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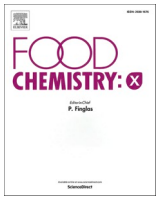

Ganoderma lucidum driven fermentation of *Rosa roxburghii* pomace: Effects on noodle physicochemical properties, digestion, and gut microbiota

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ABSTRACT

Rosa roxburghii pomace (RRP) is a high dietary fiber byproduct that is underutilized. This study investigated the effects of *Ganoderma lucidum* fermented *Rosa roxburghii* pomace (FRRP) on noodle cooking characteristics, texture, structure, *in vitro* digestion, and fermentation. The results showed that soluble dietary fiber, active components (polysaccharides, triterpenoids and dietary flavonoids), and the total cellulase (Filter paper enzymes, Carboxymethyl cellulase), β-glucosidase and laccase activity were significantly increased in FRRP ($p < 0.05$). FRRP improved the cooking and sensory properties of noodles and inhibited starch hydrolysis during *in vitro* digestion. The resistant starch content in noodles with 15 % FRRP increased by 12.63 %, and the predicted glycemic index decreased by 9.34 %. Moreover, the intestinal microbiota structure was significantly improved, promoting the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. This study contributed to the high-value and environmentally friendly utilization of RRP and provided new insights into the development of efficient noodles.

1. Introduction

Rosa roxburghii is widely distributed in southwest China and is favored by consumers because of its rich nutrients (dietary fiber, triterpenoids, dietary flavonoids, organic acids, *etc.*) (Wang et al., 2023). By the end of 2022, the output of fresh *Rosa roxburghii* in Guizhou reached 300,000 tons (Huang et al., 2024), which is mainly used for the processing of *Rosa roxburghii* juice, and 50 % of the *Rosa roxburghii* pomace (RRP) containing a variety of bioactive substances will be produced during the processing (Wang et al., 2022). RRP contains more than 80 % insoluble fiber and is difficult to use in high-value foods due to its rough taste and difficult to swallow (Chu et al., 2019). Therefore, how to make full use of high-fiber and nutrient-rich RRP to enhance its added value, and how to prepare high-quality food are hot issues that researchers and enterprises are generally concerned about. In recent years, nutritionists have improved the ratio of soluble dietary fiber (SDF) in the diet through physical, chemical, and biological treatment modification methods to enhance the function and quality of dietary fiber, making it more widely used (Zhang et al., 2018). Microbial fermentation stands out among the methods mentioned above for its reputation as a relatively safe, environmentally friendly, and cost-effective approach to producing and enhancing high-quality dietary fiber (Chu et al., 2019).

Ganoderma lucidum is a fungus used for both medicinal and food purposes, and its fruiting bodies and mycelium contain active substances such as polysaccharides and triterpenoids (Ahmad, 2018). Its advantage lies in its ability to degrade cellulose, hemicellulose, and lignin, thereby facilitating the release of nutrient-rich small molecules. (Zhu et al., 2022). Zhang et al. (2024) reported that fermentation with *Ganoderma lucidum* increased the activity of cellulase and β-glucosidase activities. Anna et al. (2013) found that laccase from *Ganoderma lucidum* has

Abbreviations: RRP, *Rosa roxburghii* pomace; FRRP, *Ganoderma lucidum* fermented *Rosa roxburghii* pomace; DF, Dietary fiber; SDF, Soluble dietary fiber; IDF, Insoluble dietary fibers; RDS, Rapidly digestible starch; SDS, Slowly digestible starch; OCT, Optimum cooking time; RS, Resistant starch; TS, Total starch; HI, Hydrolysis index; GI, Glycemic index; pGI, Predicted glycemic index; SEM, Scanning electron microscopy; XRD, X-ray diffraction; FT-IR, Fourier transform infrared spectroscopy.

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unique properties that may be important in lignocellulose conversion. In addition, Cen et al. (2024) found that fermentation using *Ganoderma lucidum* improved the processing characteristics of soya pomace as well as a significant increase in active substances such as dietary fiber, polysaccharides, triterpenes and dietary flavonoids. Therefore, based on the high fiber of *RRP* and the high cellulase of *Ganoderma lucidum*, we predicted that fermentation of RRP using *Ganoderma lucidum* as a bacterial source would have the advantages of efficiency enhancement, production of new substances, and resource saving.

Noodles are one of the most popular traditional staple foods in Asia. (Li et al., 2012). However, commercially available refined traditional noodles are often rapidly digestible foods with limited nutritional functions (Liu et al., 2020), leading to a rapid rise in blood sugar levels after meals. This is mainly due to the high proportion of rapidly digestible starch (RDS) in refined noodles, which is quickly absorbed in the intestines and is not conducive to the fermentation process in the colon (Wang et al., 2024). To inhibit the rapid digestion of noodles and promote gut health, adding high dietary fiber is a common approach. Regand et al. (2011) found that dietary fiber (DF) can reduce starch digestibility by altering the microstructure of starchy foods, thereby restricting starch pasting. Additionally, Gao et al. (2023) demonstrated that adding dietary fiber to noodles can increase the number of beneficial bacteria in the gut, such as *Bifidobacteria* and *Lactobacilli*, and improve the intestinal environment. With the changing dietary habits of consumers and the increasing demand for sustainability, innovative nutritional noodles have become a new trend. Therefore, based on the research findings that the bioactive compounds and high dietary fiber produced by efficient fermentation of *Rosa roxburghii* pomace with Ganoderma lucidum can inhibit noodle digestion and improve gut microbiota, we predict that using FRRP as a noodle quality improver can reduce the digestion rate of noodles and increase beneficial gut bacteria.

In light of the aforementioned facts, this study aimed to evaluate the effect of *Ganoderma lucidum* fermentation on RRP and develop an improved noodle product with enhanced nutritional functionality. This research determined the dietary fiber and major active ingredient contents before and after RRP fermentation, and compared the changes in cellulase, β-glucosidase, and laccase activities pre- and post-*Ganoderma lucidum* fermentation. Furthermore, the optimal amount of FRRP to be added in noodle products and the quality characteristics of FRRP noodles were investigated. Finally, the changes in *in vitro* digestibility and GI value of FRRP noodles were elucidated. This study provides a novel and comprehensive foundation for the potential health-promoting role of *Ganoderma lucidum*-fermented RRP noodles.

2. Materials and methods

2.1. Materials

RRP was supplied by Sinopharm Tongjitang (Guizhou, China) Pharmaceutical Co. Ltd.; *Ganoderma lucidum* (CBS: 139793) was provided by Minyuan Bacteria (Chongqing, China). Wheat flour was purchased from Yihai (Zhoukou, China) Cereals and Oil Industry Co. Ltd. (Shanghai, China).

2.2. Strain culture and sample preparation

Add 5 % (*w*/w) food-grade peptone as a nitrogen source, 10–20 % water, and 5 % edible lime to adjust the pH to 10–11 to the RRP. Mix thoroughly and transfer the mixture into polypropylene bags, seal with a mycorrhizal lid, and sterilize at 121 °C for 60 min. Allow the mixture to cool to room temperature to prepare the RRP culture medium. Introduce 2–3 pieces of activated *Ganoderma lucidum* (1 cm × 1 cm × 1 cm) into the medium. After inoculation, place the bags in a constant temperature incubator at 25–28 °C under dark conditions. After 20 days, when the mycelium completely covers the medium, freeze-dry the sample (FDU-2110, Tokyo, Japan), then crushed and ground through a 100-mesh sieve, and then sealed and reserved.

