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In vitro fecal fermentation characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide

Qi Li [a,1], Weijie Wu [b,c,d,1], Hangjun Chen [b,c,d], Xiangjun Fang [b,c,d], Yanchao Han [b,c,d], Mingyong Xie [a,*], Haiyan Gao [a,b,c,d,*]

[a] State Key Laboratory of Food Science and Technology, China-Canada Joint Lab of Food Science and Technology (Nanchang), Nanchang University, Nanchang, Jiangxi Province 330047, China
[b] Food Science Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[c] Key Laboratory of Post-Harvest Handing of Fruits, Ministry of Agriculture and Rural Affairs, Hangzhou 310021, China
[d] Key Laboratory of Fruits and Vegetables Postharvest and Processing Technology Research of Zhejiang Province, Hangzhou 310021, China

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ABSTRACT

The effects of Moso bamboo (*Phyllostachys edulis*) shoot polysaccharide (BSP) on the human gut microbiota composition and volatile metabolite components were investigated by *in vitro* fermentation. After fermentation for 48 h, BSP utilization reached 40.29% and the pH of the fermentation solution decreased from 6.89 to 4.57. Moreover, the total short-chain fatty acid concentration significantly ($P < 0.05$) increased from 13.46 mM (0 h) to 43.20 mM (48 h). 16S rRNA analysis revealed several differences in the gut microbiota community structure of the BSP-treated and water-treated (control) cultures. In the BSP group, the abundance of *Firmicutes*, *Actinobacteria*, and *Proteobacteria* was significantly increased, while that of *Bacteroidetes* and *Fusobacteria* significantly decreased. Moreover, the concentrations of benzene, its substituted derivatives, and carbonyl compounds in the volatile metabolites of the BSP-treated group decreased, while that of organic acids significantly increased after 48 h of fermentation. These results demonstrate that BSP improves gastrointestinal health.

Introduction

Fermentation, an important function of the colon, is recognized as the process wherein anaerobic bacteria break down complex carbohydrates into SCFAs, gases (hydrogen, methane, and carbon dioxide), and other metabolites (Wang et al., 2019). Specifically, complex polysaccharides are broken down by the colonic microbiota into fermentation products that are absorbed by the host (Felizardo, Watanabe, Dardi, Rossoni, & Câmara, 2019) and contribute to nutrition and immune development (Bedford & Gong, 2018). Moreover, bacterial diversity in the colon is beneficial because it increases the probability of including species that can break down any complex carbohydrate into fermentation products (Porter & Martens, 2017).

Polysaccharides are an important energy source for the colonic flora. Under certain intestinal conditions, anaerobic bacteria activate their mechanism, which comprises key enzymes and metabolic pathways, to metabolize complex carbohydrates and afford metabolites such as SCFAs. SCFAs are organic products, mainly composed of acetate, propionate, and butyrate (Bach Knudsen, 2015), which play key roles in regulating the host metabolism, immune system, and cell proliferation. High SCFA concentrations are found in the proximal colon, where they are used as an energy source (especially butyric acid) in colonic cells; however, they can also be transported through the portal vein to the surrounding circulation, thereby acting on the liver and surrounding tissue (Gidley, 2013). The chemical structure and physical form of polysaccharides are critical factors that determine the fermentation rates (Shi, Fu, Tan, Huang, & Zhang, 2017). Chemical characteristics include the monosaccharide and linkage connection type, molecular size, and sugar arrangement at the molecular level (Williams, Mikkelsen, le Paih, & Gidley, 2011).

Abbreviations: ANOVA, one-way analysis of variance; BSDF, bamboo shoot dietary fibre; BSP, bamboo shoot polysaccharide; GC, gas chromatography; HPGPC, high-performance gel permeation chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; PCA, principal component analysis; RT-PCR, reverse transcription-polymerase chain reaction; SCFA, short-chain fatty acid; TLC, thin-layer chromatography.

* Corresponding authors at: Food Science Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China (H. Gao).
E-mail addresses: myxie@ncu.edu.cn (M. Xie), spsghy@163.com (H. Gao).

[1] The first two authors contributed equally.

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In vivo experiments have shown that BSPs possess prebiotic properties, including antidiabetic, anti-tumor, antioxidant, and hypolipidemic properties, and can promote good intestinal health (He et al., 2018; Zhao et al., 2017; Li et al., 2021). Previous studies have also revealed that BSDF is difficult to digest under simulated gastrointestinal digestion conditions (Chen et al., 2019; Wu et al., 2020). However, to the best of our knowledge, the effects of purified BSP on the human intestinal microbiota have not been well addressed to date. The studied models generally comprise closed anaerobic environments in airtight cylinders that employ mixed cultures of human gut microbiota. The capacity of these models to metabolize BSP substrates is then measured. Such measurements are difficult to perform in human clinical trials, owing to ethical issues and thus, *in vitro* models provide a unique opportunity to study how certain substrates are used by the microbiome. Herein, we suggest the principles for the reasonable design of a BSP model with potential health benefits to the human body.

Materials and methods

Materials

Bamboo shoots (*Phyllostachys edulis* (Carrière) J. Houz) were collected from the LinAn Genhong specialized agricultural cooperative, Zhejiang Province, China. The SCFA standards for the HPLC analysis, including acetic acid, propionic acid, butyric acid, and valeric acid, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). L-cysteine, peptone, hemin, $MgSO_4$, resazurin, vitamin K, yeast extract, and calcium chloride were also obtained from Sigma-Aldrich. NaCl, KH_2PO_4, and K_2HPO_4 were obtained from Sangon Biotech (Shanghai, China). The dung ammonia detection kit (enzymatic) was purchased from Medical System Biotechnology Co., Ltd. (Ningbo, China). All chemicals used in this study were of analytical grade.

