Microbial Short-Chain Fatty Acid Production and Extracellular Enzymes Activities during in Vitro Fermentation of Polysaccharides from the Seeds of *Plantago asiatica* L. Treated with Microwave Irradiation

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Ⓢ *Supporting Information*

ABSTRACT: Effects of microwave irradiation on microbial short-chain fatty acid production and the activites of extracellular enzymes during in vitro fermentation of the polysaccharide from *Plantago asiatica* L. were investigated in this study. It was found that the apparent viscosity, average molecular weight, and particle size of the polysaccharide decreased after microwave irradiation. Reducing sugar amount increased with molecular weight decrease, suggesting the degradation may derive from glycosidic bond rupture. The polysaccharide surface topography was changed from large flakelike structure to smaller chips. FT-IR showed that microwave irradiation did not alter the primary functional groups in the polysaccharide. However, short-chain fatty acid productions of the polysaccharide during in vitro fermentation significantly increased after microwave irradiation. Activities of microbial extracellular enzymes xylanase, arabinofuranosidase, xylosidase, and glucuronidase in fermentation cultures supplemented with microwave irradiation treated polysaccharide were also generally higher than those of untreated polysaccharide. This showed that microwave irradiation could be a promising degradation method for the production of value-added polysaccharides.

KEYWORDS: *Plantago asiatica* L., polysaccharide, microwave irradiation, degradation, short-chain fatty acids, microbial extracellular enzymes

■ INTRODUCTION

Recently, applications of microwave irradiation in the food industry have attracted a great deal of attention from people.[1] For instance, microwave irradiation has been used in heating, sterilization, and material treatment.[2] Some studies have shown that microwave irradiation could induce degradation of polymeric materials.[3] Among polymeric materials, polysaccharides have been considered one of the widely used substances in food processing and preparation as a stabilizer, thickener, texturizer, or emulsifier.[4] It was reported that the viscosity, molecular weight, and rheological and solution properties of polysaccharides could be influenced by microwave irradiation.[5,6] However, to our knowledge, no study has been conducted to examine the effects of microwave irradiation on the short-chain fatty acid (SCFA) production of the polysaccharides and the microbial extracellular enzymes activities during in vitro fermentation.

SCFA production of the polysaccharides and the microbial extracellular enzyme activities during polysaccharide fermentation are increasingly paid attention to, because SCFA is beneficial for human intestinal health and microbial extracellular enzyme activity may have an indirect effect of allowing more complete degradation of particular polysaccharides.[7] It has been shown that the decrease of molecular weight, viscosity, and particle size of polysaccharides may influence the availability of polysaccharides for bacteria[8] and thus affect the SCFA production and microbial extracellular enzyme activities. In addition, the decrease of molecular weight, viscosity, and particle size of the polysaccharides was often related to the

degradation of polysaccharide.[9] Thus, our hypothesis is that the SCFA production of the polysaccharides and microbial extracellular enzyme activity may be influenced by microwave irradiation, resulting in the polysaccharide degradation.

Polysaccharides can be obtained from many kinds of plant materials. Some *Plantago* plants, such as *Plantago afra* L., *Plantago psyllium* L., *Plantago ovata* Forsk. (isabgul), *Plantago indica* L. and *Plantago major* L., are often used in traditional medicine throughout the world. The soluble fibers in the seeds of these plants are able to improve some intestinal functions.[10] Our research group has recently isolated a pure and homogeneous polysaccharide from the seeds of *Plantago asiatica* L. with a molecular weight of 1894 kDa.[11] The polysaccharide was found to be a highly branched heteroxylan that consisted of a β-1,4-linked Xyl$p$ backbone with side chains attached to O-2 or O-3. The polysaccharide also contained terminal Xyl and Ara (see Supporting Information).[12] In addition, our recent studies have also shown that this polysaccharide may induce maturation of murine dendrite cells, have antioxidant activity, promote defecation, and increase SCFA production in mouse colon.[13−16]

In the study, the effect of microwave irradiation on the polysaccharide from the seeds of *P. asiatica* L. was evaluated using viscometer, dynamic light scattering, high-performance

gel permeation chromatography, environmental scanning electron microscope, and Fourier-transform infrared spectroscopy (FT-IR). In addition, the effects of microwave irradiation on SCFA production of the polysaccharide and microbial extracellular enzyme activities during in vitro fermentation were for the first time examined.

■ MATERIALS AND METHODS

Materials and Reagents. The seeds of *P. asiatica* L. were purchased from Ji'an, Jiangxi Province, China, and dried in the sun before use. Then, the polysaccharide from *P. asiatica* L. seeds was prepared using our published method.[11]

Dextran standards (T-2000, T-500, T-70, T-40, and T-10 of molecular weight 2000, 500, 70, 40, and 10 kDa, respectively) were obtained from Pharmacia (Uppsala, Sweden). Highly pure SCFAs were used to prepare the standard solutions for gas chromatography (GC) determination. Acetic acid (≥99.5% purity) and *n*-valeric acid (≥99.5% purity) were obtained from Merck (Darmstadt, Germany). Propionic acid (≥99.5% purity) was purchased from Janssen Chimica (Belgium), while isobutyric acid (≥99.5% purity), *n*-butyric acid (≥99% purity), isovaleric acid (≥99% purity), and 4-methylvaleric (internal standard) (≥99% purity) were purchased from Sigma (St. Louis, MO). All other reagents used were of analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China).

Microwave Irradiation. The polysaccharide solution (2.5 mg/mL) was dispersed in deionized water and stirred gently at room temperature to achieve complete solubilization. Microwave irradiation was performed in a microwave oven (MDS-2002, Shanghai Xinyi Microwave Chemistry Science and Technology Corp., Shanghai, China) at 800 W (maximum oven power for this system) for 5, 10, and 15 min.[17] This unit is equipped with a temperature sensor. The polysaccharide was completely dissolved in water before microwave irradiation. After adding the solution of polysaccharide into the container of the microwave oven, the container was lidded and a temperature sensor was inserted into the container to measure and control the internal temperature, which allows technical parameters of instrumentation to prevent a temperature over 100 °C. In addition, we also carried out a control experiment addressing the thermal effects of a temperature of 100 °C on the polysaccharide from *P. asiatica* L. The polysaccharide solution was heated at 100 °C in a water bath for 5, 10, and 15 min. However, there was no change found in the polysaccharide. Thus, the thermal effects would not play a role in the change of polysaccharide during microwave irradiation treatment.