2.3. Determination of the composition of RRP

2.3.1. Dietary fiber, polysaccharide and Total triterpenes

The method was slightly modified according to Cen et al. (2024). (1) Dietary fiber: A 1.0 g sample of RRP was mixed with 40 mL of 0.05 mol/L MES-TRIS buffer. And 100 μL of heat-resistant α-amylase, 50 μL of amylglucosidase, and 20 μL of protease were added for enzyme digestion to decompose starch and protein. The mixture was centrifuged at 8000 rpm for 20 min, and the precipitate was washed sequentially with 20 mL of water at 70 °C, acetone (twice), and 78 % ethanol. The precipitate was dried at 100 °C for 24 h and considered as insoluble dietary fibers (IDF). The supernatant was mixed with 95 % ethanol (60 °C) in a 4:1 ratio and left for 12 h. The precipitate was dried at 100 °C for 24 h to obtain SDF. (2) Polysaccharide: The 1.0 g sample with 10 mL distilled water and 40 mL anhydrous ethanol were mixed, and ultrasonic extraction for 30 min. The mixture was centrifuged at 8000 rpm for 15 min. The precipitate was washed with 80 % ethanol and heated in 100 °C water bath 2 h. After cooling, the supernatant absorbance was measured at 490 nm. (3) Total triterpenes: The 1.0 g sample and 50 mL 95 % ethanol was mixed. Perform ultrasonic extraction for 1 h, centrifuge at 8000 rpm for 15 min. 0.1 mL of 5 % vanillin-glacial acetic acid solution and 0.8 mL of perchloric acid were added to the residue, then mixed and incubated at 60 °C for 20 min. The mixture was rapidly cooled in an ice-water bath, and 5 mL of glacial acetic acid was added. The sample was left to stand at 26 °C for 10 min, and absorbance was measured at 550 nm.

2.3.2. Dietary flavonoids

A 1.0 g sample was dissolved in 70 % methanol and subjected to ultrasonic extraction for 30 min, followed by centrifuged at 5000 rpm and 15 min. The supernatant was diluted with 70 % methanol, and 1 mL of aluminum nitrate and potassium acetate were added, respectively. The solution was further dilute with 70 % methanol, and let stand for 1 h at 26 °C before the absorbance was measured at 420 nm.

2.4. Determination of enzyme activity

Enzyme solutions were prepared according to Xie et al. (2021) with modifications. FRRP and RRP samples (1.0 g each) were added to 50 mL of 0.1 mol/L citrate buffer (pH 4.8), shaken for 2 h at 28 °C and 120 rpm, and the supernatant collected as crude enzyme.

Filter paper enzymes (FPase): Filter paper (50 mg) and 1 mL of crude enzyme were incubated with 1 mL of citrate buffer at 50 °C for 30 min, the absorbance was measured at 540 nm.

Carboxymethyl cellulase (CMCase): 1 mL of Crude enzyme was mixed with 2 mL of 1 % sodium carboxymethyl cellulose and incubated at 50 °C for 30 min, with glucose concentration was determined by measuring absorbance at 540 nm.

β-Glucosidase: 1 mL of the enzyme solution was incubated with 1 mL of 5 mM *p*-nitrophenyl-β-D-glucoside at 50 °C for 30 min, and the release of p-nitrophenol was measured at 400 nm.

Laccase: 30 μL of enzyme solution was reacted with 100 μL of citrate buffer and 70 μL of ABTS substrate for 3 min, with absorbance readings taken at 420 nm initially and after 3 min.

2.5. Noodles preparation

50.0 g wheat flour, 0.5 g edible salt, 4.0 g gluten and different additions of FRRP (0 %, 5 %, 10 %, 15 %, *w*/w). Add 50 mL deionized water, put it into a pasta machine, and press and knead it repeatedly to form smooth and even sheets (1 mm), and cut it into noodles of even thickness (2 mm × 1 mm). Finally, control the water and store in a sealed container (4 °C).

2.6. Noodles characterization

2.6.1. Cooking characteristics of noodles

The cooking characteristics of noodles were evaluated according to Niu et al. (2017), with slight modifications. A measured amount of fresh dry noodles was added to 400 mL of boiling water, and the cooking time was recorded. The noodle breakage rate was calculated based on the number of broken noodles. After draining, the mass of the cooked noodles was measured to assess water absorption. The remaining noodle broth was dried in an oven at 105 °C until reaching a constant weight. After cooling to 26 °C, the mass of the residue was weighed. The calculation formula is as follows:

$$\text{Noodle breakage}\ (\%) = n/30 \times 100\%$$

$$\text{Water absorption}\ (\%) = (W2 - W1)/W1 \times 100\%$$

$$\text{Cooking loss}\ (\%) = W3/W1 \times 100\%$$

Note: n is the number of broken noodles, W_1 is the weight of raw noodles (g), W_2 is the weight of cooked noodles (g) and W_3 is the weight of total residue after drying at 105 °C (g).

2.6.2. Textural properties of noodles

The textural properties of cooked noodles were measured by TA. TOUCH texture analyzer (Shanghai Baosheng Industrial Development Co., Ltd. China). The noodles were cooked the optimal cooking time, and using a TA/2 column. Pre-test and post-test speeds were set 5.00 mm/s, with a test speed of 1.00 mm/s, interval time of 5 s, 75 % of deformation and trigger force of 5.0 g. Three parallel measurements were conducted for each sample.

2.6.3. Scanning electron microscopy (SEM)

SEM (TESCAN MIRA LMS, Czech Republic) was used to observe the microstructure of the noodles. Fresh noodles were fixed on a keeper, and placed under the scanning electron microscope, and the images were observed under an accelerating voltage of 5 kV. s.

2.6.4. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra was determined using a Fourier Transform Infrared Spectrum Analyzer (Thermo Scientific Nicolet iS20, USA) according to the methods of Hong et al. (2021) with slight modification. The freeze-dried noodles were ground and dried with potassium bromide in the ratio of 1:100 in an agate mortar and pressed into thin slices. A Fourier transform infrared spectrometer was used to collect spectra in the 4000–500 cm^{-1} range.

2.6.5. X-ray diffraction (XRD)

The ground 20 mg freeze-dried noodle sample was placed on the sample plate and analyzed by X-ray diffractometer (Rigaku D/max-A, Japan). The measuring operating voltage is 40 kV, the current is 40 mA, the step size is 0.02°, and the scanning range is 5–40°.

