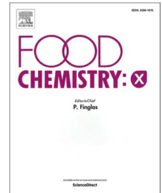
Food Chemistry: X 24 (2024) 101725

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of extraction methods of polysaccharides from *Tricholoma mongolicum* Imai on digestion and fecal fermentation *in vitro*

Bing Yang [a,*,1], Xinyu Zhang [a,1], Jingbo Zhu [a], Qunjun Wu [b], Boxiang Yang [a], Bimal Chitrakar [a], Yaxin Sang [a,*]

[a] College of Food Science and Technology, Hebei Agricultural University, 289 Lingyusi Road, Baoding, Hebei 071001, PR China
[b] Taijiyuan Biotechnology co., Ltd., Hi-tech Development Zone, 725700, Xunyang, PR China

ARTICLE INFO

Keywords:
Tricholoma mongolicum Imai
Polysaccharides
Simulated digestion
Gut microbiota
Fecal fermentation

ABSTRACT

This study employed extraction methods, namely acid, alkaline, ultrasonic-assisted, hot-water, and dual enzyme-assisted extraction to extract polysaccharides from *Tricholoma mongolicum* Imai (TMIPs), and investigated them for intestinal digestion and fecal fermentation *in vitro*. Furthermore, using fructo-oligosaccharide as a positive prebiotic control, the impact of these TMIPs as carbon sources on the growth of *Lactobacillus* and *Bifidobacterium* in liquid culture was assessed. The results showed that all fractions transit through the gastrointestinal tract without degradation. Additionally, compared to the control group, the five polysaccharides significantly promoted the growth of probiotics, with a significant increase in short-chain fatty acid production after 48 h of fermentation. Furthermore, all five polysaccharides modulated the composition of gut microbiota. This offers theoretical guidance in the rational advancement of functional products derived from edible mushrooms, aiming to enhance gastrointestinal health in humans.

1. Introduction

Tricholoma mongolicum Imai is a type of mushroom that belongs to the Tricholomataceae family, which is cultivated in various regions of northern China. This particular fungus has gained significant popularity among individuals due to its exceptional qualities in both gastronomy and traditional medicine (Wang, Zhao, Li, Wang, & Shen, 2015). The utilization of polysaccharides derived from edible mushrooms as potential prebiotics is on the rise due to their unique ability to selectively promote the growth of lactobacilli and bifidobacteria in the digestive system, while remaining resistant to easy breakdown (Rezende, Lima, & Naves, 2021). In recent years, salivary gastrointestinal digestion and fecal fermentation of edible polysaccharides simulated *in vitro* have attracted more and more attention. *In vitro* digestion simulation can simulate the human digestive environment to some extent, overcoming the complexity and difficulty of *in vitro* digestion, and has the advantages of simplicity, safety, and repeatability. β-glucan is a primary component found in most mushroom polysaccharides that remains undigested until it reaches the colon (Araújo-Rodrigues, Sousa, Relvas, Tavaria, & Pintado, 2024), where it is metabolized by gut microbiota into several beneficial metabolites, including short-chain fatty acids (SCFAs) (Hu et al., 2023). SCFAs are not only essential for nutrient absorption but also play a vital role in various metabolic mechanisms that contribute to human health. The intestinal microbiota is the core microecosystem in the human intestine, maintaining normal human functions by resisting the invasion of various viral antigens. Nevertheless, the imbalance of the intestinal microecology can give rise to numerous diseases. The polysaccharides of edible fungi have aroused extensive attention and research due to their biological activities such as regulating the structure and composition of the intestinal microbiota and protecting intestinal functions (Fei et al., 2024).

This research employed various extraction methods, such as acid-, alkali-, ultrasonic-, hot water-, and dual enzymatic-techniques, to isolate polysaccharides from *Tricholoma mongolicum* Imai (TMIPs). The objective was to examine the digestion and fermentation behavior of TMIPs, which could potentially result in alterations in their biological activity, short-chain fatty acids production, and gut microbiota composition. This study outcomes can establish a significant groundwork for a more comprehensive understanding of how digestion and fermentation impact the characteristics and biological activity of TMIPs. This insight

* Corresponding authors.
E-mail addresses: yangbing4329@126.com (B. Yang), sangyx1418@163.com (Y. Sang).
[1] Xinyu Zhang and Bing Yang contributes equally to this work.

https://doi.org/10.1016/j.fochx.2024.101725
Received 4 June 2024; Received in revised form 4 August 2024; Accepted 9 August 2024
Available online 13 August 2024
2590-1575/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

may ultimately contribute to the advancement of TMIPs as functional foods and dietary supplements.

2. Materials and methods

2.1. Preparation of TMIPs

Tricholoma mongolicum Imai was purchased from Zhangjiakou, Hebei Province, China.

Polysaccharides were extracted using five established methods in the laboratory. These methods included acid-solvent extraction, alkali-solvent extraction, Enzyme-assisted extraction, ultrasound-assisted extraction, and hot water extraction. By applying these techniques to *Tricholoma mongolicum* Imai powder, we successfully obtained five distinct types of polysaccharides, namely TMIPs-Ac, TMIPs-Al, TMIPs-E, TMIPs-U, and TMIPs-H.

2.2. Resistance to α-amylase digestion

Referring to the method described by Akbari-Alavijeh et al. To serve as a positive control, fructo-oligosaccharides (FOS) was included in the testing alongside the TMIP samples. To evaluate the resistance of the five TMIPs to α-amylase, 20 mg of α-amylase was blended with a sodium phosphate buffer containing sodium chloride and the pH was adjusted to 4–8 (Chen, Chen, Yang, Yu, & Kan, 2019). To serve as a positive control, FOS was used instead of TMIPs. Following this, 5.0 mL of TMIPs solution (10 mg/mL, dissolved in sodium phosphate buffer) was mixed with 5.0 mL of α-amylase enzyme solution at varying pH levels. These mixtures were then subjected to incubation in a water bath at 37 °C for 1 h. During the digestion process, samples were collected from the mixtures at different time intervals (0, 0.25, 0.5, 0.75 and 1 h) to assess the levels of reducing sugar and total sugar. The extent of hydrolysis was calculated using these measurements, employing a method similar to the one used for simulating human gastric juice digestion.

The degree of hydrolysis in the samples was calculated using the following equation.

$$\textbf{Hydrolysis degree} = \left(\frac{\textbf{Released reducing sugar}}{\textbf{Total sugar} - \textbf{Initial reducing sugar}}\right) \times \mathbf{100\%}$$

In this equation, "released reducing sugar" denotes the variance in reducing sugar content at a specific time during digestion and the initial reducing sugar content. The initial reducing sugar content represents the quantity of reducing sugar present in the sample before digestion. The degree of hydrolysis is expressed as a percentage, signifying the proportion of the original reducing sugar that has been released during the digestion process.

2.3. Resistance to artificial human gastric juice digestion

Referring to the method described by (Akbari-Alavijeh, Soleimanian-Zad, Sheikh-Zeinoddin, & Sarwar, 2018). This section describes a process for determining the digestibility of five TMIPs and the positive control FOS using artificial human gastric juice. First, the artificial human gastric juice was prepared by dissolving KCl, NaCl, NaH_2PO_4, $Na_2HPO_4{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, and $CaCl_2{\cdot}2H_2O$ in 1 L of deionized water and adjusting the pH to 1–5. A solution of TMIPs or FOS with a concentration of 10 mg/mL was mixed proportionally with artificial gastric juice at each pH value. The mixture should be placed in a water bath at 37 °C for 6 h. Samples of the reaction mixture were taken at 0, 0.5, 1, 2, 4 and 6 h during the digestion process. The levels of reducing sugar and total sugar were measured using the DNS method (Geng et al., 2023) and the phenol-sulfuric acid method (Chen et al., 2023), respectively. The experiment was conducted thrice to ensure accuracy and reliability.

2.4. Resistance to artificial human intestinal fluid digestion

The simulated small intestinal digestion process followed the protocols detailed in earlier research (Li et al., 2020). Firstly, an intestinal electrolyte solution was prepared by dissolving of NaCl, $CaCl_2{\cdot}2H_2O$, and KCl in deionized water. The pH of the solution was adjusted to 7. Prepare 4% (*w*/w) bile salt solution and 7% (w/w) trypsin solution with deionized water. Subsequently, a mixture of intestinal electrolyte solution, 200 g of the 4% (w/w) bile salt solution, 100 g of the 7% (w/w) pancreatin solution, and 13 mg of trypsin was thoroughly mixed. The pH of the mixture was then adjusted to 7.5. This mixture was set aside for later use. The pH of this solution was adjusted to 7. A solution of either TMIPs or FOS was prepared and mixed with the simulated intestinal fluid at a 1:1 ratio at each pH. The mixture was then incubated in a water bath at 37 °C for 6 h. At 0, 1, 2, 4 and 6 h of digestion, a portion of the reaction mixture was extracted for analysis. The DNS method was employed to determine the content of reducing sugar, while the phenol-sulfuric acid method was used to measure total sugar (Ding et al., 2019). The experiment was conducted thrice to ensure accuracy and reliability. The degree of hydrolysis of the samples was calculated using the formula described above (Chen et al., 2019).

2.5. Microorganisms, conditions for culturing, and preparation of the inoculum

In order to assess the prebiotic activity of the five TMIPs components, two strains of Bifidobacterium were procured from China Industrial Microorganism Preservation and Management Center, namely *Bifidobacterium adolescentis* (CICC 6070), and *Bifidobacterium infantis* (CICC 6069). These strains were combined with the existing strains of L. *rhamnosus* and L. *acidophilus* from the laboratory's bacterial repository for this study.

2.6. Medium preparation for experiments on TMIPs

The prepared MRS Base medium without carbon source was autoclaved for 15 min at 121 °C under high pressure. Subsequently, different extraction methods prepared TMIPs (experimental groups), glucose (the positive control groups), and FOS (the prebiotic control group) were added to the MRS base medium as carbon sources, with a final concentration of 2.0% (*w/v*) for each carbon source. To eliminate the potential effects of high-temperature sterilization on the polysaccharide components and FOS structure of TMIPs, the above carbon sources were sterilized by ultraviolet sterilization according to the method described by (Chen et al., 2019). The sterilized carbon sources were spread evenly in sterile culture dishes and placed in a laminar flow cabinet for UV sterilization. Each sterilized carbon source was dissolved in 10 mL of pre-sterilized MRS base medium and prepared into MRS base medium containing different carbon sources with a concentration of 2.0% (*w/v*).

