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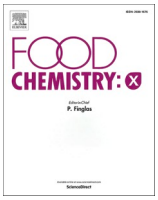

Modified dietary fiber from soybean dregs by fermentation alleviated constipation in mice

Li Wu *, Chunhong Tang, Linli Chen, Jiuyi Zhao

College of Environment and Resources, Chongqing Technology and Business University, Chongqing 400067, China

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ABSTRACT

Soybean dregs are the main by-product obtained from the processing of soy products and are good sources of dietary fiber (DF). This study showed that the soluble DF content increased from 4.97% to 18.82%, while the insoluble DF content decreased from 59.37% to 44.89% after soybean dreg fermentation using *Trichoderma* spp., without any significant change in the total DF content ($p > 0.05$). Physicochemical property and electron microscopy analysis revealed that the rehydration ratio, dissolution rate, expansion force, and oil holding capacity of DF significantly increased ($p < 0.05$) with finer microstructure. Additionally, we found that fermented DF could further promote intestinal peristalsis in mice. Furthermore, fermented DF was more effective in balancing and adjusting intestinal flora in mice and promoting the production of short-chain fatty acids. Therefore, this study provides evidence indicating a correlation between the physicochemical properties and functional benefits of DF derived from soybean dregs.

1. Introduction

The irregularity of urban lifestyle and consumption of unbalanced diets have resulted in increased intestinal problems, including constipation, in younger people (Gabrielsson et al., 2023), thereby significantly affecting their quality of life. The most common symptoms of constipation include decreased and difficult bowel movements, long duration, and abdominal bloating or pain (Aziz et al., 2020; Jin et al., 2023). The underlying cause of constipation is the inability of the digestive system to function and the less frequent bowel movements, leading to an increase in transit time and excess water absorption from food by the intestinal linings, thereby hardening the texture of stools and making their elimination difficult (Andrews & Storr, 2011). Studies have demonstrated that constipation can affect the human body in many ways. For example, in mild cases, it causes unpleasant breath, hemorrhoids, and loss of appetite; however, in severe cases, it causes irritable bowel syndrome, colon cancer, and other intestinal diseases (Jayasimhan et al., 2013; Zhang et al., 2021; Anderson & Lacy, 2014; Sundbøll et al., 2019).

Dietary fiber (DF) refers to naturally occurring, extracted, or synthetic carbohydrate polymers found in plants with a degree of polymerization ≥ 3, which cannot be digested or absorbed by the human small intestine and assists in improving intestinal function (Post et al., 2012), thereby facilitating bowel movement, ameliorating constipation, and contributing to the reduction of blood cholesterol and postprandial blood sugar levels (Ho et al., 2012; Ljubicic et al., 2017; Brownlee et al., 2017). It is frequently referred to as the "seventh class of nutrients" (Goff et al., 2018). Additionally, DF intake is believed to be effective in improving constipation (Camerotto et al., 2019). DF has dual functions regarding defecation and fecal properties, and these functions are manifested by the action of microbial metabolites, such as organic acids and unfermentable but excipient fiber components (Oshiro et al., 2022). For example, Hu et al. (2019) explained that DF facilitates bowel movement and relieves constipation by promoting mucus cell division in the colon and enhancing the expression of aquaporins, thereby increasing fecal water content and relieving constipation symptoms. Furthermore, DF acts on the glands on the surface of the intestinal lumen, promoting mucosal exudation of digestive juices while transmitting nerve signals to the enteric nervous system and increasing the peristaltic frequency of the intestine (Stephen et al., 2017).

Importantly, DF can be categorized into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF primarily includes pectin, oligosaccharides, glucan, and galactooligosaccharides, whereas IDF mainly comprises cellulose, hemicellulose, lignin, and plant waxes (Bishehsari et al., 2018; Sacranie et al., 2012). Although the physiological efficacy, physicochemical properties, and composition proportion of DF are

* Corresponding author.
E-mail address: wulili1228@163.com (L. Wu).

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closely related, studies have shown that SDF and IDF have different functionalities (Makki et al., 2018; Arayici et al., 2021). For instance, IDF's components, such as cellulose and lignin, are mostly not fermentable in the colon; they can effectively increase fecal bulk through their particle formation and water-holding capacity (Dai & Chau, 2017). In contrast, SDF ferments easily and can effectively improve the growth of gut microbiota and by-products (e.g., gases and short-chain fatty acids). Additionally, SDF positively affects constipation and gastrointestinal diseases (Mudgil & Barak, 2013; Gill et al., 2020; Staller et al., 2018; Koh et al., 2016). Based on these findings, most nutritionists and dieticians have suggested the consumption of a diet with an SDF content of 20%–30% (Elleuch et al., 2011). However, most foods have a relatively low content of SDF (Prosky et al., 1988). Consequently, we hypothesized that the DF fermentation modification method might help increase the SDF content and improve physicochemical properties, thereby enhancing its physiological effects on the human body.