2.7. In vitro digestion rate of starch

The simulated digestion of noodles was based on Goh et al. (2015) with modified slightly. 2.5 g cooked noodles were digested in 0 mL of water at 37 °C and 130 r/min. In the oral stage, 0.1 mL of 10 % α-amylase was added. Digestion was halted with 0.8 mL of 1 M HCl after 1 min. For gastric digestion, 1 mL of 10 % pepsin was added to initiate digestion, stopped by 2 mL of 1 mol/L $NaHCO_3$ and 5 mL of maleate solution (pH 6.0) after 30 min. In the intestinal stage, 0.1 mL of glucoamylase and 1 mL of 5 % trypsin were added, and volume adjusted to 55 mL with deionized water. Digest samples were collected at 0, 20, 60, 90, 120, 150, and 180 min, stopped with 3 mL of 95 % ethanol, centrifuged at 5000 r/min at 4 °C for 10 min, and 0.1 mL of supernatant was tested with a D-glucose kit. Starch content was calculated using RDS, SDS, and RS content formulas.

$$\text{RDS} = (G20 \times 0.9)/TS \times 100\%$$

$$\text{SDS} = (G120 - G20) \times 0.9/TS \times 100\%$$

$$\text{RS} = 100\% - (\text{RDS} + \text{SDS})$$

Note: G20 and G120 were the glucose content of 20 min and 120 min of digestion, respectively, and TS was the total starch content.

2.8. Hydrolysis kinetic and pGI

Noodles hydrolysis kinetics were modelled using a non-linear model for starch hydrolysis kinetics (Goñi & Alonso, 1997). Calculation of starch hydrolysis kinetics and prediction of glycemic index were based on the following equations:

$$C(\%) = C\infty\left(1 - e^{-kt}\right)$$

$$pGI = 39.71 + 0.549HI$$

Note: C (%) is the glucose concentration at t (min), C∞ (%) is the equilibrium concentration, k is the kinetic constant, and t is the time; the hydrolysis index (HI) is the ratio of the area of the sample to that of the white bread under the hydrolysis curve.

2.9. Sensory evaluation

The sensory evaluation method for the noodles was slightly modified from the approach described by Xie et al. (2023). Prior to initiating the study, we secured approval from the Ethical Review of Human Medical Experimentation at Guizhou University for all procedures involving human subjects (Number: HMEE-GZU-2024-T032). All participants provided informed consent before participating in the sensory evaluation. After the noodles were cooked to the optimal steaming time, they were numbered from 1 to 4, randomly disrupted and then evaluated by nine trained volunteers, including four females and five males.

2.10. In vitro fermentation by human gut bacteria

2.10.1. Pretreatment of fecal inocula and culture medium

The method was slightly modified from Yu et al. (2024). (1) Pretreatment of fecal inocula: Fresh fecal samples were collected from six healthy volunteers (three males and three females, aged 20–25 years, with no history of gastrointestinal disorders, following a normal diet, and not having received antibiotics within the past three months). Prior to initiating the study, we secured approval from the Ethical Review Board for Human Medical Experimentation at Guizhou University regarding all activities involving human participants (Number: HMEE-GZU-2024-T032). All participants provided informed consent before stool samples were collected. The samples were immediately transferred to an anaerobic incubation chamber, where they were diluted in PBS buffer (0.1 M, pH 7.2) and vortexed for 5 min to obtain a homogeneous fecal slurry. The slurry was then filtered through four layers of gauze to obtain the fecal inoculum. (2) Culture medium: 2.0 g/L peptone, 2 g/L yeast extract, 0.02 g/L haemin, 0.5 g/L L-cysteine, 0.5 g/L bile salt, 2.0 g/L $NaHCO_3$, 0.1 g/L NaCl、0.04 g/L KH_2PO_4, 0.04 g/L K_2HPO_4, 0.01 g/L CaCl2·6H2O, 0.01 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g vitamin K_1, 0.01 g resazur, 2 mL/L Tween-80, distilled water 1 L. and the pH was adjusted to 7.6 with 0.1 mol/L HCl solution. The medium was sterilized at 121 °C for 20 min before use.

2.10.2. In vitro fermentation

In vitro fecal fermentation with slight modifications according to Gao et al. (2023). The above medium was quantitatively dispensed (9 mL) in fermentation test tubes and four graded 2 % noodle samples (*w/v*) were added under aseptic conditions along with 1 mL of fecal inoculum, with

inulin as a positive control and carbohydrate free as a negative control. The fermentation tubes were incubated in an anaerobic incubator at 37 °C and samples were taken at 0 h, 6 h, 12 h and 24 h time intervals for further analysis. Three parallel groups were set up for each treatment group.

2.10.3. DNA extraction and 16S rRNA gene sequencing

The microbial composition of each sample was analyzed after 24 h of fermentation. Genomic DNA was extracted from the samples using the E. Z.N.A. Soil DNA Kit (Omega Bio-tek, Inc., USA) and DNA quality and concentration were checked, and the DNA samples were stored at −20 °C for subsequent experiments. PCR amplification of the bacterial 16Sr RNA gene was performed using the universal primers 338F (5'-ACTCCTACGGGGAGGCAGCAG-3′) and 806R (5'-GGACTACHVGGGTWTCTAAT-3′). V3-V4 region of the 16Sr RNA gene. The final libraries were sequenced on the Illumina Miseq/Nextseq 2000/Novaseq 6000 (Illumina, Inc., USA) platform with the sequencing strategy PE250/PE300.

2.11. Statistical analysis

All experiments were conducted in three replicates, unless stated otherwise. Data were presented as mean ± standard deviation. ANOVA, using SPSS Statistics 26.0 (IBM, New York, USA), assessed significant differences ($p < 0.05$).

3. Results and discussion

3.1. Determination of FRRP components

3.1.1. Dietary fiber

As shown in Fig. 1A, the content of total dietary fiber (TDF) in RRP after fermentation significantly decreased to 76.77 %, while the content of SDF significantly increased to 19.13 % ($p < 0.05$), indicating that *Ganoderma lucidum* degraded some of the dietary fiber during RRP fermentation and promoted the conversion of SDF. This could be attributed to the production of cellulase, β-glucosidase and laccase by *Ganoderma* fermentation, which lead to the degradation of cellulose and hemicellulose (Sitarz et al., 2013). In addition, the SDF/IDF ratio increased from 11.86 % to 33.19 % ($p < 0.05$), and the change in the SDF/IDF ratio was mainly attributed to the partial solubilization and depolymerization of hemicellulose and insoluble pectin substances, which promoted the production of SDF (Chu et al., 2019). Zhao et al. (2017) utilized *Lactobacillus* solidus to ferment wheat bran and the SDF increased by only 3.93 %. Wang et al. (2022) used *Bacillus natto* to ferment RRP and the IDF content decreased by less than 10 %. Obviously, we found that fermentation of RRP using *Ganoderma lucidum* was more significant effect. Some studies have shown that SDF has the function of lowering blood lipid levels, lowering blood pressure, improving glycemic control and reducing body weight (Surampudi et al., 2016), while *Ganoderma lucidum* fermentation can increase the content of SDF in RRP, which is a good source of SDF and provides us with a research basis for the development of products, such as noodles, for glycemic control.