Preparation of BSP and its physiochemical characteristics

The crude polysaccharide was isolated from *P. edulis* bamboo shoots using hot water, as described by Xu et al. (2014). Briefly, the dried bamboo shoots were ground into powder, filtered through a 100-mesh sieve, and extracted with 25-fold (w/v) distilled water at 85 °C for 2 h. After centrifugation for 15 min at 8000 rpm and 4 °C, the supernatant was collected and concentrated using a rotary evaporator (EYELA, Rikakikai Co., Ltd. Tokyo, Japan) at 60 °C. Precipitation was then conducted overnight with four volumes of 95% ethanol at 4 °C. The precipitate was collected, re-dissolved in distilled water (1:1), and subsequently treated with 1% high-temperature-resistant α-amylase (800 U/g, CAS: 9000-90-2, Aladdin Biochemical Technology Co., Ltd, Shanghai, China) for 30 min at 95 °C. Once the mixture was cooled to 60 °C, 0.5% papain (2000 U/g, CAS: 9001-73-4, Aladdin Biochemical Technology Co., Ltd, Shanghai, China) was added, and the samples were incubated (60 °C) for 30 min at a pH range of 4.2–4.7. Subsequently, 1% glucoamylase (10,000 U/g, CAS: 9032-08-0, Aladdin Biochemical Technology Co., Ltd, Shanghai, China) was added, followed by incubation for 30 min. Next, the enzyme was deactivated at 100 °C for 5 min. Finally, the crude polysaccharide was dialyzed (8–14 kDa, 48 h, 4 °C) and freeze-dried (48 h, −85 °C). The resulting material was labeled as BSP.

The molecular weights of the extracted and purified BSP samples were analyzed by HPGPC [Waters Technology (Shanghai) Co., Ltd., China] according to a previously reported method (Zhang et al., 2017). The total sugar, uronic acid, and total protein contents of the purified BSP were determined by the phenol sulfuric acid method (DuBois et al., 1956), m-hydroxybiphenyl method (Shi et al., 2020), and Coomassie brilliant blue method, respectively.

Collection of human intestinal microbiota

A total of 10 human stool samples were collected at the Zhejiang Academy of Agricultural Sciences from healthy fecal flora transplantation donors (five males and five females aged 22 to 28) who had not ingested antibiotics within three months prior to sampling and had no recent history of gastrointestinal disorders. All donors provided a written informed consent, and the study was approved by the Ethics Committee of Zhejiang Academy of Agricultural Sciences.

In vitro fermentation of BSP

The culture medium contained 4 g/L yeast extract, 10 g/L tryptone, 1 g/L L-cysteine, 0.9 g/L NaCl, 0.4 g/L KH_2PO_4, 0.4 g/L K_2HPO_4, 0.05 g/L hemin, 0.2 g/L $CaCl_2$, 4.5 g/L $MgSO_4$, 0.25 g/L resazurin, 0.05 g/L vitamin K, and 8 g/L BSP. A distilled water was used as the control group. The fermentation cultures were carried out in 5 mL volumes of sterilized culture medium in 10 mL fermentation bottles. The culture media were sterilized after they were added to the fermentation bottle vessels. Human feces samples were mixed with PBS buffer in a ratio of 2:1. Next, they were inoculated with 5 mL of fecal bacterial suspension (10% w/v,), prepared by mixing and homogenizing freshly voided feces in PBS at pH 7.0. Prior to fermentation, the vessels were sparged with N_2 gas to obtain an anaerobic environment. Batch fermentation was carried out anaerobically for 48 h at 37 °C (GRP-9270, Water-jacket Incubator, Shanghai Senxin experimental instrument), and samples were collected at five time points (0, 12, 24, 36, and 48 h of fermentation). The samples were then stored at − 80 °C until further analysis. At the beginning of the fermentation process and after 48 h, 1 mL samples were collected and centrifuged at 12,000 × *g* for 10 min at 4 °C. The resultant supernatants were used to determine various indicators, while the sediment was used to extract bacterial DNA.

SCFA analysis

The fermentation samples were thawed at room temperature for 30 min and centrifuged at 8000 rpm for 15 min. The supernatant, which was filtrated through a 0.22 μm membrane, was used for SCFA analysis. The SCFA standards (external standards) and supernatant contents were detected with an Agilent GC-7890B chromatograph (Agilent Technology (Beijing) Co., Ltd., China) equipped with a flame ionization detector and an HP-FFAP column (60–240 °C, 30 m × 320 μm, Agilent Technology (Beijing) Co., Ltd., China). N_2 was used as the carrier gas, with a constant flow rate set at 12.3 mL/min and a splitting ratio of 1:10, while the H_2 and air flow rates through the column were 30 mL/min and 400 mL/min, respectively. The column temperature was set at 80 °C for 5 min and subsequently raised to 250 °C at 5 °C/min. The injection port and flame ionization detector temperatures were 250 °C and 260 °C, respectively, and the injection volume was 1 μL (Song, Mao, Siu, & Wu, 2018). Each sample was examined thrice independently.

Determination of pH

The pH value was determined immediately after finishing the colonic fermentation assays (48 h) using a BPH-7200 laboratory pH meter (BPH-7200, BELL Analytical Instrument Co., Ltd., Dalian, China).

Bacterial quantification by RT-PCR from fecal and fermentation broth samples

Quantitative real-time RT-PCR (ABI StepOnePlus Real-Time PCR System, Thermo Fisher Scientific, USA) was performed to quantify the selected bacterial groups of human intestinal microbiota. Specific primers for *Bifidobacterium* and *Lactobacillus* were used as described by Nasuti et al. (2016) to quantify the respective microorganisms. The primers used were *Lac*-F (TGGAAACAGRTGCTAATACCG) and *Lac*-R

(GTCCATTGTGGAAGATTCCC) for *Lactobacillus* and *Bif*-F (GGTGTCGGCTTAAGTGCCAT) and *Bif*-R (CGGA(C/T) GTAAGGGCCGTGC) for *Bifidobacterium* (5′ to 3′) (Bartosch, Fite, Macfarlane, & McMurdo, 2004).

