

Research Article

Effect of Pretreatments on Releasing of Bound Phenolics from Dried Bamboo Shoots during Simulated *In Vitro* Colonic Fermentation

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Salt-dried bamboo shoots (SDBS), steamed dried bamboo shoots (TDBS), fumigation-dried bamboo shoots (UDBS), and fermentation-dried bamboo shoots (FDBS) are four popular types of dried bamboo shoots (DBS) enjoyed by Chinese people. In this work, we aimed at investigating the release pattern of bound phenolics from DBS prepared by different processes on simulated *in vitro* colonic fermentation. The results showed that nine phenolic compounds were identified from DBS, including seven phenolic acids and two flavonoids. Also, the contents of chlorogenic acid, *p*-coumaric acid, and ferulic acid were higher. Among the four types of DBS, FDDBS exhibited the highest release of bound phenolics during colonic fermentation, with a value of 3.619 mg GAE/g DW. In addition, all the DBS produced four short-chain fatty acids (SCFAs), with the highest content of acetic acid at 7.721 mmol/L, followed by propionic acid and valeric acid, and the lowest content of butyric acid at only 0.023 mmol/L, along with a gradual decrease in their cellulose, hemicellulose, and lignin contents. Concurrently, confocal laser scanning microscopy (CLSM) observation images showed that reduced red fluorescence exhibited a decrease in bound phenolics content, while reduced green fluorescence indicated a decrease in fiber content. A significant positive correlation ($P < 0.05$) was observed between the release of bound phenolics and total SCFA production during colonic fermentation of SDDBS, TDDBS, UDDBS, and FDDBS, with correlation coefficients of 0.632, 0.844, 0.717, and 0.916, respectively, and a significant negative correlation ($P < 0.05$) with the IC_{50} values for the inhibition of α -amylase and α -glucosidase, with correlation coefficients of -0.643 , -0.613 , -0.823 , and -0.839 , respectively. The four DBS were ranked in descending order of bound phenolic release, SCFA production, dietary fiber reduction, and inhibitory activity associated with the metabolic syndrome: FDDBS, TDDBS, SDDBS, and UDDBS. DBS released large amounts of bound phenolics and produced SCFAs during colonic fermentation, which might be potentially beneficial for maintaining intestinal microbiota balance.

1. Introduction

Phenolics are an important secondary metabolite of plants, and a large number of clinical trials have shown that they have many beneficial effects on human health [1–3]. For example, phenolics released from bamboo shoots have strong antioxidant activity, anticancer ability, and anti-tumour potential [4]. Moreover, the free phenolics are susceptible to oxidative damage, whereas bound phenolics are tightly bound to macromolecular substances with higher stability and bioaccessibility [5]. Recent studies have shown that only 5–10% of phenolics consumed by humans are

absorbed into the small intestine, and in particular, bound phenolics are able to resist digestion in the stomach and small intestine to reach the bottom of the digestive tract for colonic fermentation and release [6, 7]. Furthermore, bi-directional interaction exists between phenolics and gut microorganisms [8]. The experiments on the release patterns of bound phenolics from wheat bran during simulated *in vitro* gastrointestinal digestion and colonic fermentation have shown that the dietary fiber is partially or completely fermented in the colon; simultaneously, bound phenolics are released and absorbed by the colonic epithelial cells, which may contribute to the maintenance of a balanced intestinal

microbiota [9]. In addition, the research on changes in the bioactivity of phenolics from roasted coffee beans during colonic fermentation has indicated that phenolics affect the production of short-chain fatty acids (SCFAs) to some extent [10]. SCFAs are mainly fermented by anaerobic bacteria in the colon using indigestible carbohydrates such as dietary fiber, resistant starch, and oligosaccharides as the main products [11, 12] and play an important role in the maintenance of the normal function of the large intestine and the morphology of colonic epithelial cells [13]. Furthermore, Flint et al. [14] have demonstrated the beneficial effects of SCFAs on the control of obesity and diabetes.

Fresh bamboo shoots are processed and dried to obtain dried bamboo shoots (DBS) with different properties of bound phenolics [15, 16]. Salt-dried bamboo shoots (SDBS), steamed dried bamboo shoots (TDBS), fumigation-dried bamboo shoots (UDBS), and fermentation-dried bamboo shoots (FDBS) are four popular types of dried bamboo shoots (DBS) enjoyed by Chinese people. DBS not only contain a great deal of high-quality dietary fiber, but they are also rich in phenolics, mainly in the form of bound phenolics [4]. Xiao et al. [17] have shown that only a small portion of bound phenolics from DBS are released after gastrointestinal digestion, but it is not clear how they are further released once entering the colon. Herein, in this work, we investigate the release pattern of bound phenolics from four DBS during simulated *in vitro* colonic fermentation after gastrointestinal digestion, in order to provide guidance and reference for processing methods of DBS.

2. Materials and Methods

2.1. Material. Fresh bamboo shoots (*Phyllostachys edulis*) were obtained from Wangcheng District, Changsha City, Hunan Province. α -Amylase (10 U/mg), α -glucosidase (66 U/mg), and *p*-nitrophenyl- α -D-glucopyranoside (pNPG) were purchased from Sigma-Aldrich (St. Louis, MO). Pepsin (>3000 U/mg), trypsin (130 U/mg), and bile salt (from porcine) were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Individual phenolic standards were supplied by Solarbio Technology Co., Ltd. (Beijing, China). Folin-Ciocalteu phenol reagent, Tween-80, acarbose, metaphosphoric acid, and individual SCFA standards were purchased from Macklin Biotechnology Co., Ltd. (Shanghai, China). All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.2. Preparation and Simulated *In Vitro* Gastrointestinal Digestion of DBS

2.2.1. The Preparation Methods of DBS. The preparation methods of DBS were adopted from Xiao et al. [17]. Briefly, fresh bamboo shoots were sliced, and then, slices were soaked in 5% salt water for 24 h and dried at 60°C for 24 h to acquire SDBS. Slices were steamed 3 times for 1 h each, then flushed with cold water to 25°C, and dried at 60°C for 15 h to obtain TDBS. Slices were fumigated at high temperatures for 18 h with smoke from burning wood to provide UDBS. Slices

were added with 4% salt and sealed and naturally fermented for 7 d at room temperature in a fermentation vat and then exposed to the sun for 48 h to gain FDBS.

2.2.2. Simulated *In Vitro* Gastrointestinal Digestion of DBS. Simulated *in vitro* gastrointestinal digestion of DBS has been carried out using the methodology proposed by Ji et al. [18]. First, DBS was crushed and sieved through 180 meshes and homogenized in 80% (v/v) methanol to remove free phenolics. Second, gastric fluid, pepsin, intestinal fluid, trypsin, and bile salt were successively added to simulate *in vitro* gastrointestinal digestion. Once gastrointestinal digestion was finished, the mixture was centrifuged. Finally, the residues were freeze-dried and stored at -20°C as an initiator of *in vitro* colonic fermentation.

2.3. Simulated *In Vitro* Colonic Fermentation

2.3.1. Preparation of Colonic Fermentation Growth Medium. Preparation of colonic fermentation growth medium employed the following procedures described by Chait et al. [19] with few modifications. 2.0 g peptone, 2.0 g yeast extracts, 0.5 g L-cysteine, 0.5 g bile salt, 0.1 g NaCl, 0.04 g KH₂PO₄, 0.01 g MgSO₄·7H₂O, 0.01 g CaCl₂·6H₂O, 2 g NaHCO₃, 0.01 g vitamin K₁, 1 mL resazurin solution (1%, m/v), and 2 mL Tween-80 were weighed, dissolved with ultrapure water, and formulated into 1000 mL of colonic fermentation growth medium and then sterilized by autoclaving at 121°C for 20 min.