Soybean dregs are the main by-product derived from the processing of soy products, such as soybean oil, soy sauce, and tofu. The dried soybean dreg crude protein, crude fat, and DF contents are 25%–28%, 9%–11%, and 50%–60%, respectively; however, the percentage of SDF is only approximately 2%, which limits its application in improving intestinal function (Li et al., 2019). Therefore, in this study, we aimed to modify DF in soybean dregs using fermentation to increase the SDF content. After the modification, the physicochemical properties of DF were analyzed, and constipated mouse models were established. Additionally, the intestinal flora of mice and short-chain fatty acid concentration were analyzed and measured to provide a theoretical basis for the development of functional foods.

2. Materials and methods

2.1. Materials

Fresh bean residue was collected from Chongqing Tianrun Food Development Co., Ltd. (Chongqing, China) with moisture, protein, fat, and ash contents of 77.50%, 19.77% (dry basis percentage), 15.81% (dry basis percentage), and 3.88% (dry basis percentage), respectively. *Trichoderma* spp. was collected from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). Loperamide hydrochloride was purchased from Xi'an Janssen Pharmaceutical Ltd. (Xi'an, China).

2.2. Fermentation of soybean dregs

Trichoderma spores were inoculated into the sporulation medium and incubated (GHP-9080, Qixin Scientific Instrument Co., Ltd. Shanghai, China) at 28 °C for 3 d at a constant temperature to obtain *Trichoderma* spore suspensions (concentration, 10^7 spores/mL). Soybean dregs (initial moisture, 77.50%) were sterilized and cooled. Subsequently, they were inoculated with 3.5% *Trichoderma* spore suspensions and incubated at 27 °C for 10 d.

2.3. Measurement of DF content

The DF, IDF, and SDF contents were measured using the Association of Official Agricultural Chemists method.

2.4. Determination of DF physicochemical properties

2.4.1. Extraction of DF

The defatted soybean dregs were first milled and sieved through an 80-mesh sieve and then 1 g of the sieved powder was weighed and extracted, as described by Li et al. (2019). The extract was freeze-dried (−0.09 MPa, 70 °C) under vacuum (DZF-6050, Yiheng Scientific Instrument Co., Ltd. Shanghai, China) to a constant weight and then re-sieved through an 80-mesh sieve to obtain DF powder from soybean dregs.

2.4.2. Determination of rehydration ratio

To perform this experiment, 1 g of DF powder was soaked in 30 mL of distilled water and incubated in the water bath at 37 °C for 1 h. Subsequently, it was filtered using filter paper, with excess water aspirated, and the remaining residue weighed (Mateos-Aparicio et al., 2010). The rehydration ratio was calculated using the following formula (1) as follows:

$$R = \frac{m_2}{m_2} \tag{1}$$

where R represents the rehydration ratio; m_1 and m_2 represent the mass (g) before and after rehydration, respectively.

2.4.3. Determination of dissolution rate

In this experiment, 1 g of DF powder was weighed, and 30 mL of distilled water was added. The resulting mixture was stirred at 90 °C for 30 min and centrifuged at 2810 × *g* for 20 min. The supernatant was discarded into a Petri dish and dried to a constant mass at 105 °C (Sun et al., 2010). The dissolution rate was calculated using formula (2) as follows:

$$D = \frac{m_2 - m_1}{m} \times 100\% \tag{2}$$

where D is the dissolution rate; m represents the sample mass, g; m_1 denotes the mass of the Petri dish baked to constant weight, g; and m_2 indicates the total mass of the Petri dish and the supernatant after drying, g.

2.4.4. Measurement of expansion force

To conduct this experiment, 1 g of DF powder was weighed into a test tube, and its corresponding volume was measured and recorded. Subsequently, 10 mL of deionized water was added and shaken to thoroughly moisten the powder. After standing the mixture at 25 °C for 24 h to eliminate the presence of air bubbles, the volume of powder expansion after water absorption was recorded (Sun et al., 2010). Expansion force was calculated using formula (3) as follows:

$$E = \frac{V_2 - V_1}{m} \tag{3}$$

where E denotes the expansion force, mL/g; m represents the sample mass, g; and v_1 and v_2 indicate the sample volume (in mL) before and after expansion, respectively.

2.4.5. Measurement of oil holding capacity

To perform this experiment, 1 g of DF powder was weighed into a centrifuge tube, and 5 mL of cooking oil was added. The resulting mixture was stirred every 10 min and, after 1 h, centrifuged at 2600 × *g* for 20 min. Subsequently, the upper layer of oil was collected and weighed before it was discarded (Sun et al., 2010). Oil holding capacity was calculated using formula (4) as follows:

$$O = \frac{m_2 - m_1 - m}{m} \tag{4}$$

where O denotes oil holding capacity, g/g; m represents sample mass, g; m_1 indicates the mass of the centrifuge tube, g; and m_2 is the total mass of the centrifuge tube and residue, g.

2.4.6. Observation with an electron microscope

A small amount of DF powder from soybean dregs was extracted, and its microstructure was examined using scanning electron microscopy (×1000) (SU1510; Techcomp Scientific Instrument Co., Ltd. Shanghai, China).

2.5. Animal experiment

2.5.1. Animals and their environment

All experimental procedures involving animals were performed in strict compliance with the Animal Care and Use Guidelines and Regulations of the Ethics Committee of South China Agricultural University.

