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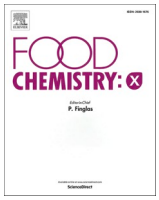

Fecal fermentation behaviors of Konjac glucomannan and its impacts on human gut microbiota

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ABSTRACT

Dietary fiber targets the regulation of the intestinal flora and thus affects host health, however, the complex relationship between these factors lacks direct evidence. In this study, the regulatory effects of Konjac glucomannan (KGM) on key metabolites of host intestinal flora were examined by using *in vitro* fermentation. The results showed that KGM could be utilized by the intestinal flora, which inhibited the relative abundance of *Paeniclostridium*, *Lachnoclostridium*, *Phascolarctobacterium*, and *Bacteroides* and enriched the relative abundance of *Desulfovibrio*, *Sutterella, etc.* Fermentation is accompanied by the production of short-chain acids, including acetic and propionic acids. Metabolomics revealed that KGM significantly promoted amino acid metabolism, lipid metabolism, and the biosynthesis of other secondary metabolites. Correlation analysis results showed that the increase of panose and N-(1-carboxy-3-carboxanilidopropyl) alanylproline content was positively correlated with the relative abundance of *Megamonas*. These results provide evidence that KGM affects host health by regulating gut microbiota and its metabolites.

1. Introduction

Recently, several studies have highlighted the strong link between gut microbiota and host health. Changes in the structural composition of some gut microbiota are accompanied by changes in host phenotypic indicators, particularly in certain characteristic microbial species. For example, *Bifidobacterium* and *Lactobacillus* are considered important sources of probiotics that play important roles in maintaining intestinal barrier function (Engevik et al., 2021), regulating host immunity (Li, Xie, et al., 2022), and regulating lipid metabolism (Liang et al., 2018). However, some microbes can cause damage to the human body. Atypical enteric-pathogenic *Escherichia coli* can induce P21-activated kinase in the intestinal epithelium, destroy the intestinal epithelial barrier, and form biofilms (Baumgartner et al., 2022). *Citrobacter rodentium* injects type III secretory system effectors into the intestinal epithelial cells that target inflammatory, metabolic, and cell survival pathways to induce infection (Mullineaux-Sanders et al., 2019). Therefore, considering the important association between gut microbes and host health, some studies have aimed to protect host health by targeting and regulating the structural composition of gut microbes (including the enrichment of probiotics and inhibition of pathogenic bacteria) (Bai et al., 2023; Wang, Bai, Chen, et al., 2023). Dietary factors are among the most important and direct factors driving the structural composition of host intestinal flora. It is rich in different functional components that can enter the human gut, leading to intestinal microbial growth, metabolism, and nutrient reproduction. In this complex process, microorganisms use functional dietary components to generate different metabolites, which then participate in regulating host health. Therefore, dietary nutritional intervention strategies targeting gut microbes are important for maintaining host health (Wang, Bai, Wang, et al., 2023).

Amorphophallus koniac is a perennial herb of the monocodonous genus *Amorphophallus koniac* that significantly reduces body weight, fat mass, and adipocyte size in high-fat diet-fed mice. Decreased serum levels of inflammatory factors upregulate the expression of tight junction proteins and regulate the expression of genes related to inflammatory proteins and energy and lipid metabolism (Kang et al., 2019). Notably, the probiotic function of konjac is largely dependent on the regulatory effect of konjac glucomannan (KGM) on the intestinal flora. Studies have shown that natural KGM better maintains microbial diversity in mouse feces and increases short-chain fatty acid (SCFA)

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contents under antibiotic interference (Mao et al., 2022). KGM improves the overall physiological status, fecal characteristics, intestinal propulsion rate, and gastric emptying rate of constipated mice by regulating intestinal flora and producing SCFAs (Zhang et al., 2021). Therefore, KGM may target gut microbiota and its metabolites for probiotic functions. However, most existing relevant studies are limited to animal experiments, and all are affected by different physiological states, such as metabolic disorders (Kang et al., 2019) and constipation (Zhang et al., 2021), which cannot provide direct evidence of a close correlation between the two. Therefore, elucidating the interaction between KGM and intestinal flora helps understand the potential probiotic function of KGM and expands its application prospects.

To clarify the KGM-intestinal flora interaction, we examined the effects of KGM on intestinal microorganisms, total sugar content, reducing sugar content, SCFAs, and pH, using *in vitro* fermentation. Non-targeted metabolomic techniques were used to detect changes in the composition of metabolites, which could provide a reference for the development of nutritional intervention strategies targeting intestinal flora.

2. Methods and materials

2.1. Materials

Peptone and yeast extract were purchased from Solarbio Science & Technology Co., Ltd. Hematin was purchased from Sangon Biotech Co., Ltd. The KH_2PO_4, $MgSO_4{\cdot}7H_2O$, NaCl, $CaCl_2$, and $FeSO_4{\cdot}7H_2O$ were of analytical grad. KGM was purchased from Ren Ai Food Technology (Shandong) Co., Ltd., with a purity of 93%. SCFA standards were purchased from Shanghai Macklin Biochemical Technology Co., Ltd.