3.1.2. Active components

As shown in the Fig. 1B, the actives content in the RRP of *Ganoderma lucidum* before and after fermentation, which showed an increase of 100.20 % in polysaccharides, 46.58 % in dietary flavonoids, and 62.14 % in triterpenoids ($p < 0.05$). The similar results in fermentation of prune pomace using a mixture of probiotic bacteria and the content of actives in fermented prune pomace was significantly higher than that of the unfermented group (Zhu et al., 2020). However, we found that *Ganoderma lucidum* fermentation of RRP was more effective. This may be due to the fact that the enzymes produced by *Ganoderma* fermentation (cellulase, β-glucosidase and laccase, *etc.*) are closely related to the

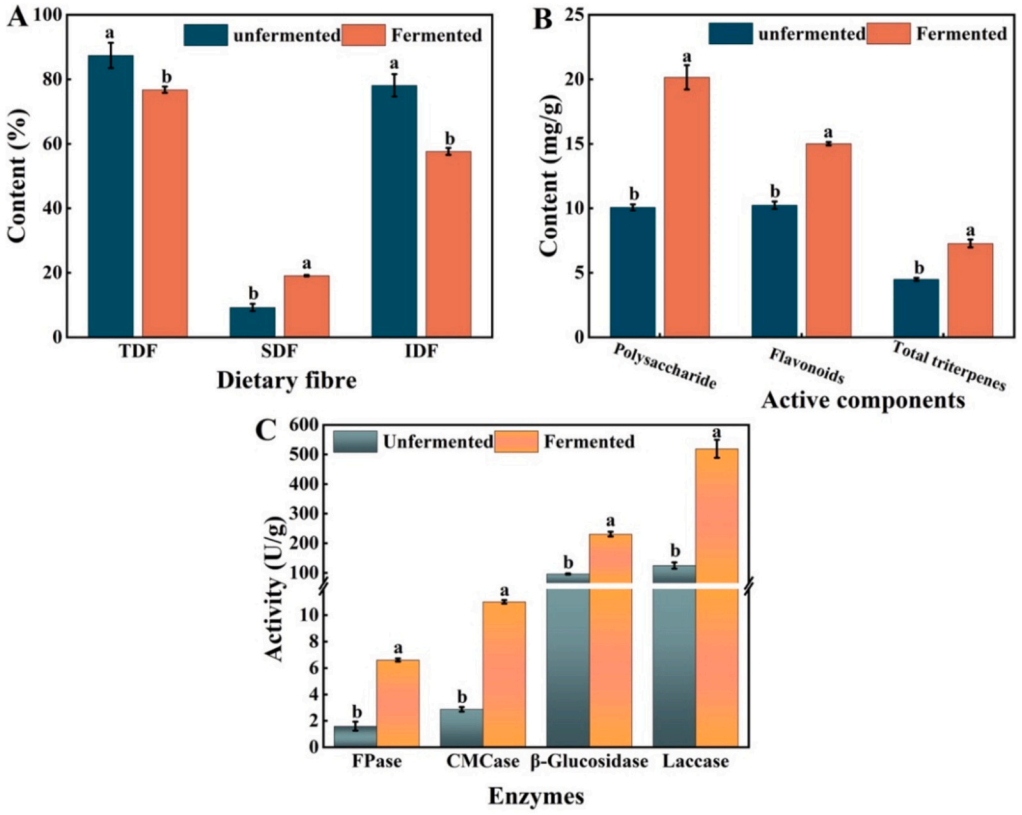

Fig. 1. Effect of *Ganoderma lucidum* fermentation on RRP characterization. Dietary fiber content in RRP (A); Active components content (B); Enzyme activity (C).

content of active substances in RRP. These enzymes hydrolyze ether, ester or glycosidic bonds and are able to effectively break down the cell wall structure to release polysaccharides and triterpenes (Zhang et al., 2024). It has also been reported that several types of esterases can be released through microbial fermentation, and esterases are involved in the transformation of dietary flavonoids structures, leading to the release of dietary flavonoids compounds (Shahidi & Yeo, 2016). Dietary flavonoids are widely found in plants and have a wide range of biological activities with significant positive effects on health (Li et al., 2023). Studies have shown that dietary flavonoid intake is negatively associated with a number of chronic diseases, including cardiovascular disease, cancer, diabetes, and other diseases such as hypertension (Zhou et al., 2022). In addition, dietary flavonoids can improve gut health by modulating the gut microbiota, promoting the growth of beneficial bacteria and inhibiting potentially pathogenic bacteria (Baky et al., 2022). Therefore, fermentation of RRP by *Ganoderma lucidum* significantly increased the RRP polysaccharides, dietary flavonoids and triterpenoids content, which facilitated the development of functional products while improving the utilization of RRP.

3.2. Enzyme activity

Cellulose is a glucose-based macromolecular polysaccharide that can be degraded by carbohydrate hydrolases such as cellulase and β-glucosidase (Xie et al., 2021). Lignin is a complex phenolic polymer that is challenging to degrade but can be oxidatively broken down by laccase (Silva et al., 2005). The effect of *Ganoderma lucidum* fermentation on the different enzymatic activities of RRP is shown in Fig. 1C. The enzymatic activities of FPase, CMCase, β-Glucosidase, laccase produced by FRRP were significantly increased by 3.13-fold, 2.82-fold, 1.39-fold, and 3.16-fold, respectively ($p < 0.05$). It indicated that cellulase and β-glucosidase as well as laccase were produced during the fermentation of *Ganoderma lucidum*. It has been shown that cellulolytic enzymes hydrolyze cellulose and hemicellulose, thereby increasing the SDF content (Jiang et al., 2024), which is consistent with the changes in dietary fiber before and after the fermentation of prickly pear pomace. Therefore, cellulase, β-glucosidase and laccase produced by *Ganoderma lucidum* could increase the added value of *RRP*.

3.3. Cooking properties

Cooking characteristics are key indicators for evaluating noodle quality. As shown in Table 1, the cooking losses for 0 %, 5 %, 10 %, and 15 % FRRP were 6.83 %, 7.17 %, 7.59 %, and 7.93 %, respectively. Noodles with cooking loss not exceeding 8 % is considered high quality (Sun et al., 2023), and the cooking loss in the 5 %, 10 %, and 15 % FRRP groups was within acceptable limits. The significant ($p < 0.05$) difference in cooking loss between the FRRP-added groups and the control (0 %) may be due to DF disrupting the starch matrix, causing surface starch to solubilize during cooking. The optimal cooking time increased to 315.00s (10 % group), likely because higher DF content interfered with starch water utilization, delaying gelatinization. FRRP, rich in dietary fiber (DF), increases water absorption due to hydroxyl groups in DF interacting with water molecules (Giuberti et al., 2015). However, excessive DF disrupts the gluten network and promotes starch granule rupture, it may destabilize the gluten protein network and lead to the disordered aggregation of protein molecules, and this amorphous aggregation may make the noodle easy to break after cooking (Housmans et al., 2023), leading to the highest cooking loss and water absorption in the 15 % group. Although steaming may cause noodles to break due to swollen starch granules, no significant difference in breakage rate was observed between FRRP-added noodles and the control. Considering the data on optimum cooking time, breakage, water absorption, and cooking loss, the best noodle quality was achieved with 10 % FRRP.

Table 1
Effect of FRRP on the Cooking Characteristics of Noodles.

Noodle samples	OCT (s)	Noodle breakage rate (%)	Water absorption (%)	Cooking loss (%)
0 %	280.00 ± 4.08^{c}	12.22 ± 1.57^{a}	118.96 ± 8.73^{c}	6.83 ± 0.19^{d}
5 %	295.00 ± 4.08^{b}	11.11 ± 1.57^{a}	140.63 ± 1.67^{b}	7.17 ± 0.14^{c}
10 %	315.00 ± 4.08^{a}	12.22 ± 1.57^{a}	152.43 ± 1.59^{a}	7.59 ± 0.03^{b}
15 %	301.67 ± 6.24^{b}	13.33 ± 2.72^{a}	162.41 ± 1.65^{a}	7.93 ± 0.06^{a}

Note: The values in the table are expressed as mean ± standard deviation (n = 3), and different letters in the same column represent significant differences ($p < 0.05$) in the cooking characteristics of noodles with different additions of FRRP.

