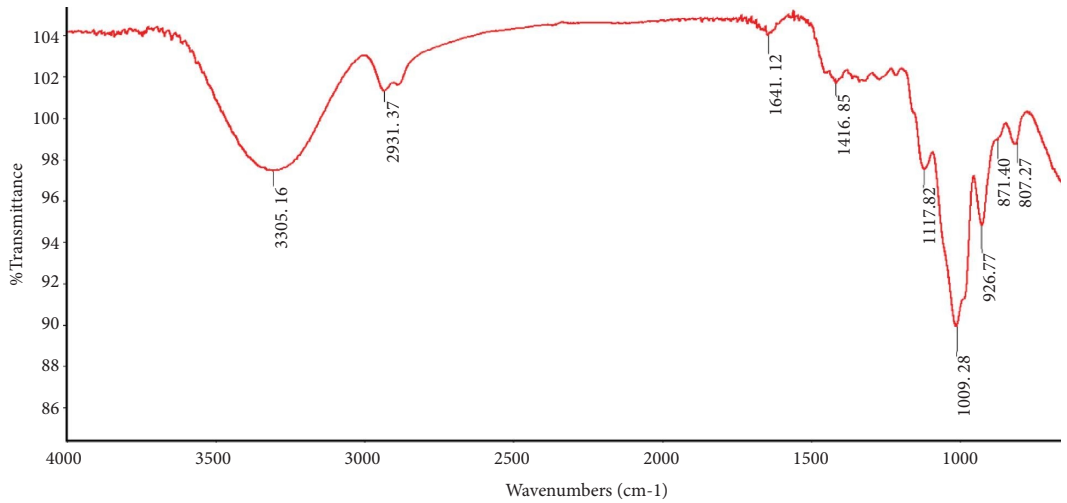

Figure 1: FT-IR spectra of PCPs. *Note*: PCPs are the polysaccharides from *Polyonatum cyrtonema*.

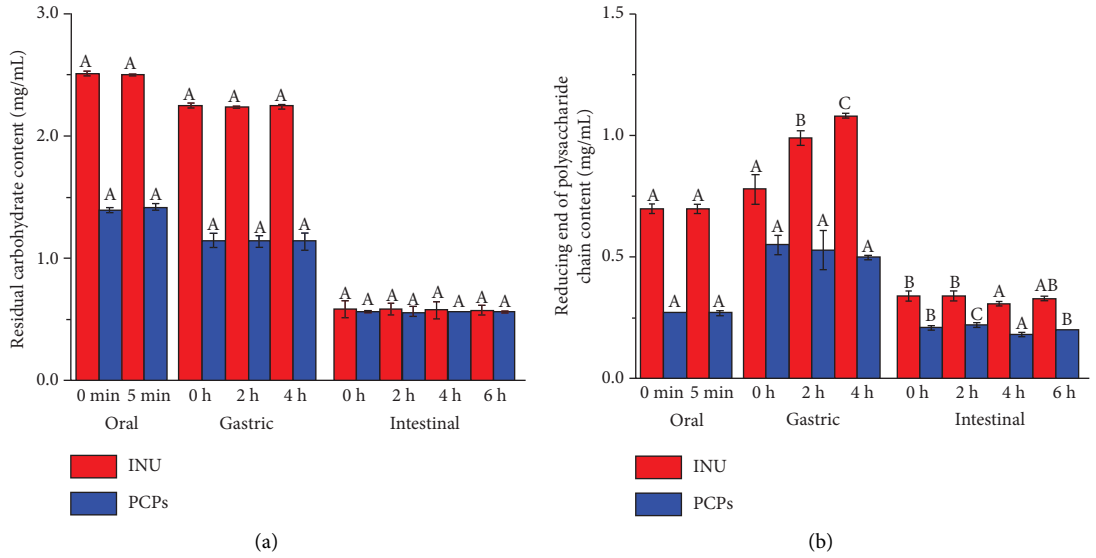

Figure 2: Residual carbohydrate content (a) and reducing end of polysaccharide chain content (b) of INU and PCPs during different phases of a multistage gastrointestinal tract digestion model. *Note*: Different letters indicate significant differences in the Duncan test at different time points in the same group. Data are expressed as mean ± SD ($n = 5$), and different letters are significantly different ($p < 0.05$). The same as below.

Table 2: Variations in Mw of PCPs throughout *in vitro* digestion and fermentation.