2.2. The monosaccharide composition

According to previous study, the monosaccharide composition of KGM was determined by Dionex ion chromatograph ICS-5000 with pulsed amperometric detector and CarboPac PA20 column (Liu et al., 2021

2.3. Fecal sample collection

Four healthy volunteers were recruited, including two males and two females (Supplemental Table 1). All volunteers completed a questionnaire survey to meet the following requirements: 1) no antibiotic intervention within 6 months; 2) not taking probiotics and related products within 1 month, 3) no history of major gastrointestinal disease, and 4) no invasive surgery or treatment. Participants will be excluded if they have any of the following: 1) Who had a severe gastrointestinal illness within the last 3 months; 2) Who suffering from endocrine, cardiovascular, thyroid, chronic liver disease or infectious diseases, *etc.*; 3) The female participant has pregnancy reaction or pregnancy; 4) Drug or alcohol dependence. In addition, the fecal sample collection process was completed with the volunteers' consent, and no other human samples were involved. All fecal samples were only used for fermentation experiments. All procedures complied with relevant laws and institutional guidelines and were supervised and approved by the Ethics Committee at Institutional Review Board of College of Food Science, Southwest University, China.

2.4. Fecal fermentation

All fecal samples were collected and placed in aseptic plastic containers, weighed, combined, and added to a pre-cooled liquid gut microbial culture medium (GMM) for homogenization, which include peptone (2 g/L), yeast extract (1 g/L), KH_2PO_4 (2 g/L), $MgSO_4{\cdot}7H_2O$ (2 mg/L), NaCl (0.08 g/L), $CaCl_2$ (8 mg/L), $FeSO_4{\cdot}7H_2O$ (0.73 mg/L), hematin (1.2 mg/L), ATCC vitamin mix (10 mL/L), ATCC trace mineral mix (10 mL/L), Tween 80 (0.5 mL/L) and L-cysteine (0.5 g/L) (pH = 7.0 ± 0.1) (Mao et al., 2014). The experimental process of *in vitro* fermentation was followed as described previously (Carlson et al., 2017; Mao et al., 2015; Sayar et al., 2007). All experimental steps were performed in an anaerobic workstation (10% H_2, 10% CO_2, 80% N_2). Please refer to the supplementary picture for flow chart (Supplemental Fig. 1).

2.5. Determination of total sugars and reducing sugars

The phenol-sulfuric acid method was used to determine the total sugar content (Yue et al., 2022) with a minor modification: 0.4 mL samples and 0.4 mL 5% phenol solution were quickly added in 5.0 mL concentrated sulfuric acid, then 2.0 mL sulfuric acid was mixed and left at room temperature for 20 min. Distilled water was set as a control, and the samples were measured at 490 nm. The 3, 5-dinitrosalicylic acid (DNS) method was used to determine reducing sugar content (Gusakov et al., 2011; Miller, 1959). After that, ultra-pure water was added to the mixture samples and was detected at 540 nm. Every sample was set to 3 parallel.

2.6. SCFA content

A total of 1 mL fermentation broth was centrifuged at 10000*g* and 4 °C for 10 min. The supernatant was collected for the determination of SCFA content. Referring to a previous method (Wang, Hu, et al., 2017), the fecal fermentation broth supernatant was collected in 2 mL tubes, and the SCFAs concentration was analyzed using gas chromatography–mass spectrometry (GC–MS). Total SCFA content was expressed as the sum of acetic, propionic, and n-butyric acids.

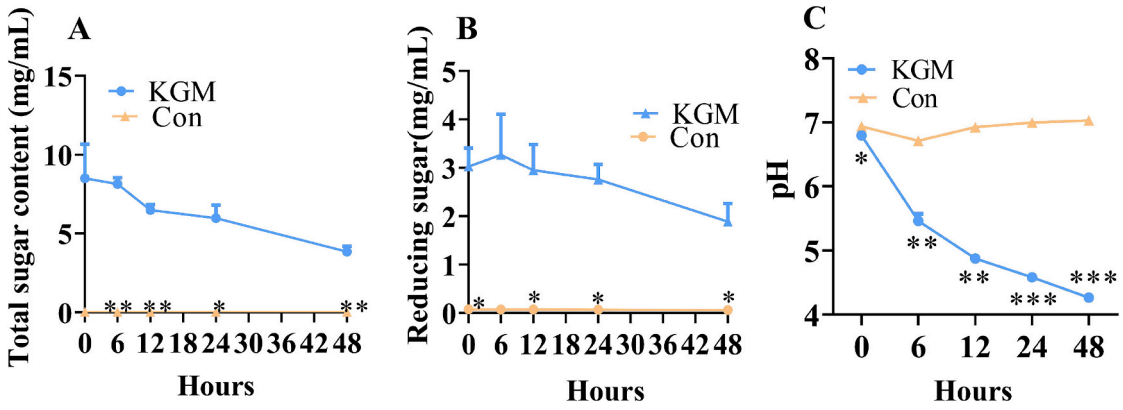

Fig. 1. Changes in the total sugar content (A), reducing sugar content (B), and pH (C).