3.4. Texture properties

The textural properties of noodles with 0 % - 15 % FRRP addition are shown in Table 2. Significant differences ($p < 0.05$) in hardness, chewiness, adhesiveness, cohesiveness, and springiness were observed between noodles with and without FRRP. Hardness and springiness increased with 5 % and 10 % FRRP, likely due to non-covalent bonding between SDF and proteins, forming a three-dimensional gel network, this gel network enables tighter cross-linking of gluten proteins, resulting in protein gel-like aggregates (Mukherjee & Gupta, 2017). And phenolic acids in IDF combining with gluten to enhance the network structure (Li et al., 2017). The dietary fiber in FRRP may disrupt protein molecular arrangement, altering the protein network structure. Studies have indicated that moderate dietary fiber enhances protein cross-linking, thereby improving noodle elasticity and toughness. However, excessive fiber can loosen the network structure and increase pore size, preventing protein cross-linking, weaking the gluten network, and ultimately reduces noodle hardness and resilience (Fan et al., 2022; Fan et al., 2024). The adhesiveness of noodles with FRRP was higher than that of the 0 % group, likely due to IDF disrupting the network structure. Generally, higher adhesiveness indicates more soluble matter on the noodle surface after cooking, consistent with the trend in steaming loss. Chewiness, which measures the energy and time needed for chewing and swallowing, also increased with more FRRP, possibly due to the enhanced protein-polysaccharide gel network (Li et al., 2022). Therefore, adding 10 % FRRP effectively increased hardness, springiness, chewiness, and adhesiveness without negatively affecting the noodles.

3.5. Characterization of noodle structure

3.5.1. SEM

The microstructure changes in noodles with varying FRRP additions are shown in Fig. 2. The 0 % group surface rough, large porous, and an irregular overall shape. With the addition of FRRP, particularly in the 5 % and 10 % groups, a dense and uniform network structure emerged. In the 10 % group, smaller gluten network holes and a uniform, dense structure were observed, likely due to SDF forming a continuous three-dimensional gel network with proteins (Zhang et al., 2020). This structure tightly encapsulated starch granules, enhancing the noodles' textural properties, consistent with previous textural findings. However, with 15 % FRRP, the gluten network became discontinuous, with larger pores and irregular surfaces. This disruption, caused by the high DF concentration diluting gluten proteins, led to a loose, porous network, making it difficult to encapsulate starch granules, which then decomposed during cooking, releasing more amylose (Sun et al., 2023). This correlates with the highest cooking loss rate observed in the 15 % group. Therefore, moderate FRRP addition improved the noodles' microstructure, similar to the effects seen with heat-treated sweet potato pomace (Sun et al., 2023). In conclusion, FRRP enhances the microstructure and overall quality of noodles.

Table 2
Effect of FRRP on textural properties of noodles.

Noodle samples	Hardness/g	Chewiness/g	Adhesiveness/g × s	Cohesiveness	Springiness
0%	213.65 ± 5.81^{d}	83.78 ± 1.44^{c}	−3.46 ± 0.12^{a}	0.60 ± 0.01^{d}	0.56 ± 0.02^{d}
5 %	279.24 ± 3.46^{b}	96.08 ± 1.59^{b}	−4.57 ± 0.20^{b}	0.67 ± 0.01^{b}	0.74 ± 0.03^{b}
10 %	318.49 ± 2.33^{a}	102.5 ± 0.82^{a}	−7.05 ± 0.48^{c}	0.72 ± 0.02^{a}	0.81 ± 0.05^{a}
15 %	258.44 ± 1.70^{c}	88.56 ± 0.92^{c}	−8.48 ± 0.38^{d}	0.63 ± 0.02^{c}	0.66 ± 0.01^{c}

Note: The values in the table are expressed as mean ± standard deviation (n = 3), and different lowercase letters in the same column represent significant differences in the texture characteristics of noodles with different addition amounts of FRRP ($p < 0.05$).

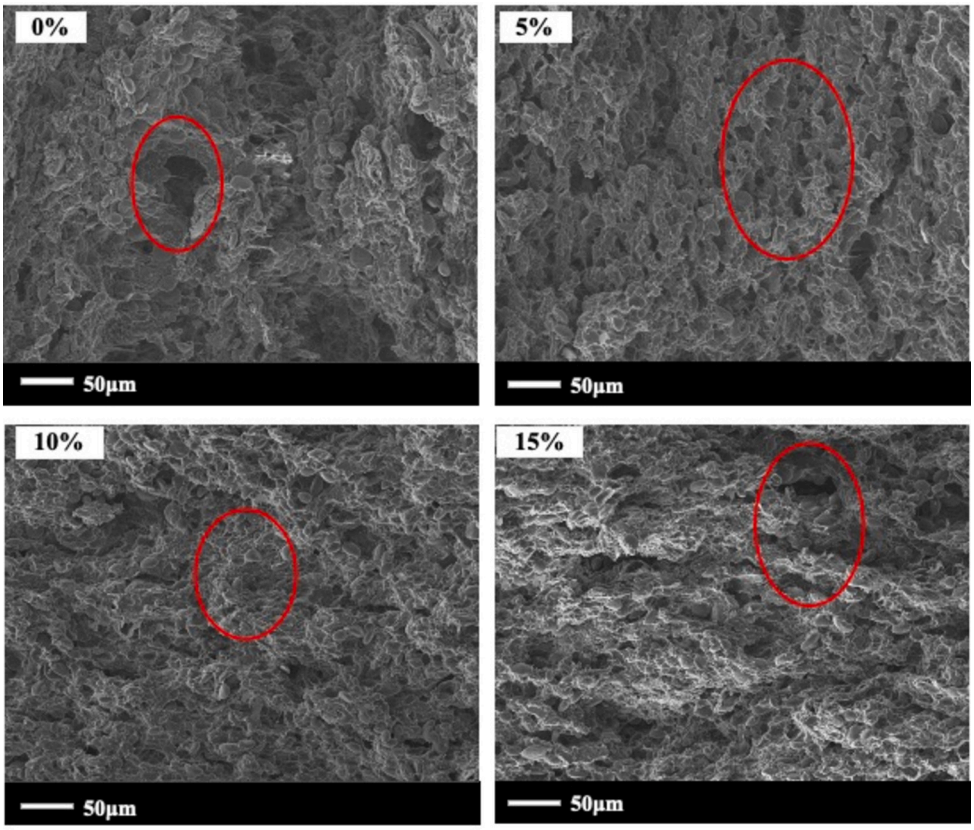

Fig. 2. Effect of FRRP on the microstructure of noodles.