Eighty male SPF-grade KM mice (20–25 g and 8 weeks old) were purchased from Liaoning Changsheng Biological Technology Co., Ltd. They were housed in different units at 20 ± 2 °C, with a 60% ± 5% relative humidity in a light/dark cycle. They underwent adaptive feeding for 7 d and were provided ad libitum access to food and water. Their body weight, food, and water intake were monitored periodically during the experiment, and their growth was analyzed.

2.5.2. Small intestinal propulsion experiment

Forty mice were randomly categorized into the following four groups, comprising 10 mice each: blank control, model control, non-fermented (NF), and fermented (F) groups. The blank, model, and the other experimental groups received a saline solution, loperamide hydrochloride, and both DF and loperamide hydrochloride by gavage, respectively. The gavage volumes administered included 0.02 mL/(g·d), 2 mg/(g·d), and 10 μg/(g·d) of physiological saline, DF, and loperamide hydrochloride, respectively. Gavage was performed once daily for 14 d, and the mice were provided ad libitum access to food and water the rest of the time. After the final gavage, the mice in each group were fasted but given ad libitum access to water for 12 h. Subsequently, the mice were fed by gavage again with loperamide hydrochloride, and after 30 min, all groups were fed by gavage with 0.2 mL of ink. Next, the mice were sacrificed by cervical dislocation after 20 min. The total length of the small intestine and the ink advancement length were measured. The ink advancement rate was calculated using Eq. (5) as follows:

$$P = \frac{S_1}{S_2} \times 100\% \quad (5)$$

where P is the small intestine ink advancement rate; S_1 denotes the ink advancement length, cm; and S_2 represents the total small intestine length, cm.

Notably, 500 mL of distilled water was added to 50 g of gum Arabic for ink preparation, and the solution was boiled until it was transparent. Subsequently, 50 g of activated carbon powder was added, and the solution was heated to the boiling point three times before being cooled and stored at 4 °C.

2.5.3. Defecation experiment

Forty KM mice were grouped as described in the small intestinal propulsion experiment. They were fed by gavage and molded in the same way. After the continuous feeding for 14 d, each group of mice was fed by gavage with 0.2 mL of ink. The time of the first black stool excretion and the number and weight of black stools excreted within 6 h were recorded for each mouse. Additionally, the water content of the stools was measured.

2.5.4. Intestinal flora analysis

High-throughput sequencing of intestinal flora 16S rRNA was performed on the feces of each group of mice. Genomic DNA was extracted from fecal samples. DNA purity, concentration, and integrity were examined using NanoDrop 2000 (Thermo Fisher, Waltham, MA) and 1% agarose gel electrophoresis. Furthermore, the V3–V4 regions of the bacterial 16S rRNA gene were amplified using polymerase chain reaction (PCR) with 338F_806R primers. The PCR products were examined and purified using 2% agarose gel electrophoresis. Moreover, PCR products were quantified and homogenized by mixing using PicoGreen fluorometry (Qubit 4, Thermo Fisher, Waltham, MA), and those between 300 and 500 bp in size were sequenced using Illumina MiSeq PE 300. Bioinformatic statistical analysis was conducted using QIIME2.0 (Shanghai Majorbio Bio-pharm Technology Co., Ltd., China).

2.5.5. Measurement of short-chain acids

The content of short-chain fatty acids in mice feces was determined using gas chromatography (7890B, Agilent Technologies Co., Ltd, Beijing, China).

2.5.5.1. Standard solution preparation. In stock solution preparation, 9940 μL of ether was added into a 15 mL centrifuge tube. Subsequently, 10 μL of acetic, propionic, butyric, isobutyric, pentanoic, and isovaleric acid standards was added into the tube. The solution was vortexed and mixed well to obtain the stock solution comprising six short-chain fatty acids.

2.5.5.2. Sample processing and analysis. In this experiment, 1 g of mouse feces was dissolved in 5 mL of pure water, then transferred into a centrifuge tube, mixed by vortexing for 2 min, and centrifuged at 4 °C for 5 min at 10744 × *g*. Subsequently, 2 mL of supernatant was taken from the mixture, and 0.2 mL of 50% sulfuric acid solution and 2 mL of ether were added to it. The resulting mixture was mixed by vortexing for 30 s and centrifuged at 4 °C 10744 × *g* for 5 min. The top layer of ether solution was filtered (using 0.45 μm mesh) into the injection vial after centrifugation at 4 °C for 2 h. Next, the sample was analyzed using gas chromatography.

Regarding the chromatographic column, an FFAP flexible quartz capillary column (30 m × 0.25 mm × 0.25 μm) was used at a temperature programmed at 100 °C (1 min), then increased to 150 °C (for 5 min) at a rate of 5 °C/min. The carrier gas used was high-purity nitrogen, with a flow rate of 2 mL/min, and the inlet temperature was set at 250 °C. The injection method used the splitless mode, with a 1.0 μL injection volume and a detector (flame ionization detector) temperature of 280 °C.

2.6. Statistical analysis

All statistical analyses were performed using SPSS 21.0 software. Mean ± standard deviation was used to indicate results, and Duncan's multiple range test and one-way analysis of variance were used to examine means differences. Statistical significance was set at $p < 0.05$.