2.7. Stimulation of probiotic growth

In the prepared test tubes, five TMIPs samples, FOS, glucose, or glucose-free MRS base medium were added, which were inoculated with active bacterial suspension at a final volume of 2% (w/v) as described in Section 2.6. Subsequently, these cultures were incubated under anaerobic conditions at 37 °C for 48 h. The bacterial growth on various carbon sources was assessed by determining the colony-forming units (CFUs) after the designated incubation period.

2.8. In vitro fermentation of TMIPs

2.8.1. In vitro fermentation with human fecal inoculum

We collected fresh fecal samples from four healthy individuals (2 female and 2 males) aged between 18 and 28 years, who had not used antibiotics in the last three months. The simulated fermentation of

TMIPs was conducted as previously, with a slight modification (Fu et al., 2023). The fecal samples were diluted in sterile centrifuge tubes using sterile modified physiological saline solution to create a 10% fecal slurry (*w/v*) (Chen et al., 2018). Subsequently after homogenization and centrifugation (at 1000 rpm and 3 °C for 4 min), the liquid portion was collected.

Under identical conditions, a control group known as the blank group was established using sterile fermentation medium. The mixtures

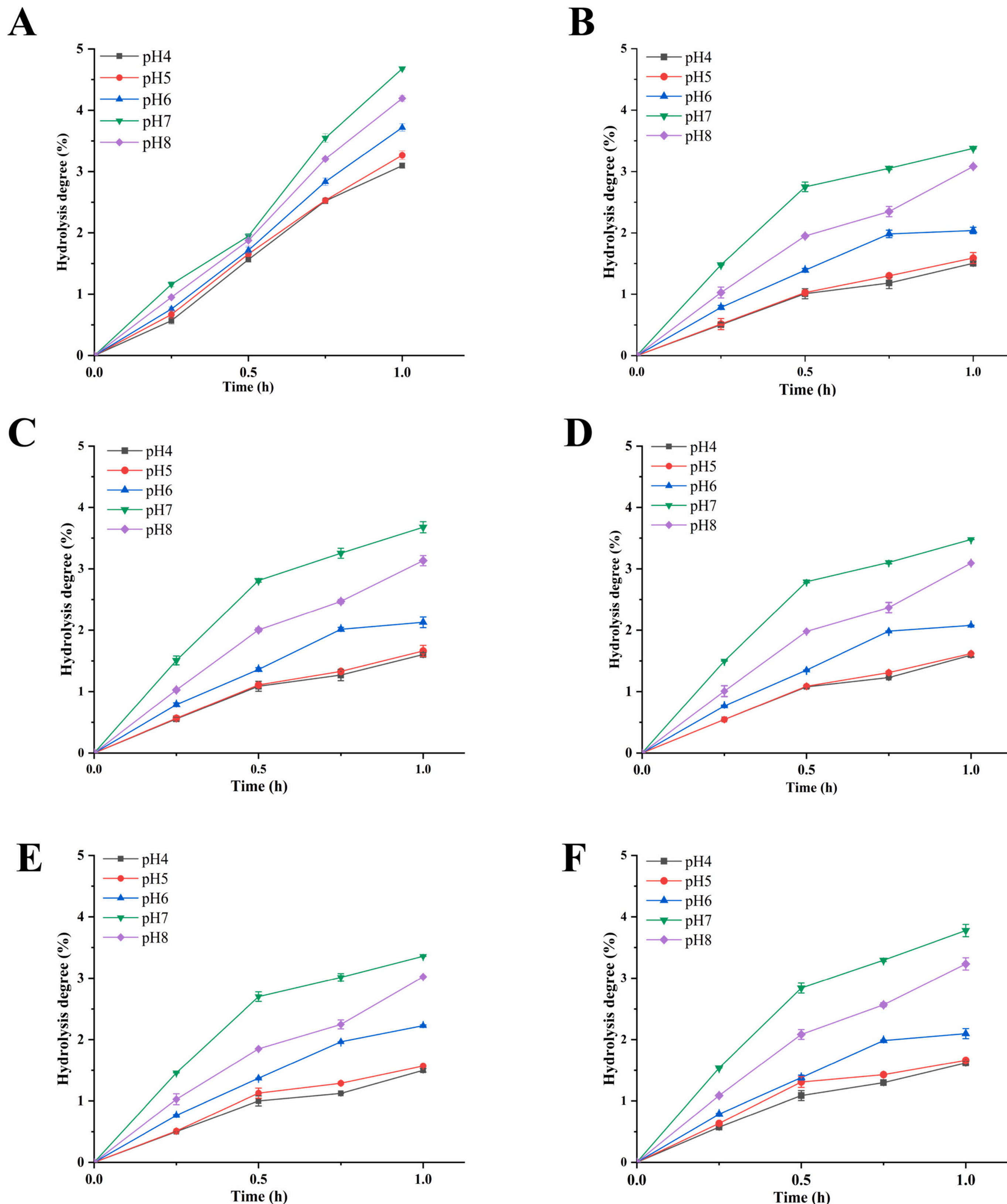


Fig. 1. Resistance of FOS and the five TMIPs to α-amylase. (A) FOS, (B) TMIPs-Ac, (C) TMIPs-Al, (D) TMIPs-E, (E) TMIPs-U, (F) TMIPs-H.

were incubated at 37 °C. Anaerobic conditions were maintained throughout the fecal fermentation process using an anaerobic box. Samples for fermentation were collected at 0, 6, 12 and 24 h to halt the fermentation process and assess the pertinent indicators. Each group was replicated three times.

2.8.2. Determination of pH and SCFAs during in vitro fermentation

The pH values of the supernatant were determined using a pH meter at fermentation times of 0, 6, 12 and 24 h (Liang et al., 2024).

Prior to measurement, the fermented samples were centrifuged at 4 °C and 4000 rpm for 15 min. At fermentation durations of 0, 6, 12 and 24 h, the concentrations of SCFAs were assessed utilizing a gas chromatography (GC) system.

2.9. Analysis of gut microbiota

Following 48 h of fermentation, the fermented solutions from each group was subjected to centrifugation, while the resulting pellets was stored at −80 °C for analysis of microbiota structure. Gene Denovo Biotechnology Co. conducted amplification analysis on the V3 - V4 region of bacterial 16S rDNA in each sample. In summary, genomic DNA was isolated from the samples, and the conserved region of rDNA was amplified using specific primers containing barcodes. Subsequently, the amplified PCR products were subjected to cutting and recovery, followed by quantification using a QuantiFluor™ fluorometer. The purified amplicons were mixed in equal amounts and used to construct sequencing libraries with sequencing adapters. Sequencing was performed using Illumina PE250. The MiSeq sequencing system (Illumina, USA) was employed for the sequencing process. Subsequently, sequence reads were demultiplexed and quality-filtered using FASTP. The evaluation of gut microbiota was carried out utilizing the QIIME microbial community analysis platform provided by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). This approach ensured a comprehensive and rigorous assessment within an academic context. To the analytical process to determine the changes in microbiota structure between the blank group, FOS group, and TMIPs groups.

2.10. Statistical analysis

Each experiment was replicated three times. Data was subjected to ANOVA analysis and deemed statistically significant at $p < 0.05$. Subsequent significance assessment was conducted using the Duncan test. Results were presented as mean ± standard deviation (SD).

3. Results and discussion

3.1. Digestibility of TMIPs by human α-amylase

To evaluate the resilience of polysaccharides in the digestive fluid, it is possible to ascertain the polysaccharide hydrolysis rate (extent of hydrolysis over time) by monitoring the changes in total carbohydrates and reducing sugars during simulated digestion. The digestion of the five TMIPs products was assessed by incubating them with α-amylase at pH 4–8. The results of this evaluation are presented in Fig. 1. As the incubation time increased, the degree of hydrolysis for both the TMIPs and FOS products also increased, reaching the maximum after 1 h of incubation. The degradation of the five polysaccharides intensified in correlation with the pH sequence: 7 > 8 > 6 > 5 > 4, suggesting that the five TMIPs isolates exhibited superior enzymatic stability when compared to FOS, while implying their robust stability during incubation with α-amylase. Additionally, there were no notable discrepancies in the degree of hydrolysis among the five polysaccharides ($p > 0.05$), indicating that the enzymatic stability of TMIPs remained unaffected by the diverse extraction processes. Therefore, these five TMIPs can enter the stomach without being substantially degraded by human saliva.

3.2. Digestibility of TMIPs by human gastric juice and small intestinal juice

Fig. 2B-F presents the findings on the digestibility of five TMIPs, highlighting their degree of hydrolysis against time in human gastric juices at varying pH levels. In contrast to FOS (Fig. 2A), all five TMIPs demonstrated an outstanding resilience against hydrolysis in simulated gastric juice conditions. While a decrease in pH in the gastric juice environment led to a marginal increase in the degree of hydrolysis for the TMIPs, their indigestibility remained significantly higher than FOS. Additionally, there was no notable disparity in the digestibility of the five polysaccharides ($p > 0.05$), suggesting that the extraction procedures did not significantly impact their degradability in the digestive system. This suggests that the five TMIPs have the potential to safely traverse the gastric tract and reach the intestine with minimal degradation by human gastric juices. This hydrolytic resilience aligns with previous observations of polysaccharides derived from another edible mushroom species (*Volvariella volvacea*), which also exhibited resistance to simulated human gastric juice (Hu et al., 2023).

Fig. 2G shows the digestion rate of the five TMIPs in simulated human intestinal fluid, expressed as the extent of hydrolysis over time. Compared to the FOS, all five TMIPs exhibited strong resistance to artificial intestinal digestion, with hydrolysis rates increasing with time. However, the maximum value was significantly lower than that of the FOS group. Moreover, there was no significant difference in the digestion rates of the five polysaccharides ($p > 0.05$), indicating that the five extraction processes did not significantly affect the digestion rates of TMIPs. This suggested that these five TMIPs can safely reach the intestine and be utilized by intestinal microbiota.

3.3. Beneficial properties of TMIPs

3.3.1. Effects of different TMIPs on probiotic growth

As shown in Fig. 3A-D, after 24 h of fermentation, the population size of four probiotics on a glucose-free MRS base medium showed only a marginal increase compared to 0 h. This finding suggested that the glucose-free MRS base medium employed in this research was a dependable option for conducting prebiotic activity experiments (Choque Delgado & Tamashiro, 2018). In contrast to the control, the five TMIPs and FOS exhibited a notable enhancement in the growth of these four probiotics during the 24 h fermentation period ($p < 0.01$). Yet, the rapid metabolism of glucose by probiotics led to reduced growth of these four bacterial species in MRS medium supplemented with TMIPs or FOS in comparison to the glucose-supplemented medium after 24 h ($p < 0.05$). After 48 h of fermentation, the size of bacterial populations had obviously decreased in media supplemented with glucose, whereas there was only a slight decrease in populations growth in TMIPs. The heightened cell mortality in the glucose-containing medium can be ascribed to the swift exhaustion of glucose, resulting in an acidic environment that is unsuitable for the proliferation of probiotic bacteria because they are highly susceptible to pH fluctuations during fermentation (Wu et al., 2022). On the other hand, the delayed metabolism of TMIPs can be attributed to its intricate structure, triggering diverse metabolic pathways and extending the survival of probiotic bacteria.