Experiments were conducted three times with different solutions of polysaccharide in order to verify the repeatability of the microwave process. Portions of the solutions after microwave irradiation were lyophilized for further analyses.

Analysis of Polysaccharide after Microwave Irradiation.
Apparent Viscosity. The apparent viscosity of polysaccharide solution was measured with a Brookfield DV-IIIUltra programmable rheometer (Brookfield, Stoughton, MA) at 25 °C, equipped with a CP52 spindle (spindle multiplier constant = 9.83, shear rate constant = 2).

Particle Size (PS) and Distribution Determination. The average particle size and distribution of polysaccharide solutions were determined by a Nicomp 380/ZLS zeta potential/particle sizer (PSS Nicomp, Santa Barbara, CA) based on dynamic light scattering (DLS). The solutions were diluted to a concentration of 0.5 mg/mL with deionized water, and all measurements were carried out at 25 °C.[9]

Determination of Molecular Weight. Molecular weight (M_w) was determined by high-performance gel permeation chromatography (HPGPC). Specifically, it was determined in a Waters high-performance liquid chromatography (HPLC) system (UK6 injector and 515 HPLC pump) equipped with a Waters Ultrahydrogel linear column (7.8 mm × 300 mm) and a Waters 410 refractive index (RI) detector, connected in series with a Millennium 32 workstation (Waters, Milford, MA). A sample solution (20 μL) was injected in each run, with distilled water as the mobile phase at a flow rate of 0.5 mL min^{−1}.[18] Dextran standards (T-2000, T-500, T-70, T-40, and T-10,

Sigma-Aldrich, Shanghai, China) and glucose (M_w 180 Da, Sigma-Aldrich, Shanghai, China) were used to calibrate the column and establish a standard curve.

Determination of Reducing Sugar Content (C_R). The amount of reducing sugars in polysaccharide was determined using the dinitrosalicylic acid (DNS) method.[19] Xylose at different concentrations was used to generate a calibration curve. A modified DNS reagent (1.5 mL) consisting of 1% DNS, 0.2% phenol, 0.5% sodium sulfite, and 1% NaOH was added to 2 mL of sample. The mixture was then heated for 5 min at 100 °C. Then, 1 mL of a 40% solution of Rochelle salts was added to the mixture subsequently with the development of the color and prior to cooling. Lastly, the samples were detected by a double beam UV/vis spectrophotometer (TU-1901, PGENENAL, Beijing, China) at a wavelength of 540 nm.

Scanning Microscopy Analysis. Polysaccharide samples were taken after freeze-drying, and samples were prepared by sticking the polysaccharide onto double-sided adhesive tape attached to a circular specimen stub. The samples were viewed using an environmental scanning electron microscope (ESEM) (Quanta200F, FEI Deutschland GmbH, Kassel, Germany) at 30 kV voltage and 3.0 spot size. Low vacuum mode was used for the ESEM.

FT-IR Spectroscopy. The FT-IR spectra of polysaccharide samples were recorded on a Nicolet 5700 spectrometer (Thermo Co., Madison, WI). The dried samples were ground to fine powder with KBr (spectroscopic grade) and pressed into pellets for spectra measurement in the frequency range of 4000−400 cm^{−1}.[18] The data were collected and plotted as transmittance (%) as a function of the wavenumber (cm^{−1}) and analyzed with Ominic 7.2 software.

In Vitro Fermentation of Polysaccharides. *Fecal Slurry Preparation.* The fresh fecal samples were collected from four healthy donors who never had large bowel disease before. The donors also had followed normal diets and not been treated with antibiotics for at least 3 months. Collected fecal samples were mixed with an equal amount of feces from each donor. The mixed fecal samples were immediately stored in an anaerobic jar before use.[20]

Preculture. Mixed fecal samples (120 g) was precultured in 1 L of preculture medium (10 g of tryptone, 5 g of yeast, 10 g of NaCl, 5 g of glucose, and 6 g of maltose) anaerobically. After overnight growth, 120 mL of the preculture was filtered through sterile gauze sponges to remove large particles and stored in an anaerobic jar before use.[21]

Fermentation. The composition of 1 L of growth medium was 4.5 g of NaCl, 4.5 g of KCl, 1.5 g of NaHCO$_3$, 0.69 g of MgSO$_4$·H$_2$O, 0.8 g of L-cysteine HCl·H$_2$O, 0.5 g of KH$_2$PO$_4$, 0.5 g of K$_2$HPO$_4$, 0.4 g of bile salt, 0.08 g of CaCl$_2$, 0.005 g of FeSO$_4$·7H$_2$O, 1 mL of 80, and 4 mL of resazurin solution (0.025%, w/v) as anaerobic indicator.[20] Growth medium was sterilized at 121 °C for 15 min before use and divided into sterile anaerobic vessels.

The polysaccharide solution samples were allowed to hydrate overnight at 4 °C before the fecal culture was introduced. Then, the polysaccharide samples were subjected to the in vitro fermentation with the precultured human fecal microbiota. The total volume of the fermentation slurry was 50 mL. For the tested samples, the final fermentation volume was 40% growth medium and 40% human fecal preculture, and the last 20% was from polysaccharide solution (100 mg polysaccharide in total) or distilled water (control). All samples and the fecal preculture were introduced in different anaerobic sealed tubes containing growth medium, under the condition of 10% H$_2$, 10% CO$_2$, and 80% N$_2$ in a Forma Anaerobic System (Thermo Electron Crop., Marietta, OH).[22] Separate tubes were prepared for each replicate at each time point (0, 6, 12, and 24 h during fermentation). The anaerobic sealed tubes were then incubated at 37 °C for 24 h in a TC-2112B thermostat shaker (160 rpm, Shanghai Nuoji Corp., Shanghai, China).

