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Wheat bran soluble fibers enhance intestinal barrier function and shape gut microbiota via digestion-resistant bioactivities

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ABSTRACT: Wheat bran represents an abundant milling byproduct with untapped value for developing functional food ingredients. In this study, a soluble fiber fraction (WBDF) was isolated from wheat bran, composed mainly of glucose ($12.23 \pm 0.18\%$), xylose ($41.94 \pm 0.28\%$) and arabinose ($35.49 \pm 0.26\%$), with a molecular weight of 9.35×10^5 Da. Combined structural and functional analyses confirmed the digestion resistance of WBDF through its retained monosaccharide composition, antioxidant activity and α -glucosidase inhibition. When evaluated in an LPS-stimulated Caco-2/RAW 264.7 co-culture system, WBDF alleviated inflammation-associated epithelial dysfunction by restoring transepithelial electrical resistance, reducing paracellular permeability, consistent with strengthened tight-junction expression and reduced pro-inflammatory cytokines. Subsequent *in vitro* fermentation confirmed that WBDF served as a fermentable substrate for gut microbiota, increasing short-chain fatty acid production and promoting the growth of beneficial taxa such as *Bifidobacterium* and *Prevotella*, while reducing *Fusobacterium* relative abundance. Overall, this work establishes a mechanistic framework that connects digestive stability, intestinal barrier protection, and microbiota modulation, highlighting WBDF as a promising functional ingredient derived from grain-processing byproducts.

Keywords: Wheat bran; Milling byproducts; Dietary fiber; Simulated digestion; Intestinal barrier function; Gut microbiota

1. Introduction

Maintaining intestinal health is essential for overall physiological balance. The gut functions not only as a digestive organ but also as a key interface that regulates nutrient absorption, immune responses, and metabolic homeostasis^[1]. The intestinal barrier and the gut microbiota are critical, interdependent components in this process. While the intestinal barrier serves as a physical shield, preventing harmful substances from entering the systemic circulation, the gut microbiota facilitates immune regulation. This regulatory activity, in turn, contributes to the production of bioactive metabolites that support overall host health^[2]. A stable barrier structure and a balanced microbial community are central to sustaining host health. However, inflammation,

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stress, and inappropriate dietary patterns can disrupt this system. Damage to the epithelial layer weakens its protective role, while disturbances in microbial composition may promote the growth of harmful bacteria and reduce beneficial metabolites such as short-chain fatty acids (SCFAs). These changes can further aggravate inflammation and increase the risk of chronic disorder^[1]. As a result, dietary strategies that help preserve barrier integrity and support microbial balance have gained increasing attention.

Dietary fibers that resist degradation by upper gastrointestinal enzymes can reach the colon largely intact, where they interact closely with the gut microbiota and shape its ecological balance^[3]. Subsequently, these fibers serve as fermentable substrates for colonic microorganisms, promoting the production of beneficial metabolites such as SCFAs. In addition to supporting nutrient metabolism and immune regulation, both SCFAs and the selective fermentation process contribute to modulating gut microbial composition, enhancing the growth of beneficial taxa and strengthening colonization resistance against pathogens. For instance, β -glucans from oats and barley effectively lower serum cholesterol and improve glycemic control by increasing SCFAs production and enriching *Bifidobacterium* and *Lactobacillus*^[4]. An *in vitro* infant gut microbiota model revealed that wheat, sorghum, rice, and oat cereals selectively increased fiber-degrading taxa (e.g., *Bifidobacteriaceae*, *Ruminococcaceae*) while reducing *Enterobacteriaceae*^[5]. These findings underscore the health relevance of cereal-derived bioactivity and their interaction with gut microbial communities.