Degradation rate, gas production, and gas composition analysis

The degradation rate of the polysaccharides after fermentation was determined by TLC. The unfermented and fermented media were sampled (0.2 μL), stained, and colored (120 °C), and a scanner was used to calculate the proportion of gray scale reduction. The degradation rate was calculated using the equation: (Proportion of gray scale after fermentation)/ (Gray scale before fermentation) × 100%. An intestinal microbial fermentation gas analyzer (HL-QT01, Suzhou Hailu Biotechnology Co., Ltd., Jiangsu Province, China) was used to determine the components and content of the gas produced after fermentation.

Gut microbiota studies

The total bacterial genomic DNA of the fermentation samples (1 mL) was extracted using the QIAamp Fast DNA Stool Mini kit (QIAGEN, Valencia, CA, United States) according to the manufacturer's manual. The DNA integrity was confirmed by 1% agarose gel electrophoresis (Fu et al., 2019), and the microorganism DNA concentrations were subsequently verified using a Nanodrop 3300 fluorospectrometer (Thermo Fisher Scientific, Massachusetts, USA). The primers 515F and 806R (Caporaso et al., 2012) were used to amplify the V3–V4 variable region of the bacterial 16S rRNA gene. DNA samples were stored at − 20 °C until sequencing. All amplified DNA samples were sent to Personalbio Biotechnology Co., Ltd. (Shanghai, China) for the high-throughput sequencing and analysis of microflora diversity. R statistical software was used for the bioinformatics analysis.

Production of volatile gut metabolites

Analysis of the volatile metabolites from the fermentation solution was conducted on a TRACE 1300 gas chromatograph coupled to a TSQ 9000 mass selective detector (Thermo Fisher Scientific Technologies, Shanghai, China). Compounds were separated on a TG-624Si MS column (30 m × 0.25 mm × 0.14 μm, Thermo Fisher Scientific Technologies). Mass spectroscopic conditions were as follows: Injector temperature of 250 °C in splitless mode. Initial temperature gradient of 40 °C for 2 min subsequently raised to 300 °C at 6 °C/min and held at this temperature for 5 min. The mass spectra were generated at an electron energy of 70 eV, an acceleration voltage of 3 kV, and a solvent delay of 3 min. Helium (99.999%) was used as the carrier gas with a constant starting flow rate of 1 mL/min. The total ion chromatograms were collected by scanning from *m/z* 35 to 350 at 1 scan/s (Zamora-Gasga et al., 2018). The volatile metabolites of fermentation liquor were determined at 300℃ for 40 min. The volatile compounds were identified by comparing the retention times with those in the MS library (NIST, USA). The peak area values of all the metabolites were calculated and normalized according to internal standards. The levels of all confirmed metabolites are shown in the PCA using R software (version 4.0.0).

Statistical analysis

Experiments were conducted in triplicate. All data are expressed as the mean ± standard deviation. The experimental data were evaluated by ANOVA followed by Duncan's test using SPSS 23.0 software. Correlations were considered significant at $P < 0.05$.

Results and discussion

Physiochemical characteristics of BSP

The basic physical and chemical properties of the purified BSP samples were elucidated from the HPGPC spectra (Fig. 1), which show the BSP components before and after purification. In this study, BSP was obtained by enzymolysis extraction and ethanol precipitation in 8.41% yield, and comprised 83.71% saccharide, 0.96% protein, and 34.09% uronic acid contents. The average molecular weight of BSP was 72.2 kDa. The water-soluble dietary fiber purity was 98%, and the monosaccharides of BSP were arabinose, glucose, and galactose.

pH and SCFA profiles during in vitro colonic fermentation

The SCFA concentrations in the fermentation cultures at 0, 12, 24, 36, and 48 h for the samples using BSP as the fermentation substrate (BSP group) and water (control group) (Table 1) were comparatively studied. The total SCFA concentration increased from 13.46 ± 0.8 mM (0 h) to 43.20 ± 1.16 mM (48 h) in the BSP group ($P < 0.05$), with the latter value being markedly higher than that observed in the control group (24.83 ± 1.0 mM at 48 h). As shown in Fig. 2A, the pH variation was consistent with the differences in the SCFA concentrations. Thus, the pH variation in the BSP group was significant ($P < 0.05$), decreasing from 6.89 at the beginning of the process (0 h) to 4.90 after 48 h. On the other hand, there were no marked changes in the pH of the control group (6.86 at 0 h and 6.70 after 48 h). As shown in Fig. 2B, after fermentation for 48 h, BSP utilization reached 40.29%. We then proceeded to observe the changes in the essential SCFA contents in the BSP group. Thus, after 48 h fermentation, the acetic acid (22.48 ± 3.11 mM), propionic acid (14.35 ± 1.48 mM), and valeric acid (0.42 ± 0.26 mM) concentrations in the BSP group were significantly higher ($P < 0.05$) than those in the control group (13.81 ± 2.51 mM, 7.72 ± 0.48 mM, and 0.18 ± 0.10 mM, respectively). The butyric acid concentration in the BSP group (5.95 ± 0.48 mM) was also markedly higher than that in the control group (2.99 ± 0.57 mM).

SCFAs produced by polysaccharide fermentation provide the host with many health benefits. Indeed, after 48 h of fermentation, the acetic acid, propionic acid, butyric acid, valeric acid, and total SCFA yields in the BSP group were significantly higher than those in the control group. Moreover, SCFAs derived from polysaccharide fermentation have been identified as a major source of nutrition and energy for the colonic cells and bacteria and are usually considered conducive to intestinal health by enhancing mineral absorption, regulating pathogens by lowering the pH in the intestinal lumens, being the main energy source for colonocytes, and most importantly, maintaining the intestinal barrier intact (Wang et al., 2019). Acetic acid, as an energy source for both gut and liver cells, plays an important role as a signaling molecule in

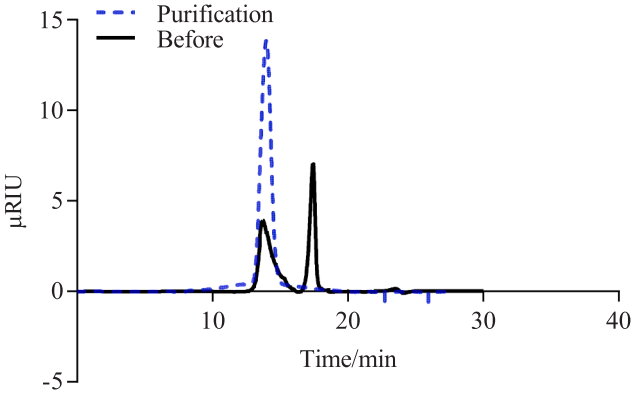

Fig. 1. High-performance gel permeation chromatography spectra of bamboo shoot polysaccharide before and after purification.