Samples	Mw (kDa)
PCPs	4.20 ± 0.04a
PCPs-S	4.17 ± 0.22a
PCPs-G	4.14 ± 0.17a
PCPs-I	4.09 ± 0.09a
PCPs-6 h	3.53 ± 0.19b
PCPs-12 h	2.26 ± 0.13c
PCPs-24 h	1.56 ± 0.43 d

Data are presented as mean ± SD ($n = 5$), with statistical significance observed among variables a–d ($p < 0.05$) based on a Duncan test within the same group but at varying time points. PCPs-S, PCPs-G, and PCPs-I refer to samples of PCPs subjected to digestion with different fluids, namely, saliva, gastric, and intestinal, respectively. PCPs-6 h, PCPs-12 h, and PCPs-24 h represent PCPs undergoing fermentation by human fecal microbiota for 6, 12, and 24 hours, respectively.

to the production of acetic acid [44]. These results indicated that PCPs might have a greater impact on promoting the growth of acetic-acid bacteria than INU. Acetic acid may prevent the buildup of lipids in the liver and reduce the amount of belly fat [45]. With the progress of *in vitro* fermentation, the steady rise of the PCPs group in propionic acid levels had a critical role in weight reduction, cholesterol-lowering, and the prevention of diet-induced obesity [46]. Contents of butyric, n-valeric, and isovaleric acids in the three groups were at a low level, despite significant differences. The total acid content in the PCPs group was significantly increased after 24 h *in vitro* fermentation ($p < 0.05$), which was similar to that in the INU group. The accumulation of SCFAs could lead to a decrease in pH in the intestinal environment and be beneficial for the gut [47]. These results indicated that PCPs could be degraded and

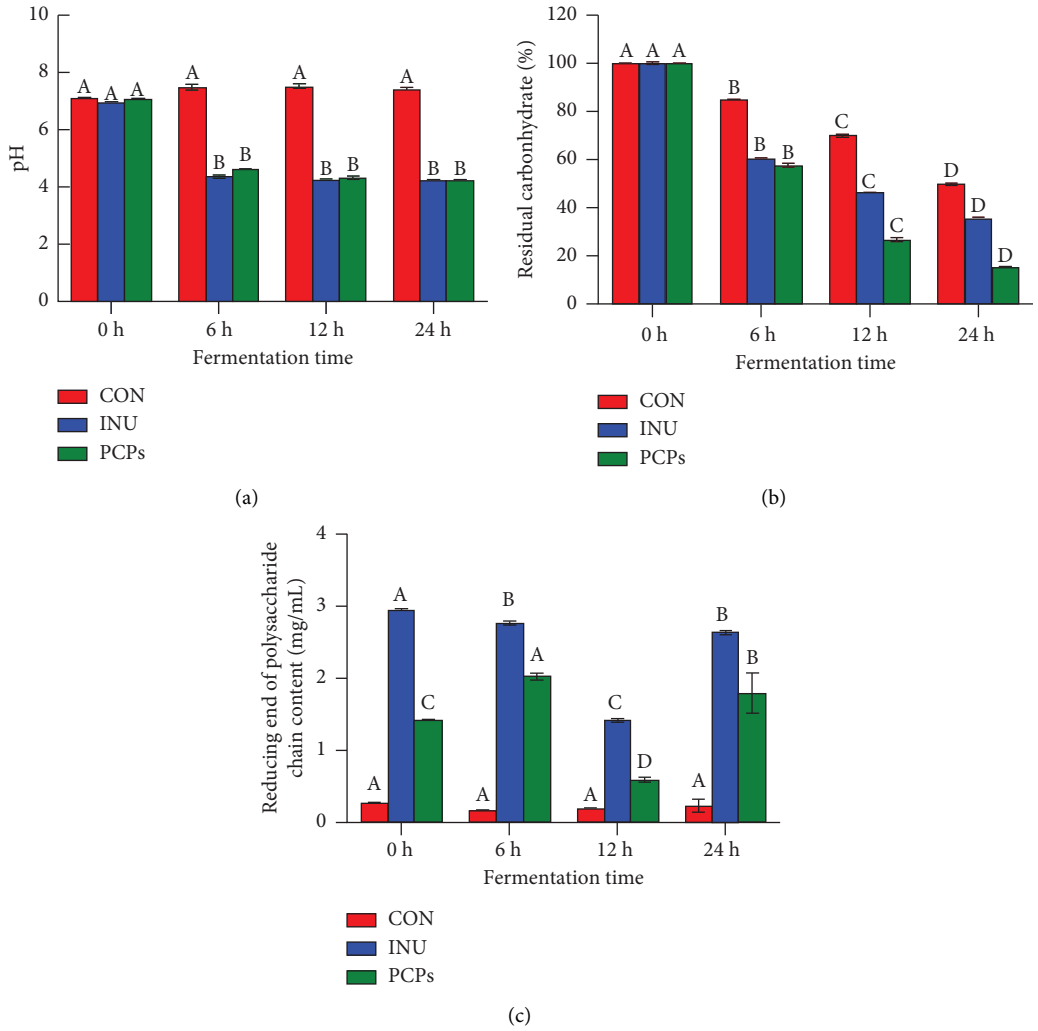

Figure 3: pH value (a), residual carbohydrate (b), and reducing end of polysaccharide chain content (c) were assessed throughout *in vitro* fermentation. The results are presented as mean ± SD ($n = 5$), with a significant difference observed in the (a–c) mean ($p < 0.05$).