Microbial Enzyme Activities. Samples were analyzed at 0, 6, 12, and 24 h of fermentation. The fermentation was stopped by extracting the fermentation cultures at different time points into vials and then plunging the vials into iced water for 20 min. Glucuronidase activity in the fermented cultures was measured by microtiter plate analysis according to the method of Fisher and Woods.[23] Xylanase, arabinofuranosidase, and xylosidase activities were assayed using the

Table 1. Apparent Viscosity (η), Average Molecular Weight (M_w), Content of Reducing Sugars (C_R), and Particle Size (PS) of Polysaccharide from the Seeds of *P. asiatica* L. after Different Microwave Irradiation Treatments (power = 800 W)[a]

sample[b]	apparent viscosity (η)	M_w (kDa)	C_R (mM)	PS (μm)
U	55.31 ± 2.18 a[c]	1903.1 ± 93.0 a	0.157 ± 0.007 a	4.83 ± 0.09 a
M-5	41.64 ± 1.77 b	1755.7 ± 45.0 b	0.164 ± 0.010 a	2.85 ± 0.06 b
M-10	33.24 ± 1.44 c	1444.0 ± 41.0 c	0.233 ± 0.009 b	1.83 ± 0.04 c
M-15	21.09 ± 0.60 d	1208.0 ± 29.0 d	0.355 ± 0.016 c	0.63 ± 0.02 d

[a]Data are presented as the mean ± standard deviation of triplicate measurements. [b]U = untreated sample; M-5 = sample treated with microwave irradiation for 5 min; M-10 = sample treated with microwave irradiation for 10 min; M-15 = sample treated with microwave irradiation for 15 min. [c]Values in the same column with different letters are significantly different ($p < 0.05$).
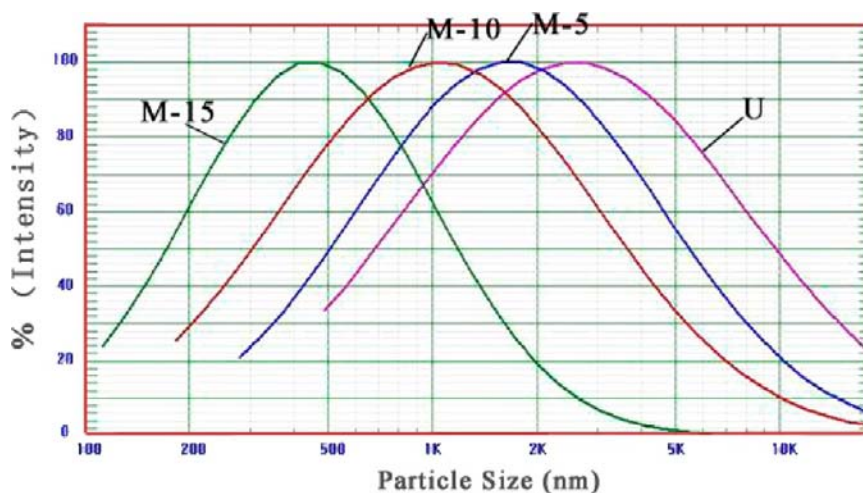

Figure 1. Effect of microwave irradiation (power = 800 wW) on the particle size of the polysaccharide. U = untreated sample; M-5 = sample treated with microwave irradiation for 5 min; M-10 = sample treated with microwave irradiation for 10 min; M-15 = sample treated with microwave irradiation for 15 min.

method of Pollet et al.[24] Briefly, xylanase activity was determined using an adaptation of the Xylazyme AX method described in the Megazyme T-XYZ200 03/06 data booklet. Arabinofuranosidase and xylosidase activities were determined using p-nitrophenyl-α-L-arabinofuranoside and p-nitrophenyl-β-D-xylopyranoside.

SCFA Measurement. The fermentation cultures were centrifuged at 4800g for 15 min. The supernatants were used for determination of SCFA. Chromatographic analysis was carried out using the Agilent 6890 N GC system according to our published method.[16] A GC column (HP-INNOWAX, 190901N-213, J & W Scientific, Agilent Technologies Inc.) of 30 m × 0.32 mm i.d. coated with 0.50 μm film thickness was used. Nitrogen was supplied as the carrier gas at a flow rate of 19.0 mL/min with a split ratio of 1:10. The initial column temperature was held at 100 °C for 0.5 min and then programmed at a rate of 4 °C/min to 180 °C. The temperatures of the FID and injection port were 240 °C. The flow rates of hydrogen and air were 30 and 300 mL/min, respectively. The volume of injected standard SCFA and sample for GC analysis was 0.2 μL, and the running time for each analysis was 20.5 min. The independently replicated determinations were performed three times for each sample. Data handling was carried out with a HP Chem Station Plus software (A.09.xx, Agilent).

Statistical Analysis. Microwave irradiation treatment on polysaccharide and each property analysis was all done in triplicate. Results are expressed as the mean ± standard deviation (SD). Data for each property of different polysaccharide samples and data for SCFA production of different polysaccharide samples and control (distilled water) or the microbial extracellular enzyme activities at the same time point during in vitro fermentation were evaluated by one-way analysis of variance using SPSS 10.0 software (Version 16.0, Chicago, IL). The difference between data for different polysaccharide samples was evaluated by the SNK test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Apparent Viscosity and Molecular Weight. In our study, the apparent viscosity of polysaccharide solutions changed significantly after microwave irradiation. The apparent viscosity (shear rate = 200 s^{-1}) was reduced the most (61.9%) for samples being microwave irradiation treated for 15 min, followed by 10 min (39.9% reduction), and 5 min (24.7% reduction), respectively (Table 1).

Tiwari et al. reported a bigger apparent viscosity decrement (about 90%) for polysaccharide solutions sonicated at 10.1 W/cm^2. They proposed the change was due to molecular weight decline.[25] In this study, a reduction of average molecular weight was also observed (Table 1). Polysaccharide treatment by microwave irradiation at different time points (5−15 min) resulted in significant differences in molecular weight. After polysaccharide was treated by microwave irradiation at 5, 10, and 15 min, its average molecular weight was reduced by 7.8%, 24.1%, and 36.5%, respectively.