Table 1
Comparison of the short-chain fatty acid (SCFA) concentrations in the fermentation cultures at 0, 12, 24, 36, and 48 h for the samples with bamboo shoot polysaccharide as the fermentation substrate (BSP group) and water (control group).

Sample	Time (h)	SCFAs (mM)						
		Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Valeric acid	Isovaleric acid	Total acid
BSP	0	8.40 ± 0.50^{e}	3.15 ± 0.53^{e}	1.51 ± 0.45^{d}	0.28 ± 0.12^{b}	0.05 ± 0.02^{c}	0.06 ± 0.04^{b}	13.46 ± 0.8^{d}
	12	15.66 ± 2.46^{b}	7.73 ± 1.82^{d}	3.11 ± 0.70^{b}	0.30 ± 0.12^{b}	0.06 ± 0.04^{c}	0.11 ± 0.03^{ab}	26.98 ± 1.3^{c}
	24	21.16 ± 2.04^{a}	9.38 ± 0.90^{c}	3.87 ± 0.45^{b}	0.43 ± 0.17^{b}	0.11 ± 0.04^{b}	0.13 ± 0.04^{ab}	35.06 ± 0.9^{b}
	36	20.95 ± 2.28^{a}	11.13 ± 0.53^{b}	4.32 ± 0.55^{a}	0.52 ± 0.12^{a}	0.19 ± 0.02^{ab}	0.15 ± 0.03^{a}	37.16 ± 2.1^{b}
	48	22.48 ± 3.11^{a}	14.35 ± 1.48^{a}	5.33 ± 0.51^{a}	0.62 ± 0.15^{a}	0.25 ± 0.09^{a}	0.17 ± 0.02^{a}	43.20 ± 1.5^{a}
Control	0	7.90 ± 0.50^{e}	3.12 ± 0.50^{e}	1.51 ± 0.45^{d}	0.22 ± 0.13^{b}	0.02 ± 0.02^{c}	0.04 ± 0.02^{b}	12.87 ± 0.7^{d}
	12	10.83 ± 1.32^{d}	$6.46 \pm .84^{d}$	2.27 ± 0.87^{c}	0.24 ± 0.03^{b}	0.03 ± 0.04^{c}	0.05 ± 0.04^{b}	19.86 ± 1.1^{c}
	24	12.00 ± 0.87^{c}	6.99 ± 0.67^{d}	2.05 ± 0.30^{c}	0.26 ± 0.05^{b}	0.04 ± 0.05^{c}	0.08 ± 0.04^{ab}	22.13 ± 1.5^{b}
	36	13.61 ± 2.24^{c}	7.43 ± 0.56^{d}	2.41 ± 0.17^{c}	0.29 ± 0.02^{c}	0.04 ± 0.05^{c}	0.09 ± 0.02^{ab}	22.90 ± 1.2^{b}
	48	13.81 ± 2.51^{c}	7.72 ± 0.48^{d}	2.67 ± 0.23^{c}	0.32 ± 0.01^{b}	0.05 ± 0.01^{c}	0.13 ± 0.03^{a}	24.83 ± 1.0^{b}

Note: Different superscript letters in the same column indicate significant differences ($P < 0.05$).

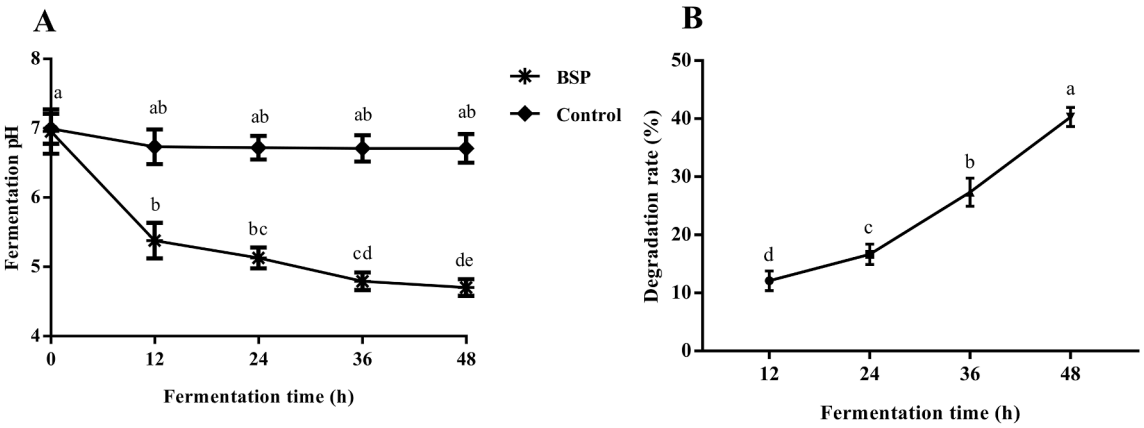

Fig. 2. Changes in pH and degradation rates of bamboo shoot polysaccharide. (A) pH shifts, (B) rates of BSP degradation.

gluconeogenesis and lipogenesis (Fu et al., 2019). The metabolic pathway of propionic acid has been related to polysaccharide fermentation (Al-Lahham, Peppelenbosch, Roelofsen, Vonk, & Venema, 2010). Up to 90% propionic acid was transmitted to the liver via the portal vein, with the majority used for gluconeogenesis and inhibition of cholesterol synthesis (Al-Lahham et al., 2010).