Table 3: Total and individual short-chain fatty acid concentration (mmol/mL ± SD) after 24 h fermentation.

SCFAs (mM/L)	Group	Fermentation time (h)			
		0	6	12	24
Acetic acid	CON	2.61 ± 0.17a, D	4.91 ± 0.12c, A	3.98 ± 0.13b, C	4.65 ± 0.23c, B
	INU		6.87 ± 0.16b, C	10.32 ± 0.02a, B	28.32 ± 0.17b, A
	PCPs		10.32 ± 0.19a, C	11.64 ± 0.08a, B	32.90 ± 0.15a, A
Propionic acid	CON	0.69 ± 0.05a, D	1.53 ± 0.13c, C	2.95 ± 0.02c, B	4.17 ± 0.21c, A
	INU		9.97 ± 0.20a, C	11.90 ± 0.24a, B	15.53 ± 0.21a, A
	PCPs		7.23 ± 0.29b, C	9.16 ± 0.12b, B	12.13 ± 0.32b, A
Butyric acid	CON	0.54 ± 0.03a, D	0.64 ± 0.13b, C	1.25 ± 0.17a, A	0.75 ± 0.27b, B
	INU		1.09 ± 0.04a, B	1.23 ± 0.08b, A	1.09 ± 0.11a, C
	PCPs		0.68 ± 0.10b, C	0.67 ± 0.15c, A	0.72 ± 0.14b, B
N-valerate acid	CON	ND	ND	0.63 ± 0.31b, B	0.63 ± 0.31b, A
	INU			0.65 ± 0.22b, B	1.01 ± 0.50a, A
	PCPs			0.85 ± 0.21a, A	ND
Isovaleric acid	CON	ND	ND	ND	ND
	INU			0.87 ± 0.15a, A	ND
	PCPs			0.69 ± 0.13b, A	0.65 ± 0.12a, B
Total acid	CON	3.84 ± 0.25a, D	7.08 ± 0.38b, C	8.81 ± 0.63c, B	10.20 ± 1.02b, A
	INU		17.93 ± 0.40a, C	24.97 ± 0.71a, B	45.95 ± 0.99a, A
	PCPs		18.23 ± 0.58a, C	23.01 ± 0.69b, B	46.40 ± 0.73a, A

The data show significant differences ($p < 0.05$) among different times denoted by capital letters (A–D) within the same group, and among different groups denoted by lowercase letters (a–d) at the same time. ND indicates values not detected.

utilized by microorganisms in the gut to generate a large number of SCFAs, which was consistent with the results of *in vitro* fermentation of other herbaceous polysaccharides [25, 48].

3.4. Regulatory Effect of PCPs on Intestinal Microbiota. The present investigation analyzed samples subjected to high-throughput sequencing following 24 hours of fecal fermentation, with the aim of evaluating the influence of indigestible PCPs on microbial composition. A comprehensive total of 838,471 valid reads were generated from 15 samples, each yielding an average sequence length of 421 base pairs postfiltration of suitable reads. Notably, the rarefaction curves for the Sobs index in each sample displayed a plateau, suggesting sufficient sequencing depth for species detection and indicating satisfactory coverage of microbial diversity (Figure 4(a)).

α-diversity analysis indicates the evenness and richness of intestinal microflora. The Chao index is commonly used to reflect the abundance of microflora, while the Shannon index and Simpson index can be able to reflect the diversity of microflora. Compared with the CON group, the Chao index of the PCPs group was significantly decreased, indicating that PCPs could affect the richness of intestinal flora after 24 h of fermentation (Figure 4(b)). The Shannon index in the PCPs group was significantly reduced, and the Simpson index was significantly increased, indicating that PCPs could enrich the flora diversity (Figures 4(c) and 4(d)). Compared with the INU group, PCPs had a stronger effect on the diversity of intestinal flora during the fermentation. In general, the risk of getting illness decreases with increasing gut microbial diversity [49]. Thus, it can be speculated that PCPs might have a positive effect on human health by regulating the diversity of flora.

In this work, the principal component analysis (PCoA) and cluster analysis at the ASV level were done to visualize the β-diversity, and the -diversity analysis was utilized to assess the variations in species complexity from different treatments (Figures 4(e) and 4(f)). As Figure 4(e) displays, there was a significant difference in intestinal flora between any two groups from the CON, PCPs, and INU groups. From the PC1 axis (Figure 4(f)), the PCPs and INU group were significantly different from the CON group, indicating that PCPs and INU had notable regulatory effects on intestinal flora diversity. It can be seen from the PC2 axis that the PCPs and INU groups are different to some extent, but the PC2 axis occupies a lower proportion, indicating that PCPs and INU have similar regulatory effects on the composition of the microbial community, accompanied by a few differences.