2.3.2. Preparation of Fecal Slurries. The preparation of fecal slurries was performed based on previously reported procedures with slight modifications [20]. Fresh fecal samples were obtained from 3 healthy male donors aged 20–25 years, who did not have an intestinal disease, maintained a poly-phenol-free diet, and had not received probiotic or antibiotic treatment for 1 month. Fecal samples were collected within 1 h of defecation and mixed in a ratio of 1:1:1 under the N₂ atmosphere, subsequently, diluted and blended with 10% sterile phosphate-buffered saline (PBS) at a ratio of 1:5 (m:v), and finally filtered through double layers of sterile cotton gauze to obtain 200 g/L fecal slurries.

2.3.3. Construction of Colonic Fermentation Models. Construction of the colonic fermentation model was conducted as described by Dong et al. [21] with slight adaptations. In short, 0.5 g DBS obtained after the gastrointestinal digestion step was mixed in 11 mL of growth medium and 9 mL of fecal slurries. After that, the mixtures were incubated at 37°C, 160 rpm under anaerobic conditions, and taken out at 0.5, 6, 12, 24, and 48 h, respectively. In addition, 11 mL of growth medium and 9 mL of fecal slurries were added to a blank group in the same case to correct for interference from colonic microbiota and growth medium. As soon as colonic fermentation was completed, the mixtures were immediately centrifuged (4°C, 15 min, and 7100 g). Additionally, the colonic fermentation supernatant was stored as

the sample solution at -20°C for further analysis, and the residues were freeze-dried for dietary fiber determination and CLSM testing.

2.4. Determination of Total Phenolic Content. The content of bound phenolics released from DBS at different colonic fermentation stages was detected according to the Folin–Ciocalteu assay by Li et al. [22] with some adaptations. In brief, supernatants from each colonic fermentation stage were used as sample solutions to be determined. Also, the results were indicated as milligrams of gallic acid equivalents per gram of sample dry weight (mg-GAE/g DW).

2.5. Determination of Composition and Content of Phenolic Compounds. The composition and content of phenolic compounds were analyzed, as previously described method in Jakobek et al. [23] with light modifications. First of all, the sample solution was concentrated in a vacuum at 40°C and reconstituted with 80% (v/v) methanol and then filtered through a $0.45\ \mu\text{m}$ membrane filter. Similarly, nine standards were prepared in the same way.

Quantitative and qualitative analyses of phenolic compounds in colonic fermentation supernatants were quantified using an LC-30A HPLC system (Shimadzu Inc., Kyoto, Japan), equipped with an Agilent Zorbax SB-C18 $250 \times 4.6\ \text{mm}$, $5\ \mu\text{m}$ column (Palo Alto, CA, USA), and a UV-VIS detector. Briefly, mobile phases were HPLC grade methanol (A) and ultrapure water with 0.1% formic acid solution (B). The elution program is as follows: Solution B: 0–5 min, 90%; 5–18 min, 80–65%; 18–22 min, 65–50%; 22–30 min, 50–35%. Besides, other HPLC conditions included a flow rate of 1 mL/min, injection volume of $10\ \mu\text{L}$, column temperature of 40°C , and detection wavelength of 280 nm.

2.6. Measurement of SCFAs and pH

2.6.1. Determination of pH Value. The pH values simulated *in vitro* fermentation samples were measured by Fu et al. [24] with some modifications. In brief, *in vitro* fermentation samples of different groups were collected at 0.5, 6, 12, 24, and 48 h, respectively, and immediately centrifuged at 4°C , 8000 rpm for 15 min to segregate the supernatants. The pH value of each supernatant was determined by a pH meter (Mettler Toledo, Switzerland).

2.6.2. Measurement of SCFAs Production. The SCFAs production was measured by Tang et al. [25] with minor adaptations. Centrifuged supernatants at each stage of colonic fermentation were used as the sample solution for this experiment. First, 0.2 mL of 25% (m/v) metaphosphoric acid was mixed with 1 mL of sample solution. After acidification in an ice bath for 1 h, the mixtures were centrifuged at 10,000g for 10 min under 4°C and finally filtered through $0.22\ \mu\text{m}$ membrane filters. Likewise, four standards were dissolved in ultrapure water and also passed through membrane filters.

The analysis of SCFAs in colonic fermentation supernatants was carried out with the utilization of an HS-GC system (Shimadzu Inc., Kyoto, Japan) equipped with a flame ionization detector (FID) and a Rtx-Wax column ($30\ \text{m} \times 0.25\ \text{mm}$, $0.25\ \mu\text{m}$). Nitrogen was used as carrier gas at a flow rate of 24 mL/min with a split ratio of 8:1, and hydrogen and air at a flow rate of 40 and 400 mL/min, respectively. The oven temperature was initially set at 100°C for 0.5 min, then ramped up to 180°C at $5^{\circ}\text{C}/\text{min}$, and eventually held at 200°C for 5 min with the rate of $20^{\circ}\text{C}/\text{min}$. Besides, the temperatures of the FID detector and injection port were set at 230°C and 220°C , respectively. All results were expressed in mmol/L for further statistical analysis.

2.7. Determination of Dietary Fiber Content

2.7.1. Cellulose Content Analysis. The analysis of cellulose content in bamboo shoot residues from each colonic fermentation stage was adopted from Li et al. [26] with a few modifications. 4 mL of mixture solution of 2% acetic acid and 2% HNO_3 in a ratio of 1:1 (v:v) was added to 0.05 g of residues and incubated in a boiling water bath for 25 min, and then, precipitates were washed 3 times. Subsequently, 10 mL of 10% H_2SO_4 and 0.1 M $\text{K}_2\text{Cr}_2\text{O}_7$ in a ratio of 1:1 (v:v) were added and incubated in boiling water for 10 min. After cooling, 5 mL of 20% (m/v) KI and 1 mL of 0.5% (m/v) starch solution were added, and then, 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ was added until the mixture just turned blue and did not change color for 0.5 min. In addition, a blank experiment was set up as a control. Cellulose content was calculated using the following equation:

$$\text{cellulose content (\%)} = \frac{K(a-b)}{24n}, \quad (1)$$

where “24” is the titration constant of 1 mol of cellulose (characterized as $\text{C}_6\text{H}_{10}\text{O}_5$) equivalent to $\text{Na}_2\text{S}_2\text{O}_3$; “K” is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$, mol/L; “a” is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ consumed in blank control, mL; “b” is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ consumed in test, mL; and “n” is the mass of DBS, g.

2.7.2. Hemicellulose Content Analysis. The analysis of hemicellulose content in bamboo shoot residues from each colonic fermentation stage has been carried out using the methodology proposed by Wu et al. [27] with some adaptations. 4.5 mL of 80% (m/v) $\text{Ca}(\text{NO}_3)_2$ was added to 0.05 g of residues and incubated in a boiling water bath for 5 min and then centrifuged. Next, 10 mL of 2 M HCl was added and incubated in boiling water for 45 min. After cooling, phenolphthalein was added, and the mixture solution was neutralized with 2 M NaOH until it was rose-colored, and then diluted to 35 mL and filtered. Subsequently, 1.5 mL of color reagent was mixed with 2 mL of filtrate and then boiled in a water bath for 5 min. Finally, the mixture solution was fixed to 15 mL, and absorbance was recorded at 540 nm. The results were compared with the glucose standard curve to calculate the hemicellulose content.

TABLE 1: The content of bound phenolics released from DBS during colonic fermentation (mg-GAE/g DW).