3.5.2. FTIR

Fig. 3A presents the FTIR spectra of noodles with varying amounts of FRRP. The spectra of noodles with FRRP addition were similar to those without, showing no new absorption bands, but the intensity of some of the absorption peaks changed, indicating that the addition of FRRP did not form new chemical bonds in the starch. From the infrared spectra of the noodles with different amounts of FRRB, it can be seen that the peak at 3438 cm^{-1} corresponds to the absorption peak caused by the stretching vibration of the hydroxyl group, and the comparison can be seen that with the increase of the amount of FRRP added, its intensity is getting smaller, which indicates that its content is decreasing, the peak at 2931 cm^{-1} corresponds to the stretching vibration of the C—H bond in the methyl group or the methylene group, and the peak at 1605 cm^{-1} corresponds to the C=C-H bond in the methyl group. The peak at 2931 cm^{-1} corresponds to the stretching vibration of C—H bond in methyl or methylene group; the peak at 1605 cm^{-1} corresponds to the stretching vibration of C═C bond, and the comparison shows that the intensity of the vibration peak is the largest when the FRRP addition is 5 %; the peak

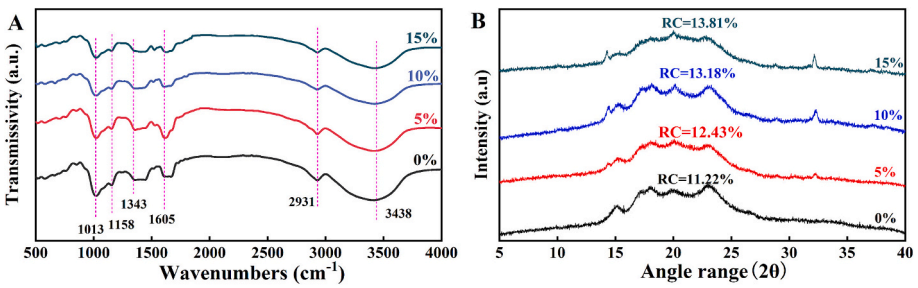

Fig. 3. Effect of different concentrations of FRRP on noodles structure. FTIR (A); XRD (B);

at 1343 cm^{-1} is the absorption peak caused by the stretching vibration of the C—N bond in the amide structure; the peaks at 1158 cm^{-1} and 1013 cm^{-1} correspond to the stretching vibration of the C—O bond.

3.5.3. XRD

The X-ray diffraction (XRD) patterns of noodles with varying levels of FRRP supplementation are illustrated in Fig. 3B. It is noteworthy that the addition of FRRP increased the relative crystallinity (RC) of the noodles. The reasons for this phenomenon may be (1) FRRP was surrounded by the reticulation of starch during heating, which inhibited its pasty form. Some of these small granular starches did not swell and collapse completely (Fig. 2), preserving their crystal structure and increasing the crystallinity of the starch mixture. (2) The addition of FRRP promoted the recrystallisation of starch during pasta retorting, which could be attributed to the fact that the double helixes of dietary fibers and straight-chain starch or branched-chain starch in FRRP were intertwined and interacted with each other, which enhanced the network structure of the starch gel. (3) Protein gels are three-dimensional network structures formed by protein molecules. With the increase of FRRP addition, the protein content in the noodles gradually decreased, resulting in the weakened protein gel network and increased pore size, which enhanced intermolecular cross-linking and resulted in more organized arrangement of protein molecules. This arrangement promotes the formation of intermolecular hydrogen bonds between starch and recrystallization of starch, which in turn increases the relative crystallinity of the noodles (Gui et al., 2020; Wang et al., 2020). In addition, it has been shown that a higher RC value indicates a more stable structure, which enhances the resistance of noodles to digestion (Zhou et al., 2024).

3.6. Sensory properties

Sensory evaluation is one of the indicators for evaluating the quality of noodles during the development of noodle products. As shown in Fig. 4C, the sensory evaluation of noodle samples made with different fermented RRP additions was performed. Noodles with FRRP addition (5 % and 10 %) scored well in terms of taste and stickiness. The variation in flavor was attributed to the unique flavor and sweetness of *Rosa roxburghii*. However, with excessive addition of FRRP (15 %), the noodles scored significantly lower in terms of stickiness and palate. This is consistent with the change in textural properties of the noodles. When FRRP was added at 15 %, the sensory score of the noodles was 80.28 ± 1.75, which showed poor sensory characteristics. However, when FRRP was added at 0 %, 5 % and 10 %, the sensory composite scores of noodles were 87.22 ± 1.36, 88.11 ± 1.93 and 85.39 ± 2.08, respectively, and the highest sensory evaluation of noodles with 5 % FRRP was found to be the most acceptable, thus noodles with 5 % FRRP were the most preferred.

3.7. In vitro digestion rate of starch

Fig. 4A shows that adding FRRP significantly decreased the *in vitro* starch digestion rate of noodles ($p < 0.05$). All samples exhibited similar hydrolysis trends, but the hydrolysis rate for the 15 % FRRP addition was significantly lower than that of the 0 % group. The starch digestion rate increased rapidly up to 20 min. Subsequently, the release of glucose gradually decreased with the increase of digestion time, showing a gradual slowing down trend, and finally maintaining a lower rate in the late stage of digestion, reaching 78.78 % in the 0 % group and only to 65.19 % in the 15 % group at 180 min. This may be due to the fact that DF in FRRP inhibits starch hydrolysis and glucose release to some extent (Dhingra et al., 2012). Thus, the reducing the digestion rate.