16S rRNA gene sequencing further demonstrated that PCPs based on *in vitro* fermentation could change intestinal flora composition. Compared with the CON group, the PCPs and INU groups exhibited a notable rise in the relative abundance of *Firmicutes* and *Actinobacteria*, whereas the relative abundance of *Proteobacteria* decreased (Figure 5(a)). In addition, compared with the INU group, the relative abundance of *Actinobacteria* in the PCPs group was significantly increased. Similar results showed that INU could be metabolized by some bacteria of *Firmicutes* and *Actinobacteria* [50]. *Firmicutes* are usually able to use polysaccharides to produce SCFAs, creating acidic conditions conducive to the proliferation of butyric-producing bacteria, such as *Megamonas*, and inhibiting the growth of pathogens [51]. *Actinobacteria* contain probiotics such as *Bifidobacterium*, which can protect hosts from intestinal pathogenic infections through the production of acetate [52]. *Proteobacteria* include many pathogens, such as *Klebsiella*, *Enterobacter*, and *Escherichia-Shigella*, which are prone to cause intestinal microbial disorders and are positively correlated with diabetes, inflammation, and cancer, thus affecting the body health of the host [53].

Figure 5(b) displays the categorization of microbial communities at the genus level. *Escherichia-Shigella*, *Fusobacterium*, and *Klebsiella* relative abundances were considerably lower in the INU and PCPs groups compared to the CON group, which may indicate an inflection point and chronic illnesses [54]. An impact on the host-gut metabolism, immune system, and the structure of the gut intestinal microbiota may result from the relative abundance of health-promoting gut microbiota in the PCPs group being much greater than that in the CON group [48].

Based on the findings of the ASVs, a linear discriminant analysis effect size (LEfSe) study was conducted to investigate the distinct microbiota in the fermentation groups. There was a major difference between the three groups (Figure 5(c)). Notably, *Bifidobacterium* and *Lactobacillus* were enriched in the PCPs group as observed compared to the control group, which was consistent with the results of the single-strain culture model in previous literature [22]. *Bifidobacterium* has a variety of probiotic functions, which can improve intestinal diseases caused by immune system disorders, secret antimicrobial peptides to enhance the intestinal barrier, and improve the activity of host antioxidant enzymes to alleviate oxidative stress damage [55]. While *Lactobacillus* can maintain microecological balance, inhibit the growth of conditioned bacteria in the intestinal tract, and improve body immunity [56]. These two probiotics can be rapidly grown using fructans like INU [57]. PCPs have similar structures as INU [58], and therefore, the relative abundance of *Bifidobacterium* and *Lactobacillus* can also be increased after the intervention of PCPs.

These results indicated that PCPs could improve the composition of intestinal flora by increasing beneficial bacteria and decreasing the relative abundance of conditioned pathogens.

3.5. Correlation between SCFAs and Microbiota. Figure 6 displays correlations between SCFA concentrations and gut microbiota. Results showed that *Megamonas* and *Bifidobacterium* correlated positively with total SCFAs. *Megamonas* had a very high link with propionic acid and a close positive correlation with acetic acid, while *Bifidobacterium* had a highly significant positive correlation with acetic acid. According to a survey, *Bifidobacterium* species produce primarily acetate and lactate when they ferment