Reducing Sugars Content. The decrease of viscosity and average molecular weight are often explained by the breakdown of glycosidic bonds inside the polymer chain when polymer was degraded,[26] and they could also be used to monitor depolymerization of polysaccharides. In addition, the amount of reducing sugars could also provide some information about the depolymerization of polysaccharides. As shown in Table 1, there was no significant difference in reducing sugars content of samples being microwave irradiation treated for 5 min. When the treatment time was further increased from 5 to 15 min, a significant increase in content of reducing sugars was observed (from 0.157 ± 0.007 to 0.355 ± 0.016 mM). The increase of
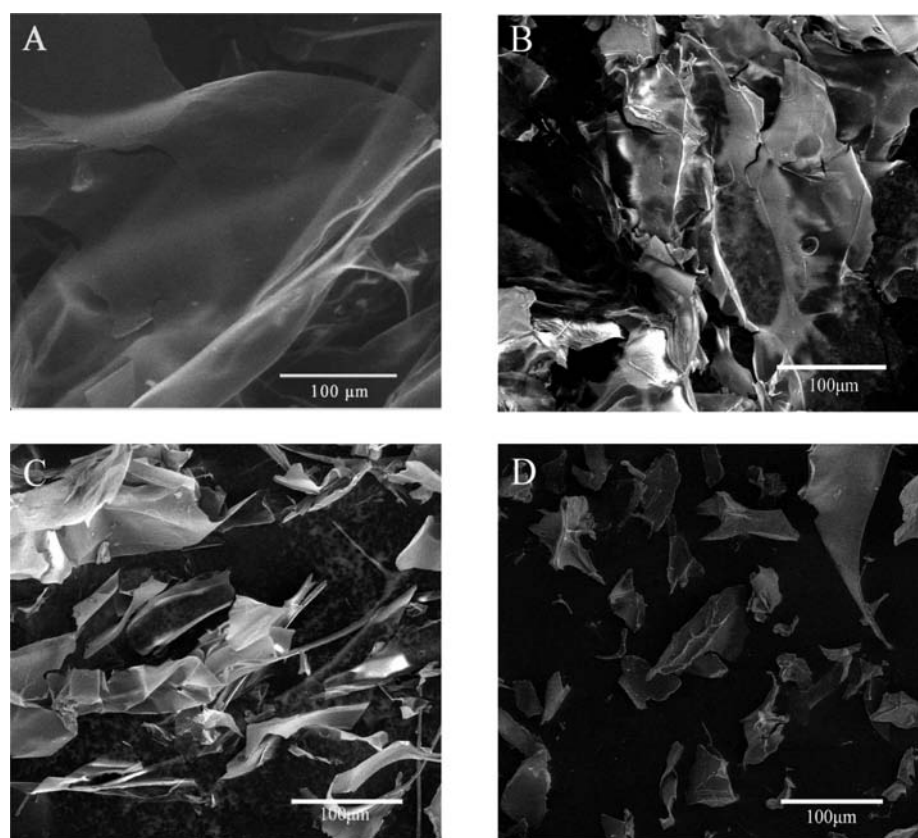
Figure 2. Effect of microwave irradiation (power = 800 W) on the environmental scanning electron micrograph of polysaccharide from *P. asiatica* L. seeds: (A) untreated sample, (B) sample treated with microwave irradiation for 5 min, (C) sample treated with microwave irradiation for 10 min, and (D) sample treated with microwave irradiation for 15 min.

reducing sugars content may due to the breakdown of glycosidic bonds during microwave irradiation.

Particle Size and Surface Topography. The particle size and distribution of the polysaccharide from *P. asiatica* L. seeds before and after being treated by microwave irradiation are shown in Table 1 and Figure 1. After microwave irradiation, the distribution profile of particle size shifted left and narrowed, indicating that the particle size of the polysaccharide decreased. The size of the polysaccharide decreased from 4.83 ± 0.09 (untreated) to 2.85 ± 0.06, 1.83 ± 0.04, and 0.63 ± 0.02 μm, respectively, after 5, 10, and 15 min microwave irradiation.

As shown by ESEM, the untreated polysaccharide was big flakelike lamella (Figure 2A). However, after being microwave irradiation treated, the original flakelike structure was changed to smaller fragments. In addition, with the time of the microwave irradiation increased, the fragments of the polysaccharide samples became smaller (Figure 2B−D).

FT-IR Spectroscopy. The primary functional groups in the untreated and microwave irradiation treated polysaccharide samples were analyzed by FT-IR spectroscopy (Figure 3). The strong absorption at 1047 cm^{-1} was due to the stretching vibration of the pyranose ring. In the anomeric region (950−700 cm^{-1}) the spectrum exhibited the characteristic absorption at 810 cm^{-1} due to the presence of mannose.[27] There are two types of end carbon−glucoside bonds (α and β) that can be distinguished by IR. In IR spectra the α-type C−H bond has an absorption peak near 844 cm^{-1}, while that of the β-type C−H bond is near 891 cm^{-1}.[27] A characteristic absorption at 899 cm^{-1} indicated that the β configuration of the sugar units was also observed, but there was no absorption near 844 cm^{-1}

corresponding to the α configuration. The absorption band at 1629 cm^{-1} indicated the presence of uronic acid. The band at 3421 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide. The bands at 2924 and 1404 cm^{-1} were due to C−H stretching vibration.[18] For untreated and microwave irradiation treated polysaccharide samples, the spectra were rather similar and there was no difference for characteristic absorption bands as described above between them. These results showed that microwave irradiation did not alter the primary functional groups in the polysaccharide.

Enzyme Activities in the Fermentation. The polysaccharide from *P. asiatica* L. was characterized by high contents of xylose, arabinose, and glucuronic acid.[12] Therefore, we investigated the activities of xylanase, arabinofuranosidase, xylosidase, and glucuronidase in fermented cultures, which were closely related to the fermentation of xylose, arabinose, and glucuronic acid in polysaccharide (Figure 4).