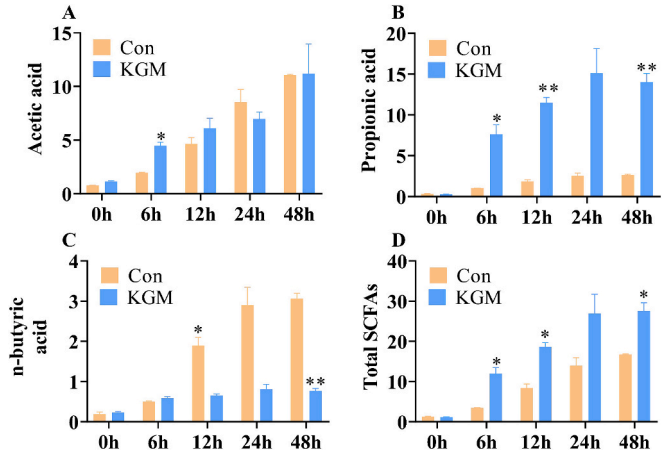

Fig. 2. Acetic acid (A), propionic acid (B), n-butyric acid (C), and total short-chain fatty acids (SCFAs) (D).
Note: Total SCFAs are the sum of acetic, propionic, and n-butyric acids.

2.7. 16S rRNA analysis

One milliliter of the fermentation solution was centrifuged, and precipitates were collected using the Fast DNA SPIN Kit (MP Biomedicals, Carlsbad, CA, USA) to extract total DNA from the precipitate. A previous study on intestinal flora detection was used as a reference (Wang et al., 2017), the V3-V4 region genes were amplified and collected products were used for sequencing the structure of gut microbiota. The online data visualization reference (https://www.microbiomeanalyst.ca/MicrobiomeAnalyst/home.xhtml) was analyzed.

2.8. Metabolome analysis

To detect the changes of metabolites in the fermentation supernatant (Bai et al., 2021; Xu et al., 2020): 1 mL of the fermentation supernatant was added to 4 mL of acetonitrile-methanol mixed solution to extract metabolites. After ultrasonic treatment at 40 kHz for 10 min, the metabolites were placed at −20 °C for 1 h. The samples were then centrifuged at 4 °C and 8000 *g* for 15 min, and the supernatant was used for metabolomic analysis.

2.9. Data statistics and analysis

All graphs were generated using GraphPad Prism 8, and the data were represented as the mean ± standard error of mean (SED). Student's *t*-test was used to analyze statistically significant differences, and $p < 0.05$ was considered significant.

3. Results

3.1. Total sugar, reducing sugar content and pH

As shown in Fig. 1A, the total sugar content of the Con group did not change significantly during the fermentation process. In contrast, the initial KGM content in the fermentation liquid was approximately 10 mg/mL, and the content of KGM continuously decreased during the fermentation process. The KGM content in the fermentation liquid was approximately 3.84 mg/mL after 48 h. As shown in Fig. 1B, the reduced sugar content in the Con group did not change significantly during fermentation. In contrast, at 0–6 h, the reducing sugar content in the fermentation solution showed a slight increase. Thereafter, the content of reduced sugar continued to decrease, and after 48 h, the content of reduced sugar decreased to 1.89 mg/mL. Polysaccharide degradation was accompanied by a decrease in the pH of the fermentation solution. As shown in Fig. 1C, the initial pH values of the Con and KGM groups were close to 7.0. With continuous fermentation, the pH of the Con group did not change significantly, whereas that of the KGM group continued to decrease, which was consistent with the changing trend in total sugar content.

3.2. SCFA content

The fermentation process was accompanied by a continuous increase

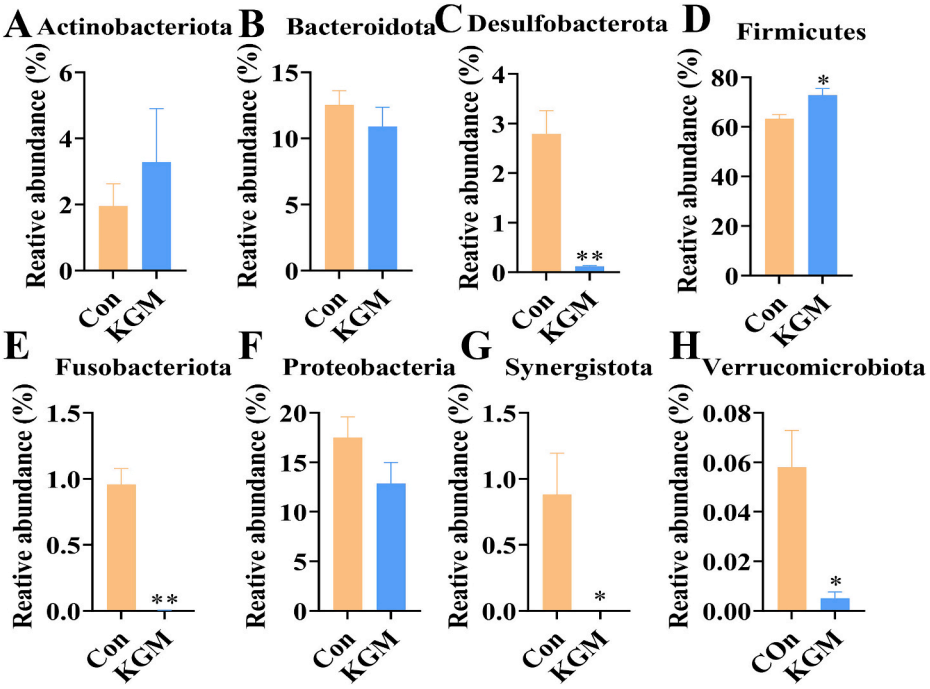

Fig. 3. Relative abundance of Actinobacteriota (A), Bacteroidota (B), Desulfobacterota (C), Firmicutes (D), Fusobacteriota (E), Proteobacteria (F), Synergistota (G), and Verrucomicrobiota (H).