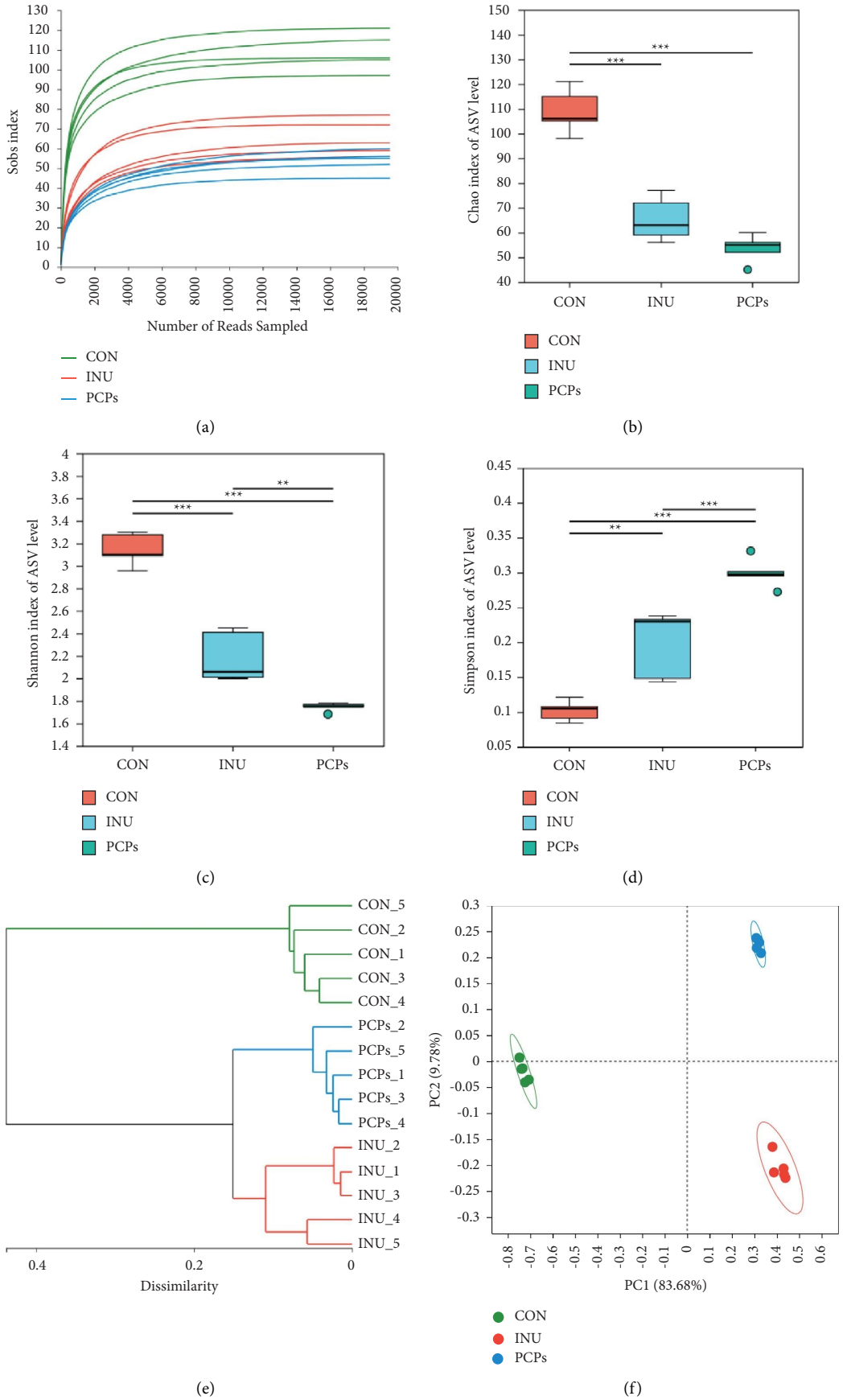

Figure 4: Analyses of the differences in each group's total bacterial composition. (a–d): Alpha diversity indexes for fermentation microbiota. Sobs index (a), Chao index (b), Shannon index (c), and Simpson index (d). Statistical significance within each treatment group was analyzed using the Kruskal–Wallis test and denoted as significant with two asterisks (** $p \leq 0.01$) or two asterisks (*** $p \leq 0.001$). (e) Sample level clustering tree based on Bray–Curtis; (f) PCoA based on Bray–Curtis.