After 6 h incubation, xylanase activities (Figure 4A) increased 2−3 times the value at time 0 h [0.65 ± 0.08 xylanase units/ (h·mL)] in all samples. Then, it continued to increase for all samples upon further incubation to 12 h, and the levels for microwave irradiation treated samples were all significantly higher than that for untreated polysaccharide ($p < 0.05$). With the microwave irradiation time increased, the xylanase activities in fermentation cultures of polysaccharide samples also increased. After 24 h incubation, xylanase activities for all samples decreased. However, the values for microwave irradiation treated samples were still significantly higher than that for untreated polysaccharide ($p < 0.05$).
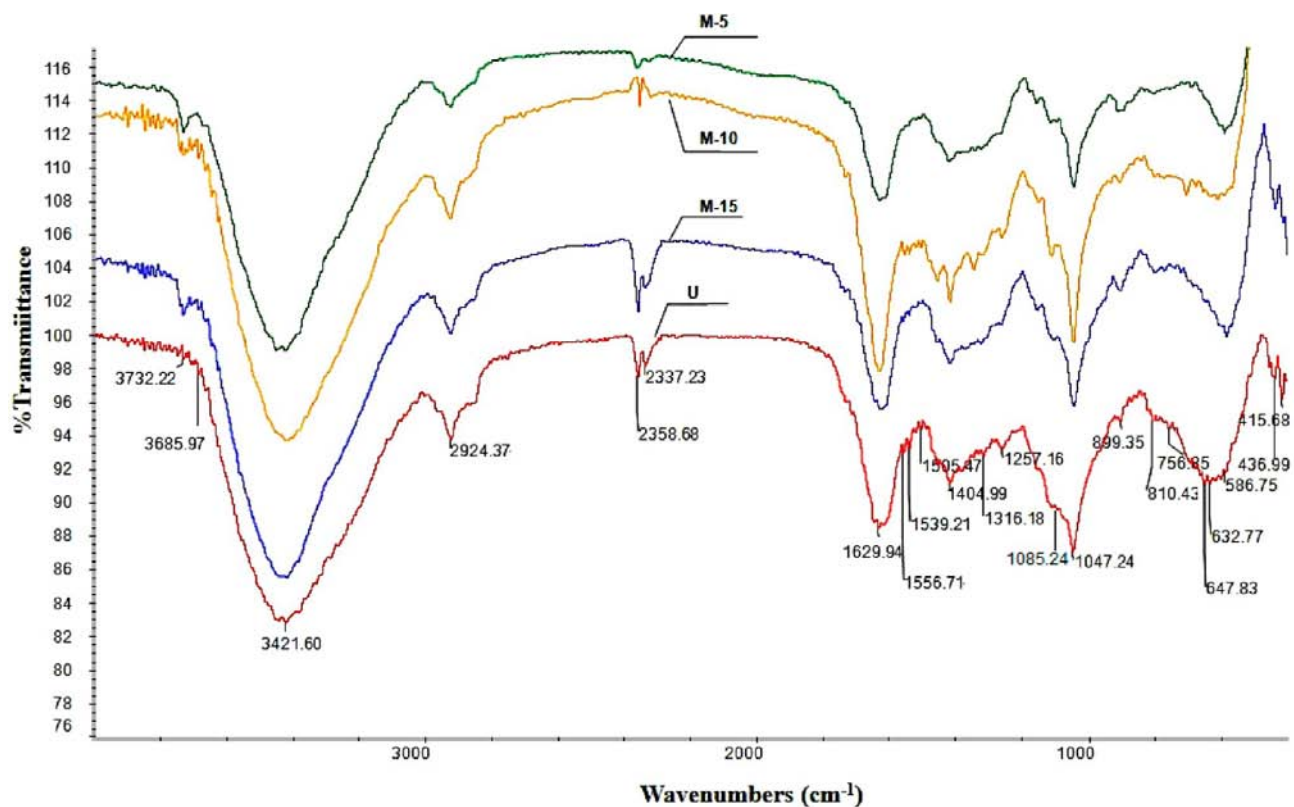
Figure 3. Effect of microwave irradiation (power = 800 W) on FT-IR spectra of polysaccharide from *P. asiatica* L. seeds: U = untreated sample, M-5 = sample treated with microwave irradiation for 5 min, M-10 = sample treated with microwave irradiation for 10 min, and M-15 = sample treated with microwave irradiation for 15 min.

Arabinofuranosidase activities (Figure 4B) also significantly increased during the initial incubation phase for all samples except untreated polysaccharide. After 6 h incubation, the microwave irradiation treated polysaccharide samples showed activities that were on average 1.5 times higher than activities in the untreated sample and 2−3 times higher than the value at the start of the experiment. For all samples, arabinofuranosidase activities continued to increase upon further incubation to 12 h, and activities for microwave irradiation treated samples were all significantly higher than that for untreated polysaccharide ($p <$ 0.05). After 24 h incubation, arabinofuranosidase activities of all samples decreased to levels similar to the values at the start of the experiment ($p > 0.05$), and there was no significant difference between untreated and microwave irradiation treated samples.

Xylosidase activities (Figure 4C) were not significantly increased during the initial 6 h incubation for all samples compared to the start of the experiment (0.012 ± 0.004 nkat p-nitrophenol/mL) except the polysaccharide being microwave irradiation treated for 15 min. After 12 h incubation, xylanase activities increased to 4−8 times the value at time 0 h in all samples ($p < 0.05$). The levels for samples treated with microwave irradiation for 10 and 15 min were all significantly higher than that for untreated polysaccharide ($p < 0.05$). With the microwave time increased, the xylosidase activities for the polysaccharide samples also increased. After 24 h, arabinofur-anosidase activities of all samples decreased to levels similar to the values at the start of the experiment, and there was no significant difference among different samples ($p > 0.05$).

Glucuronidase activities (Figure 4D) were significantly increased during the initial 6 h incubation for all samples compared to the start of the experiment [0.005 ± 0.001 glucuronidase units/(h·mL)], but there was no significant difference among different polysaccharide samples ($p > 0.05$). After 12 h, xylanase activities increased to over 10 times the value at time 0 h in all samples ($p < 0.05$), and the levels for samples treated with microwave irradiation for 10 and 15 min were all significantly higher than that for untreated poly-saccharide ($p < 0.05$). The glucuronidase activities for polysaccharide samples also increased with an increase of microwave irradiation time. After 24 h incubation, glucur-onidase activities decreased to levels similar to the values at the start of the experiment, and there was no significant difference among different samples ($p > 0.05$).