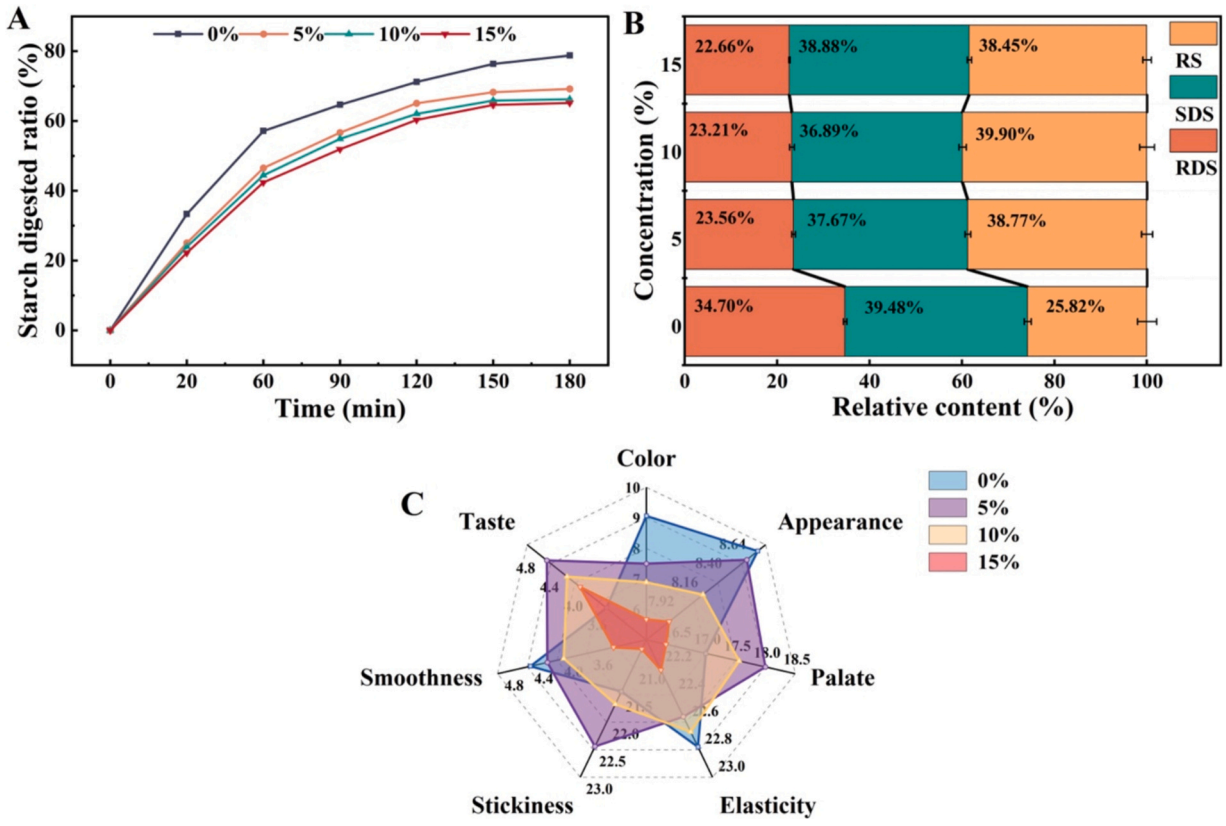

Fig. 4. Effect of different concentrations of FRRP on starch digestibility of noodles and sensory evaluation. *In vitro* digestion curve of cooked noodles (A); The contents of RDS, SDS, and RS of cooked noodles (B); Sensory properties of noodles (C).

The proportions of RDS, SDS and RS in the pasta starch were changed by the addition of FRRP. As shown in Fig. 4B, the RDS content of noodles with FRRP addition (23.56 %, 23.21 %, and 22.66 %) decreased significantly ($p < 0.05$) compared to RDS (34.70 %) in the 0 % group. SDS was significantly higher (40.05 %) in the 5 % FRRP noodles added than in the 0 % group (37.88 %) ($p < 0.05$). The RS content of the noodles with added FRRP (34.90 %, 37.96 %, 39.68 %) was significantly higher ($p < 0.05$) than that of the 0 % group (28.83 %). This may be due to the fact that DF is not easily hydrolyzed by digestive enzymes in added FRRP, DF is an excellent starch coating material that protects starch from swelling and binding to amylase, therefore reducing the action of the enzyme in order to inhibit starch hydrolysis, which resulted in a significant increase in the percentage of RS ($p < 0.05$) (Yin et al., 2021).

3.8. Hydrolysis kinetic and pGI

To predict the glycemic response of FRRP noodles, the digestion process was simulated using a first-order kinetic function. The correlation results are shown in Table 3. The C_∞ of the noodles decreased significantly from 77.39 % to 69.07 %, The hydrolysis index (HI) of the noodles significantly decreased from 84.92 % to 67.90 %. Interestingly, the postprandial glycemic index (pGI) of the noodles significantly decreased from 86.33 to 76.99. The k value of noodles was also significantly reduced as compared to the control. The result further indicated that the addition of FRRP reduced the starch digestibility of the noodles. This may be related to the main components of FRRP, as more FRRP was added thereby replacing the starch matrix and decreasing the total starch content, the noodle starch was replaced by FRRP, the DF in the noodles increased, and the dietary fiber in the FRRP contained a large number of hydrophilic groups, which absorbed water and swelled to impede the action of digestive enzymes, thus inhibiting the starch digestion (Xie et al., 2023). Moreover, the addition of DF to starch formulations can lead to enhanced overlap between molecular chains through non-covalent bonding interactions between DF and starch, limiting starch pasting and therefore reducing starch digestibility (Zhang et al., 2022). Thus, the addition of FRRP has a great potential for digestive resistance function.

3.9. Analysis of the vitro simulated fermentation

3.9.1. Alpha and beta diversity analysis

In order to estimate the effect of ingesting FRRP-containing noodles on the human gut microbiota, we analyzed the α-diversity of the microbial community *in vitro*. The community richness was expressed by the Chao1 index, and the community diversity was described by the Shannon index. As shown in Fig. 5A and Fig. 5B, after 24 h of fermentation, the microbial diversity of the FRRP-added noodle group decreased significantly ($p < 0.05$) compared with the control group, and there was not significant difference compared with the inulin group. This is consistent with the findings of Yi et al. (Yi et al., 2022). The addition of FRRP noodles appears to reduce the richness and diversity of the human gut microbiota. In addition, the β-diversity of the microbial community was analyzed using PCoA. As shown in Fig. 5C, PCoA1 accounted for 60.42 % of the variance, whereas PCoA2 accounted for 22.13 % of the variance, which indicates that the microbial structure of the control group changed significantly from the other groups (inulin group or noodle group) after fermentation. These results indicated a significant separation of the intestinal communities, which lead to the conclusion that FRRP-enriched noodles significantly altered the compositional structure of the intestinal community.

Table 3
Effect of FRRP on the degree of hydrolysis extent, kinetic constants, hydrolysis index and predicted glycaemic index of noodles.

Samples	C_∞ (%)	k	HI (%)	pGI
0%	77.39 ± 0.79^a	0.023 ± 0.002^a	84.92 ± 0.24^a	86.33 ± 0.13^a
5 %	72.00 ± 0.30^b	0.018 ± 0.001^b	73.43 ± 0.30^b	83.33 ± 0.18^b
10 %	69.10 ± 0.07^c	0.018 ± 0.001^b	70.46 ± 0.19^c	78.39 ± 0.11^c
15 %	69.07 ± 0.28^c	0.166 ± 0.001^c	67.90 ± 0.14^d	76.99 ± 0.08^d

Note: The values in the table are expressed as mean ± standard deviation (n = 3), different lowercase letters in the same column represent values that are significantly different ($p < 0.05$).

3.9.2. Structure of the intestinal microbiota

To further investigate the effect of FRRP-enriched noodles on the composition of the intestinal community, we analyzed the microbial community structure of different treatment groups at the phylum and genus levels. At the phylum level (Fig. 5D), the main intestinal community in the FRRP-enriched noodle group was dominated by *Bacteroidetes*, *Actinobacteriota*, and *Firmicutes*. It was observed that the *Actinobacteria* and *Firmicutes* were able to degrade complex polymers, thereby enhancing their utilization (Xia et al., 2022). When the FRRP addition amount is 5 %, the relative abundance of the *Bacteroidetes* in the noodle group is the highest. The *Bacteroidetes* can degrade polysaccharides and oligosaccharides in the substrate and provide nutrients for the host and other intestinal microorganisms (Zafar & Saier Jr., 2021). At the genus level (Fig. 5E), when the FRRP addition was 5 %, the relative abundances of *Bifidobacterium* and *Lactobacillus* in the noodle group were higher than those in the control group (Con) and other addition groups. This finding similar with the research conducted by Gao et al. (2023). *Bifidobacteria* and *Lactobacilli* are recognized as beneficial bacteria. Studies have demonstrated that an increase in the abundance of these microorganisms is negatively correlated with levels of inflammatory cytokines and the incidence of obesity (Michael et al., 2020). In addition, the relative abundance of Escherichia-Shigella in the noodle group without FRRP addition (FRRP0) was higher than that in the control group (without carbon source, treated with pure fecal fermentation). This increase may be due to the high protein content in the noodle digestion products added to the fermentation system. These protein degradation products can provide nutrients for *Escherichia-Shigella*, thereby promoting its growth. Fan et al. (2017) observed in animal models that a high-protein diet promoted the growth of this bacterial group. Similarly, Gao et al. (2023) observed in an experiment with whole-wheat noodles containing added soluble dietary fiber that the relative abundance of *Escherichia-Shigella* was higher in the fiber-added noodle group compared to the control group. However, as the amount of FRRP in the noodles increased, the groups with 5 % and 15 % FRRP addition showed a significant reduction in *Escherichia-Shigella* abundance. When the FRRP addition reached 15 %, the relative abundance of *Escherichia-Shigella* was significantly reduced by 11.62 % compared to the FRRP0 group ($p < 0.05$). No significant difference in *Escherichia-Shigella* abundance was observed with 10 % FRRP addition. These results suggest that adding FRRP to noodles does not increase the risk of *Escherichia-Shigella* infection and may even have a potential inhibitory effect. Additionally, using linear discriminant analysis (LDA) effect size (LEfSe), we explored the significant differences in microbial communities across noodle groups with varying FRRP additions. As shown in Fig. 5F and Fig. 5G, LEfSe analysis identified 35 significantly different intestinal community ($p < 0.05$). When the FRRP addition was 5 %, the dominant flora in the noodle group were Bifidobacterium and Actinomycetes. This suggests that the 5 % FRRP addition in noodles can significantly increase the abundance of Bifidobacterium in the intestinal community, thereby enhancing the composition of the gut microbiota. Therefore, when FRRP was added at 5 % in noodles, it was able to significantly enhance the abundance of Bifidobacteria in the intestinal community, which improved the composition of the intestinal microbiota.