3. Results and analysis

3.1. Measurement of dietary fiber content in soybean dregs

The SDF content of soybean dregs increased by 13.85% after fermentation, which was 3.8 times higher; however, the IDF content decreased by 14.48%, and the TDF content did not change significantly ($p > 0.05$). The increase in SDF was similar to the decrease in IDF. The increase in SDF was primarily due to the breakdown of IDFs, such as cellulose and hemicellulose, by fermentation.

3.2. Determination of dietary fiber physicochemical properties

As presented in Table 1, the physicochemical properties of DF from soybean dregs all significantly increased after fermentation ($p < 0.05$), among which the dissolution rate had the greatest increase (i.e., 13.32%). As the SDF content increased after fermentation, most SDFs dissolved in water, thereby increasing the dissolution rate. However, as IDF decomposed, the surface area of fibers and intermolecular gaps increased. Nevertheless, as the hydrophilic groups of SDF also played a role, the rehydration ratio, expansion force, and the oil holding capacity of DF from soybean dregs increased correspondingly after fermentation (Lyu et al., 2021).

Table 1
Physicochemical properties of dietary fiber from soybean dregs.

Group	Rehydration ratio	Dissolution rate (%)	Expansion force (g/mL)	Oil holding capacity (g/g)
Non-fermented	3.23 ± 0.12^{b}	6.55 ± 0.21^{b}	5.58 ± 0.11^{b}	1.97 ± 0.18^{b}
Fermented	3.87 ± 0.15^{a}	19.87 ± 0.33^{a}	6.33 ± 0.16^{a}	2.41 ± 0.13^{a}

Note: The data between groups were all significantly different ($p < 0.05$).

3.3. Electron microscopy analysis

As shown in Fig. 1, the unfermented DF surface is relatively even and smooth, with a tight structure as shown in Fig. 1a. In contrast, the DF surface is rougher with finer fiber and a looser structure after fermentation as shown in Fig. 1b. Particularly, after soybean dregs were fermented with *Trichoderma* spp., some IDF degraded into SDF with smaller molecular weights, thereby decreasing polymerization and molecular weight and increasing particle surface area (Ullah et al., 2017).

3.4. Growth of mice

After 14 d of feeding and gavage, the mice gradually showed an increasing normal trend in body weight, indicating that the gavage amount of soybean residue DF was reasonable and the growth was good. However, the initial and final weights of each group of mice were not significantly different ($p > 0.05$).

3.5. The effect of soybean residue fermentation modification on intestinal propulsion of mice

The blank group had the highest ink propulsion rate of 72.99% in the intestinal propulsion experiment. However, the ink propulsion rate of the model group was 36.24%, which was approximately 36.75% lower than that of the blank group, indicating that the peristaltic strength of the intestines became weaker in mice fed by gavage with loperamide hydrochloride and that the constipation model was successfully established. In comparison, the ink propulsion rates of mice in the NF and F groups were 52.03% and 59.40%, respectively, and both increased compared with the model group, indicating that different DF from soybean dregs could promote intestinal peristalsis in mice. The ink advancement rate in the F group was 7.37% higher than that in the NF group. Therefore, we hypothesized that the higher SDF content in the fermented soybean dregs and the increased viscosity of SDF dissolved in water are more likely to delay gastric emptying and promote small intestinal peristalsis.

3.6. The effect of different DF from soybean dregs on mice defecation

Under the different doses administered, each group of mice had good fecal formation and no diarrhea. As presented in Table 2, compared with the blank group, the time to defecate the first black stool increased by 96.51 min, the number of defecated pellets decreased by 11.20 in 6 h, the total amount of defecation decreased by 0.22 g in 6 h, and the fecal water content decreased by 3.40%. Notably, the differences were significant ($p < 0.05$), indicating that the mouse constipation model was successfully established. However, compared with the model group, the time to defecate the first black stool was reduced in the other experimental groups. The number of black stools, the total number of stools, and the water content of stools within 6 h increased in the other experimental groups because the IDF in soybean dregs was not soluble in water or fermented by microorganisms in the large intestine. Additionally, IDF might have reduced the residence time of excreta in the intestine, increased stool volume, and had a laxative effect. Furthermore, compared with the NF group, the time to defecate the first black stool was shortened by 15.37 min, the number of defecated pellets in 6 h was increased by 3.70, the total amount of defecated stool in 6 h was increased by 0.08 g, and the water content of stool was increased by 2.05% in the F group (All differences were significant, $p < 0.05$). As SDF content was higher after the fermentation of soybean dregs, SDF was soluble in water and could increase bowel movement and stool water content. Moreover, soybean dregs can be fermented and degraded by microorganisms in the large intestine, promoting the synthesis of short-chain fatty acids, significantly decreasing the pH value, and effectively maintaining the balance of intestinal flora (Makki et al., 2018).

Table 2
Effects of different dietary fibers from soybean dregs on defecation in mice.

Group	Time to defecate the first black stool/min	Number of defecated pellets in 6 h	Total amount of defecation/g	Fecal water content/%
Blank	112.25 ± 23.63^{d}	20.30 ± 3.23^{a}	0.38 ± 0.06^{a}	17.06 ± 1.77^{b}
Model	208.76 ± 17.56^{a}	9.10 ± 2.62^{d}	0.16 ± 0.05^{c}	13.66 ± 1.87^{c}
NF	151.40 ± 13.26^{b}	13.40 ± 2.73^{c}	0.24 ± 0.05^{b}	16.79 ± 1.96^{b}
F	136.03 ± 13.60^{c}	17.10 ± 3.75^{b}	0.32 ± 0.07^{a}	18.84 ± 1.51^{a}

Note: Different small letters in the same column indicated significant differences between groups ($p < 0.05$).