It could be seen above that the activities of xylanase, arabinofuranosidase, xylosidase, and glucuronidase in fermenta-tion cultures supplemented with the microwave irradiation treated polysaccharide were generally higher than those with untreated polysaccharide. In addition, activities of these enzymes in fermentation cultures supplemented with the microwave irradiation treated polysaccharide were increased with the increase of microwave irradiation time. It was found that the microbiota possess a broad spectrum of enzyme activities. However, colonic bacteria appear to be subjected to regulation. Their activities and in particular their polysacchar-idase activities can be induced by exposure to substrate. Thus microflora can exhibit preferential utilization of certain substrates and release certain enzymes.[28] In this study, the polysaccharide was characterized by high contents of xylose, arabinose, and glucuronic acid;[12] thus, activities of the related enzymes (xylanase, arabinofuranosidase, xylosidase, and glucur-onidase) can be induced by exposure to the polysaccharide.
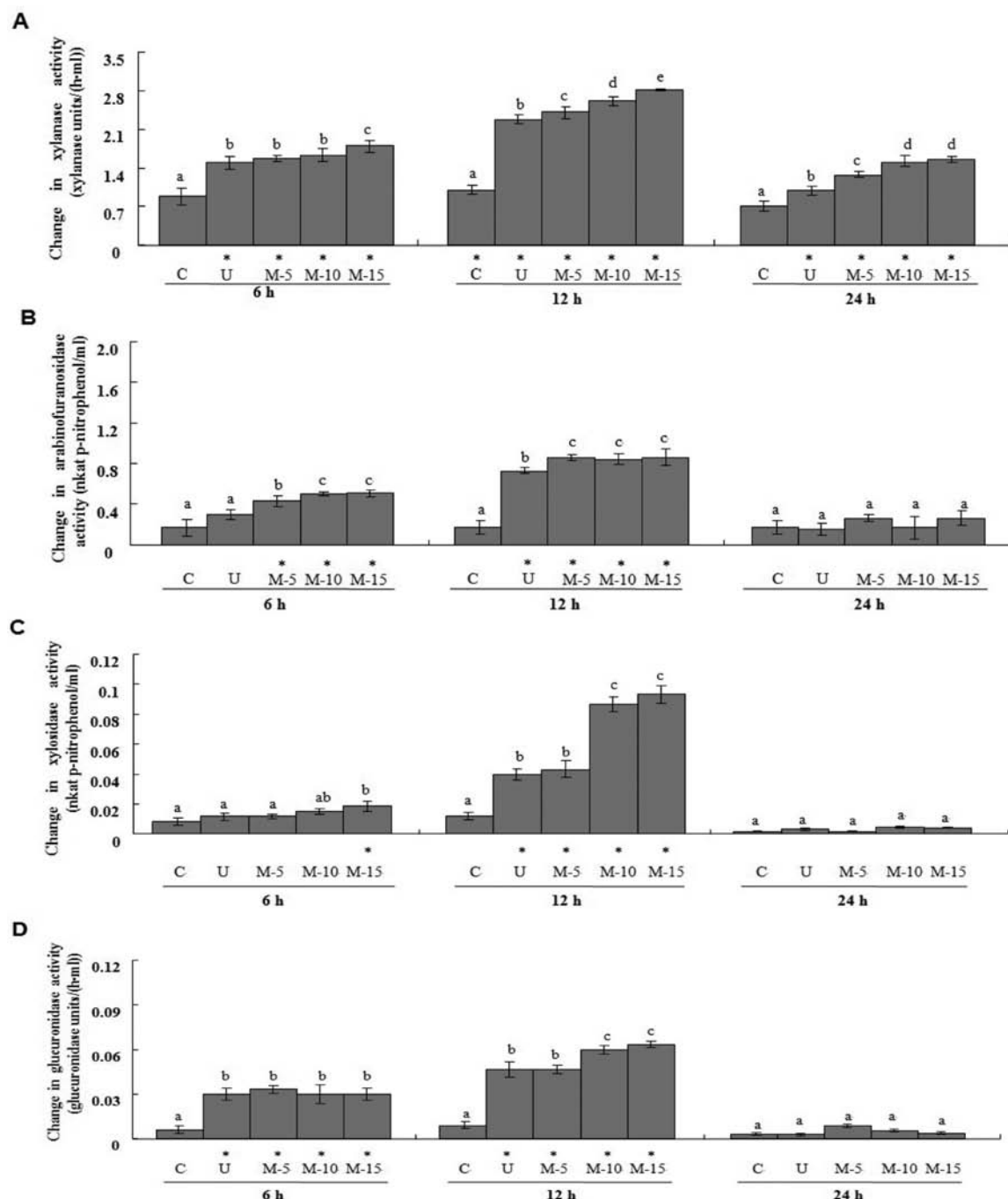
Figure 4. Change in activities of microbial extracellular enzymes (A) xylanase [xylanase units/(h·mL)], (B) arabinofuranosidase (nkat p-nitrophenol/mL), (C) xylosidase (nkat p-nitrophenol/mL), and (D) glucuronidase activity [glucuronidase units/(h·mL)] after 6, 12, and 24 h fermentation (37 °C, anaerobic) of different microwave irradiation treated (power = 800 W) polysaccharide samples and control (distilled water) as compared to time 0 h: C = control, U = untreated sample, M-5 = sample treated with microwave irradiation for 5 min, M-10 = sample treated with microwave irradiation for 10 min, and M-15 = sample treated with microwave irradiation for 5 min. Data are expressed as the mean ± standard deviation. Values from the same time point differ significantly ($p < 0.05$) when not sharing a common letter. Values with an asterisk differ significantly ($p < 0.05$) from the start of the experiment [time 0 h; 0.65 ± 0.08 xylanase units/(h·mL), 0.15 ± 0.04 nkat p-nitrophenol/mL, 0.012 ± 0.004 nkat p-nitrophenol/mL, and 0.005 ± 0.001 glucuronidase units/(h·mL), for parts A, B, C, and D respectively].