Butyric acid is an energy source for colonic epithelial cells, providing 60–70% of their total body energy requirement (Wu et al., 2020). It also inhibits cell apoptosis and therefore aids in the prophylaxis of colon cancer, decreases bacterial transposition and inflammation, and enhances intestinal barrier functioning. Thus, together with the production of SCFAs, the benefits of polysaccharides fermentable in the large intestine have been intensively studied, showing protective effects in the colon. The results of the current study using BSP suggest that the generation of high concentrations of SCFAs is beneficial to human health.

Gas production, and gas composition analysis

The food ingested by the human body produces a small amount of gas (e.g. CO_2, H_2, CH_4, H_2S, NH_3) during digestion and absorption. These gases are collectively called intestinal gas. Once the gas-producing bacilli are out of balance, excessive gas will be produced, leading to an increase of the pressure in the intestinal tract (Zhu, Zhou, Liu, Pi, Zhou, Li, et al., 2021). During polysaccharide digestion, rapid decomposition in the large intestine causes abdominal distension and discomfort (Amorim et al., 2020). As shown in Fig. 3A, there was no obvious change in sample pressure during fermentation. The ammonia concentrations of the BSP and control groups measured after 0 h (BSP 0 and control 0, respectively) and 48 h (BSP 48 and control 48, respectively) are shown in Fig. 3B. For the BSP sample, the results show a reduction in the final ammonia concentration from 32.07 mmol/g (BSP 0) to 15.54 mmol/g (BSP 48). Two other gas components were also detected during fermentation. Compared with the control group, the BSP group, slight increases in the carbon dioxide [from 1.0 2 ± 0.31 mmol/L (12 h) to 1.58 ± 0.18 mmol/L (24 h); Fig. 3C] and hydrogen gas (from 1.16 ± 0.55 mmol/L (12 h) to 1.39 ± 0.5 mmol/L (24 h); Fig. 3D] contents were observed. The fermentation of prebiotics by human fecal inoculation also affects gas production. For the BSP group, CO_2 production increased over time so that the CO_2 concentration after 48 h fermentation was higher than the concentration detected after 12 h. On the other hand, methane production was not detected, indicating that methanogenic bacteria were absent from the original microflora.

Increased SCFA production due to prebiotic fermentation leads to a decrease in pH, which in turn promotes a decrease in intestinal pathogenic factors and ammonia production. It is well known that there are three main sources of intestinal gas: swallowed air, intraluminal gas production (including chemical reactions and bacterial metabolism), and diffusion from the bloodstream into the lumen of the gastrointestinal tract (Wang et al., 2019). In terms of the intestinal gas production mechanism, CO_2, H_2, and CH_4 are the main gases produced in the gastrointestinal tract. Of these, large quantities of CO_2 are generated by the reaction between hydrogen and bicarbonate ions, while the intestinal bacteria ferment produces small CO_2 and H_2. Most of these gases, which are closely related to health and disease, are expelled directly from the gastrointestinal tract or are absorbed into the systemic circulation and expelled from the lungs. Although these experiments produced carbon dioxide and hydrogen gases, the amounts were too small to be harmful to the body.

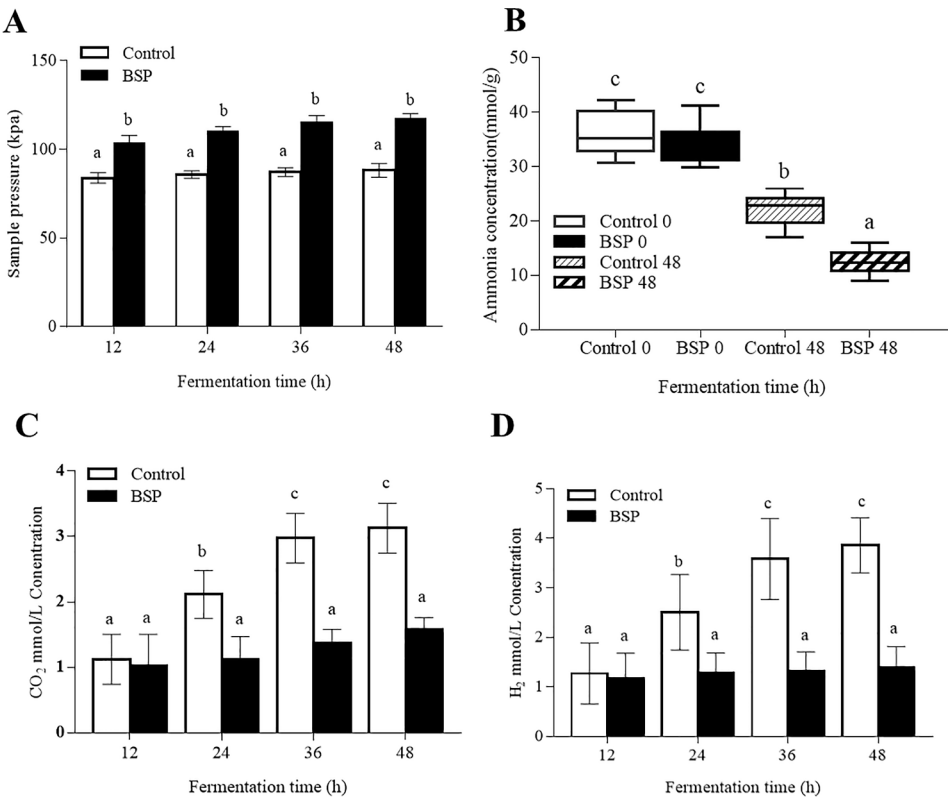

Fig. 3. Total pressure of the fermentation samples and analysis of the produced gas components of these samples. (A) Change in the total pressure of the fermentation samples. (B) Concentration of ammonia in the fermentation broth after 48 h. (C) CO_2 and (D) H_2 gas contents generated during fermentation.