3.7. Diversity of intestinal flora

3.7.1. Alpha diversity

The richness and diversity of a species within an ecological community can be obtained by evaluating the alpha diversity metrics. In this

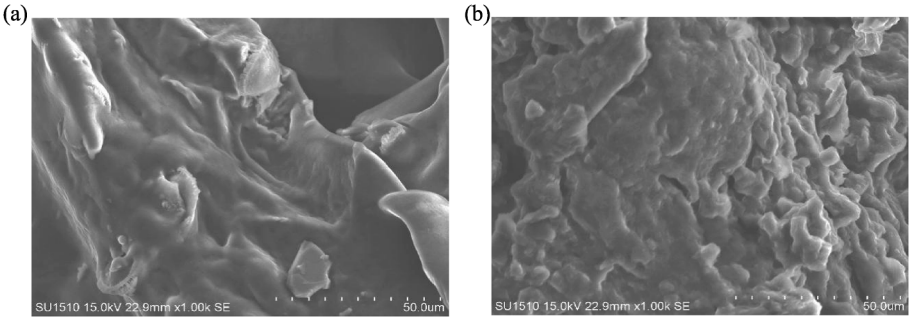

Fig. 1. Scanning electron micrograph of dietary fiber from soybean dregs (a) Non-fermented, (b) Fermented.

study, we used Simpson's index to reflect community diversity.

As Simpson's index decreased, community diversity increased. As shown in Fig. 2(a), Simpson's index of the model group was the largest, followed by the NF, then the blank and F groups. Therefore, this demonstrates that the blank and F groups have relatively high community diversity. After modeling, the model group's community diversity decreased, which means that constipation can decrease intestinal flora diversity in mice. Interestingly, we found that DF from soybean dregs after gavage and fermentation could increase the intestinal flora diversity to a level of no significant difference with the blank group ($p > 0.05$). Notably, the F group had higher community diversity than the NF group with a significant difference ($p < 0.05$), implying that DF from soybean dregs can further increase intestinal flora diversity in mice.

Therefore, by evaluating alpha diversity metrics, we found that community diversity decreased in the intestines of constipated mice, whereas DF from soybean dregs increased the diversity, among which fermented DF from soybean dregs had the best effects.