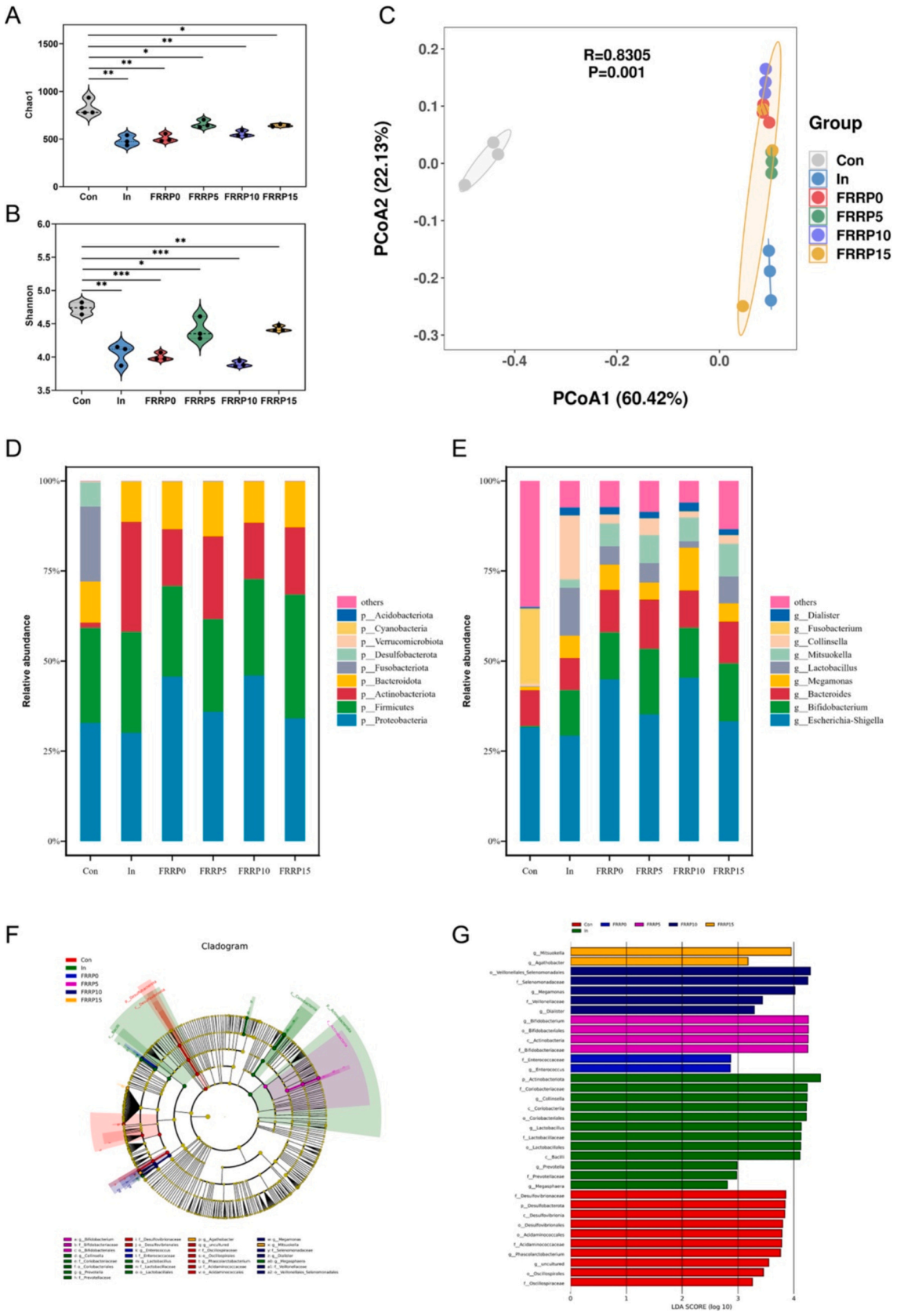

Fig. 5. Chao1 index (A); Shannon index (B); Principal Coordinate (PCoA) Analysis (C); phylum level (D); genus level (E); LEfSe analysis (F); LDA score histogram (D).

4. Conclusion

This study examined the impact of *Ganoderma lucidum* fermentation on the composition of *RRP* and the effect of FRRP on noodles quality characteristics. The activities of FPase, CMCase, β-Glucosidase and laccase of RRP were significantly enhanced by *Ganoderma* fermentation. These enzymes promoted the degradation of cellulose and lignin on the surface of *RRP*, improving the content of SDF, polysaccharides, triterpenoids and dietary flavonoids, and other active ingredients. Meanwhile, the addition of FRRP improved the orderliness of the starch structure in the noodles was enhanced, resulting in a tighter starch structure and higher RC value, which improved the texture and cooking characteristics of the noodles. As the proportion increased, the RS of noodles gradually increased and the pGI value of starch gradually decreased, and effectively limited the interaction between starch digestive enzymes and starch. In addition, the addition of 5 % FRRP to the noodles significantly affected the diversity and composition of the intestinal microbiota in the *in vitro* fermentation results. This was especially true for beneficial bacteria such as Bifidobacteria and Lactobacilli. In conclusion, *Ganoderma lucidum*-fermented *RRP* showed positive changes in nutrition and application, which provides a new pathway and theoretical basis for the comprehensive development and utilization of *RRP*, as well as a good prospect and potential.

CRediT authorship contribution statement

Mingzhu Liu: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Kai Yan:** Writing – original draft, Resources. **Shan Yu:** Visualization. **Fuyao Tan:** Resources, Investigation, Formal analysis. **Wenkang Hu:** Resources. **Ziru Dai:** Methodology. **Huaimao Tie:** Visualization. **Xuefeng Zeng:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest in the publication of this manuscript.

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Data availability

Data will be made available on request.

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