Wheat (*Triticum aestivum* L.) ranks among the top three cereal crops globally, supplying around 20% of the world's dietary protein and caloric needs^[6]. Wheat bran, constituting approximately 15–20% of the grain's dry weight, is generated during the milling process and consists of the outer pericarp, the aleurone layer, and residual endosperm. Growing evidence has emphasized the functional value of wheat bran, transforming its status from a milling byproduct into a recognized functional food ingredient^[7]. In particular, arabinoxylan-rich fractions from wheat bran have been extensively documented for their prebiotic and gut barrier-protective properties^[8]. However, much of the existing literature focuses on either insoluble fiber fractions prevalent in cereals or on purified, commercially available arabinoxylans. Critical gaps remain regarding the specific bioactivity and functional fate of the soluble dietary fiber fraction derived directly from wheat bran processing streams, especially its resilience to gastrointestinal digestion and the consequent implications for intestinal health.

To address these gaps, this study diverges from prior work by specifically isolating and characterizing a soluble dietary fiber (WBDF) from wheat bran, thereby emphasizing its higher value utilization. We first establish that WBDF maintains its core structure and functional attributes under simulated digestive conditions. This confirmed stability provides the rationale for probing its dual role in mucosal protection and microbial modulation. Furthermore, its protective effects were assessed using an inflammation-induced Caco-2/RAW 264.7 co-culture model, and its capacity to modulate gut microbiota was examined through *in vitro* fermentation. This approach provides new mechanistic insight into how soluble cereal-derived fibers

contribute to gut health and strengthens the scientific basis for the high-value utilization of wheat bran processing by-products.

2. Materials and methods

2.1 Materials and reagents

Yangmai No.25 wheat bran was provided from Jiangsu University of Science and Technology, Nanjing, China. Caco-2 and RAW 264.7 cell were obtained from National Collection of Authenticated Cell Culture. Dulbecco's modified eagle medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Thermo Fisher Scientific Co., Ltd. (Waltham, USA). Trypsin-EDTA solution (0.25%, w/v) was bought from Sigma-Aldrich Co., Ltd. (Milwaukee, USA). Fluorescein isothiocyanate (FITC)-dextran was obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Transwell inserts with 0.4 μm pores were bought from Corning Co., Ltd. (New York, USA). All other chemicals employed in this study were sourced from either Macklin or Aladdin Co., both located in Shanghai, China. Detailed information on all enzymes used in this study, including enzyme source, manufacturer, activity units, and lot numbers, were listed in Table S1. All reagents utilized were of analytical purity.

2.2 Preparation of wheat bran dietary fiber

Dry Yangmai No.25 wheat bran was milled into powder and smashed through a 40-mesh sieve. The sample was then treated with absolute ethanol at a ratio of 1:15 (w/v) for defatting overnight, after which it was filtered and air-dried. The degreased powder was heated and stirred with ultrapure water (1:20, w/v) at 95 °C, fully gelatinized for 10 min. Subsequently, 3% (w/w) heat-stable α -amylase (≥ 2100 U/g) was added and incubated at 90 °C for 30 min. After cooling and filtering, the precipitation was added to 0.5 M NaOH solution (1:15 w/v) at 40 °C for 2 h, then ultrasounded with the frequency of 40 kHz for 30 min. The supernatant was adjusted to pH 7.0, after which papain was introduced at a dosage of 4% (w/w) relative to the sample mass and incubated at 50 °C for 2 h. The enzyme was then inactivated by heating at 100 °C for 15 min, and the mixture was subsequently centrifuged to remove the precipitated protein. A four-fold volume of ethanol was added to the supernatant to concentrate overnight. The precipitate was collected by centrifugation (8000 g, 15 min), then redissolved in ultrapure water, and dialyzed with 10 kDa membrane for desalting. After freeze-drying, WBDF was obtained.

2.3 Physicochemical property analysis

2.3.1. Chemical composition

The total sugar content was determined by the phenol-sulfuric acid method with glucose as the standard. Protein content was mainly determined by the bicinchoninic acid kit. The total phenolics were determined by Folin-Ciocalteu method^[9].

2.3.2 Determination of molecular weight

The WBDF samples were filtered through 0.22 μm filter and analyzed by high performance size exclusion chromatography (HPSEC) (7.8 $\times$ 300 nm, SB-806 HQ and SB-804 HQ column). The procedure was

commenced with a 50 μL volume, and elution was performed with NaCl solution containing 0.02% proclin at a flow rate of 0.5 mL/min. Data analysis were carried out using Astra software (Wyatt Technologies Co., CA, USA).