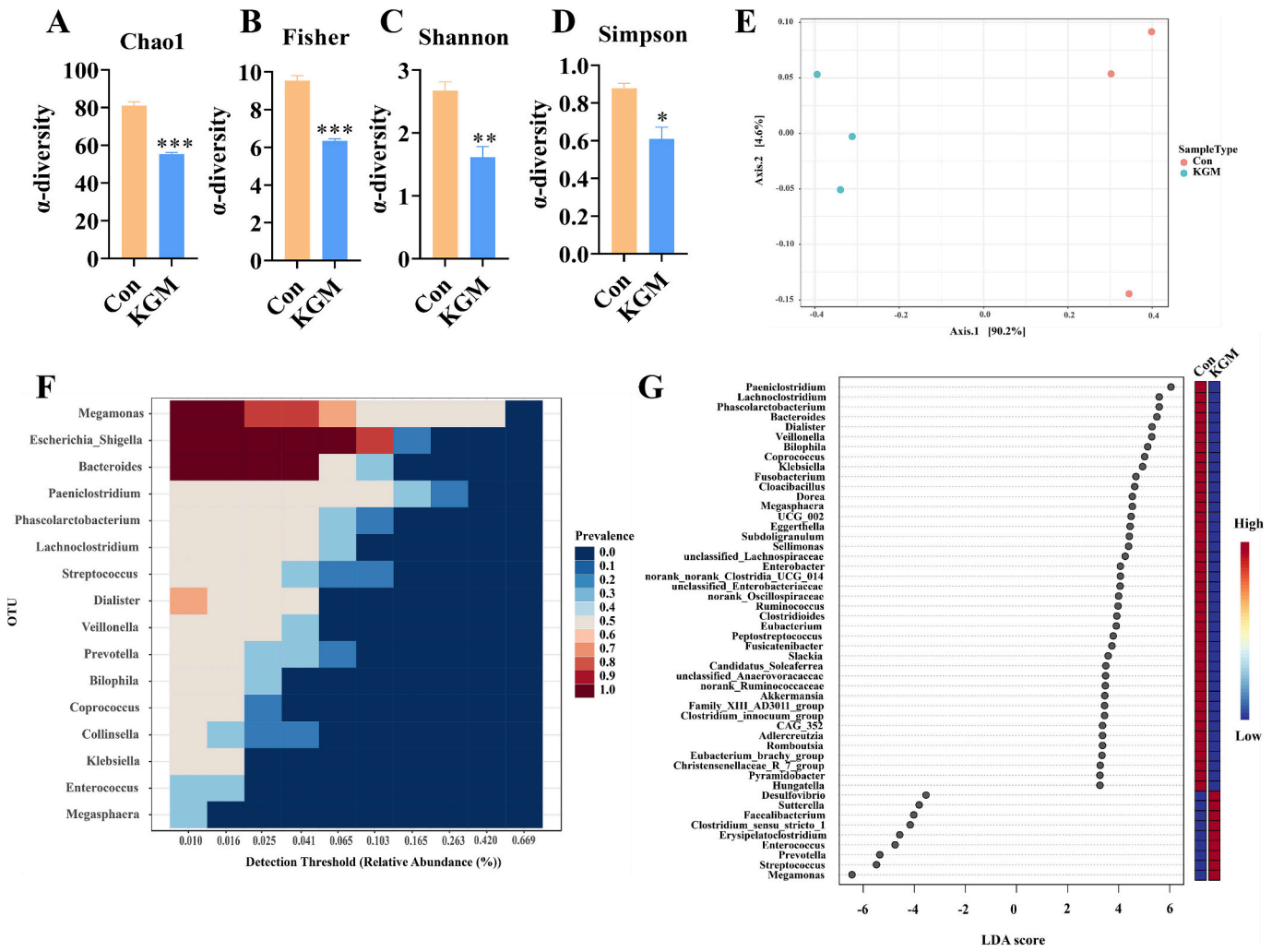

Fig. 4. Alpha diversity is indicated by the Chao1 (A), Fisher (B), Shannon (C), and Simpson (D) indices. Principal component analysis (PCA) (E), core species (F), and LEfSe analyses of different groups (G).

in the SCFA content, with the total SCFA content being the highest at 48 h (Fig. 2D). A slight increase in total SCFAs was also observed in the Con group without KGM, which could be attributed to small carbohydrate fermentation carried out by the fecal inoculum or the interference caused by SCFAs. The total SCFA content increased sharply in the first 24 h of fermentation and then slowly in the next 24 h, suggesting that the fermentation rate reached its maximum at 24 h. Compared to the Con group, KGM fermentation produced more SCFAs. Specifically, acetic and propionic acids were important end products of KGM fermentation (Fig. 2A-B), whereas the butyric acid content did not increase significantly (Fig. 2C).

3.3. Changes in bacterial community structure

At the phylum level, KGM significantly affected the structural composition of the flora compared to the Con group. KGM significantly enriched Firmicutes (Fig. 3D) while significantly suppressed Desulfobacterota, Fusobacteriota, Synergistota, and Verrucomicrobiota (Fig. 3C, E, G, H).