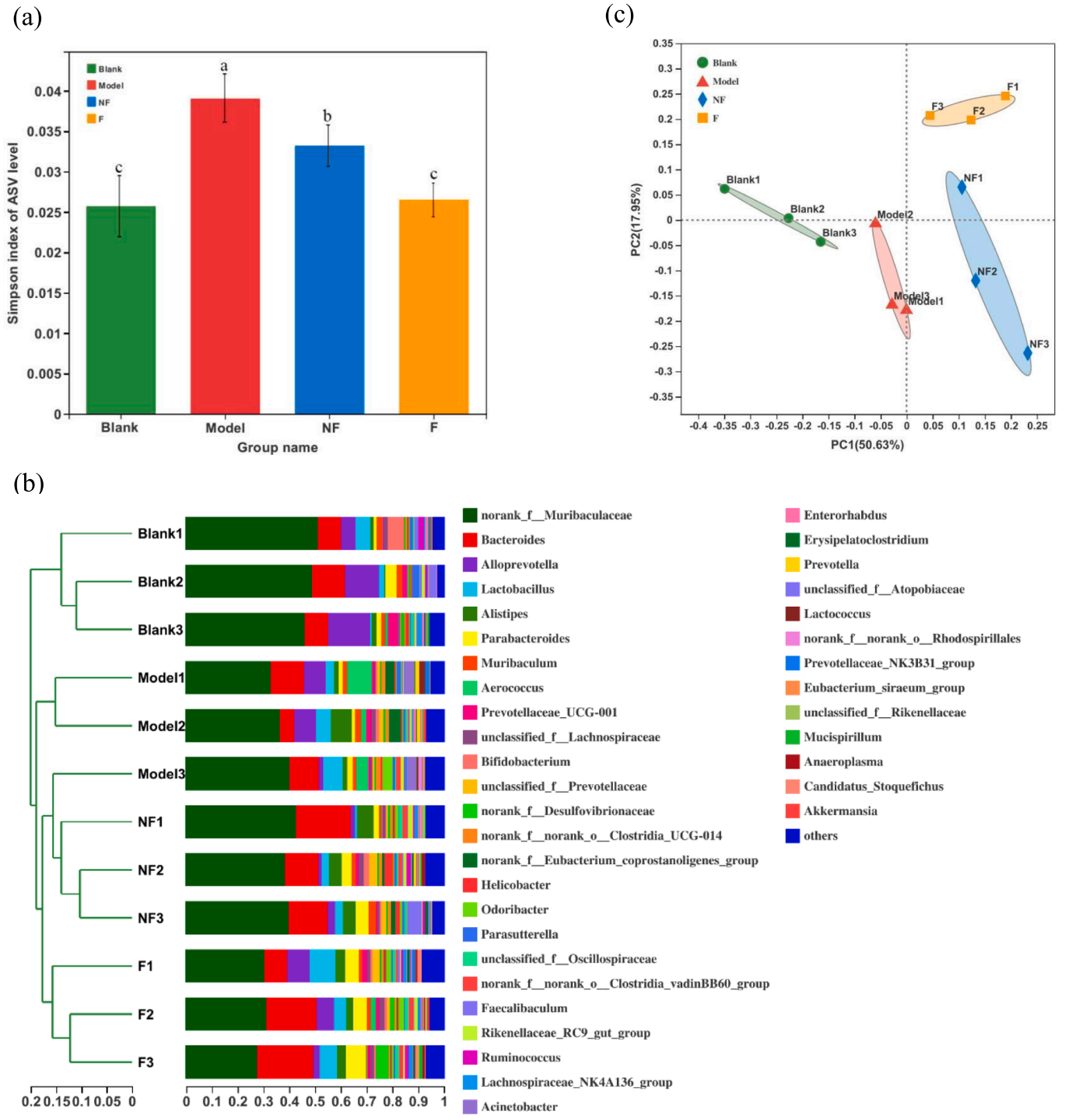

Fig. 2. Diversity of intestinal flora. (a) Index of Simpson, (b) Hierarchical clustering tree, (c) PCoA analysis. Note: Different small letters indicated significant differences between groups ($p < 0.05$).

3.7.2. Beta diversity

3.7.2.1. Sample-level clustering analysis. As shown in Fig. 2(b), in the dendrogram at the genus classification level, the blank, model, NF, and F groups had similar sample distances, implying that relatively minor differences existed within groups. However, the blank and the other three groups were not on the same branch, implying that the intestinal flora in constipated mice might have changed. Moreover, the NF, F, and model groups were on different secondary branches, meaning that the DF from soybean dregs could significantly affect intestinal flora diversity in constipated mice ($p < 0.05$). Among them, the distribution of genera in the NF and model groups was more similar in distance than that in the F group, indicating that the DF from soybean dregs could significantly affect intestinal flora diversity in constipated mice.

3.7.2.2. Principal coordinate analysis (PcoA). As shown in Fig. 2(c), the groups were relatively close together in the PCoA plots, indicating that less variation existed within the groups, while significant differences existed in the species distribution between the groups. Among them, the distance between the F and model groups was further than that of the NF group, implying that the DF from soybean dregs could significantly affect intestinal flora diversity in constipated mice. This finding is comparable to the conclusion from the sample-level clustering analysis.

3.8. Composition of intestinal flora in mice

3.8.1. Distribution of microbial community at the phylum level

As shown in Fig. 3(a), the studied samples comprised the following six main phyla: Bacillota, thick-walled Bacteroidota, Actinomycetes, Ascomycota, Thermodesulfobacteriota, and Campilobacterota. Among them, Bacillota and thick-walled Bacteroidota were the most abundant