3.3.2. TMIPs on the acid production (pH) of probiotics

When carbohydrates are fermented by probiotic bacteria as a carbon source, acidic metabolites such as SCFAs (lactic acid and acetic acid) are produced. This leads to a decrease in the pH of culture medium. Therefore, pH value of the medium can to some extent reflect the utilization of carbon sources by probiotic bacteria (Shang et al., 2018). The drop in pH value is mainly due to the acid produced by biotic fermentation. It was observed that the initial pH value of culture medium with TMIPs as a carbon source (extracted using different methods) was significantly higher than that of the blank control group and the culture medium with FOS and glucose as a carbon source ($p < 0.05$). This could

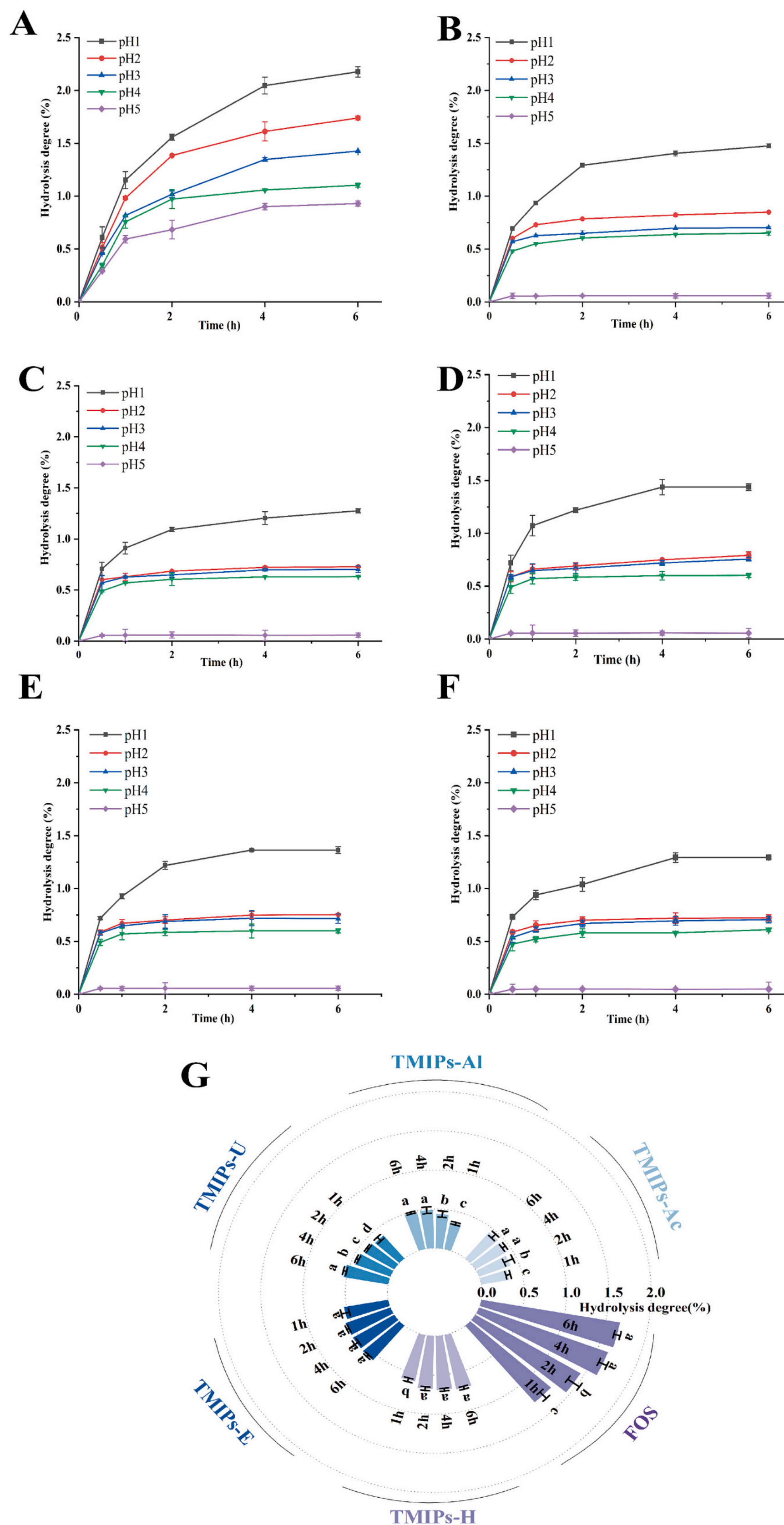


Fig. 2. Resistance of FOS and the five TMIPs to artificial gastric juice (A-F) and small intestinal juice (G). The results are presented for (A) FOS, (B) TMIPs-Ac, (C) TMIPs-Al, (D)TMIPs-E, (E)TMIPs-U, (F) TMIPs-H, (G) Resistance of FOS and the five TMIPs fractions to small intestinal juice.

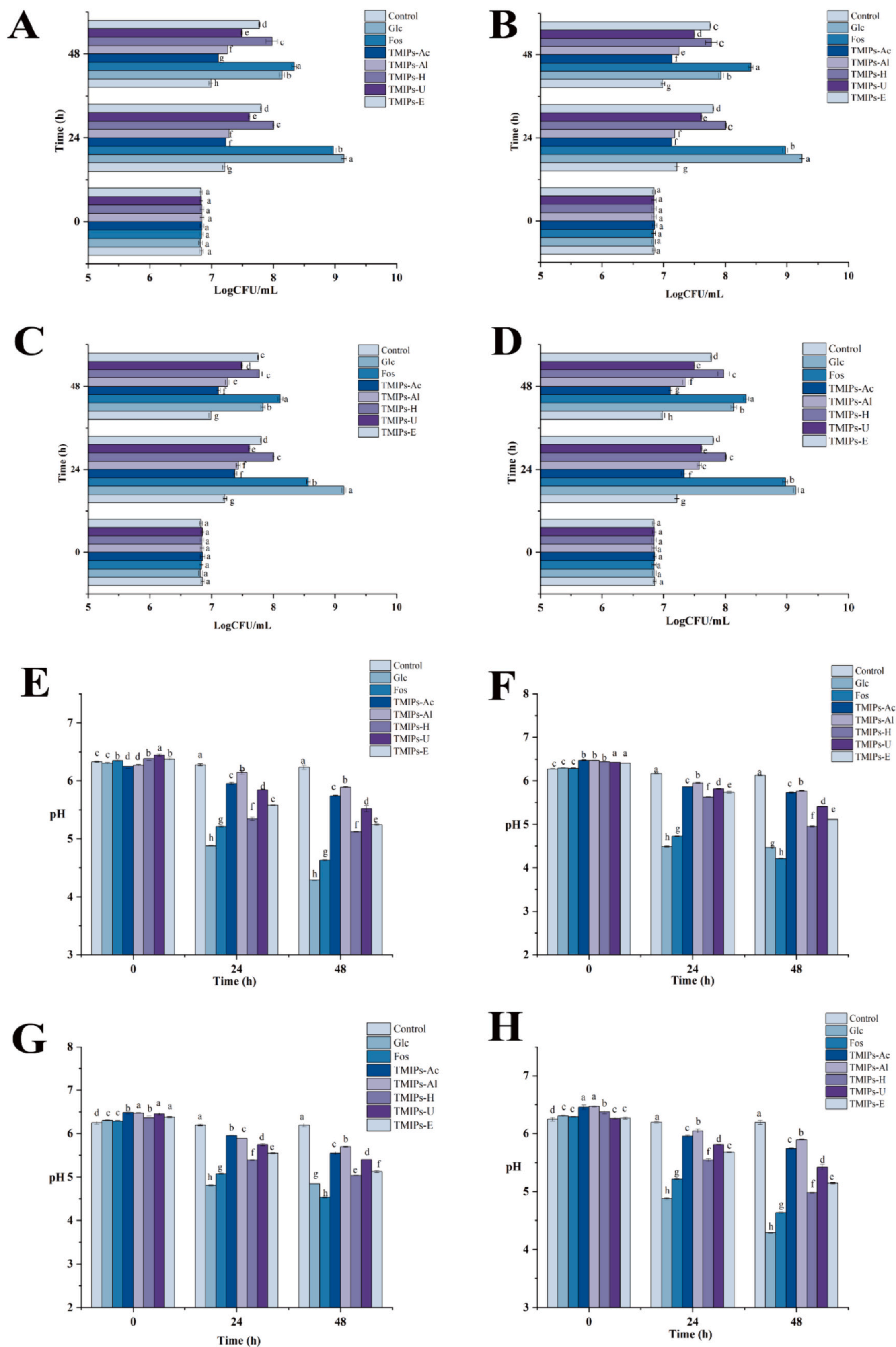


Fig. 3. The impact of TMIPs on the proliferation (A-D) and pH (*E*-H) of probiotics in a glucose-free MRS base medium in comparison to FOS. (A) *L. rhamnosus*, (B) *B. infantis*, (C) *B. adolescentis*, and (D) *L. acidophilus.* (E) *L. rhamnosus*, (F) *B. infantis*, (G) *B. adolescentis*, and (H) *L. acidophilus.*

be because of the presence of a certain amount of protein in the TMIPs (Zhang et al., 2022). After 24 h of fermentation, pH value of the blank control group only slightly decreased, while pH values of the glucose and FOS groups significantly decreased ($p < 0.05$). Particularly, the pH value of the glucose group decreased sharply. However, the pH reduction in the five TMIPs groups was significantly lower than that of the glucose and FOS groups, indicating that glucose and FOS were more easily utilized by probiotic bacteria. Comparing with pH value of the culture medium before fermentation, pH value of the blank control group only slightly decreased after 48 h of fermentation. In contrast, pH values of the FOS, glucose, and five TMIPs groups all significantly decreased ($p < 0.05$). However, pH values of the five TMIPs were significantly higher than those of the FOS and glucose groups. Additionally, among the five TMIPs, the TMIPs-H and TMIPs-E groups showed a greater decrease in pH value ($p < 0.05$). These results indicated that FOS, glucose, and the five TMIPs can be utilized by the four probiotic strains. In summary, the results in Fig. 3 *E*-H clearly indicated that the above five TMIPs were not only non-toxic to the tested probiotic bacteria, but were also actually good substrates that promoted the growth of the four probiotic bacteria.