After the polysaccharide was treated by the microwave irradiation, the apparent viscosity, average molecular weight, and particle size of the polysaccharide decreased, and the surface topography of the polysaccharide changed from large flakelike structure to smaller chips. This degradation of the polysaccharide could make it easier for the microbiota to get close to the polysaccharide and utilize it more efficiently.

Therefore, xylanase, arabinofuranosidase, xylosidase, and glucuronidase activities could be improved when the fermentation cultures were supplemented with the microwave irradiation treated polysaccharide compared to the untreated one. With the microwave irradiation time increasing, the degradation of the polysaccharide was more serious, resulting in more increased enzyme activities.
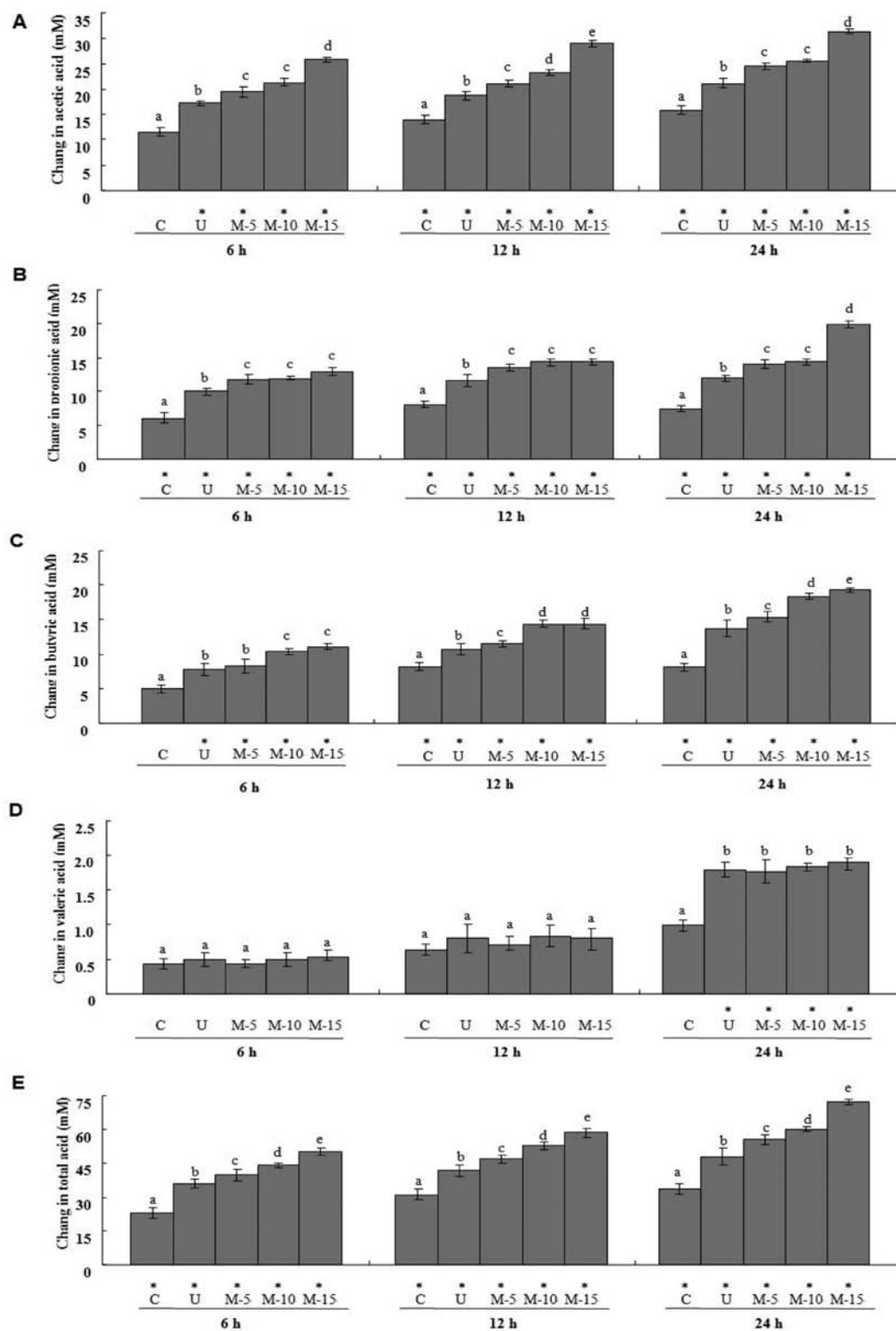
Figure 5. Change in (A) acetic acid, (B) propionic acid, (C) butyric acid, (D) valeric acid, and (E) total acid concentrations after 6, 12, and 24 h fermentation (37 °C, anaerobic) of different microwave irradiation treated (power = 800 W) polysaccharide samples and control (distilled water) as compared to time 0 h: C = control, U = untreated sample, M-5 = sample treated with microwave irradiation for 5 min, M-10 = sample treated with microwave irradiation for 10 min, and M-15 = sample treated with microwave irradiation for 15 min. Data are expressed as the mean ± standard deviation. Values from the same time point differ significantly ($p < 0.05$) when not sharing a common letter. Values with an asterisk differ significantly ($p < 0.05$) from the start of the experiment (time 0 h; 10.1 ± 0.3, 3.7 ± 0.3, 4.0 ± 0.2, 0.5 ± 0.1 and 18.2 ± 0.8 mM for parts A, B, C, D, and E, respectively).

SCFA Production of the Fermentation. Acetic, propionic, and *n*-butyric acids were found as the dominant components in total SCFA during polysaccharide fermentation (Figure 5). They were also reported to be beneficial for human

health. Acetate could be oxidized in brain, heart, and peripheral tissues. Propionic acid was reported to affect the liver and cholesterol metabolism. Butyric acid serves as an energy source for colonic epithelium, regulates growth and apoptosis of epithelial and immune cell, and provides protection against colonic cancer and colitis.[29]

As shown in Figure 5A, acetic acid concentrations strongly increased for all polysaccharide samples upon incubation with human fecal microbiota, with the increase being the strongest in the first 6 h of incubation. After 24 h, acetic acid concentrations of the polysaccharide fermented cultures were always significantly higher than that at the time 0 h (10.1 ± 0.3 mM). At all time points, fermentation of microwave irradiation treated samples resulted in significantly ($p < 0.01$) higher concentrations of acetic acid than fermentation of the untreated polysaccharide. With the microwave irradiation time increased, acetic acid produced from the polysaccharide fermentation also increased.