At the genus level, KGM significantly reduced the α-diversity of the flora (Fig. 4A-4D) and altered the β-diversity (Fig. 4E) compared to the Con group. *Megamonas*, *Escherichia_Shigella*, and *Bacteroides etc.* are the core species of fermentation products (Fig. 4F). Notably, compared with the Con group, KGM significantly reduced the relative abundances of *Paeniclostridium*, *Lachnoclostridium*, *Phascolarctobacterium*, *Bacteroides*, *Dialister*, and other genera, and significantly increased the relative abundances of *Desulfovibrio*, *Sutterella*, *Faecalibacterium*, *Clostridium sensu stricto_1*, *Erysipelatoclostridium*, *Enterococcus*, *Prevotella*, *Streptococcus*, and *Megamonas* (Fig. 4G).

3.4. Metabolite composition

In the S-plot generated using OPLS-DA (Fig. 5A), the red ones at both ends are used to identify those variables that contribute most to class differentiation. These differential metabolites were selected for further analysis of VIP values (Fig. 5B). The top five metabolites with the most significant differences between the groups were Phe-Leu, panose, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, 5-hydroxytetradecanedioylcarnitine, and higher content of D-galactose. The distribution of the differential metabolites is shown in the clustering heat diagram (Fig. 5C). Compared to the Con group, 21 metabolites were significantly increased in the KGM group, including panose, Phe-Leu, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, 5-hydroxytetradecanedioylcarnitineh and D-galactose. The result of Venn plot showed that (Fig. 5D) 65 different metabolites were unique to the KGM group and 28 were unique to the Con group. There were 1451 common metabolites between the KGM and Con groups.

According to the bar and bubble charts of the top 20 metabolic pathways significantly enriched using KEGG (Fig. 6A, B), differential metabolite enrichment of KGM mainly affected tropane, piperidine, and

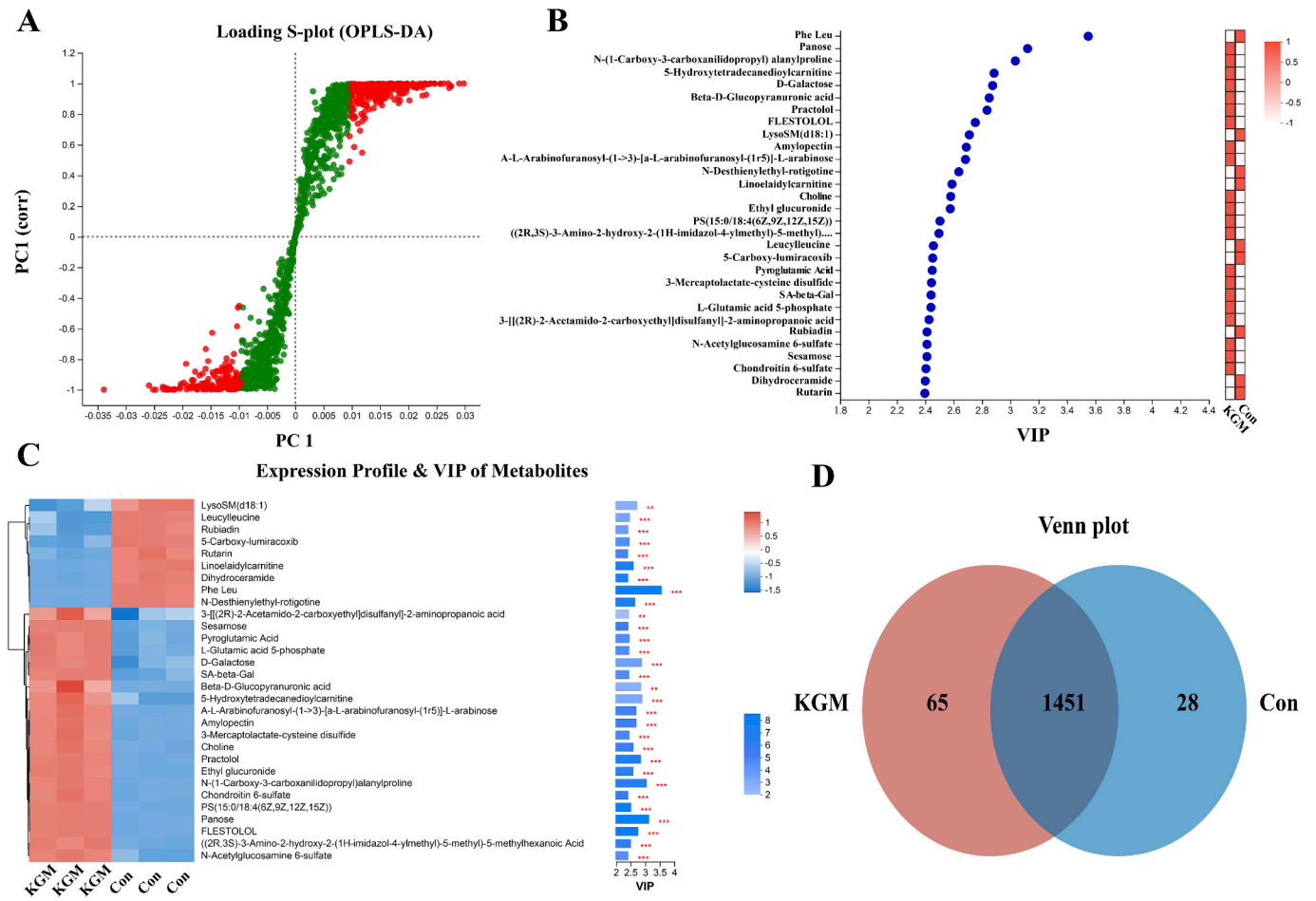

Fig. 5. S-plot based on the OPLS-DA score chart (A), VIP value analysis (B), distribution profile of differential metabolites (C), and Venn plot of differential metabolites (D).