Fermentation time (h)	SDBS	TDBS	UDBS	FDBS
0.5	0.370 ± 0.021 ^b	0.431 ± 0.034 ^a	0.366 ± 0.003 ^b	0.430 ± 0.021 ^a
6	1.144 ± 0.018 ^c	1.536 ± 0.015 ^a	0.683 ± 0.058 ^d	1.431 ± 0.028 ^b
12	2.146 ± 0.034 ^b	2.599 ± 0.028 ^a	0.875 ± 0.111 ^c	2.528 ± 0.027 ^a
24	2.844 ± 0.026 ^b	3.119 ± 0.109 ^a	1.118 ± 0.030 ^c	3.034 ± 0.022 ^a
48	3.054 ± 0.126 ^c	3.352 ± 0.018 ^d	1.242 ± 0.050 ^b	3.619 ± 0.089 ^a
Control	5.630 ± 0.096 ^b	5.406 ± 0.092 ^b	2.590 ± 0.056 ^c	6.156 ± 0.322 ^a

a, b, c, and d indicate that there was a significant difference in release amounts of bound phenolics from four DBS at the same fermentation stage, $P < 0.05$; "Control" indicate the content of bound phenolics from the four kinds of DBS before colonic fermentation.

2.7.3. Lignin Content Analysis. The analysis of lignin content in bamboo shoot residues from each colonic fermentation stage was conducted using the method reported by Javier-Astete et al. [28] with few modifications. 5 mL of 1% acetic acid was mixed with 0.05 g of DBS pellets and soaked for 1 h, and then, precipitates were washed 3 times. Next, 4 mL of a mixture solution of ethanol and ether was added, soaked, washed, and evaporated in a boiling water bath. Afterwards, 3 mL of 72% H_2SO_4 was added and soaked for 16 h, and then, 5 mL of distilled water was added and boiled for 5 min. After cooling, 5 mL of distilled water and 0.5 mL of 10% (m/v) $BaCl_2$ were continued to be added and mixed, and precipitates were washed and dried. Subsequently, 10 mL of a mixture solution of 10% H_2SO_4 and 0.05 M $K_2Cr_2O_7$ in a ratio of 1:1 (v:v) was added and incubated in a boiling water bath for 15 min. After cooling, 5 mL of 20% (m/v) KI and 1 mL of 0.5% (m/v) starch solution were added. Finally, 0.2 M $Na_2S_2O_3$ was added until the mixture solution just turned blue and did not change color for 0.5 min. In addition, a blank experiment was set up as a control. Lignin content was calculated using the following equation:

$$\text{lignin content (\%)} = \frac{K(a-b)}{48n}, \quad (2)$$

where "48" is the titration constant of 1 mol of lignin (characterized as $C_{11}H_{12}O_4$) equivalent to $Na_2S_2O_3$; "K" is the concentration of $Na_2S_2O_3$, mol/L; "a" is the volume of $Na_2S_2O_3$ consumed in blank control, mL; "b" is the volume of $Na_2S_2O_3$ consumed in test, mL; and "n" is the mass of DBS, g.

2.8. Inhibitory Activities of Enzymes Associated with Metabolic Syndrome. The α -amylase and α -glucosidase inhibition assays adopted the method of Burgos-Edwards et al. [29] with minor modifications. Centrifuged supernatants at each stage of colonic fermentation were used as the sample solution for this experiment.

2.8.1. α -Amylase Inhibition Assay. In short, 100 μ L of sample solution was mixed with 100 μ L of 1% (m/v) soluble starch solution and incubated at 37°C for 5 min. Next, 100 μ L of α -amylase solution (8 U/mL) was added and incubated at 37°C for 20 min, and then, 200 μ L of color reagent was added and boiled for 15 min. Finally, 40 μ L of the mixture solution was diluted with 210 μ L of PBS, and absorbances were recorded at 540 nm. Acarbose served as a positive control for

both assays. The inhibitory activity of α -amylase was calculated using the following equation:

$$\alpha - \text{amylase inhibitory activity (\%)} = \left(1 - \frac{A_1 - A_0}{A_2 - A_0}\right) \times 100, \quad (3)$$

where " A_0 " is the blank control group without adding sample solution and enzyme, " A_1 " is the sample group, and " A_2 " is the control group without adding sample solution.

The sample solution concentration and the α -amylase or α -glucosidase inhibition rate were used as the regression equation to calculate the sample solution concentration at 50% inhibition, and the results were expressed as IC_{50} values.

2.8.2. α -Glucosidase Inhibition Assay. Likewise, 20 μ L of sample solution was mixed with 20 μ L of α -glucosidase solution (0.2 U/mL) for 3 min at 37°C, and 40 μ L of *p*-nitrophenyl- α -D-glucopyranoside (10 mM) was added and incubated for 10 min. Finally, 100 μ L of 0.2 M Na_2CO_3 was added, and absorbances were recorded at 540 nm upon completion of the reaction. The calculation manner was similar to the α -amylase inhibition assay.

2.9. Confocal Laser Scanning Microscopy. The observation confocal laser scanning microscopy (CLSM, Leica TCS SP8, Leica Microsystems Inc., Heidelberg, Germany) was performed with the method described by Zhang et al. [9]. The residues from different colonic fermentation stages were stained with a fluorescent dye consisting of 1 mg/mL Congo red. Images of fibers or spontaneous fluorescent phenolics were captured at 488 nm or 408 nm excitations, respectively.

2.10. Statistical Analysis. The above tests were conducted in triple parallel trials, except for special instructions. Correlations between datasets were estimated by the Pearson correlation coefficient. One-way analysis of variance (ANOVA) with Duncan post hoc test was used to analyze the significance of the difference between groups by SPSS 22. Plots were carried out using Origin 2018.

3. Results and Discussion

3.1. Release of Bound Phenolics from DBS during In Vitro Colonic Fermentation. The bound phenolics were further released from DBS after gastrointestinal digestion, and the

TABLE 2: Chemical compositions of bound phenolics released from DBS during colonic fermentation (mg/10 g).