3.3.3. Production of SCFAs

The accumulation of SCFAs in cultures of the four probiotic strains under various carbon sources after 48 h of fermentation is summarized in Table 1. These SCFAs represented the main metabolic end products, which indicated both the proliferation of probiotics and the utilization of carbon sources by them. The results revealed that, compared to the blank control group, the use of TMIPs as a carbon source in the culture medium altered the SCFAs profile, leading to the production of lactic acid, acetic acid, propionic acid, and butyric acid. A significant increase in the total concentration of SCFAs ($p < 0.05$) was observed in the cultures using TMIPs as the carbon source, compared to the control cultures. This confirms that probiotic bacteria were able to utilize TMIPs to sustain their survival and metabolic activity.

3.4. Impacts of TMIPs in vitro fecal fermentation

Multiple research studies have indicated that numerous plant polysaccharides are resistant to digestion in the human gastrointestinal tract but can be fermented by colonic microbiota, resulting in the beneficial production of SCFAs. Previous *in vitro* digestion experiments have demonstrated that TMIPs exhibit strong stability when exposed to α-amylase, simulated gastric and intestinal fluid tests suggest that TMIPs have a potential to reach the colon. However, there is still uncertainty regarding the extent to which intestinal microbiota utilize TMIPs. Therefore, further investigation into the *in vitro* fermentability of TMIPs is essential.

3.4.1. Impacts of TMIPs in fecal fermentation on pH

Change in pH value are crucial indicators for monitoring the fermentation process. During the fermentation of polysaccharides by intestinal flora, acidic fermentation end-products, such as lactic acid and various SCFAs were predominantly produced. The production of these products led to a reduction in intestinal pH, thereby impacting the composition of gut microbiota.

From Fig. 4, compared with the control group, the fermentation of

Table 1
SCFAs profile in liquid cultures of four probiotic strains after fermentation for 48 h with different carbon sources.

Bacteria	Carbon source	Lactic acid(mmol/L)	Acetic acid(mmol/L)	Propionic acid(mmol/L)	Butyric acid(mmol/L)	Total(mmol/L)
L. rhamnosus	Control	4.66 ± 0.25^{e}	1.55 ± 0.02^{g}	0.92 ± 0.01^{f}	0.72 ± 0.01^{e}	7.85 ± 0.25^{g}
	Glucose	52.68 ± 1.31^{a}	4.38 ± 0.30^{f}	3.04 ± 0.16^{c}	3.02 ± 0.25^{b}	63.12 ± 2.02^{a}
	FOS	16.55 ± 0.87^{bc}	15.77 ± 0.49^{a}	5.52 ± 0.36^{a}	2.86 ± 0.37^{b}	40.70 ± 2.09^{b}
	TMIPs-Ac	7.19 ± 0.69^{d}	5.88 ± 0.19^{d}	2.12 ± 0.23^{e}	2.21 ± 0.08^{c}	17.41 ± 1.19^{f}
	TMIPs-Al	6.58 ± 0.54^{d}	5.35 ± 0.26^{e}	2.83 ± 0.36^{d}	1.86 ± 0.06^{d}	16.62 ± 1.22^{f}
	TMIPs-E	17.91 ± 0.75^{b}	8.07 ± 0.24^{b}	3.54 ± 0.14^{b}	2.48 ± 0.29^{bc}	32.00 ± 1.42^{c}
	TMIPs-U	6.13 ± 0.27^{d}	7.83 ± 0.32^{b}	3.06 ± 0.17^{c}	3.75 ± 0.11^{a}	20.77 ± 0.87^{e}
	TMIPs-H	15.80 ± 0.99^{c}	6.54 ± 0.29^{c}	2.97 ± 0.25^{c}	2.69 ± 0.28^{b}	27.00 ± 1.81^{d}
L.acidophilus	Control	5.20 ± 0.46^{f}	1.43 ± 0.01^{g}	0.96 ± 0.01^{f}	0.38 ± 0.01^{d}	7.97 ± 0.49^{g}
	Glucose	55.07 ± 1.09^{a}	3.63 ± 0.35^{f}	2.86 ± 0.26^{d}	2.48 ± 0.11^{b}	64.04 ± 1.81^{a}
	FOS	12.46 ± 0.89^{b}	12.56 ± 0.35^{a}	4.37 ± 0.24^{a}	2.33 ± 0.17^{b}	32.72 ± 1.65^{b}
	TMIPs-Ac	8.30 ± 0.85^{d}	5.02 ± 0.37^{d}	2.23 ± 0.22^{e}	2.00 ± 0.43^{bc}	17.55 ± 1.87^{e}
	TMIPs-Al	6.85 ± 0.79^{e}	4.67 ± 0.01^{e}	2.19 ± 0.17^{e}	1.76 ± 0.14^{c}	15.17 ± 1.11^{f}
	TMIPs-E	13.05 ± 1.09^{b}	7.99 ± 0.30^{b}	3.89 ± 0.14^{b}	3.72 ± 0.32^{a}	28.43 ± 1.85^{c}
	TMIPs-U	8.67 ± 0.24^{c}	6.12 ± 0.16^{c}	3.32 ± 0.09^{c}	2.44 ± 0.06^{b}	20.55 ± 0.55^{d}
	TMIPs-H	12.19 ± 0.89^{b}	7.69 ± 0.20^{b}	3.41 ± 0.32^{c}	3.92 ± 0.25^{a}	27.21 ± 1.68^{c}
B. adolescentis	Control	1.80 ± 0.02^{f}	1.50 ± 0.01^{f}	1.24 ± 0.01^{f}	0.81 ± 0.02^{f}	5.35 ± 0.06^{f}
	Glucose	19.91 ± 0.61^{a}	7.19 ± 0.23^{e}	5.16 ± 0.34^{b}	3.64 ± 0.12^{e}	35.18 ± 1.30^{b}
	FOS	11.53 ± 0.48^{b}	19.01 ± 1.14^{a}	5.74 ± 0.48^{a}	6.91 ± 0.21^{b}	43.19 ± 2.31^{a}
	TMIPs-Ac	8.79 ± 0.21^{d}	7.74 ± 0.12^{e}	2.14 ± 0.02^{e}	1.80 ± 0.36^{f}	20.47 ± 0.71^{e}
	TMIPs-Al	4.09 ± 0.13^{e}	10.22 ± 0.37^{c}	3.04 ± 0.41^{d}	4.70 ± 0.37^{d}	22.05 ± 1.28^{e}
	TMIPs-E	10.69 ± 0.42^{b}	13.48 ± 0.94^{b}	5.96 ± 0.23^{a}	7.70 ± 0.06^{a}	31.83 ± 0.75^{c}
	TMIPs-U	9.18 ± 0.47^{c}	8.81 ± 0.06^{d}	4.12 ± 0.64^{d}	3.40 ± 0.15^{e}	25.71 ± 1.62^{d}
	TMIPs-H	11.50 ± 0.68^{b}	9.47 ± 0.38^{c}	4.40 ± 0.06^{c}	5.85 ± 0.35^{c}	37.22 ± 1.65^{b}
B. infantis	Control	2.36 ± 0.06^{f}	1.52 ± 0.01^{g}	1.52 ± 0.02^{g}	0.89 ± 0.02^{f}	6.29 ± 0.11^{e}
	Glucose	20.74 ± 0.59^{a}	4.33 ± 0.26^{f}	3.54 ± 0.13^{d}	2.05 ± 0.17^{d}	30.66 ± 1.35^{a}
	FOS	10.52 ± 0.38^{b}	11.92 ± 0.89^{a}	5.31 ± 0.18^{a}	5.63 ± 0.21^{a}	33.38 ± 1.66^{a}
	TMIPs-Ac	5.67 ± 0.25^{d}	4.68 ± 0.22^{f}	2.42 ± 0.26^{f}	1.39 ± 0.34^{e}	14.16 ± 0.87^{d}
	TMIPs-Al	4.53 ± 0.11^{e}	5.05 ± 0.17^{e}	2.54 ± 0.34^{f}	1.29 ± 0.03^{e}	13.23 ± 0.65^{d}
	TMIPs-E	7.27 ± 0.53^{c}	7.23 ± 0.04^{b}	4.93 ± 0.16^{b}	2.72 ± 0.30^{b}	22.15 ± 1.03^{c}
	TMIPs-U	9.83 ± 0.57^{b}	5.59 ± 0.08^{d}	3.22 ± 0.17^{e}	2.44 ± 0.30^{c}	21.08 ± 1.12^{c}
	TMIPs-H	10.64 ± 0.85^{b}	6.05 ± 0.17^{c}	4.40 ± 0.06^{c}	2.92 ± 0.25^{b}	24.01 ± 1.33^{b}

Note:All results are reported as the mean ± standard deviation of three independent experiments.
Means in the same column with different letters indicate significant differences for each probiotic strain. ($p < 0.05$).

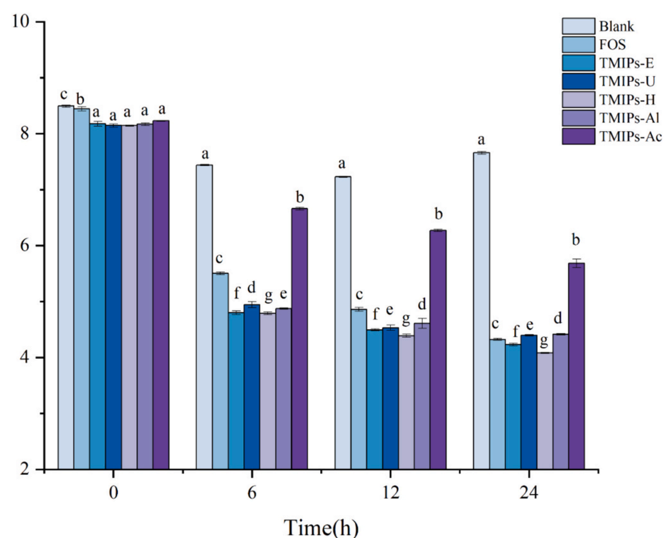


Fig. 4. Change in pH value during fermentation.