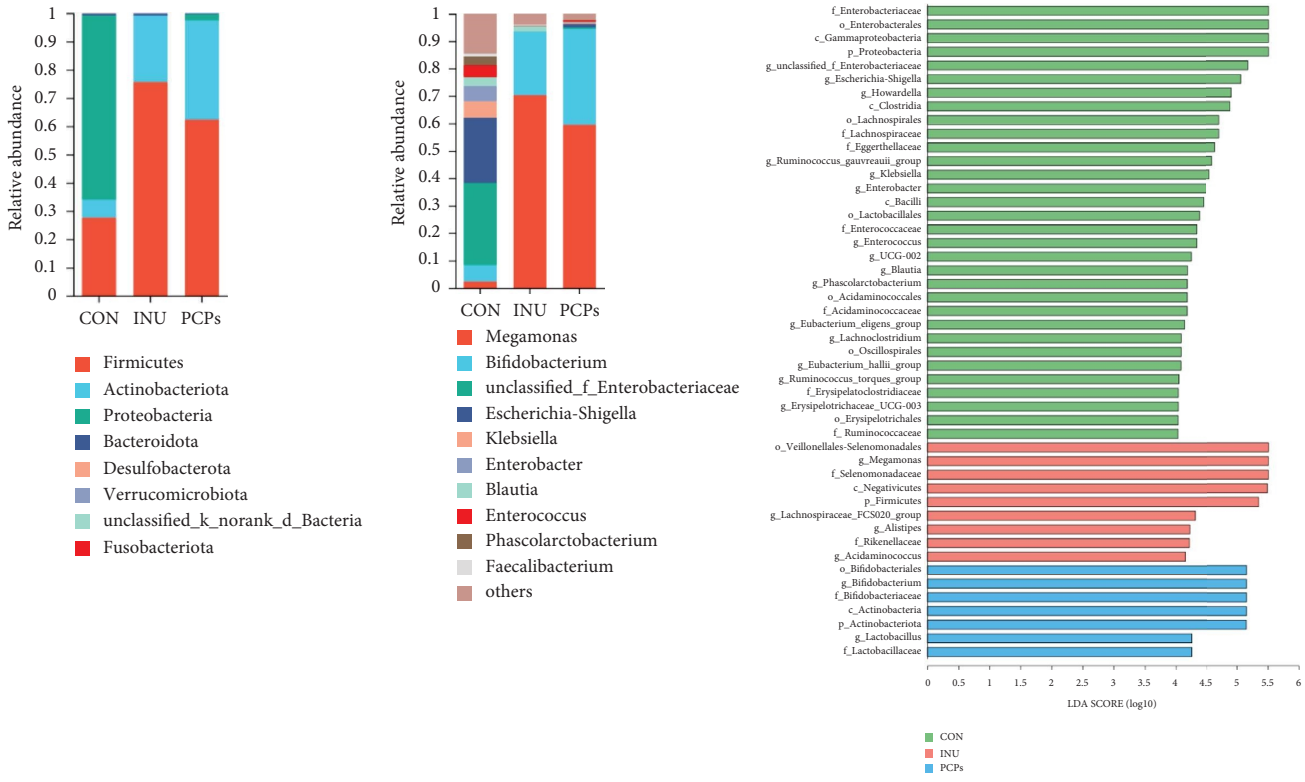

Figure 5: Phylum composition histogram (a), genus composition histogram (b), and LDA score histogram (c) in microbial community structure of different groups. Species that lacked annotations at the designated classification level and those with a representation of less than 0.5% in the sample were aggregated under the category of others.

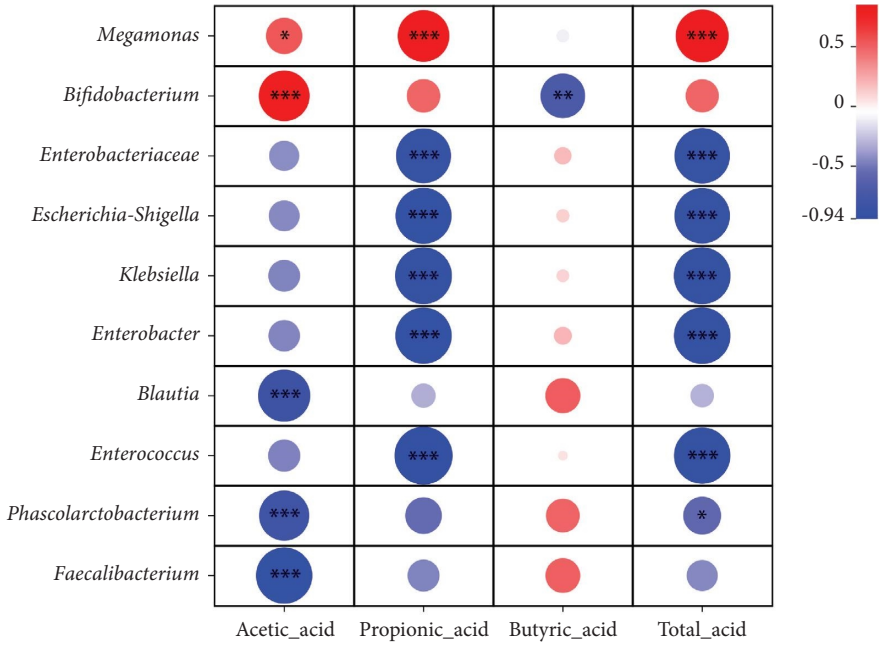

Figure 6: Spearman rank correlation related to microbial metabolites of short-chain fatty acids and species ASVs from microbiota.

carbohydrates [59]. Acetic acid was generally regarded as the fermentation product of beneficial bacteria such as *Bifidobacterium*, which could regulate the environment of intestinal flora and was mainly used for the synthesis of dry lipids and cholesterol to provide energy for surrounding tissues [60]. During the breakdown of complex