Standards	0.5 h				6 h				12 h				24 h				48 h			
	SDBS	TDBS	UDBS	FDBS	SDBS	TDBS	UDBS	FDBS	SDBS	TDBS	UDBS	FDBS	SDBS	TDBS	UDBS	FDBS	SDBS	TDBS	UDBS	FDBS
Gallic acid	0.239 ± 0.045 ^a	0.499 ± 0.041 ^a	0.559 ± 0.040 ^b	0.659 ± 0.037 ^c	0.396 ± 0.044 ^b	0.516 ± 0.041 ^a	0.537 ± 0.040 ^a	0.578 ± 0.044 ^b	0.353 ± 0.044 ^b	0.447 ± 0.042 ^b	0.291 ± 0.046 ^d	0.651 ± 0.037 ^a	0.333 ± 0.037 ^b	0.314 ± 0.016 ^c	0.304 ± 0.016 ^b	0.469 ± 0.014 ^a	0.642 ± 0.042 ^b	0.522 ± 0.042 ^a	0.290 ± 0.041 ^c	0.275 ± 0.047 ^c
Catechin	ND	0.176 ± 0.014	0.363 ± 0.014	ND	ND	ND	0.406 ± 0.035 ^a	0.408 ± 0.035 ^a	ND	ND	0.347 ± 0.036 ^b	0.621 ± 0.029 ^a	0.325 ± 0.032 ^b	0.341 ± 0.036 ^b	0.384 ± 0.035 ^b	0.579 ± 0.031 ^a	0.439 ± 0.034 ^a	0.371 ± 0.035 ^a	0.371 ± 0.035 ^a	0.335 ± 0.036 ^b
<i>p</i> -Hydroxybenzoic acid	0.605 ± 0.111	0.567 ± 0.112	0.593 ± 0.111	0.595 ± 0.111	0.584 ± 0.111	0.609 ± 0.111	0.555 ± 0.014 ^a	0.601 ± 0.013 ^a	ND	0.534 ± 0.013 ^b	0.558 ± 0.014 ^b	0.626 ± 0.011 ^a	0.759 ± 0.018 ^a	0.442 ± 0.013 ^c	0.342 ± 0.014 ^c	1.220 ± 0.010 ^a	0.360 ± 0.014 ^a	0.365 ± 0.014 ^a	0.601 ± 0.013 ^a	0.996 ± 0.008 ^b
Chlorogenic acid	0.536 ± 0.087	0.531 ± 0.087	0.524 ± 0.087	0.522 ± 0.087	0.530 ± 0.087	0.613 ± 0.087	0.535 ± 0.087	0.557 ± 0.087	0.721 ± 0.108 ^a	0.606 ± 0.111 ^a	0.643 ± 0.101 ^a	0.628 ± 0.111 ^a	0.759 ± 0.108 ^a	0.789 ± 0.107 ^a	0.763 ± 0.107 ^a	0.599 ± 0.111 ^a	0.866 ± 0.105 ^a	0.951 ± 0.104 ^a	0.951 ± 0.104 ^a	0.634 ± 0.110 ^a
Caffeic acid	0.286 ± 0.029	0.285 ± 0.029	0.289 ± 0.029	0.344 ± 0.029	0.297 ± 0.029	0.295 ± 0.029	0.315 ± 0.029	0.311 ± 0.030	0.527 ± 0.087 ^a	0.286 ± 0.029 ^a	0.327 ± 0.087 ^a	0.341 ± 0.087 ^a	0.556 ± 0.086 ^a	0.358 ± 0.087 ^a	0.503 ± 0.087 ^a	0.524 ± 0.087 ^a	0.523 ± 0.087 ^a	0.539 ± 0.087 ^a	0.439 ± 0.087 ^a	0.527 ± 0.087 ^a
Syringic acid	0.403 ± 0.045	0.385 ± 0.045	0.396 ± 0.045	0.387 ± 0.045	0.642 ± 0.043 ^b	0.515 ± 0.041 ^a	0.656 ± 0.043 ^a	0.620 ± 0.044 ^a	1.583 ± 0.036 ^a	0.935 ± 0.041 ^a	0.905 ± 0.041 ^a	0.807 ± 0.042 ^a	1.966 ± 0.033 ^a	1.292 ± 0.038 ^a	1.435 ± 0.038 ^a	0.383 ± 0.029 ^a	0.327 ± 0.029 ^a	0.439 ± 0.029 ^a	0.325 ± 0.028 ^a	0.361 ± 0.029 ^a
<i>p</i> -Coumaric acid	0.796 ± 0.052	0.706 ± 0.053	0.739 ± 0.053	0.711 ± 0.053	0.832 ± 0.052 ^b	1.239 ± 0.052 ^b	0.752 ± 0.052 ^a	0.752 ± 0.052 ^a	0.783 ± 0.053 ^a	1.500 ± 0.051 ^a	0.761 ± 0.051 ^a	0.887 ± 0.053 ^a	0.756 ± 0.053 ^a	2.272 ± 0.049 ^a	0.773 ± 0.053 ^a	0.722 ± 0.052 ^a	0.796 ± 0.052 ^a	1.093 ± 0.052 ^a	0.890 ± 0.051 ^a	0.944 ± 0.051 ^a
Ferulic acid	0.148 ± 0.016	0.125 ± 0.016	0.127 ± 0.016	0.233 ± 0.014 ^a	0.178 ± 0.015 ^a	0.192 ± 0.015 ^a	0.111 ± 0.017 ^a	0.256 ± 0.017 ^a	0.438 ± 0.019 ^a	0.163 ± 0.019 ^a	ND	0.391 ± 0.017 ^a	0.110 ± 0.019 ^a	0.077 ± 0.019 ^a	0.077 ± 0.019 ^a	0.113 ± 0.019 ^a	0.205 ± 0.019 ^a	0.075 ± 0.019 ^a	0.888 ± 0.052 ^a	0.691 ± 0.053 ^a
Rutin	3.110 ± 0.079	3.475 ± 0.064 ^a	3.723 ± 0.069 ^a	3.421 ± 0.070 ^a	3.459 ± 0.070 ^a	4.881 ± 0.064 ^a	4.209 ± 0.136 ^a	4.465 ± 0.071 ^a	4.747 ± 0.055 ^a	5.108 ± 0.070 ^a	4.147 ± 0.081 ^a	5.806 ± 0.084 ^a	5.476 ± 0.137 ^a	6.421 ± 0.208 ^a	4.886 ± 0.067 ^a	6.278 ± 0.103 ^a	4.646 ± 0.066 ^a	6.465 ± 0.338 ^a	3.916 ± 0.185 ^a	4.763 ± 0.150 ^b
Sum																				

^aND: not detected. ²a, b, c, and d indicate that there was a significant difference in the release amount of individual phenolic from four DBS fermentation supernatants at the same fermentation stage, $P < 0.05$.