TMIPs had a lower initial pH value. This might be due to the presence of a small amount of uronic acid in TMIPs. With the progress of fermentation, pH value of the TMIPs fermentation solution continued to decrease, while pH value of the blank fermentation solution first decreased and then slightly increased. Throughout the entire fermentation process, the pH value of the TMIPs fermentation solution is always lower than that of the blank fermentation solution. According to the research, the production of SCFAs reduces the pH of surrounding environment, which is beneficial for the growth of bifidobacteria and lactic acid bacteria (Liu et al., 2021).

These results indicated that the fermentation process of TMIPs had a significant impact on pH value of the gut microbiota environment. The significant decrease in pH value in the TMIPs fermentation solution was related to the production of lactic acid and SCFAs by the gut microbiota during the TMIPs fermentation process (Luo et al., 2023). Polysaccharides can gradually and continuously regulate the intestinal microenvironment to produce SCFAs (Li et al., 2023; Liu et al., 2021).

3.4.2. Impacts of TMIPs on production of SCFAs

Many types of polysaccharides cannot be digested by the human upper digestive tract but can reach the cecum and colon, where they are consumed and utilized by specific gut microbiota to produce SCFAs (Sivaprakasam, Prasad, & Singh, 2016), mainly including lactic acid, acetic acid, propionic acid, and butyric acid, which play an important role in maintaining the normal morphology of colonic epithelial cells and the normal function of the colon (Liu et al., 2021). Acetic acid has been recognized as a valuable energy source for the gut microbiota (Chen et al., 2023; Tang et al., 2024), while propionic acid has demonstrated its efficacy in reducing serum cholesterol levels, regulating blood lipid levels, and enhancing tissue insulin sensitivity (Han et al., 2022). Furthermore, n-butyric acid plays diverse physiological roles such as preserving the integrity and functionality of the intestinal barrier; suppressing immune responses, and exhibiting anti-cancer and anti-inflammatory properties (Ge et al., 2024; Liu et al., 2021). Therefore, in this experiment, the concentrations of SCFAs were measured during the degradation process of TMIPs extracted with different methods, in order to further confirm that TMIPs were decomposed and utilized by gut microbiota. From Table 2, it can be seen that lactic acid, acetic acid, and propionic acid are the major SCFAs in the blank, FOS, and TMIPs groups. However, the concentrations of these three SCFAs were significantly different in the three different experimental groups.

Comparing the TMIPs group with the blank group in Table 2, it was observed that although the concentrations of lactic acid, acetic acid, propionic acid, and total SCFAs increased with fermentation time in both groups, the TMIPs group had significantly higher concentrations of lactic acid, acetic acid, propionic acid, and total SCFAs during the 6–24 h fermentation period, compared to the blank control group ($p < 0.05$). This indicated that TMIPs significantly promoted the production of SCFAs by gut microbiota, with lactic, acetic, and propionic acid being the main SCFAs products.

The differences in SCFAs concentration among different groups are caused by the inherent properties of the carbon compounds provided in the fermentation medium (Lv et al., 2022). Due to the difference of material structure and molecular weight, polysaccharides from different sources can promote the growth of certain bacteria; produce different metabolites and exhibit different fermentation characteristics during *in vitro* fermentation (Ou et al., 2022). It is known that the control group, due to the lack of carbon compound supply, undergoes a slow fermentation process, resulting in insufficient production of SCFAs. FOS are considered to be a type of prebiotic that can stimulate the growth of beneficial bacteria in the human body and help maintain a healthy balance of gut microbiota (Markowiak-Kopeć & Śliżewska, 2020). The five TMIPs were acted upon by intestinal microbiota during fermentation process, breaking down into monosaccharides. These liberated monosaccharides are utilized by microorganisms to produce SCFAs as the end products.

These results indicated that both TMIPs-E and TMIPs-H exhibited significant probiotic activity, and served as potential carbon sources for the prebiotics, primarily exerting beneficial effects on human health through the production of SCFAs.

3.4.3. Impacts of TMIPs on gut microbiota

Human intestinal microbiota plays a critical role in human health, nutrition, and energy regulation (Thomson, Garcia, & Edwards, 2021). Understanding the relationship between TMIPs, the gut microbiota and its metabolites play a pivotal role in enhancing human health and preventing diseases, primarily through modulating the composition and function of the microbiota (Payling, Fraser, Loveday, Sims, & Mcnabb, 2020). To evaluate the effects of TMIPs on the gut microbiota, we performed high-throughput sequencing analysis on samples from both the TMIPs and FOS groups after a 48 h fermentation period.

3.4.3.1. Gut microbiota α and β diversity of samples cultivated in vitro. The relationship between TMIPs and the human gut microbiota was studied, aiming to regulate gut health and prevent diseases through the use of TMIPs. The diversity of the gut microbiota can be reflected by alpha diversity analysis of each sample's community, which assesses their richness and diversity. As shown in Fig. 5, several statistical metrics were used to quantify the species richness and diversity of the gut microbiota, following the fermentation of different extracts of TMIPs. These metrics included the Simpson index, Shannon index, and Sobs index, all of which can reflect the microbial community's richness and biodiversity (Zhao et al., 2024). The Shannon index and Simpson index are commonly used to evaluate the richness and evenness of the species, providing a comprehensive measure of diversity (Xu et al., 2025). Higher values of these indices indicate increased species richness and more balanced distributions, indicating greater diversity. The Sobs index primarily focuses on the richness of a sample, specifically the count of detected OTUs. Additionally, due to the completely different calculation methods of different statistical analysis indices, it is normal that the change trends may not be completely consistent (Zhang et al., 2024). The PCoA plot demonstrates clear clustering of gut microbiota compositions obtained from various sample groups, indicating distinct differences. Across various carbohydrate groups, it was evident that the gut microbiota structure was more similar within the five TMIPs groups. This indicated that gut microbiota composition changed under FOS and the five TMIPs, respectively.

3.4.3.2. Influence of TMIPs on gut microbiota. According to Fig. 6A, the

Table 2
The molar concentration of SCFAs in different fermentation cultures.

Sample	Time (h)	Lactic acid (mmol/L)	Acetic acid (mmol/L)	Propionic acid (mmol/L)	n-butyric acid (mmol/L)	*i*-butyric acid (mmol/L)	n-valeric acid (mmol/L)	*i*-valeric acid (mmol/L)	Total (mmol/L)
Blank	0	0.59 ± 0.04[d]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.59 ± 0.04[d]
	6	0.89 ± 0.12[c]	2.73 ± 0.10[c]	0.58 ± 0.03[c]	0.36 ± 0.01[c]	N.D.	N.D.	N.D.	4.56 ± 0.26[c]
	12	1.28 ± 0.10[b]	3.78 ± 0.43[b]	1.27 ± 0.04[b]	0.59 ± 0.02[b]	N.D.	N.D.	N.D.	6.92 ± 0.59[b]
	24	1.59 ± 0.06[a]	6.03 ± 0.21[a]	1.54 ± 0.19[a]	0.75 ± 0.01[a]	N.D.	N.D.	N.D.	9.91 ± 0.47[a]
FOS	0	0.59 ± 0.04[d]	1.37 ± 0.22[d]	1.73 ± 0.01[d]	0.95 ± 0.06[b]	N.D.	N.D.	N.D.	4.64 ± 0.33[d]
	6	5.94 ± 0.41[c]	9.36 ± 0.54[c]	3.82 ± 0.41[c]	1.23 ± 0.29[a]	N.D.	N.D.	N.D.	20.35 ± 1.65[c]
	12	9.67 ± 1.03[b]	13.21 ± 0.63[b]	4.31 ± 0.26[b]	1.54 ± 0.34[a]	N.D.	N.D.	N.D.	28.73 ± 2.26[b]
	24	12.56 ± 0.42[a]	15.26 ± 0.31[a]	5.87 ± 0.69[a]	1.43 ± 0.14[a]	N.D.	N.D.	N.D.	35.12 ± 1.56[a]
TMIPs-Ac	0	0.59 ± 0.01[d]	1.96 ± 0.40[d]	0.87 ± 0.06[d]	0.60 ± 0.19[b]	N.D.	N.D.	N.D.	4.02 ± 0.68[d]
	6	3.65 ± 0.48[c]	3.51 ± 0.08[c]	1.37 ± 0.09[c]	0.86 ± 0.29[a]	N.D.	N.D.	N.D.	9.39 ± 0.94[c]
	12	4.85 ± 0.14[b]	5.36 ± 0.25[b]	2.31 ± 0.16[b]	1.20 ± 0.12[a]	N.D.	N.D.	N.D.	13.72 ± 1.67[b]
	24	6.82 ± 0.23[a]	8.45 ± 0.21[a]	3.88 ± 0.13[a]	1.26 ± 0.26[a]	N.D.	N.D.	N.D.	20.41 ± 2.83[a]
TMIPs-Al	0	0.59 ± 0.01[d]	1.92 ± 0.35[d]	0.76 ± 0.26[d]	0.75 ± 0.15[c]	N.D.	N.D.	N.D.	4.02 ± 0.77[d]
	6	1.38 ± 0.28[c]	3.43 ± 0.24[c]	1.08 ± 0.39[c]	0.93 ± 0.18[b]	N.D.	N.D.	N.D.	6.82 ± 1.09[c]
	12	3.30 ± 0.39[b]	5.37 ± 0.34[b]	2.05 ± 0.17[b]	1.28 ± 0.11[a]	N.D.	N.D.	N.D.	12.00 ± 1.01[b]
	24	5.59 ± 0.26[a]	7.27 ± 0.61[a]	3.12 ± 0.39[a]	1.23 ± 0.30[a]	N.D.	N.D.	N.D.	17.21 ± 1.56[a]
TMIPs-E	0	0.96 ± 0.04[d]	2.05 ± 0.17[d]	0.82 ± 0.12[c]	0.95 ± 0.17[c]	N.D.	N.D.	N.D.	4.78 ± 0.50[d]
	6	4.38 ± 0.39[c]	3.12 ± 0.39[c]	1.02 ± 0.14[c]	1.52 ± 0.20[b]	N.D.	N.D.	N.D.	10.04 ± 1.12[c]
	12	6.08 ± 0.58[b]	6.35 ± 0.20[b]	3.26 ± 0.09[b]	1.64 ± 0.08[a]	N.D.	N.D.	N.D.	17.33 ± 2.95[b]
	24	8.08 ± 0.27[a]	13.75 ± 0.03[a]	4.57 ± 0.23[a]	1.78 ± 0.14[a]	N.D.	N.D.	N.D.	28.18 ± 1.67[a]
TMIPs-U	0	0.74 ± 0.06[d]	1.42 ± 0.31[d]	0.92 ± 0.12[c]	0.63 ± 0.03[c]	N.D.	N.D.	N.D.	3.71 ± 0.52[d]
	6	2.86 ± 0.13[c]	4.36 ± 0.34[c]	1.10 ± 0.10[c]	0.97 ± 0.25[b]	N.D.	N.D.	N.D.	9.29 ± 0.82[c]
	12	5.22 ± 0.21[b]	7.62 ± 0.28[b]	2.18 ± 0.26[b]	1.72 ± 0.21[a]	N.D.	N.D.	N.D.	16.74 ± 2.96[b]
	24	6.53 ± 0.52[a]	9.56 ± 0.35[a]	4.04 ± 0.14[a]	1.83 ± 0.48[a]	N.D.	N.D.	N.D.	21.96 ± 3.49[a]
TMIPs-H	0	0.71 ± 0.07[d]	1.99 ± 0.72[d]	0.95 ± 0.03[d]	0.69 ± 0.25[c]	N.D.	N.D.	N.D.	3.80 ± 1.07[d]
	6	3.03 ± 0.17[c]	4.34 ± 0.43[c]	1.10 ± 0.1[c]	0.97 ± 0.05[b]	N.D.	N.D.	N.D.	9.44 ± 1.75[c]
	12	4.51 ± 0.19[b]	7.57 ± 0.85[b]	2.18 ± 0.26[b]	1.42 ± 0.13[a]	N.D.	N.D.	N.D.	15.68 ± 2.43[b]
	24	8.97 ± 0.41[a]	15.46 ± 0.54[a]	4.04 ± 0.14[a]	1.51 ± 0.09[a]	N.D.	0.17 ± 0.02	N.D.	29.98 ± 4.18[a]