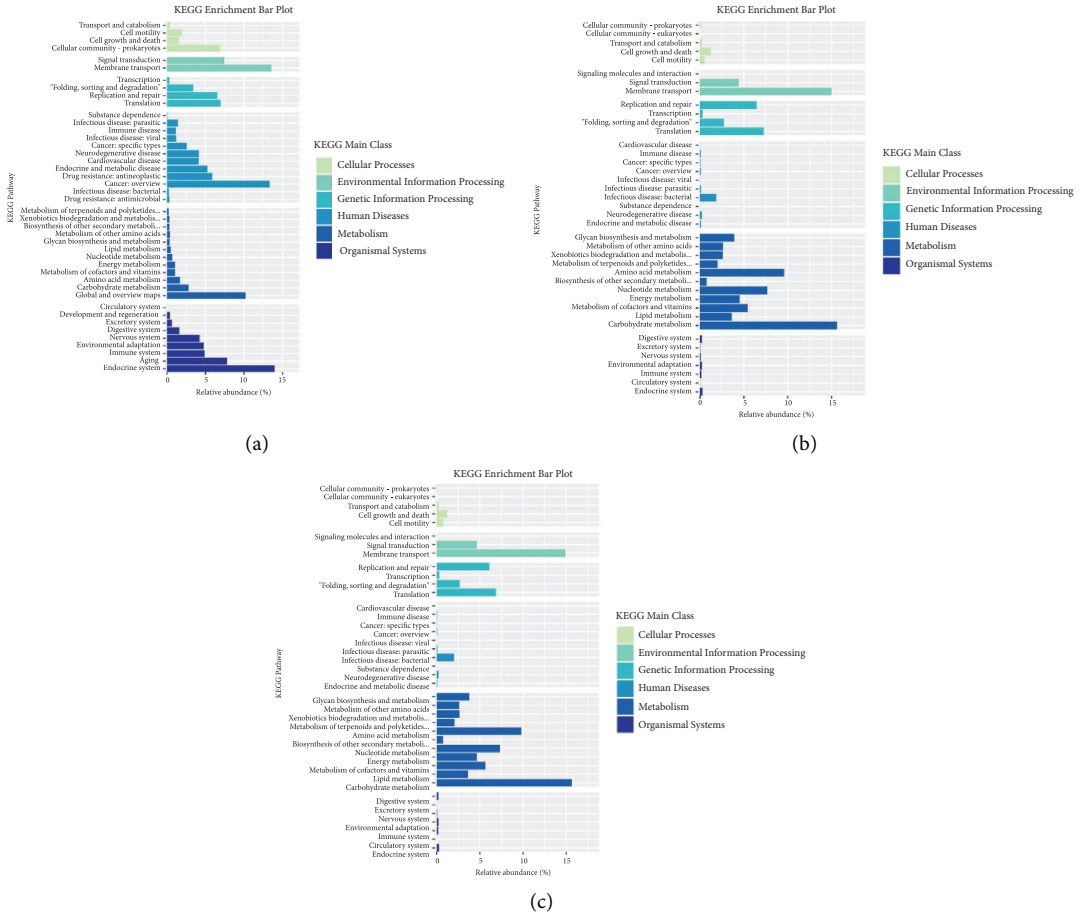

Figure 7: KEGG analysis of the relative abundance of metabolic pathways of CON (a), INU (b), and PCPs (c) group ($n = 5$).

carbohydrates, bacterial cross-feeding among microorganisms may contribute to the formation of SCFAs [61]. Additionally, it was discovered that conditional harmful bacteria and total acid concentration had a negative association, such as *Enterobacteriaceae, Escherichia-Shigella, Klebsiella, Enterobacter,* and *Enterococcus*. As mentioned above, PCPs could maintain the balance of gut flora, increase the generation of acetic and propionic acid, as well as the growth of beneficial bacteria.

3.6. Functional Alterations of Gut Microbiota by PICRUSt2 Analysis. The 16S rRNA sequencing of the fecal samples produced the ASVs, which were then connected to the KEGG pathway. The predicted alterations in metabolic function within the microbiome following PCPs intervention were derived based on the abundance distribution of each metabolic pathway in each sample as a result of the intervention. The six categories of metabolism, genetic information processing, environmental information processing, cellular activities, organismal systems, and human illnesses are used by the KEGG Pathway Database to categorize metabolic pathways. Multiple levels are further split within each metabolic pathway. The metabolic route map corresponds to the second level, which has 45 metabolic pathway subfunctions.