pyridine alkaloid biosynthesis, protein digestion and absorption, and D-amino acid metabolism compared to the Con group. Among the seven KEGG metabolic pathways (metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases, and drug development), KGM promoted amino acid metabolism, lipid metabolism, and the biosynthesis of other secondary metabolites (Fig. 6C). Compound classification analysis was performed on these differential metabolites (Fig. 6D), which mainly included steroids, peptides, organic acids, nucleic acids, lipids, and carbohydrates. Among these, phospholipids belonging to lipids and amino acids belonging to peptides were the most differentially expressed.

3.5. Correlation analysis between the gut flora and key metabolites

As shown in Fig. 7, the content of panose, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, and D-galactose was positively correlated with the relative abundance of *Megamonas*. 5-hydroxytetradecanedioylcarnitine was positively correlated with *Sutterella*.

4. Discussion

The strong association between dietary polysaccharides and their hosts has been widely recognized. However, the effect of different diets on the host depends on various internal and external factors, such as the polysaccharide species and structure of the inherent microbes of the gut. Therefore, elucidating the interactions between polysaccharides and flora is helpful in predicting the potential probiotic functions of dietary polysaccharides. In this study, during the fermentation of KGM by the intestinal flora, the total sugar content continued to decrease with increasing pH, indicating that KGM was continuously utilized and acidic substances were produced. The SCFA test results explain this phenomenon. Compared to the Con group, the total SCFA content in the KGM group increased continuously.

Effects of dietary polysaccharides on host phenotypes are mediated by intestinal microbes. Dietary polysaccharides enter the host intestine and serve as nutrients for the growth, metabolism, and reproduction of intestinal microorganisms. However, differences in the structural composition of intestinal microorganisms may produce different responses to dietary polysaccharides, which may depend on differences in genes such as the innate glycoside hydrolases of different microorganisms. Therefore, elucidating the interactions between different dietary polysaccharides and the intestinal flora is helpful for analyzing and predicting the potential probiotic functions of dietary polysaccharides. According to previous studies, KGM comprises β-1,4 linked D-glucose and d-mannose residues with a reported ratio of 1:1.6 or 1:1.4 (Zhang, 2014). In this study, the ratio of glucose to mannose in KGM was 1:1.3. Notably, the ratio differs with konjac breeds (Smith & Srivastava, 1959), purity or extraction strategy, and other factors. This may partly explain the difference in the ratio of glucose and mannose. In this study, KGM significantly increased the relative abundance of Firmicutes. As an important component of the intestinal flora, Firmicutes includes *Lactobacillus*, *Weissella*, *Streptococcus*, *Faecalibacterium*, and other microbial genera. Interestingly, at the genus level, *Desulfovibrio*, *Sutterella*, *Faecalibacterium*, *Clostridiumsensustricto1*, *Erysipelatoclostridium*, *Enterococcus*, *Prevotella*, *Streptococcus*, and *Megamonas* were detected. It is worth