microbial communities in each group primarily consisted of Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, which was consistent with a previous literature (Han et al., 2023). Following 48 h of fermentation, there were significant individual variations in phylum levels among the groups. Compared to the blank group, the abundances of Proteobacteria and Bacteroidota were increased after TMIPs and FOS treatment. Compared with the blank group, the abundance of Proteobacteria in TMIPs decreases. Proteobacteria are a distinct category of bacteria and are commonly found in the fecal microbiota of healthy individuals. An excessive abundance of Proteobacteria in the gut

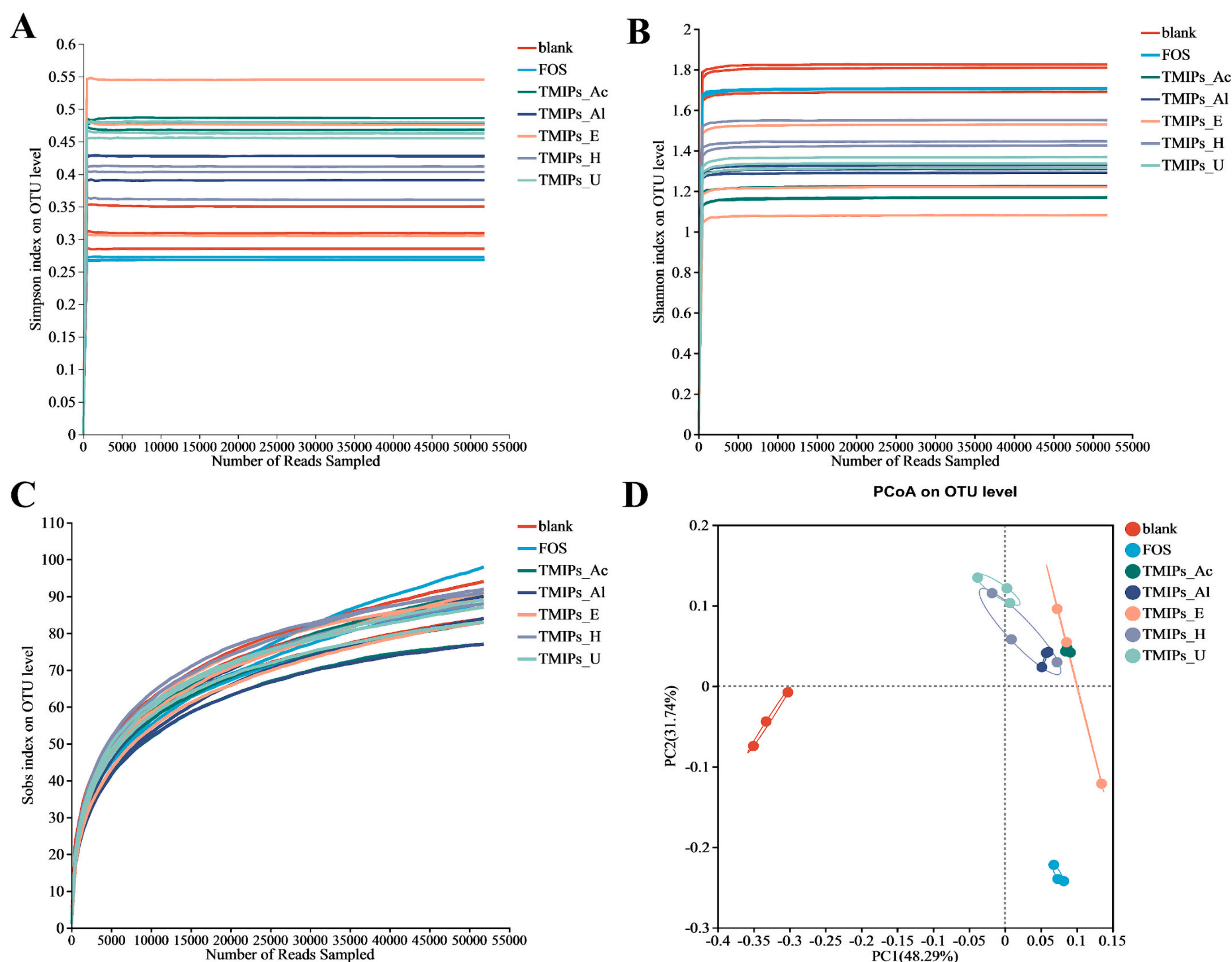


Fig. 5. Gut microbiota α and β diversity of samples cultivated *in vitro*. (A) Simpson; (B) Shannon; (C) Sobs indices, and (D) Principal coordinates analysis based on Bray-Curtis distance.

microbiome can lead to an imbalance in the microbial community, resulting in low-grade inflammation or, in some cases, chronic colitis (Turnbaugh et al., 2006). Therefore, the reduction in Proteobacteria abundance in the TMIPs group was considered beneficial for human health. It is noteworthy that compared with the blank group, the abundance of Bacteroidetes significantly increased after adding TMIPs, clearly indicating that TMIPs have a significant promoting effect on the growth of Bacteroidetes. This discovery not only highlights the probiotic potential of TMIPs, but also reveals their possible positive impact on maintaining human health. This is because the Bacteroidetes phylum plays a crucial role in the intestine, serving as the main producer of propionic acid, a substance that plays a key role in regulating blood lipids and cholesterol bacteria (Liang et al., 2024).

The distribution of the relatively dominant species across different sample groups is visually represented in Fig. 6B through a heatmap, revealing the pattern of species variation between the control and treatment groups, showing the dominant taxa at the genus level in the TMIPs group as Escherichia-Shigella, Bifidobacterium, Klebsiella, Megamonas, Prevotella, and Bacteroides. The TMIPs and FOS group had significant effects on the key genera, compared to the blank group. Following a 48 h fermentation period, Bifidobacteria emerged as the prevailing bacterial species in both the FOS group and TMIPs group, while Klebsiella was significantly reduced. In the TMIPs group, Bacteroides was the main bacteria involved in the hydrolysis and utilization of TMIPs, which might be attributed to Bacteroides' endogenous phospholipases ability to degrade the lactose side chain of TMIPs, stimulating its growth. (Liu et al., 2021). Bacteroides have the ability to enzymatically break down polysaccharides, resulting in the production of beneficial metabolites, such as SCFAs, which prevent gastrointestinal diseases and inflammation (Xia, Liu, Li, Ren, & Liu, 2023). Moreover, Bacteroides is crucial for improving the metabolism and immune dysregulation in obese patients (Wu et al., 2022). However, we also observed an increase in the relative abundance of Escherichia-Shigella in TMIPs, which may be caused by the ability of Escherichia-Shigella to maintain its growth in the fermentable culture medium by using oligosaccharides generated from TMIPs (Luo et al., 2023). Ultimately, TMIPs bring about changes in original gut microbiota composition by promoting beneficial microbial communities and reducing harmful ones.

With the LEfSe multilevel species hierarchy tree in Fig. 6C and the LDA discriminant column in Fig. 6D, we can observe species and their LDA scores that differ significantly between groups. A higher LDA score for a species indicates its greater importance in a particular group. Blank group is mainly composed of Bacteroidetes, Actinobacteria and Firmicutes dominant species of the door. In particular, the FOS group significantly showed an increase in the number of some probiotics in the