The average bacterial abundance in the three groups corresponded to the secondary functional pathway of the KEGG database (Figure 7). It is obvious that the enrichment degree of human disease and organism system pathway was affected in the CON group (Figure 7(a)). PCPs and INU both obviously decreased the enrichment degree of human disease and the organism system pathways and increased some metabolism pathways, including amino acid metabolic and carbohydrate metabolism (Figures 7(b) and 7(c)). The metabolism of both PCPs and the INU group is the most crucial pathway of gut microbiota. By increasing the production of SCFAs in the intestines, nonstarch polysaccharides have been proven to aid in the metabolism of starch, sucrose, and oligosaccharides [62]. The abundance of pathways under the headings of environmental information processing, genetic information processing, and metabolism was getting much higher, whereas the abundance of pathways under the heading of organismal system was significantly lower in the PCPs group. Among the secondary pathways of metabolism, amino acid metabolic, and carbohydrate metabolism were significantly ($p < 0.05$) rich in the PCPs group. Amino acid metabolism included phenylalanine metabolism and biosynthesis of phenylalanine, tyrosine, and tryptophan, which were essential for the proper functioning of the body [63]. Moreover, improving

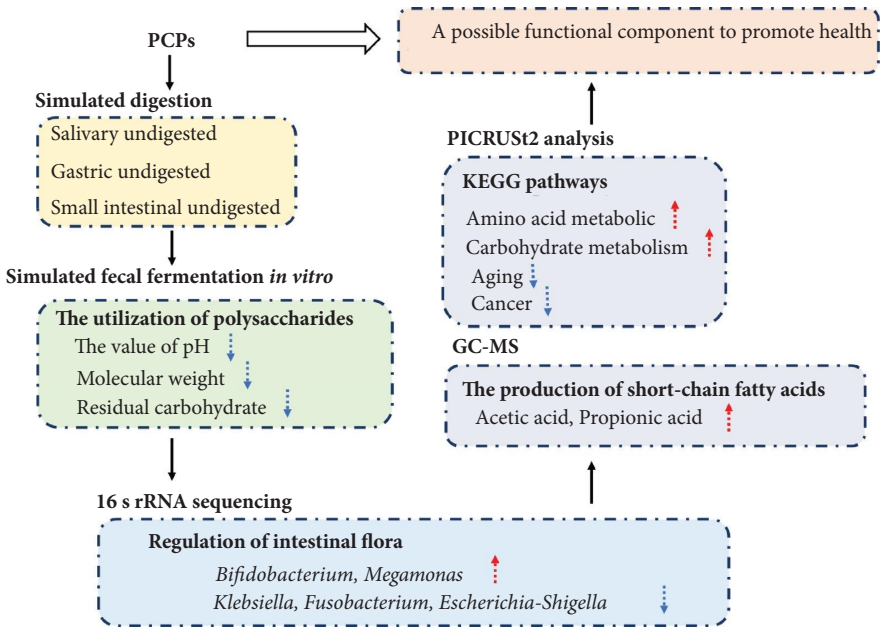

Figure 8: The flowchart and related changes of PCPs digestion in simulated oral-gastrointestinal tract and its *in vitro* fermentation.

the carbohydrate metabolism pathway could make better use of the intake of carbohydrates and provide enough energy for the body [64]. The gut microbiota is connected to host insulin sensitivity, poor amino acid metabolism associated with diabetes, and glucose metabolism [65]. As shown in Figure 8, PCPs might affect the metabolic pathways in the gut microbiota in order to perform its biological activity. The subsequent examination of the biological activity of PCPs may be guided by the functional hypotheses made by the gut flora. However, PICRUSt2 analysis could only predict the KEGG pathways using data from the 16S rRNA of gut microbiota. Future research should use other methods, such as meta-genomics analysis, to gain a more precise understanding of how PCPs affect the functional activity of the gut microbiota.

4. Conclusion

In conclusion, PCPs were undigested after passing the simulated digestive tract (oral cavity, stomach, and small intestine). Throughout the process of *in vitro* fermentation, PCPs were degraded and utilized by gut microbiota. Additionally, the concentrations of SCFAs, particularly acetic and propionic acids, were markedly raised. At the phylum level, the PCPs and INU group dramatically boosted the relative abundance of *Firmicutes* and *Actinobacteriota* while significantly decreasing the relative richness of *Proteobacteria*. In contrast to the INU group, the relative abundance of *Bifidobacterium* at the genus level was considerably increased in the PCPs group. The specialized metabolic processes of gut microbiota, such as amino acid metabolism and carbohydrate metabolism, may also benefit from PCPs intervention. Based on the above results, PCPs were utilized by intestinal flora to produce SCFAs, regulate the structure of intestinal flora, and exert biological activities by affecting the human disease, organism system, and metabolism pathway of intestinal flora, possibly resulting in beneficial effects on health. Therefore, our results imply that PCPs are anticipated to be a functional component for promoting health.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Ya Gao was responsible for investigation, data analysis, and writing the original draft. Ying Xiao was responsible for the methodology, writing, review, and editing, and formal analysis. Ling Yu and Qingjiu Tang were responsible for resources and validation. Lin Ye and Jinyan Wang were responsible for supervision and project administration. Yipeng Wang was responsible for conceptualization. Fangfang Zhang was responsible for supervision. Jianjin Zhou was responsible for funding acquisition. All the authors read and approved the final manuscript.

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