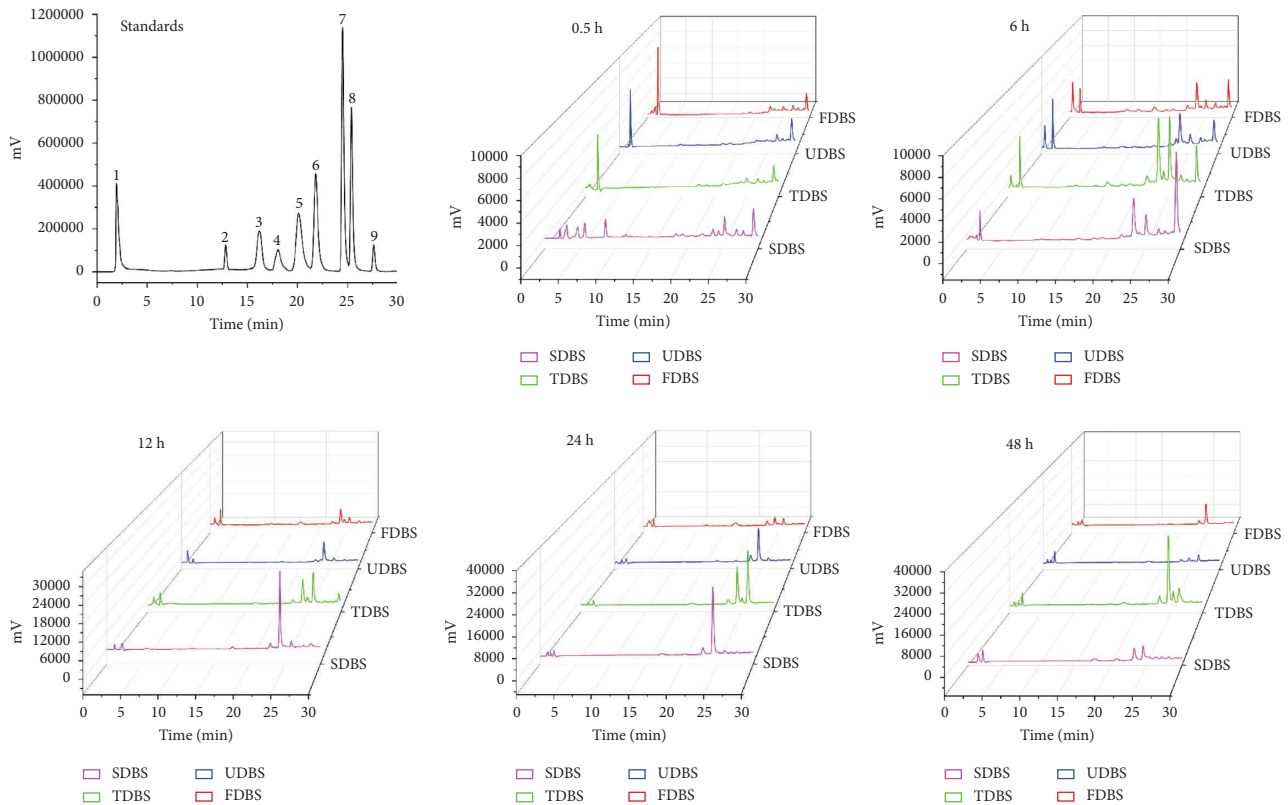


FIGURE 1: HPLC chromatograms of bound phenolics released from DBS during colonic fermentation. (1) Gallic acid; (2) catechin; (3) *p*-hydroxybenzoic acid; (4) chlorogenic acid; (5) caffeic acid; (6) syringic acid; (7) *p*-coumaric acid; (8) ferulic acid; (9) rutin acid.

release regularity of bound phenolics from four kinds of DBS during *in vitro* colonic fermentation is presented in Table 1. It was evident from Table 1 that the release amount of bound phenolics from all DBS increased gradually. In particular, the release amount of bound phenolics in SDBS, TDBS, UDBS, and FDBS was reduced from 5.630, 5.406, 2.590, and 6.156 mg-GAE/g DW to 3.054, 3.352, 1.242, and 3.619 mg-GAE/g DW, respectively.

Besides, bound phenolics in DBS were further released by the action of microorganisms during colonic fermentation. Most interestingly, the release amount of bound phenolics from DBS at 48 h colonic fermentation ranged from high to low: FDBS, TDBS, SBDS, and UDBS. The reason may be that UDBS were prepared by smoking at high temperatures, resulting in the destruction of large amounts of bound phenolics. However, FDBS were prepared under relatively mild conditions so that bound phenolics were minimally destroyed. Besides, the action of enzymes produced by lactic acid bacteria may be attenuated by the hydrophobic interactions between bound phenolics and fibers, which in turn promotes the release of bound phenolics from FDBS [30, 31].

3.2. Chemical Compositions of Bound Phenolics Released from DBS. The main chemical compositions and contents of bound phenolics released from DBS during *in vitro* colonic fermentation are reported in Table 2 and Figure 1. According

to Table 2, SDBS, TDBS, UDBS, and FDBS released nine phenolic compounds during colonic fermentation, including seven phenolic acids and two flavonoids, with high levels of chlorogenic acid, *p*-coumaric acid, and ferulic acid. However, Miranda-Hernández et al. [32] detected mainly proanthocyanidins as phenolics in lychee pericarp, and Soufi et al. [33] detected mainly hydroxytyrosol, trichothecenes, and caffeic acid in olives, suggesting that the type of phenolics is related to the food matrix. In addition, the sum release amounts of chlorogenic, *p*-coumaric, and ferulic acid liberation from SDBS, TDBS, UDBS, and FDBS at 48 h fermentation were 50.2%, 64.3%, 59.5%, and 47.6% of their total, respectively. Moreover, much higher levels of polyphenol compounds were detected during colonic fermentation than gastrointestinal digestion [17].

In addition, there was a certain discrepancy in the release amount of bound phenolics from four DBS at the same fermentation stage. We also discovered that the release amount of bound phenolics was significantly larger in FDBS and TDBS than in SDBS and UDBS before 24 h fermentation ($P < 0.05$) whereas it was significantly larger in SDBS, TDBS, and FDBS than in UDBS at 48 h fermentation ($P < 0.05$). The reason may be that the other three types of dried bamboo shoots had relatively dense structures, and their bound phenolics were released slowly. While UDBS was smoked at high temperatures for a long time during processing, therefore, it had a looser structure, so its bound phenolics were susceptible to rapid release.

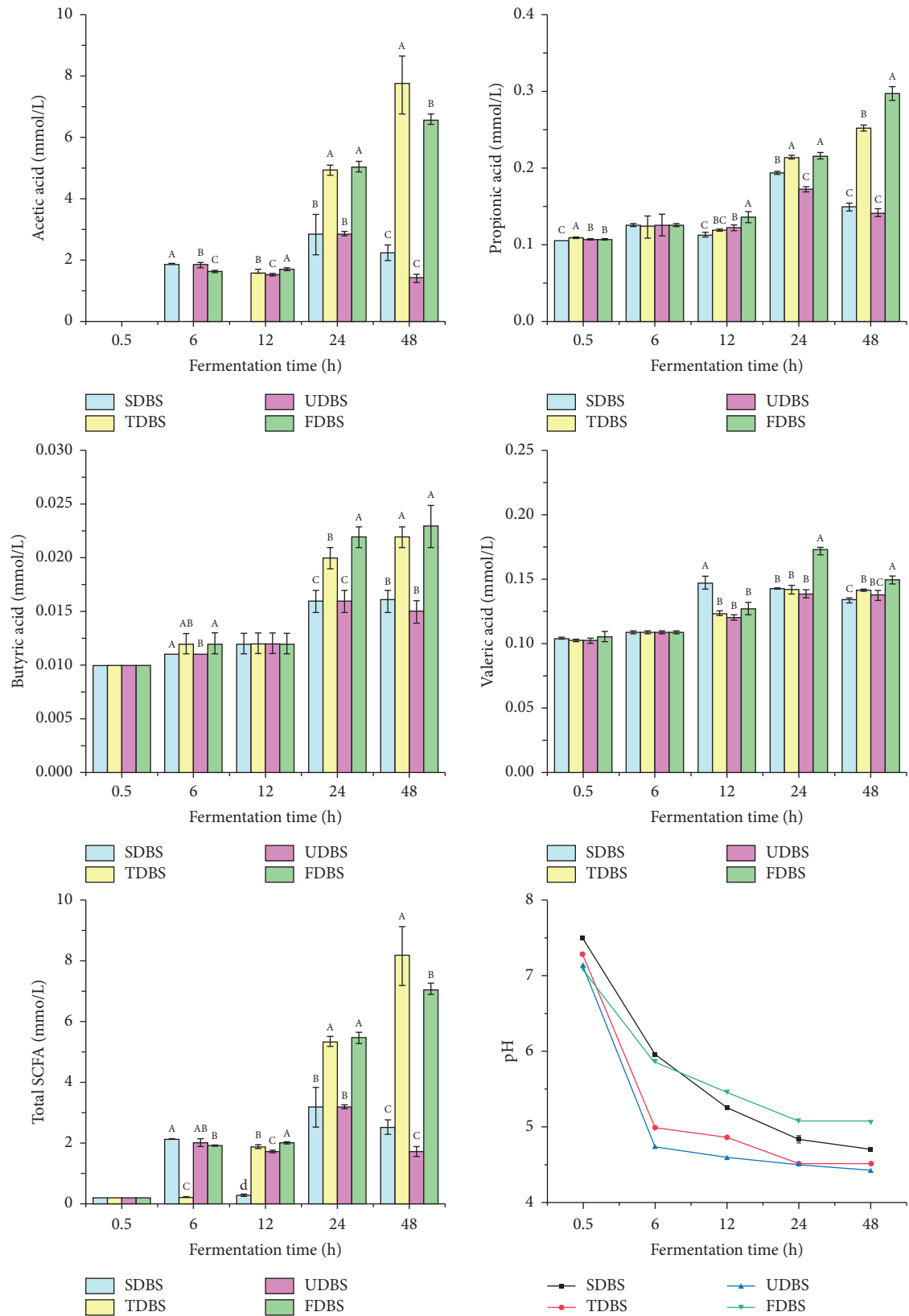


FIGURE 2: Production of SCFAs and change of pH values in DBS during colonic fermentation. A, B, C, and D indicated that there was a significant difference in the production of SCFAs in the fermentation supernatant of four DBS at the same fermentation stage, $P < 0.05$.