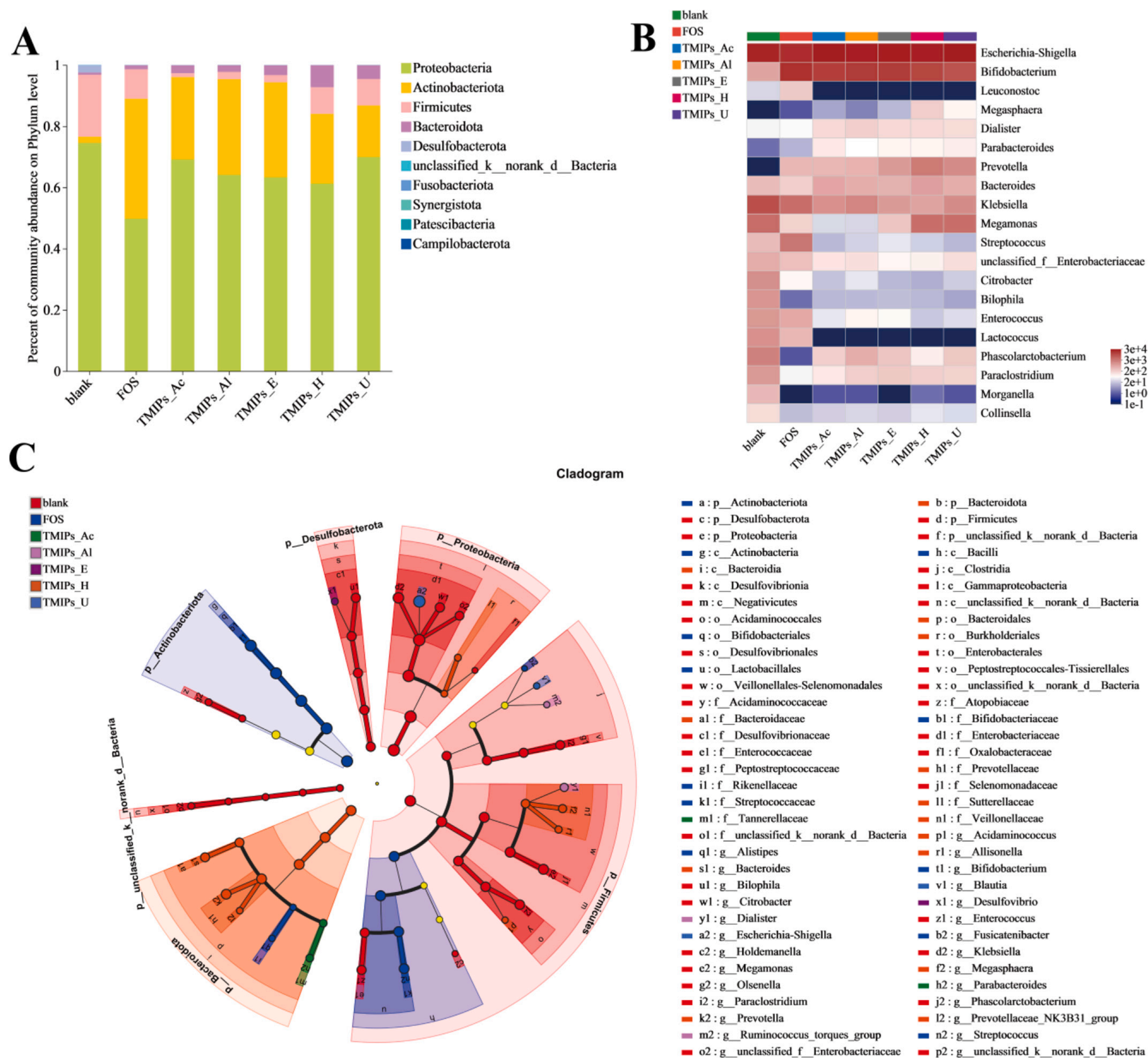


Fig. 6. Bacterial taxonomic profiling at (A) phylum and (B) heatmap analysis of the relative abundance of the bacterial community at the genus level; (C) The evolutionary branch plot for LEfSe analysis; (D) Histogram of LDA values (log 10) higher than 2 for differential bacteria.

phylum Bacteroidetes, suggesting that FOS promotes these bacterial groups. TMIPs groups, on the other hand, revealed significant changes in specific phyla such as Proteobacteria and Actinobacteria. Combination of Fig. 6C and D comprehensive analysis, we found that in the blank group klebsiella bacteria and enterobacteriaceae bacteria in the low poly fructose and TMIPs group were significantly decreased, which suggests that low poly fructose and TMIPs has inhibitory effect on the potential pathogens. Clearly, the TMIPs group showed significant effects in promoting the growth of probiotics and inhibiting potential pathogens. As a result, this proves that TMIPs treatment can effectively improve the intestinal flora, and promote the intestinal health.

4. Conclusion

This study revealed that the digestibility of the five polysaccharide components in *Tricholoma mongolicum* Imai was minimally impacted by different extraction processes. The five TMIPs components exhibited a high degree of resistance to digestion by α-amylase, simulated gastric fluid, and intestinal fluid. Nonetheless, the prebiotic potential of TMIPs was significantly influenced by various extraction processes. When used as a substitute for glucose as a carbon source in *in vitro* fermentation of four probiotic strains, the polysaccharide components, namely TMIPs-E and TMIPs-H were found to enhance the proliferation of probiotics and stimulate the production of increased SCFAs. The *in vitro* fermentation results showed that TMIPs significantly lowered the pH of the fermentation broth and increased the concentrations of SCFAs; promoted the

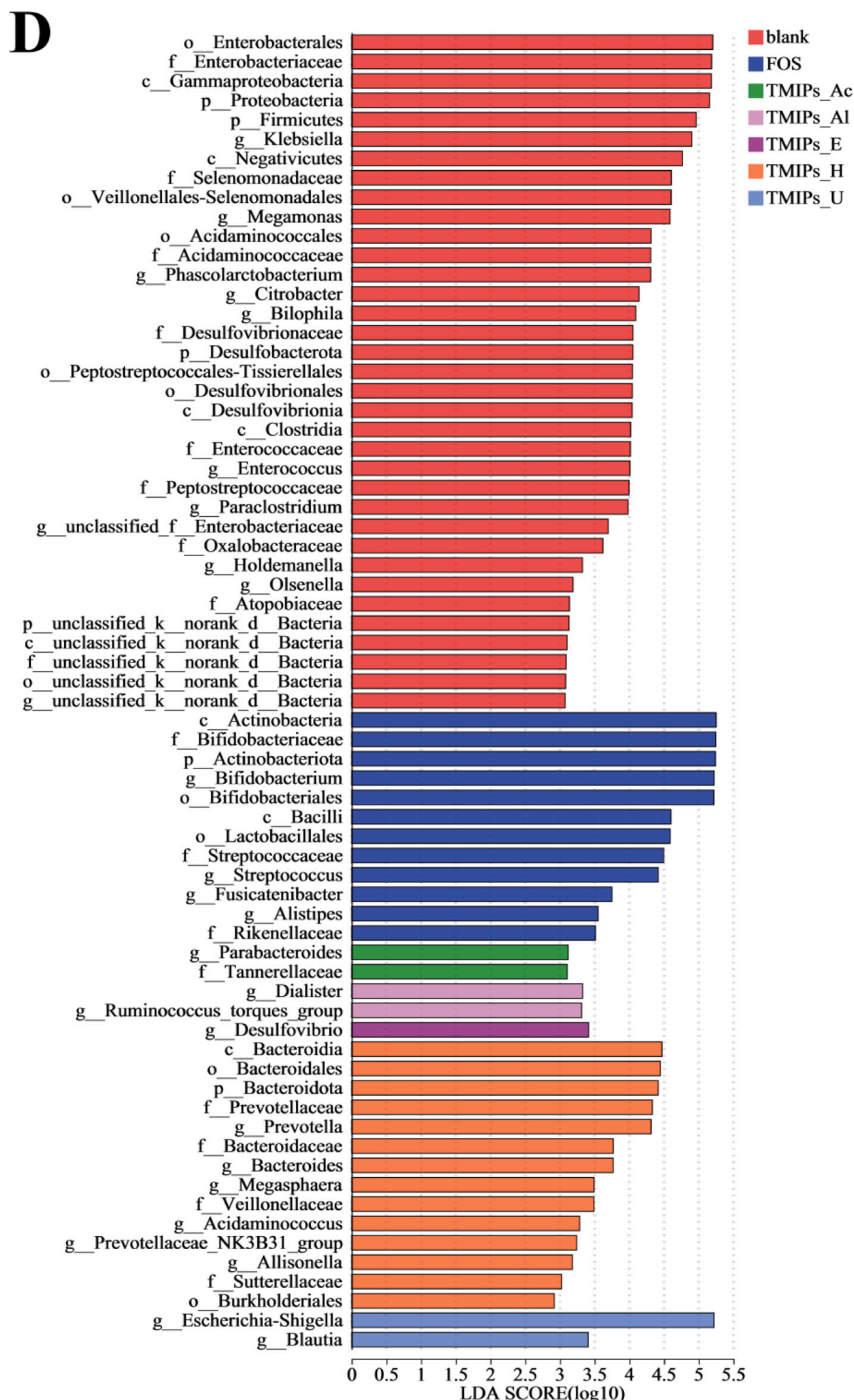


Fig. 6. (*continued*).

proliferation of beneficial bacteria; and inhibited the growth of harmful bacteria. This study provides a foundation for understanding how TMIPs can improve gut health and prevent diseases by modulating the gut microbiota.

CRediT authorship contribution statement

Bing Yang: Data curation. **Xinyu Zhang:** Writing – review & editing, Writing – original draft, Conceptualization. **Jingbo Zhu:** Project administration, Conceptualization. **Qunjun Wu:** Software. **Bimal Chitrakar:** Validation. **Yaxin Sang:** Investigation.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

Acknowledgements

This research was supported by the Key Research and Development Program of Shaanxi (Program No. 2024GX-YBXM-175), Introduced Talent Research Project of Hebei Agricultural University (YJ2021007).

References

Akbari-Alavijeh, S., Soleimanian-Zad, S., Sheikh-Zeinoddin, M., Hashmi, & Sarwar. (2018). Pistachio hull water-soluble polysaccharides as a novel prebiotic agent. *International Journal of Biological Macromolecules: Structure, Function, 107*(Pt.A), 808–816. https://doi.org/10.1016/j.ijbiomac.2017.09.049

Araújo-Rodrigues, H., Sousa, A. S., Relvas, J. B., Tavaria, F. K., & Pintado, M. (2024). An overview on mushroom polysaccharides: Health-promoting properties, prebiotic and gut microbiota modulation effects and structure-function correlation. *Carbohydrate Polymers, 333*, Article 121978. https://doi.org/10.1016/j.carbpol.2024.121978

Chen, G., Chen, X., Yang, B., Yu, Q., & Kan, J. (2019). New insight into bamboo shoot (Chimonobambusa quadrangularis) polysaccharides: Impact of extraction processes on its prebiotic activity. *Food Hydrocolloids, 95*. https://doi.org/10.1016/j.foodhyd.2019.04.046

Chen, G., Xie, M., Wan, P., Chen, D., Ye, H., Chen, L., Zeng, X., & Liu, Z. (2018). Digestion under saliva, simulated gastric and small intestinal conditions and fermentation in vitro by human intestinal microbiota of polysaccharides from Fuzhuan brick tea. *Food Chemistry, 244*, 331–339. https://doi.org/10.1016/j.foodchem.2017.10.074

Chen, M., Chen, X., Guo, Y., Liu, N., Wang, K., Gong, P., Zhao, Y., & Cai, L. (2023). Effect of in vitro digestion and fermentation of kiwifruit pomace polysaccharides on structural characteristics and human gut microbiota. *International Journal of Biological Macromolecules, 253*, Article 127141. https://doi.org/10.1016/j.ijbiomac.2023.127141

Choque Delgado, G. T., & Tamashiro, W. M. D. S. C. (2018). Role of prebiotics in regulation of microbiota and prevention of obesity. *Food Research International, 113*, 183–188. https://doi.org/10.1016/j.foodres.2018.07.013