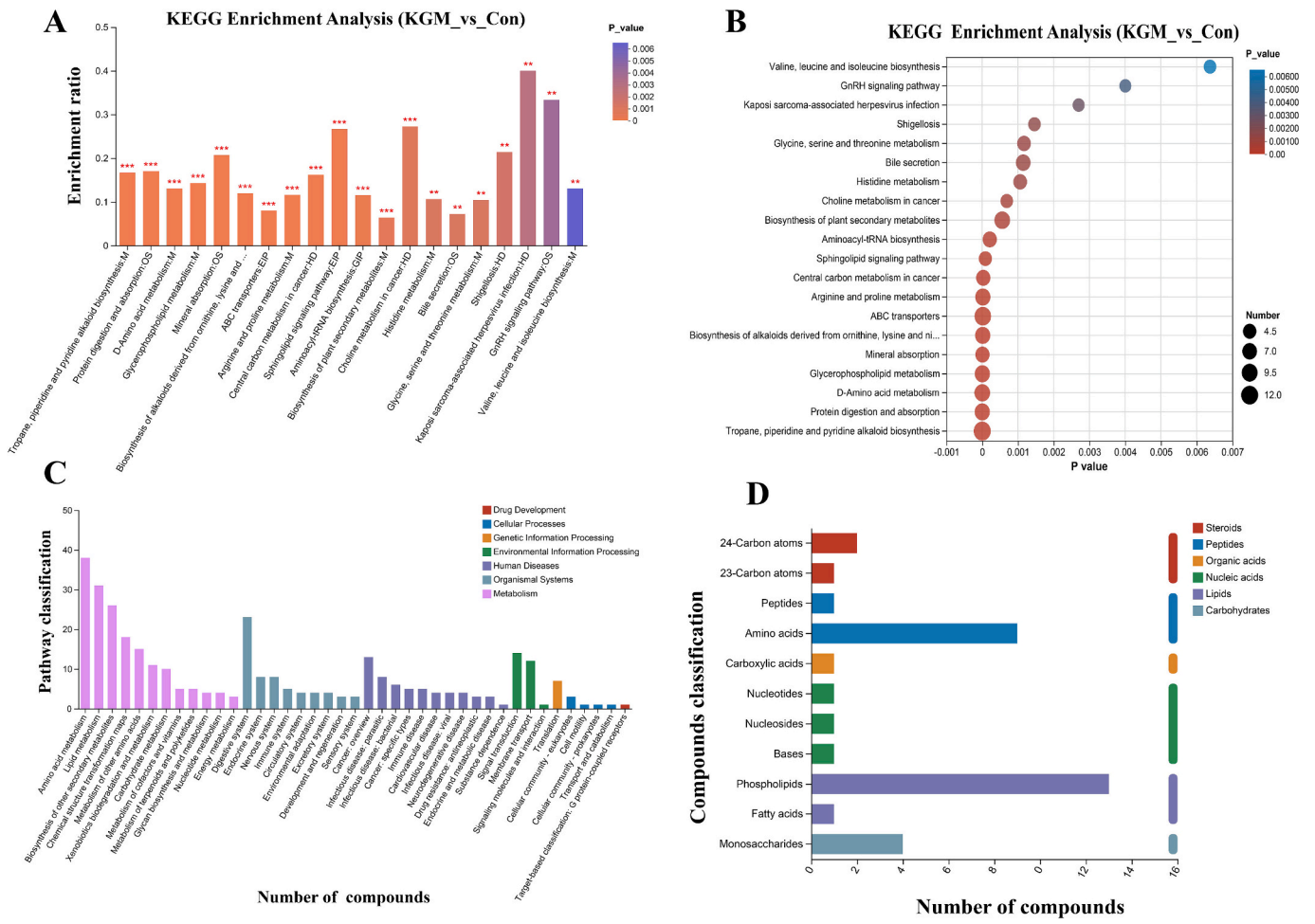

Fig. 6. KEGG enrichment analysis bar (A), bubble (B), KEGG pathway classification (C), and KEGG compound classification (D).

noting that *Faecalibacterium* can reduce the symptoms of chronic kidney disease in mice, reduce the content of various uremic toxins in the serum, and improve intestinal inflammation, permeability, and intestinal flora composition (Li et al., 2022). *Streptococcus thermophilus* can induce apoptosis and stagnation of colorectal cancer cells and inhibit the growth of colorectal cancer grafts in mice (Li et al., 2021). Meanwhile, KGM significantly inhibited the relative abundances of *Paeniclostridium*, *Lachnoclostridium*, *Phascolarctobacterium*, *Bacteroides*, and *Dialister*. The relative abundance of *Lachnoclostridium* was associated with depressive symptoms (Radjabzadeh et al., 2022). These results suggest that KGM may exert potential probiotic functions by enriching beneficial microorganisms in the gut and inhibiting potentially pathogenic bacteria.

The effects of the gut microbes on host health are largely determined by metabolite production. SCFA stones are an important product of dietary polysaccharides used by intestinal microorganisms and are an important material basis for probiotics. Acetate supplementation reversed social impairment in mice and led to significant changes in gene expression, such as synaptic signaling, in the medial prefrontal cortex (Osman et al., 2023). Propionic acid supplementation increases the number of regulatory T cells and immunosuppressive function, reduces proinflammatory helper T cells (Th)17 and Th1, and reduces the annual recurrence rate and brain atrophy in patients with multiple sclerosis (Duscha et al., 2020). Butyric acid promotes intestinal barrier function, accelerates the repair of intestinal epithelial cell damage, and maintains intestinal homeostasis (Wang et al., 2020). Therefore, SCFA content is an important index for evaluating the probiotic function of microorganisms. In this experiment, KGM significantly increased the acetic and propionic acid contents in the fermented liquor, suggesting that they may be important substances for KGM to exert its probiotic function. The production of SCFAs depends on the composition of the gut microbiota, so the benefits of KGM may vary from different microbiota compositions. In this study, SCFAs concentrations showed an upward trend in the Con group (no carbon source added), which may be attributed to metabolic cross-feeding among bacteria (Bunesova et al., 2018; Cockburn & Koropatkin, 2016) and little carbon source remaining in fecal filtrate can serve as a nutrient source for microorganisms for SCFAs production.

In recent years, advancements in metabolomics technology have deepened our understanding of intestinal flora metabolites. In this study, we analyzed the metabolite composition of the fermentation broth using a non-targeted metabolome. The results showed that Phe-Leu, panose, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, 5-hydroxytetradecanedioylcarnitineh, and D-galactose levels increased in the KGM group. Panose is used as a nutrient to promote the reproduction of *Bifidobacterium* while increasing the production of butyrate and acetate. These findings suggest that it is a novel prebiotic candidate (Mäkeläinen et al., 2009). D-galactose is widely used to induce senescence in cells (Wang et al., 2022) and hosts (Azman et al., 2021). KEGG analysis showed that the differential metabolite enrichment of KGM mainly affected the tropane, piperidine, and pyridine alkaloid biosynthesis, protein digestion and absorption, and D-amino acid metabolism. These results suggest that microbes influence host health and disease by using KGM to produce metabolites.