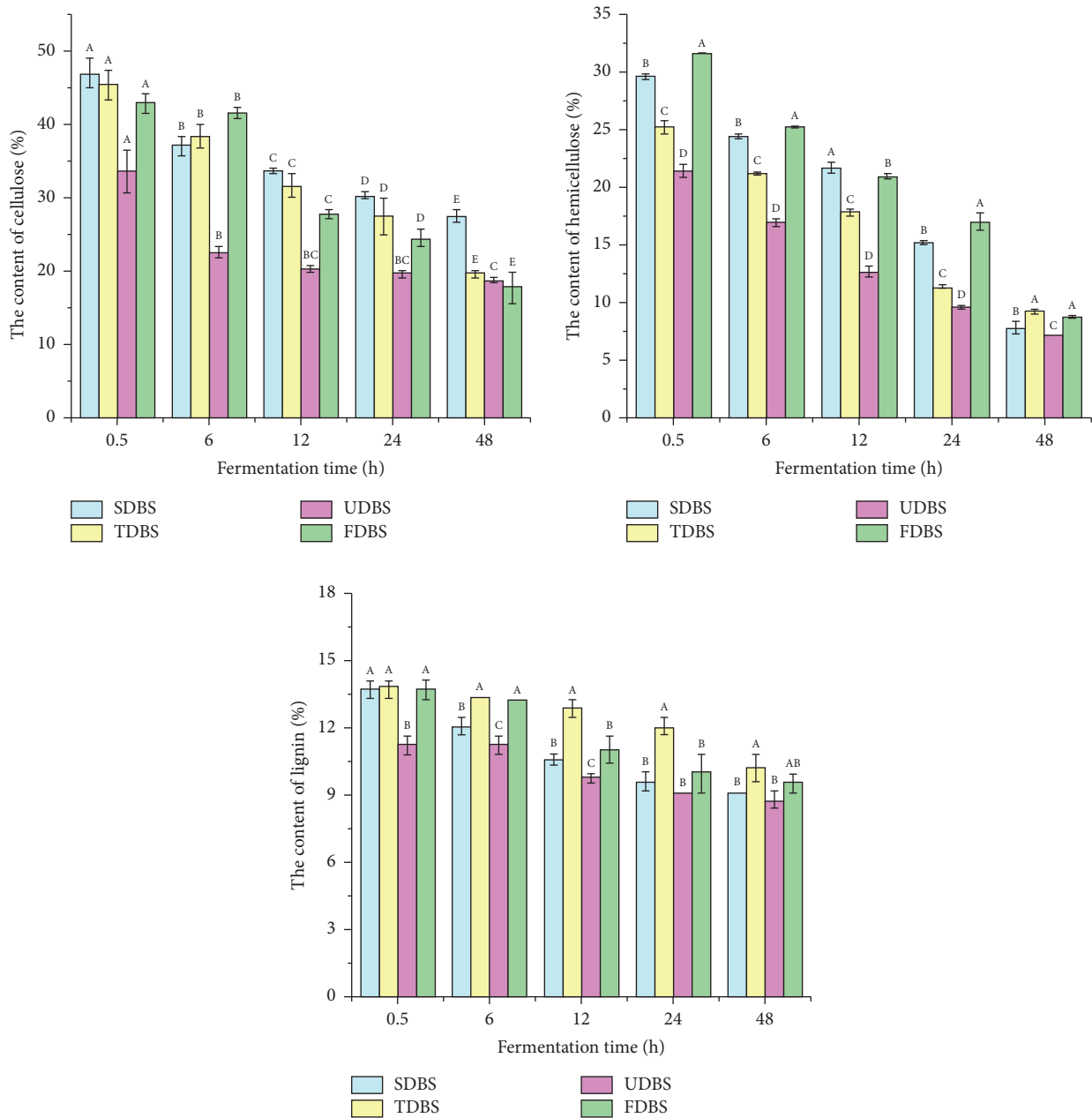


FIGURE 3: Changes in cellulose, hemicellulose, and lignin contents of DBS during colonic fermentation. A, B, C, D, and E indicate that there was a significant difference in dietary fiber contents of the same DBS at different fermentation stages, $P < 0.05$.

Besides, the phenolics without dietary fiber protection were easily degraded or transformed by intestinal microorganisms, and the longer the exposure, the greater the risk. Bermúdez-Soto et al. [34] confirmed that the presence of digestive enzymes and intestinal microorganisms could induce structural transformation or degradation of phenolic compounds in wild cherry juice during *in vitro* gastrointestinal digestion.

3.3. Production of SCFAs and pH Values in DBS. SCFAs were the main terminal products of dietary fiber during colonic fermentation with the action of intestinal microorganisms,

which played an important role in regulating the osmotic pressure and maintaining the normal function of the colon [35]. The production of SCFAs and change of pH during *in vitro* colonic fermentation of DBS prepared by different processes are illustrated in Figure 2.

The DBS produced SCFAs, with the largest amount of acetic acid at 7.721 mmol/L, followed by propionic acid and valeric acid, and the lowest amount of butyric acid at 0.023 mmol/L. We also found a lower production of SCFAs in the precolonic fermentation period; however, the production of SCFAs was higher at 24 h and 48 h fermentation. In addition, the production of SCFAs was significantly higher in FDDBS and TDDBS than in SDDBS and UDDBS ($P < 0.05$).

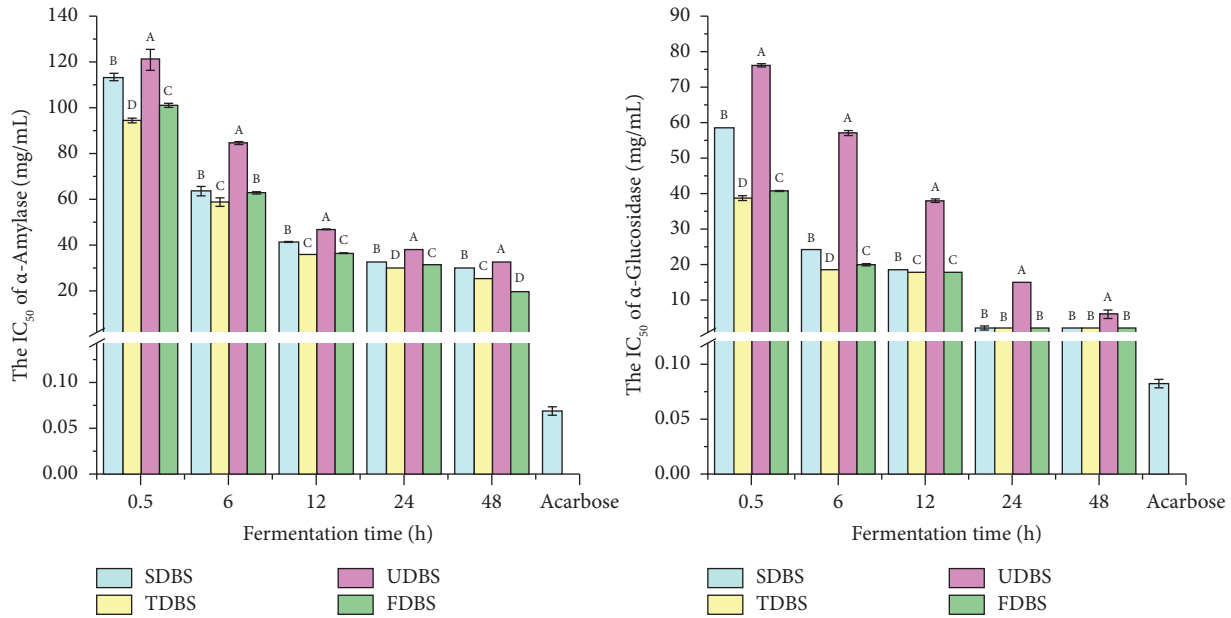


FIGURE 4: Inhibition of enzyme activities related to the metabolic syndrome by fermentation supernatants of DBS during colonic fermentation. A, B, C, and D indicate that there was a significant difference in enzyme inhibitory activities of fermentation supernatants from four DBS at the same fermentation stage, $P < 0.05$.