Effects of BSP on intestinal microbiota diversity

Fig. 4A and B show the log values of the selected bacterial group counts detected at two fermentation time points (0 h and 48 h). The results reveal a significant increase in microorganisms in the BSP group after 48 h of fermentation compared to the values observed at the starting point (0 h) and for the control group (48 h). Indeed, both *Bifidobacterium* and *Lactobacillus* showed a significant increasing trend after 48 h of BSP fermentation. Thus, compared to the unfermented samples, *Bifidobacterium* and *Lactobacillus* were stimulated to increase by 2.21 log (CFU/mL) and 2.90 log (CFU/mL), respectively ($P < 0.05$). Compared with the BSP (0 h) group, the counts for *Bifidobacterium* and *Lactobacillus* increased by 3.07 log (CFU/mL) and 2.53 log (CFU/mL), respectively, after 48 h of BSP fermentation ($P < 0.05$). *Bifidobacterium* and *Lactobacillus* are some of the most common microorganisms in the gastrointestinal tract of humans and some animals and are well known to play an important role in maintaining the health of their host.

In this study, we used high-throughput sequencing to assess the dynamics of different intestinal bacterial populations during fermentation, the 16S sequencing results were submitted to the NCBI database (SAMN21164041- SAMN21164060). Different polysaccharides generated different metabolite profiles, and the microbial communities changed accordingly, especially at low phylogenetic levels. The relative abundances of the intestinal microbiota in the BSP and control groups are illustrated by histograms in Fig. 4C and D. The control and BSP groups comprised *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria*, with the first three groups being the dominant phyla. Generally, *Bacteroidetes* became less abundant for both groups, while *Actinobacteria* and *Proteobacteria* became more abundant in the BSP group after 48 h of fermentation, when compared to the intestinal microbiota samples from the unfermented group. As shown in Fig. 4C, *Firmicutes* and *Actinobacteria* were significantly ($P < 0.05$) more abundant in the BSP group than in the control group after 48 h of fermentation. Conversely, the abundance of *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria* in the BSP group was significantly ($P < 0.05$) lower than that observed in the control group. The BSP group at 48 h of fermentation significantly increased the relative abundance of beneficial gut bacteria, such as *Firmicutes* and *Actinobacteria*. Some *Firmicutes* in the gut generate SCFAs by fermenting indigestible carbohydrates, thereby promoting health. In particular, probiotics such as the butyrate-producing colonic *Firmicutes* have been found to increase colonic butyrate concentrations and improve gut function (Xu et al., 2019). Actinomycetes, gram-positive bacteria represented by *Bifidobacteria*, have also been associated with good gut health (Wu et al., 2020). Acid conditions are also reported to be beneficial to the growth of *Firmicutes*, which produce butyrate and inhibit the growth of the acid-sensitive *Bacteroidetes*. A previous study revealed that during fecal fermentation, the pH of the BSP group (pH = 4.57) was significantly lower than that of the control group (pH = 6.70), inhibiting the relative abundance of *Bacteroidetes* in the former group. These experimental results are consistent with the results reported herein. The presence of *Fusobacteria* and *Proteobacteria* may lead to gastric cancer, imbalance of the intestinal flora, and inflammatory colitis (Fu et al., 2019; Wu et al., 2020). As shown in Fig. 4D, the control group was mainly composed of *Prevotella*, *Lachnospiraceae*, *Faecalibacterium*, *Veilloplla*, *Bacteroides*, and *Escherichia − Shigella* ($P < 0.05$). However, the relative abundance of *Lactobacillus*, *Bifidobacterium*, and *Megamonas* in the BSP group was significantly higher ($P < 0.05$). *Lactobacillus* has beneficial effects on the intestinal flora composition; improves the function of the human gastrointestinal tract; restores the balance of intestinal flora; forms an antibacterial biological barrier; and maintains human health by inhibiting cholesterol absorption, lowering blood lipids and blood pressure, exerting immunomodulatory effects, and enhancing human immunity and resistance (Zhao, Liu, Kwok, Cai, & Zhang, 2020). *Bifidobacterium* is a member of the class *Actinobacteria*, which comprises probiotics that are highly beneficial to human intestinal health (Han et al., 2020; Nasuti et al.,