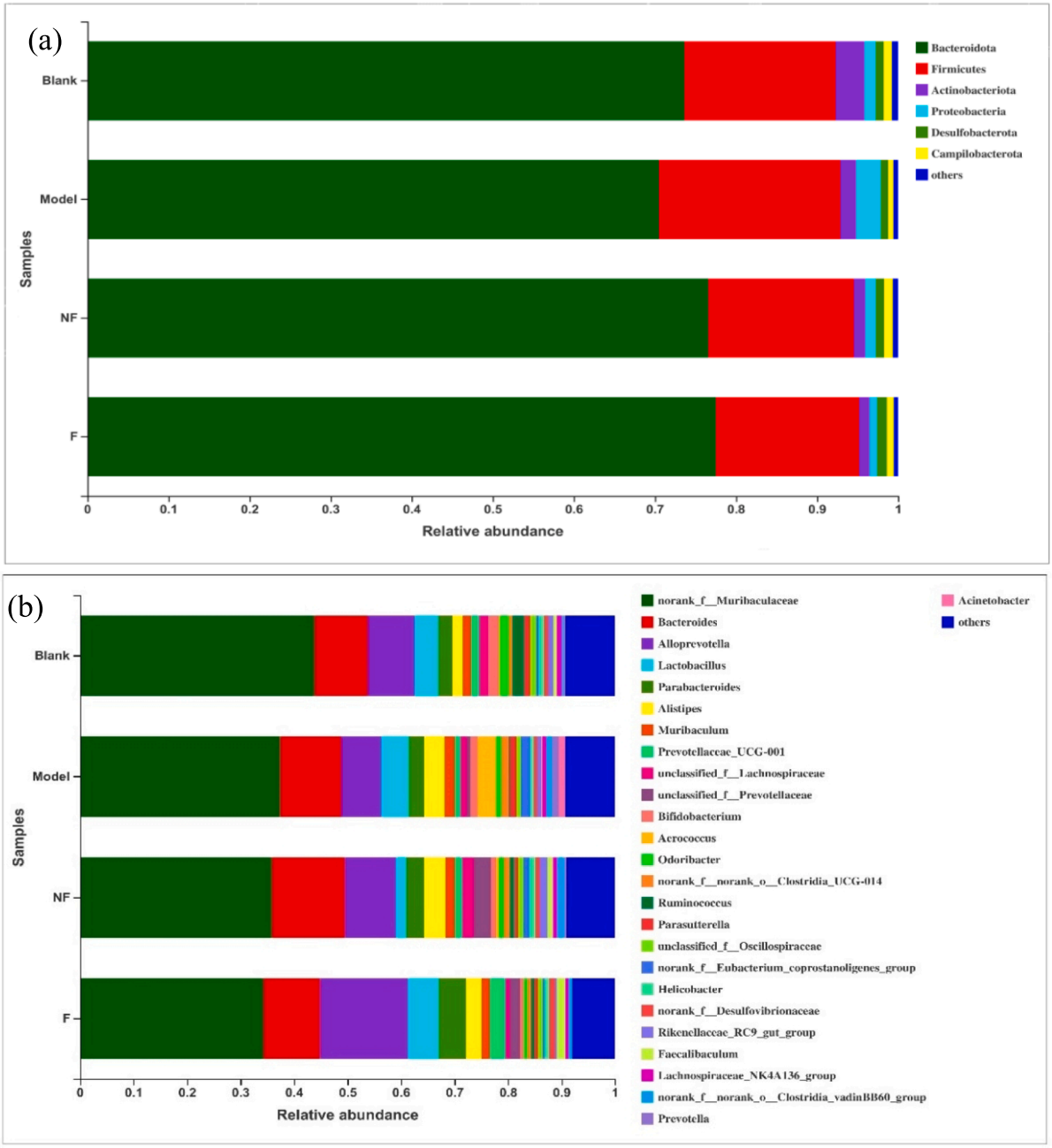

Fig. 3. Bat chart of relative abundance of microbiota in each group. (a) Phylum level and (b) genus level.

phyla, with relative abundances above 70% and 17%, respectively. Specifically, studies have demonstrated that an imbalance in Bacillota/thick-walled Bacteroidota ratio could lead to metabolic syndromes, such as obesity and diabetes; therefore, its relatively high ratio could help balance the intestinal flora and maintain a relatively good body and health condition (Turnbaugh et al., 2006). Notably, the difference between the model and the blank group was significant ($p < 0.05$), which showed a decrease in the phylum Bacillota and an increase in the thick-walled Bacteroidota, indicating that the Bacillota/thick-walled Bacteroidota ratio decreased in constipated mice. However, the NF and F groups showed a significant increase ($p < 0.05$) compared with the model group, indicating that DF from soybean dregs could increase the Bacillota/thick-walled Bacteroidota ratio; however, no significant difference was found between the NF and F groups. The blank group had the highest abundance of Actinomycetes (3.58%), whereas the other three groups had a lower abundance. The abundance of the phylum Ascomycota in the model group was 3.05%, which was 1.68% higher than that in the blank group, with a significant difference ($p < 0.05$). This indicates that the abundance of the phylum Ascomycota in the intestines of constipated mice would increase, thereby increasing the risk of disease. Notably, the abundance of Ascomycota was significantly lower in the NF and F groups than in the model group ($p < 0.05$), indicating that DF from soybean dregs could reduce the abundance of Ascomycota. However, Thermodesulfobacteriota and Campilobacterota phyla had no significant differences among the four groups.

3.8.2. Distribution of microbial community at the genus level

As shown in Fig. 3(b), the most abundant genera in the intestines of the experimental mice were *norank_f_Muribaculaceae, Mycobacterium*, and *Prevotella*. Compared to the blank group, the abundance of *norank_f_Muribaculaceae* in the model, NF, and F groups significantly decreased ($p < 0.05$), indicating that the abundance of this genus would decrease in the intestines of constipated mice. However, the abundance of *Mycobacterium* in the F group was significantly lower than that in the NF group ($p < 0.05$), indicating that the fermented DFs from soybean dregs would reduce the abundance of *Mycobacterium* in the intestines of constipated mice. Generally, *Mycobacterium* normally resides in human and animal intestines, and when immune dysfunction or dysbiosis occurs in the body, it can easily cause endogenous infection. The abundance of *Prevotella* spp. in group F was 16.48%, which was significantly higher than that in the other three groups ($p < 0.05$). Notably, this genus helped to reduce the risk of cardiovascular disease, ulcerative colitis, and irritable bowel syndrome, indicating that the fermented DF from soybean dregs was more effective in increasing *Prevotella* spp. in the intestine of mice (Xue & Ren, 2019).