There were significant positive correlations between release amounts of bound phenolics from SDBS, TDBS, UDBS, and FDBS (Table 1) and their total SCFA productions (Figure 2) during *in vitro* colonic fermentation, with correlation coefficients of 0.632, 0.844, 0.717, and 0.916, respectively. This seems to indicate that the release of phenolics from DBS promoted the production of SCFAs. Furthermore, the total SCFA productions of SDBS, TDBS, UDBS, and FDBS were significantly and negatively correlated with the pH values of their fermentation supernatants, with correlation coefficients of -0.643 , -0.613 , -0.823 , and -0.839 , respectively, reflecting that the decrease in pH could be accounted for the high production of SCFAs. It was also found that the pH of the fermentation supernatant decreased significantly ($P < 0.05$) with longer fermentation time due to the production of large amounts of SCFAs [25].

3.4. The Content of Cellulose, Hemicellulose, and Lignin in DBS. The major dietary fibers of DBS included cellulose, hemicellulose, and lignin, and their reduction during *in vitro* colonic fermentation is depicted in Figure 3. It could be noted from Figure 4 that the dietary fiber contents in DBS decreased during this period. Interestingly, cellulose and hemicellulose contents of four DBS decreased faster than lignin ($P < 0.05$). Meanwhile, cellulose and hemicellulose contents in FDBS dropped more quickly than SDBS, TDBS, and UDBS ($P < 0.05$). The reason could be that dietary fiber was available to intestinal microorganisms during colonic fermentation [36], particularly cellulose and hemicellulose in FDBS appeared to be easier for microorganisms to utilize as fermentation substrates and consequently decreased more and faster.

Most interestingly, the negative correlation between the dietary fiber content and the total SCFA production during colonic fermentation is presented in Table 3. As shown in Table 3, cellulose, hemicellulose, and lignin contents of DBS were negatively correlated with their total SCFA productions ($P < 0.05$), while the correlation coefficients were higher for TDBS and FDBS than for SDBS and UDBS. Besides, anaerobic bacteria in the colon utilized dietary fiber as a substrate for fermentation and producing metabolites such as SCFAs, which had a critical role in maintaining a balanced intestinal flora and inhibiting the growth of undesirable bacteria [13, 14].

3.5. Inhibitory Activities of α -Amylase and α -Glucosidase by the Fermentation Supernatants from DBS during *In Vitro* Colonic Fermentation. α -Amylase and α -glucosidase play key roles in carbohydrate digestion, and inhibition of their activities can suppress the rapid rise of blood glucose in the body. Hemalatha et al. [37] showed that acarbose is a common α -amylase and α -glucosidase inhibitor and was used as a positive control in this experiment. The results of the inhibitory ability of fermentation supernatants during *in vitro* colonic fermentation are shown in Figure 4.

The IC₅₀ values for both α -amylase and α -glucosidase inhibition in DBS gradually decreased along with the increasing duration of colonic fermentation in Figure 4, which indicated a progressive enhancement in their inhibitory capacities. In general, the fermentation supernatants of four DBS exhibited a certain ability to inhibit α -amylase after 12 h fermentation, with the IC₅₀ values for α -glucosidase inhibition in TDBS and FDBS significantly lower than that in SDBS ($P < 0.05$), which in turn were significantly lower than UDBS ($P < 0.05$). Moreover, the IC₅₀ values for α -glucosidase inhibition in SDBS, TDBS, and FDBS were significantly lower

TABLE 3: Correlation coefficient of dietary fiber contents in DBS and their SCFA productions during colonic fermentation.

Standards	SDBS			TDDBS			UDDBS			FDDBS		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Acetic acid	-0.867**	-0.907**	-0.921**	-0.922**	-0.947**	-0.954**	-0.798**	-0.668**	-0.631*	-0.896**	-0.958**	-0.912**
Propionic acid	-0.808**	-0.892**	-0.850**	-0.881**	-0.934**	-0.910**	-0.672**	-0.741**	-0.750**	-0.888**	-0.948**	-0.893**
Butyric acid	-0.833**	-0.914**	-0.855**	-0.899**	-0.952**	-0.912**	-0.714**	-0.886**	-0.905**	-0.862**	-0.891**	-0.889**
Valeric acid	-0.521*	-0.431	-0.576*	-0.941**	-0.982**	-0.839**	-0.786**	-0.955**	-0.966**	-0.837**	-0.769**	-0.880**
Total SCFA	-0.875**	-0.915**	-0.927**	-0.919**	-0.948**	-0.950**	-0.801**	-0.680**	-0.643**	-0.897**	-0.958**	-0.913**

**Indicate that the correlation coefficient between the content of dietary fiber and the production of SCFAs was significant, $P < 0.05$.