Ding, Y., Yan, Y., Peng, Y., Chen, D., Mi, J., Lu, L., Luo, Q., Li, X., Zeng, X., & Cao, Y. (2019). In vitro digestion under simulated saliva, gastric and small intestinal conditions and fermentation by human gut microbiota of polysaccharides from the fruits of Lycium barbarum. *International Journal of Biological Macromolecules, 125*, 751–760. https://doi.org/10.1016/j.ijbiomac.2018.12.081

Fei, Z., Xie, H., Xie, D., Wang, M., Du, Q., & Jin, P. (2024). Structural characterization and high-efficiency prebiotic activity of the polysaccharide from Tremella aurantialba endophytic bacteria. *International Journal of Biological Macromolecules, 260*, Article 129347. https://doi.org/10.1016/j.ijbiomac.2024.129347

Fu, C., Ye, K., Ma, S., Du, H., Chen, S., Liu, D., Ma, G., & Xiao, H. (2023). Simulated gastrointestinal digestion and gut microbiota fermentation of polysaccharides from Agaricus bisporus. *Food Chemistry.*. https://doi.org/10.1016/j.foodchem.2023.135849

Ge, Q., Hou, C., Rao, X., Zhang, A., Xiao, G., Wang, L., Jin, K., Sun, P., & Chen, L. (2024). In vitro fermentation characteristics of polysaccharides from coix seed and its effects on the gut microbiota. *International Journal of Biological Macromolecules, 262*, Article 129994. https://doi.org/10.1016/j.ijbiomac.2024.129994

Geng, X., Guo, D., Bau, T., Lei, J., Xu, L., Cheng, Y., Feng, C., & Meng, j., & Chang, M.. (2023). Effects of in vitro digestion and fecal fermentation on physico-chemical properties and metabolic behavior of polysaccharides from Clitocybe squamulosa. *Food Chemistry: X, 18*, Article 100644. https://doi.org/10.1016/j.fochx.2023.100644

Han, X., Zhou, Q., Gao, Z., Bianca Xu, G., Chen, H., Chitrakar, B., Sun, Y., Zhao, W., Lin, X., Zhou, K., & Zhang, Z. (2023). Characterization of procyanidin extracts from hawthorn (Crataegus pinnatifida) in human colorectal adenocarcinoma cell line Caco-2, simulated digestion, and fermentation identified unique and novel prebiotic properties. *Food Research International, 165*, Article 112393. https://doi.org/10.1016/j.foodres.2022.112393

Han, X., Zhou, Q., Gao, Z., Lin, X., Zhou, K., Cheng, X., Chitrakar, B., Chen, H., & Zhao, W. (2022). In vitro digestion and fecal fermentation behaviors of polysaccharides from Ziziphus Jujuba cv. Pozao and its interaction with human gut microbiota. *Food Research International, 162*, Article 112022. https://doi.org/10.1016/j.foodres.2022.112022

Hu, W., Di, Q., Liang, T., Zhou, N., Chen, H., Zeng, Z., Luo, Y., & Shaker, M. (2023). Effects of in vitro simulated digestion and fecal fermentation of polysaccharides from straw mushroom (Volvariella volvacea) on its physicochemical properties and human gut microbiota. *International Journal of Biological Macromolecules: Structure, Function and Interactions, 239*. https://doi.org/10.1016/j.ijbiomac.2023.124188

Li, J., Pang, B., Yan, X., Shang, X., Hu, X., & Shi, J. (2020). Prebiotic properties of different polysaccharide fractions from Artemisia sphaerocephala Krasch seeds evaluated by simulated digestion and in vitro fermentation by human fecal microbiota. *International Journal of Biological Macromolecules, 162*, 414–424. https://doi.org/10.1016/j.ijbiomac.2020.06.174

Li, X., Chen, Y., Song, L., Wang, J., Song, Z., Zhao, X., Zhou, C., & Wu, Y. (2023). Partial enzymolysis affects the digestion of tamarind seed polysaccharides in vitro: Degradation accelerates and gut microbiota regulates. *International Journal of Biological Macromolecules: Structure, Function and Interactions, 237*. https://doi.org/10.1016/j.ijbiomac.2023.124175

Liang, X., Liu, M., Yao, A., Cui, W., Wei, Y., Guo, S., Duan, J.-L., Kang, H., Zhou, X., Su, S., Jin, H., Zhang, F., & Duan, J.-A. (2024). In vitro fermentation characteristics and interaction of neutral and acidic polysaccharides from Lycii fructus on human gut microbiota. *Food Hydrocolloids, 152*, Article 109940. https://doi.org/10.1016/j.foodhyd.2024.109940

Liu, Y., Li, Y., Ke, Y., Li, C., Zhang, Z., Wu, Y., Hu, B., Liu, A., Luo, Q., & Wu, W. (2021). In vitro saliva-gastrointestinal digestion and fecal fermentation of Oudemansiella radicata polysaccharides reveal its digestion profile and effect on the modulation of the gut microbiota. *Carbohydrate Polymers, 251*, Article 117041. https://doi.org/10.1016/j.carbpol.2020.117041

Luo, Q., Li, X., Li, H., Kong, K., Li, C., Fang, Z., Hu, B., Wang, C., Chen, S., Wu, W., Li, X., Liu, Y., & Zeng, Z. (2023). Effect of in vitro simulated digestion and fecal fermentation on boletus auripes polysaccharide characteristics and intestinal flora. *International Journal of Biological Macromolecules, 249*, Article 126461. https://doi.org/10.1016/j.ijbiomac.2023.126461

Lv, K., Yuan, Q., Li, H., Li, T., Ma, H., Gao, C., Zhang, S., Liu, Y., & Zhao, L. (2022). Chlorella pyrenoidosa polysaccharides as a prebiotic to modulate gut microbiota: Physicochemical properties and fermentation characteristics in vitro. *Foods, 11*(5), 725. https://doi.org/10.3390/foods11050725

Markowiak-Kopeć, P., & Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients, 12*(4). https://doi.org/10.3390/nu12041107

Ou, J., Wang, Z., Liu, X., Song, B., Chen, J., Li, R., Jia, X., Huang, R., Xiang, W., & Zhong, S. (2022). Regulatory effects of marine polysaccharides on gut microbiota dysbiosis: A review. *Food Chemistry: X, 15*, Article 100444. https://doi.org/10.1016/j.fochx.2022.100444

Payling, L., Fraser, K., Loveday, S. M., Sims, I., & Mcnabb, W. (2020). The effects of carbohydrate structure on the composition and functionality of the human gut microbiota. *Trends in Food Science & Technology, 97*. https://doi.org/10.1016/j.tifs.2020.01.009

Rezende, E. S. V., Lima, G. C., & Naves, M. M. V. (2021). Dietary fibers as beneficial microbiota modulators: A proposed classification by prebiotic categories. *Nutrition, 89*, Article 111217. https://doi.org/10.1016/j.nut.2021.111217

Shang, Q., Wang, Y., Pan, L., Niu, Q., Li, C., Jiang, H., Cai, C., Hao, J., Li, G., & Yu, G. (2018). Dietary polysaccharide from Enteromorpha Clathrata modulates gut microbiota and promotes the growth of Akkermansia muciniphila, Bifidobacterium spp. and Lactobacillus spp. *Marine Drugs, 16*(5). https://doi.org/10.3390/md16050167

Sivaprakasam, S., Prasad, P. D., & Singh, N. (2016). Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. *Pharmacology & Therapeutics, 164*, 144–151. https://doi.org/10.1016/j.pharmthera.2016.04.007

Tang, Z., Bao, P., Ling, X., Qiu, Z., Zhang, B., & Hao, T. (2024). In vitro digestion under simulated saliva, gastric and small intestinal conditions and fermentation of nicotinamide mononucleotide, and its effects on the gut microbiota. *Food Research International, 177*, Article 113779. https://doi.org/10.1016/j.foodres.2023.113779

Thomson, C., Garcia, A. L., & Edwards, C. A. (2021). Interactions between dietary fibre and the gut microbiota. *Proceedings of the Nutrition Society, 80*(4), 398–408. https://doi.org/10.1017/S0029665121002834

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*(7122), 1027–1031. https://doi.org/10.1038/nature05414

Wang, J., Zhao, Y., Li, W., Wang, Z., & Shen, L. (2015). Optimization of polysaccharides extraction from Tricholoma mongolicum Imai and their antioxidant and antiproliferative activities. *Carbohydrate Polymers, 131*, 322–330. https://doi.org/10.1016/j.carbpol.2015.06.009

Wu, D.-T., An, L.-Y., Liu, W., Hu, Y.-C., Wang, S.-P., & Zou, L. (2022). In vitro fecal fermentation properties of polysaccharides from Tremella fuciformis and related modulation effects on gut. *Food Research International.*. https://doi.org/10.1016/j.foodres.2022.111185

Xia, B., Liu, X., Li, Z., Ren, J., & Liu, X. (2023). The effects of microbiota-targeted approaches in inflammatory bowel disease: Probiotics, probiotic foods, and prebiotics. *Current Opinion in Food Science, 49*, Article 100956. https://doi.org/10.1016/j.cofs.2022.100956

Xu, B., Sun, P., Lu, J., Wang, Y., Lin, X., Chen, C., Zhu, J., Jia, H., Wang, X., Shen, J., Yu, C., & Feng, T. (2025). Studies of peach gum polysaccharide on gut microbiota in vitro fermentation by human feces. *Journal of Future Foods, 5*(1), 79–87. https://doi.org/10.1016/j.jfutfo.2024.01.007

Zhang, F., Zheng, H., Zheng, T., Xu, P., Xu, Y., Cao, Y., Jia, F., Zeng, Y., Fan, Y., He, K., Dai, X., Hou, F., & Yang, Y. (2024). Adsorption, in vitro digestion and human gut microbiota regulation characteristics of three Poria cocos polysaccharides. *Food Science and Human Wellness, 13*(3), 1685–1697. https://doi.org/10.26599/FSHW.2022.9250195

Zhang, N., Yang, B., Mao, K., Liu, Y., Chitrakar, B., Wang, X., & Sang, Y. (2022). Comparison of structural characteristics and bioactivity of Tricholoma mongolicum Imai polysaccharides from five extraction methods. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.962584

Zhao, Y., Wang, D., Wang, P., Zhao, W., Zhao, S., Ma, Y., Liu, Y., & Zhao, X. (2024). Microbiota response of pectin determined by its structural characteristics during in vitro fecal fermentation: A comparative study of various pectin sources. *Food Hydrocolloids, 150*, Article 109730. https://doi.org/10.1016/j.foodhyd.2024.109730