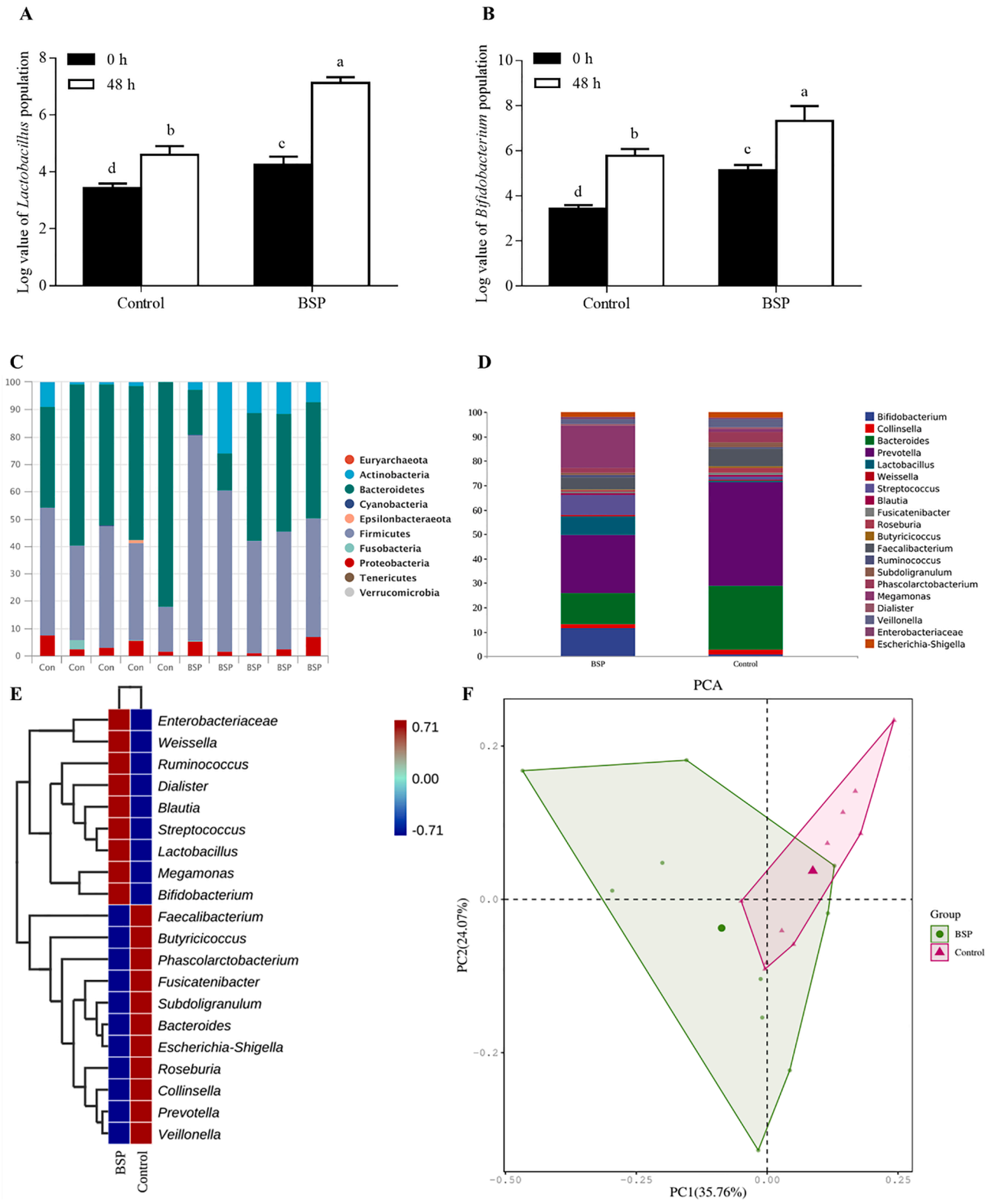

Fig. 4. Intestinal microbial community structure after 48 h of fermentation. (A) *Lactobacillus* abundance. (B) *Bifidobacterium* abundance. (C) Bar plots of the prevalence at the phylum level. (D) Bar plots of the prevalence at the genus level. (E) Heatmap analysis of the relative abundance of the bacterial community at the genus level. (F) Principal component analysis (PCA) from 48 h of fermentation.

2016). It controls intestinal diseases, regulates the immune system, and exhibits anticancer activity (Di Gioia, Aloisio, Mazzola, & Biavati, 2014). In the BSP group, the relative abundance of *Fusobacterium* was significantly reduced, while those of *Megasphaera* and *Collinsella* increased when compared to the values of the control group. *Fusobacterium* is related to gastric cancer occurrence (Han et al., 2020), while *Collinsella*, which is associated with abnormal lipid metabolism and type 2 diabetes, produces gases in the intestine. Finally, *Megasphaera* ferments different types of carbohydrates to afford acetic, propionic, and lactic acids. The above results indicate that BSP stimulates

the growth of beneficial bacteria such as the genera *Bifidobacterium*, *Megamonas*, and *Lactobacillus*, inducing SCFA production. This phenomenon is consistent with those reported in previous studies (Fu et al., 2019).

Production of volatile gut metabolites

To further understand the utilization of BSP by intestinal microorganisms, we employed GC-MS to elucidate volatile metabolite changes in the fermentation solution at 0 h and 48 h. This study analyzed a total of 46 different intestinal microbiota metabolites, including sulfur compounds, organic acids, hydrocarbons, indoles and their derivatives, esters, benzene and its substituted derivatives, amines, alcohols and polyols, carbonyl compounds, heterocyclic compounds, and ethers. The data in Table 2 (Supplementary Material) reveal that after BSP fermentation for 48 h, the compounds that were produced at relatively high levels during the fermentation process were mainly organic acids (including acetic acid, propionic acid, butyric acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, and *n*-decanoic acid) and fatty acid esters (including butanoic acid methyl ester, butanoic acid 3-methyl-, pentanoic acid 4-methyl-, dodecanoic acid methyl ester, and hexadecanoic acid methyl ester). Compounds that declined during the fermentation process mainly comprised trimethylamine, dimethyl trisulfide, benzaldehyde, phenol, 4-methylphenol, and acetophenone. According to the experimental results, the increase in these metabolites correlated with the decrease in pH. This condition is also considered beneficial to health. To predict the metabolic function of gut microbiota, related KEGG pathways were analyzed. As demonstrated in Fig. 5E and 5F, BSP affected 12 metabolism pathways, and 2 of them (fatty acid biosynthesis and glycolysis gluconeogenesis) were significantly ($P < 0.05$) enriched. Fatty acid biosynthesis and glycolysis gluconeogenesis pathways were closely associated with the production of SCFAs, which could promote the levels of organic acids and modulate the gut microbiota compositions.

Notable increases were observed in the relative concentrations of the sulfur compounds, alcohols and polyols, and benzene and its substituted derivatives during fermentation (48 h) of the control group. Other compounds showed reductions in both groups compared to the amounts observed at 0 h (Fig. 5). The comparison of the 0 h and 48 h time points in the BSP group showed a decrease in the concentrations of benzene and its derivatives, carbonyl compounds, indole and its derivatives, esters, and hydrocarbons and an increase in those of the fatty acid esters