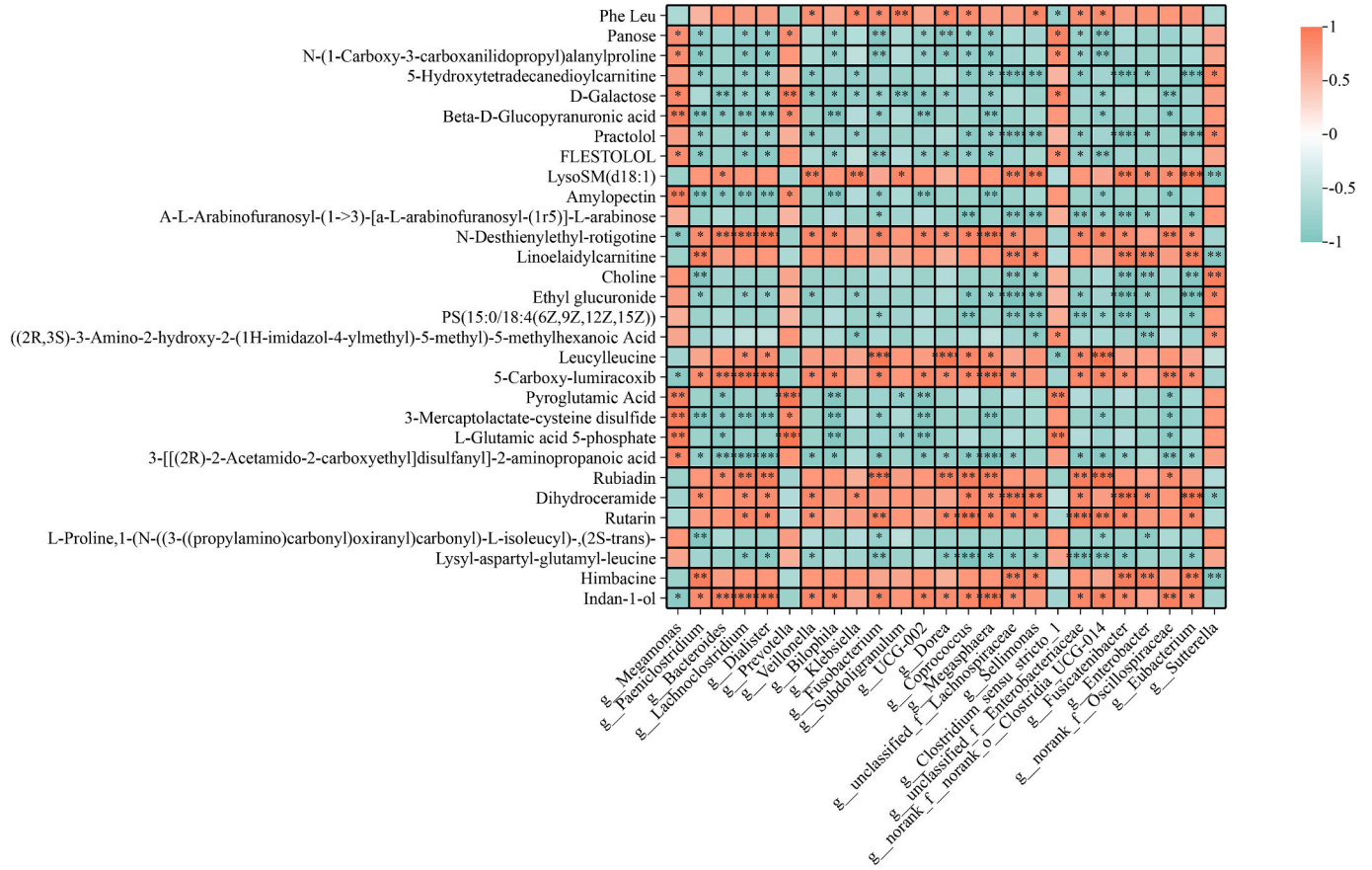

Fig. 7. Correlation analysis between the gut flora and key metabolites.

5. Conclusion

KGM can be used as a nutrient source for intestinal microbes to enrich the relative abundance of *Desulfovibrio*, *Sutterella*, and *Faecalibacterium*, and inhibit the relative abundance of *Paeniclostridium*, *Lachnoclostridium*, *Phascolarctobacterium*, and *Bacteroides*. Meanwhile, acetic and propionic acids were enriched in the KGM group when compared with the Con group. Besides, metabolomic results showed that KGM significantly increased panose, Phe-Leu, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, 5-hydroxytetradecanedioylcarnitineh and D-galactose contents. The content of panose, N-(1-carboxy-3-carboxanilidopropyl) alanylproline, and D-galactose was positively correlated with the relative abundance of *Megamonas*. 5-hydroxytetradecanedioylcarnitineh was positively correlated with *Sutterella*. These results indicate that KGM is mainly metabolized by intestinal microorganisms in the colon, and a large number of metabolites are involved in host metabolism.

CRediT authorship contribution statement

Xiang Tan: Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Botao Wang:** Writing – review & editing, Methodology, Conceptualization. **Xu Zhou:** Writing – review & editing, Methodology, Conceptualization. **Cuiping Liu:** Formal analysis. **Chen Wang:** Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Junying Bai:** Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

None.

Data availability

All data included in this study are available upon request by contact with the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101610.

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