Concentration of propionic acid also increased for all polysaccharide samples with incubation time (Figure 5B). Values after 6, 12, and 24 h incubation differed significantly ($p < 0.05$) from that at the start (3.7 ± 0.3 mM), and the highest level (at 24 h) was 3−6 times higher than that at time 0 h. At all time points, fermentation of microwave irradiation treated samples resulted in significantly ($p < 0.05$) higher concentrations of propionic acid than fermentation of the untreated polysaccharide. The concentration of propionic acid produced by the polysaccharide increased with the increase in microwave irradiation time.

Butyric acid concentration differed significantly ($p < 0.05$) from that at the start of the experiment (4.0 ± 0.2 mM) for all polysaccharide samples after 6 h incubation (Figure 5C). It continued to increase upon further incubation, and values after 12 and 24 h incubation were 2−3 and 4−6 times higher than that at time 0 h, respectively. Fermentation of microwave irradiation treated samples resulted in significantly ($p < 0.05$) higher butyric acid concentrations than fermentation of untreated polysaccharide after 12 and 24 h incubation. With the microwave irradiation time increased, the concentration of butyric acid produced by the polysaccharide also increased.

For valeric acid concentration (Figure 5D), there was no significant difference between the polysaccharide samples and the start of the experiment (0.5 ± 0.1 mM) within 12 h incubation. However, concentrations of the polysaccharide samples after 24 h incubation differ significantly from that at time 0 h ($p < 0.05$), but there was still no significant difference among different polysaccharide samples ($p > 0.05$).

Concentrations of total SCFA at 6, 12, and 24 h during fermentation are presented in Figure 5E. The microwave irradiation treated polysaccharide fermentation cultures contained significantly higher concentrations of total SCFA than the untreated polysaccharide at all time points ($p < 0.05$). With the microwave irradiation time increased, the total SCFA produced by the polysaccharide increased and the highest value was for the polysaccharide sample treated for 15 min with microwave irradiation.

It could also be recognized from the results above that production of total SCFA and acetic, propionic, and butyric acids of the polysaccharide during in vitro fermentation significantly increased after microwave irradiation. The SCFA production of polysaccharides during fermentation was found to be related to some properties of polysaccharides, including particle size, structural features, molecular weight, surface area characteristics, solubility, and viscosity.[28]

Previous studies have shown that the particle size of the polysaccharide determining the surface available to bacteria may influence the fermentation and thus affect the SCFA production.[8] To degrade polysaccharides, microbial glycosidases must have access to their substrates. One factor important for the control of accessibility is particle size. Decreasing particle size may increase the external surface area and so increases the area exposed to bacteria. In the present study, the particle size of the polysaccharide decreased significantly after microwave irradiation (Table 1 and Figure 1). The accessible surface area of the polysaccharide for the microbiota to contact was increased after microwave irradiation, thus increasing the SCFA production.

Other parameters, such as structural properties, of the polysaccharides are also important factors. The nature of monomers and of the glycosidic linkages and the presence and distribution along the backbone of some functional groups or side chains can modulate their utilization by bacteria.[30] For arabinoxylans from some cereal, their extent of fermentation has been shown to depend on their degree of branching.[31] In our study, the degradation of the polysaccharide after microwave irradiation may derive from the rupture of the glycosidic bond (Table 1). Therefore, the structural properties of the polysaccharide from *P. asiatica* L. may be changed to some extent after microwave irradiation, and the SCFA production was influenced.

It was found that polysaccharides being degraded to some extent or with lowered molecular weight could be more efficiently utilized by the microbiota.[28] For example, some polysaccharides derived from alga (alginates, ulvans, carrageenan) were poorly fermented while their constitutive elements (monomers, dimers) were easily fermented.[32] In this study, the molecular weight of the polysaccharide was decreased and the rupture of glycosidic bond was found after microwave irradiation. Therefore, the degraded polysaccharide from *P. asiatica* L. may be more efficiently utilized by the microbiota, and the SCFA production was increased after microwave irradiation.

The available surface was found to influence the fermentation of dietary fiber (availability to microbial degradation). The surface available for bacteria or molecular probes such as enzymes will depend on the surface topography of the fiber, which is related to its processing conditions.[8] In this study, the surface topography of the polysaccharide changed from large, flakelike structure to smaller chips (Figure 2); thus, the available surface for microbial degradation was also changed, which may influence the utilization of the polysaccharide by microbiota, resulting in the changes of the SCFA production.

Previous studies have also shown that the solubility and viscosity could affect the fermentation of the polysaccharide by microbiota. A lower viscosity of the polysaccharide often resulted in a higher solubility, and the more soluble polysaccharides were generally more rapidly fermented than equivalent polysaccharides with low solubility or high viscosity, which made the microbiota more efficiently utilize the polysaccharides.[32] In this study, the viscosity of the polysaccharide was significantly decreased (Table 1). Therefore, the polysaccharide from *P. asiatica* L. may be better utilized by the microbiota and the SCFA production was increased after microwave irradiation.

Our results showed the effects of microwave irradiation on the SCFA production of the polysaccharide and the microbial extracellular enzyme activities during in vitro fermentation for the first time. The polysaccharide from *P. asiatica* L. was degraded after microwave irradiation. However, short-chain fatty acid production of the polysaccharide during in vitro fermentation significantly increased after being microwave irradiation treated. Activities of microbial extracellular enzymes in fermentation cultures supplemented with microwave irradiation treated polysaccharide were also generally higher than those with untreated one added. These results showed that microwave irradiation could be a promising degradation method to the production of value-added polysaccharides.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The structure of the polysaccharide from the seeds of *P. asiatica* L. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS USED

SCFA, short-chain fatty acid; GC, gas chromatography; FID, flame ionization detector; ESEM, environmental scanning electron microscope; HPGPC, high-performance gel permeation chromatography; HPLC, high-performance liquid chromatography.

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