Among the less abundant genus, *Lactobacillus* spp. was significantly higher in the F group than in the NF group ($p < 0.05$). Particularly, previous studies have shown that *Lactobacillus* spp. play important roles in improving the intestine, promoting intestinal peristalsis, and helping digestion and absorption of food (Huang et al., 2022). Similarly, the abundance of *Paramecium* spp. in the F group was significantly higher than that in the other three groups ($p < 0.05$), at 5.06%. Specifically, it was 1.75% higher than that in the NF group. Furthermore, studies have demonstrated that *Paramecium* spp. could reduce intestinal inflammation and enhance mitochondrial and ribosomal activity in colon cells (Lai et al., 2022). The abundance of *Alistipes* spp. was significantly higher in the model and NF groups than in the blank and F groups ($p < 0.05$), implying that the DF could only decrease this genus after fermentation. Notably, research has shown that *Alistipes* were found in the intestinal flora of mice with cancer, and decreasing the abundance of this genus can improve the body's immunity and inhibit tumor growth (Goubet et al., 2018). The abundance of Prevotellaceae_UCG-001 in the F group was significantly higher than in the other three groups ($p < 0.05$). When studying how inulin could alleviate glucose and lipid metabolism disorders, Song et al. (2019) found that this genus can be metabolized using DFs, thereby improving metabolism disorders. Notably, the abundances of *unclassified_f_Lachnospiraceae*, *unclassified_f_Prevotellaceae*, *Rikenellaceae_RC9_gut_group*, and *norank_f_norank_o_Clostridia_vadinBB60_group* were significantly lower in the F group than in the NF group ($p < 0.05$), implying that the fermented DF from soybean dregs could decrease the abundance of these genera.

3.9. Intestinal short-chain fatty acid content in mice

As shown in Table 3, the total acid content was reduced by 44.11 μmol/g ($p < 0.05$) in the model group compared with the blank group. Additionally, both the NF and F groups significantly increased in total acid content by 12.06 μmol/g and 21.49 μmol/g, respectively, compared to the model group. Notably, the main metabolites of DF when fermented by gut bacteria are short-chain fatty acids. These fatty acids play key roles in regulating host metabolism, lowering colonic pH, improving intestinal barrier function, and lowering cholesterol levels (Wang et al., 2021). In this study, the total acid content of the F group was significantly higher than that of the NF group. As SDF content was higher in fermented DF from soybean dregs, it was more easily fermented and degraded by gut bacteria, thereby producing more acid. Studies have demonstrated that short-chain fatty acids can stimulate intestinal peristalsis (Sun et al., 2018). Particularly, the acetic acid content in the F group was significantly higher than that in the NF group. Therefore, we speculated that this could be attributed to an increase in *Prevotella* spp. abundance because the main fermentation products of *Prevotella* spp. are acetic and succinic acids. Additionally, butyric acid was significantly higher in the F group than in the NF group. Butyric acid-producing bacteria mainly reside in the cecum and colon and belong to the genera *Clostridium*, *Eubacterium*, and *Fusobacterium*. This implies that the fermented DF from soybean dregs can improve the growth and reproduction of these bacteria. However, no significant difference was observed between the two groups regarding propionic, isobutyric, valeric, and isovaleric acids.

4. Conclusion

In this study, soybean dregs were bio-transformed through solid-state fermentation using *Trichoderma* spp., and changes in content and physicochemical properties of DF from soybean dregs before and after fermentation were analyzed. Additionally, the laxative effects of DF from soybean dregs were evaluated using a mouse model, and changes in the intestinal flora of mice and short-chain fatty acids were analyzed. We found that the content of SDF in soybean dregs increased after fermentation compared to before fermentation, whereas that of IDF decreased. Furthermore, the rehydration ratio, dissolution rate, expansion force, and the oil holding capacity of DF significantly increased with finer microstructure, indicating that the DF could further promote intestinal peristalsis in mice, delay defecation, and increase the amount of defecation and fecal moisture content. Moreover, we observed that the

Table 3
Content of short-chain fatty acids in mice feces (μmol/g).

Name	Blank	Model	NF	F
Acetic acid	28.24 ± 1.79^{a}	13.68 ± 2.97^{d}	18.90 ± 3.48^{c}	22.31 ± 2.24^{b}
Propionic acid	15.60 ± 1.11^{a}	5.07 ± 1.65^{c}	7.57 ± 1.03^{b}	8.13 ± 1.99^{b}
Isobutyric acid	2.74 ± 0.59^{a}	1.43 ± 0.40^{c}	1.59 ± 0.33^{bc}	2.20 ± 0.67^{ab}
Butyric acid	26.96 ± 1.04^{a}	11.64 ± 0.96^{d}	14.91 ± 1.02^{c}	19.86 ± 1.03^{b}
Isovaleric acid	2.35 ± 0.47^{a}	1.43 ± 0.37^{b}	1.69 ± 0.31^{b}	1.72 ± 0.33^{b}
Valeric acid	2.91 ± 0.65^{a}	1.46 ± 0.38^{b}	2.12 ± 0.30^{b}	1.97 ± 0.84^{b}
Total acid	78.81 ± 5.65^{a}	34.70 ± 6.73^{d}	46.76 ± 6.47^{c}	56.19 ± 7.09^{b}

Note: Different small letters in the same row indicated significant differences between groups ($p < 0.05$).

fermented DF from soybean dregs was more effective in balancing and regulating intestinal microbiota in mice and increasing the content of short-chain fatty acids in mice intestines. Therefore, in future research, other than developing a series of functional products comprising DF from soybean dregs to improve its social and economic benefits, its physiological functions and mechanism of action should be further studied.

CRediT authorship contribution statement

Li Wu: Conceptualization, Methodology, Investigation, Visualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Chunhong Tang:** Conceptualization, Methodology, Project administration, Funding acquisition, Writing – review & editing. **Linli Chen:** Resources, Supervision, Formal analysis. **Jiuyi Zhao:** Validation, Investigation, Data curation, Writing – original draft.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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