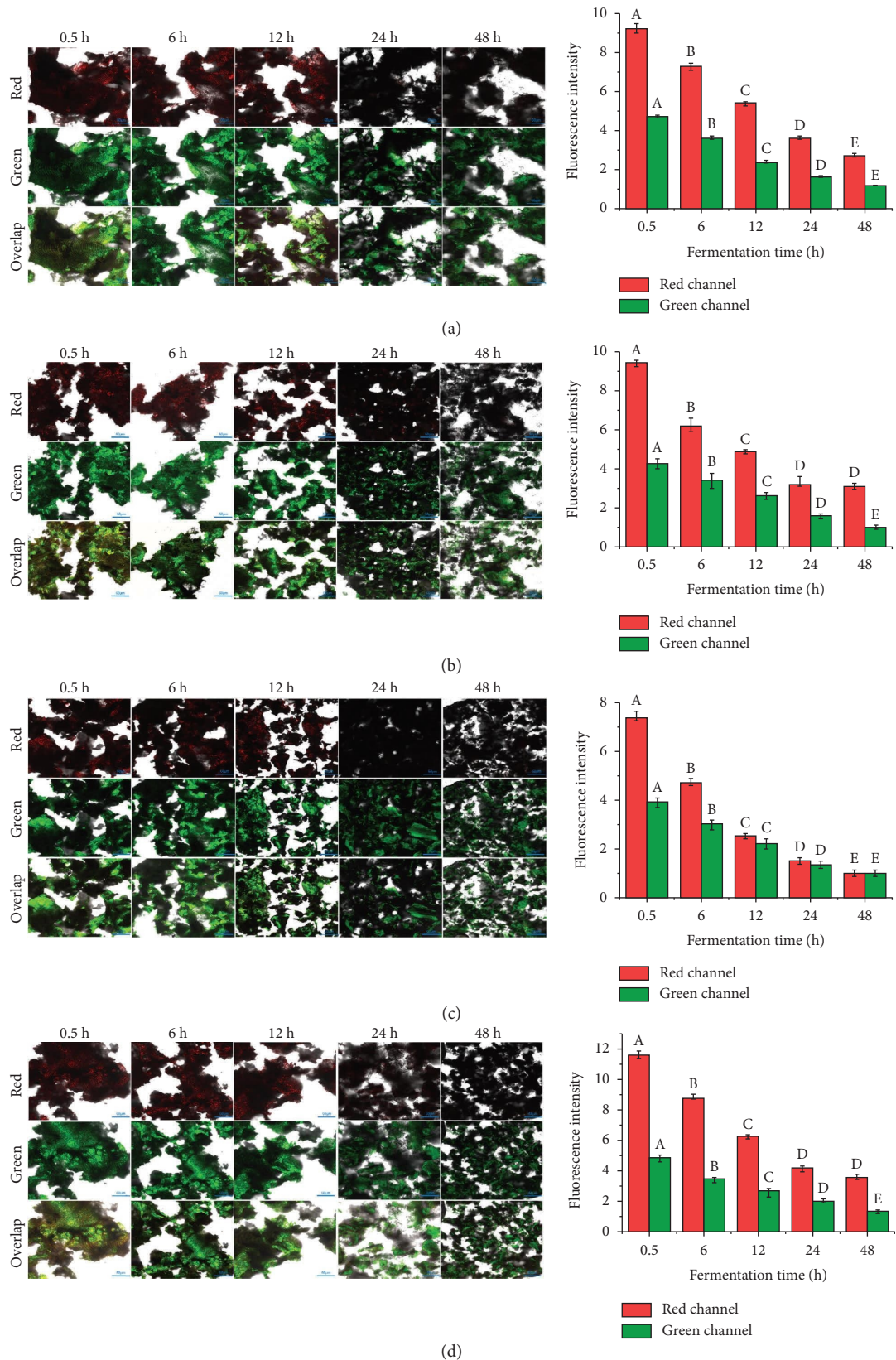


FIGURE 5: CLSM images of SDBS, TDBS, UDDBS, and FDBS residues at different colonic fermentation stages (scale was 60 μm). A, B, and C on the same color histogram indicate significant differences in fluorescence intensities of the same DBS at different fermentation stages, $P < 0.05$. (a) SDBS, (b) TDBS, (c) UDDBS, and (d) FDBS.

than that in UDBS after 24 h fermentation ($P < 0.05$). Adisakwattana et al. [38] confirmed that ferulic acid was a strong α -glucosidase inhibitor; meanwhile, the results in Table 1 showed that ferulic acid was present in all four DBS fermentations; therefore, the inhibition activity of α -glucosidase was probably attributed to the release of a certain amount of ferulic acid from DBS during colonic fermentation.

On the one hand, the fermentation supernatants of SDBS, TDBS, UDBS, and FDBS exhibited a significant negative correlation between their IC_{50} values for inhibition activities of α -amylase and α -glucosidase and the release amount of bound phenolics during *in vitro* colonic fermentation ($P < 0.05$), with correlation coefficients of -0.945 , -0.987 , -0.953 , and -0.981 and -0.952 , -0.952 , -0.982 , and -0.952 , respectively. As a result, DBS fermentation supernatants showed the ability to inhibit sugar metabolism in organisms and delay the carbohydrate digestion process [39]. Interestingly, the inhibition capacity of four DBS for metabolic syndrome-related enzymes was in descending order of FDBS, TDBS, SDBS, and UDBS, and this sequence was generally consistent with the ranking of bound phenolic release shown in Table 1. Espín et al. [12] clarified that biotransformed polyphenols under the action of intestinal microflora could reach the small intestine again through hepatoenteral circulation, which could help control the postprandial blood glucose.

On the other hand, the fermentation supernatants of SDBS, TDBS, UDBS, and FDBS observed a significant negative correlation between their IC_{50} values for α -amylase and α -glucosidase inhibition and their total SCFA productions during *in vitro* colonic fermentation ($P < 0.05$), with correlation coefficients of -0.840 , -0.767 , -0.737 , -0.849 and -0.829 , -0.828 , -0.683 , -0.927 , respectively. Therefore, it could be concluded that the inhibitory activities of DBS on α -amylase and α -glucosidase were also correlated with their total SCFA productions. Furthermore, it was proven that an increased risk of diabetes mellitus type 2 (T_2D) was associated with a deficiency of SCFAs in the colon [40, 41]. However, maybe we need *in vivo* experiments to further confirm this effect.

3.6. CLSM. In order to research the release pattern of bound phenolics from DBS at different fermentation stages, fluorescence images were carried out using CLSM after Congo red staining. The results of CLSM observation and fluorescence intensity quantification of different DBS during colonic fermentation are presented in Figure 5. Tang et al. [42] showed a positive correlation between fluorescence intensity and phenolic content. The red fluorescence observed by CLSM was caused by the autofluorescence of phenolic compounds such as ferulic acid [43], and the green fluorescence was presented by the fiber particles [9].

The red fluorescence and green fluorescence of DBS gradually diminished with the increase of colonic fermentation, indicating the continuous release of bound phenolics and the decrease of dietary fiber in DBS. The red fluorescence intensity was consistent with the changing pattern of bound phenolic content of four DBS particles during *in vitro*

colonic fermentation. FDBS particles had the strongest red fluorescence, followed by SDBS and TDBS, and UDBS was the weakest.

As seen in the figure, although the structure of DBS fiber particles was relatively intact during the precolonic fermentation period, the fiber structure began to disintegrate. At the late fermentation stages, especially after 24 h of colonic fermentation, the DBS particles broke into small fragments, and there was an obvious cell wall structure disintegration, which indicated that a variety of enzymes produced by the intestinal microflora had acted to break down the plant cell wall structure, leading to the DBS particles decreasing and the green fluorescence diminishing gradually, and our results are in agreement with Zhang et al. [9].

4. Conclusions

In summary, the bound phenolics in DBS prepared by different processes were further released under the actions of intestinal microorganisms after simulated *in vitro* gastrointestinal digestion into the colon. The results exhibited that nine phenolic compounds were identified in DBS, including seven phenolic acids and two flavonoids, with higher contents of chlorogenic acid, *p*-coumaric acid, and ferulic acid. In addition, DBS produced SCFAs, including acetic, propionic, butyric, and valeric acids, while their dietary fiber contents gradually decreased. All four DBS produced fewer SCFAs in the early stage of fermentations, however, and generated more SCFAs at 24–48 h fermentation. Moreover, a significant positive correlation ($P < 0.05$) was found between the release amount of bound phenolics and the production of total SCFA, and a significant negative correlation ($P < 0.05$) was discovered between the release amount of bound phenolics and the IC_{50} values for inhibition of enzyme activities associated with metabolic syndrome. Furthermore, the release amount of bound phenolics, the production of SCFAs, the decrease in dietary fibers, and the inhibition of enzymes related to metabolic syndrome for DBS were in descending order: FDBS, TDBS, SDBS, and UDBS. Among four DBS, FDBS provided a better performance during simulated *in vitro* colonic fermentation.

However, we only provided a simple qualitative analysis of the bound phenolic species released from DBS and did not provide a detailed description regarding the utilization of phenolics by gut microorganisms for metabolism or transformation during colonic fermentation. Therefore, the interaction between phenolics in DBS and intestinal microorganisms can be explained in more depth with 16S rRNA. Besides, we only investigated the release of bound phenolics from DBS during simulated *in vitro* colonic fermentation, but *in vivo* experimental results can be further verified using animal tests in subsequent research.

Data Availability

The original or processed data of these findings are not currently available for sharing, since the data also form a part of our ongoing research.

Ethical Approval

Ethics approval was not required for this research.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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