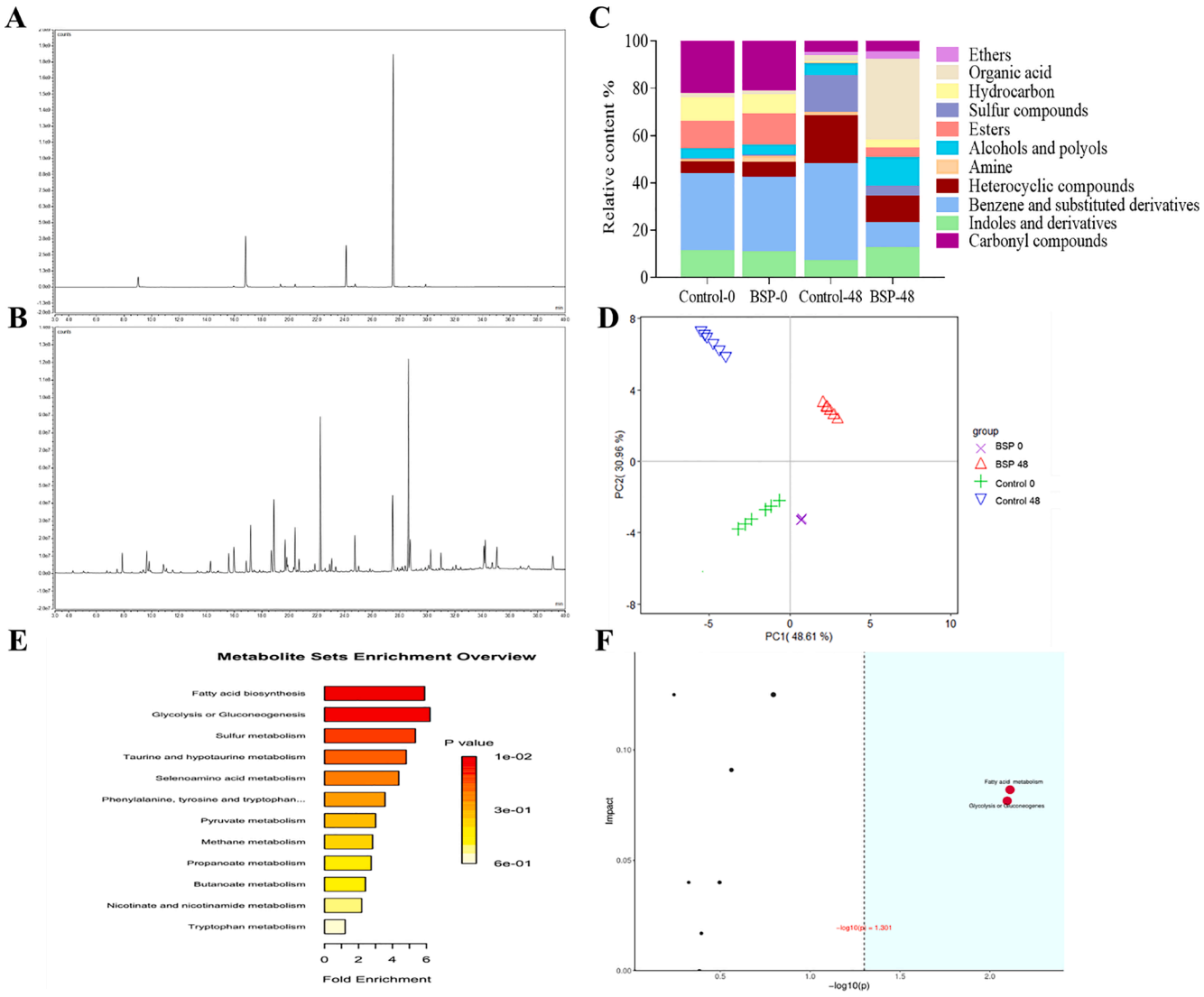

Fig. 5. Effects of BSP on volatile intestinal metabolites production. Gas chromatography-mass spectrometry (GC-MS) chromatograms of the (A) control (water, 48 h) and (B) bamboo shoot polysaccharide (BSP, 48 h) fermentation solutions. (C) Relative cumulative concentration (%) of the volatile metabolites in the control and BSP groups after *in vitro* fermentation for 0 h and 48 h. (D) Principal component analysis (PCA) of the volatile metabolites after 0 h and 48 h of fermentation. (E) ORA enrichment analysis of metabolic pathways. (F) Topological analysis of metabolic pathways.

and organic acids. The control group (48 h) mainly consisted of indole and its derivatives, benzene and its derivatives, carbonyl compounds, alcohols and polyols, sulfur compounds, and esters. Compared to that of the control group, the fermentation of the BSP group resulted in a significant increase in the concentrations of organic acids and fatty acid esters, while the production of the metabolites of indole and its derivatives, benzene and its substituted derivatives, sulfur compounds, and carbonyl compounds was significantly reduced. Intestinal microbiota ferment to produce organic acids that are beneficial to the host, including acetic, propionic, and butyric acids. These organic acids are produced by polysaccharide fermentation by intestinal bacteria, especially members of the *Firmicutes* phylum (Medrano, Gangoiti, Simonelli, & Abraham, 2020). On the other hand, indole and its derivatives are products of amino acid metabolism by bacteria such as *Bacteroides* (Fu et al., 2019). It has been reported that amino acid fermentation products such as phenol and p-cresol produce intestinal toxins (Lee, Wood, & Lee, 2015). BSP fermentation caused acidification and production of organic acids in the medium. Thus, we extrapolated that the biological activity of BSP can be partly attributed to its ability to be fermented by microbiota to produce beneficial organic acids, indicating its potential prebiotic activity in the intestine.

Conclusion

The aim of this study was to generate a new insight into *in vitro* fermentation of BSP by human gut microbiota for its potential use as a functional food ingredient. The addition of BSP increased the production of SCFAs and reduced the pH and ammonia concentrations. Fermentation of BSP increased the diversity of the bacterial communities and increased the relative abundance of *Firmicutes* and *Actinobacteria*, while reducing the abundance of *Proteobacteria*. SCFAs were produced during the fermentation of BSP, with acetic acid, propionic acid, and butyric acid as the primary metabolites. GC-MS results demonstrated that BSP can be fermented by the human intestinal microbiota to produce organic acid metabolites that are beneficial to intestinal health. This study strongly suggests that BSP has potential prebiotic properties that are beneficial to human health, thereby showing great commercial potential as a functional food ingredient. However, further *in vivo* studies should be carried out to obtain more realistic models of its probiotic nature and determine accurate recommended daily doses.

CRediT authorship contribution statement

Qi Li: Methodology, Formal analysis, Data curation, Writing – original draft. **Weijie Wu:** Writing - review & editing, Supervision. **Hangjun Chen:** Writing - review & editing, Supervision. **Xiangjun Fang:** Writing - review & editing, Supervision. **Yanchao Han:** Writing - review & editing, Supervision. **Mingyong Xie:** Writing - review & editing, Supervision. **Haiyan Gao:** Writing - review & editing, Supervision.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100129.

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