2.3.3 Monosaccharide composition analysis

1 mL WBDF samples (3 mg/mL) were hydrolyzed in sealed ampoules with the same volume of 4 mol/mL trifluoroacetic acid (TFA) at 110 °C for 7 h, which was later treated with nitrogen and methanol to remove the TFA. Afterwards, the samples (450 μL) were combined with 450 μL 0.3 M NaOH and 450 μL 0.5 M 1-phenyl-3-methyl-5-pyrazolone (PMP) - methanol solution, reacting at 70 °C for 30 min in the thermostat water bath for derivatization, followed by neutralizing with 0.3 mol/mL HCl solution. After adding trichloromethane for PMP extraction, the upper aqueous layer was passed through a 0.22 μm filter.

A volume of 10 μL of the sample was analyzed using a high-performance liquid chromatography (HPLC) system (Waters e2695, USA) and a Zorbax Eclipse XDB-C18 column. The mobile phase consisted of solvent A, which contained 0.05 M KH_2PO_4 buffer with 15% acetonitrile (pH 6.8), and solvent B, which comprised the same KH_2PO_4 buffer concentration with 40% acetonitrile (pH 6.8). The flow rate was set at 1 mL/min throughout the 45 min analysis. The gradient elution protocol started with 0% solvent B, ramped to 15% over the initial 10 min, and further increased to 25% over the subsequent 20 min.

2.3.4 Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FT-IR) spectroscopy was employed to characterize the functional groups of WBDF. Freeze-dried samples were finely ground and thoroughly mixed with spectroscopic-grade potassium bromide (KBr) at a mass ratio of 1:200 (w/w). The mixture was compressed into transparent pellets under constant pressure. Infrared spectra were recorded using an FT-IR spectrometer (Nicolet iS10, Thermo Fisher Scientific, USA) in the range of 4000-400 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans per sample. Background spectra were collected using pure KBr and automatically subtracted prior to analysis.

2.4 Simulated gastrointestinal digestion of WBDF

According to the previous method^[10] with slight modifications, WBDF was dissolved in the simulated salivary fluid (SSF) (32 mL) to 16 mg/mL and held at 37 °C for 2 min. Samples (6 mL) were collected at 0 min, 2 min and were immediately boiled to inhibit enzymatic activities, respectively.

The remained 20 mL solution from the oral digestion was mixed with 20 mL simulated gastric fluid (SGF) added with CaCl_2 and pepsin. After that, the system was buffered to pH 3.0 and held at 37 °C. Samples (8 mL) were collected at 0, 1, and 2 h and boiled for 5 min.

Afterwards, sodium hydroxide solution was used to buffer the simulated gastric digestive solution to pH 7.0. Then neutralized solution (16 mL) was added with an equal amount of simulated small intestinal fluid (SIF) and maintained at 37 °C. Samples (10 mL) were collected at 0, 1, and 2 h and boiled for 5 min.

At the same time, the simulated digestion solution without WBDF was taken as control group B, and WBDF solution without the digestion solution was taken as control group C. The samples collected from each state were then dialyzed with 10 kDa membrane for desalting and freeze-drying.

2.5 Antioxidant activity analysis

2.5.1 DPPH radical scavenging activity

The free radical scavenging capacity of WBDF and its digestates was evaluated using the DPPH assay with minor modifications. Briefly, 2.0 mL of sample solution was mixed with 2.0 mL of freshly prepared DPPH solution (0.2 mmol/L in ethanol). The reaction mixture was vortexed and incubated in the dark at ambient temperature for 30 min to allow completion of the reaction. Absorbance was subsequently measured at 517 nm. Ethanol was used as the blank, while the DPPH solution without sample served as the control. The DPPH radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_s - A_\beta)/A_0] \times 100$$

where A_s , A_β and A_0 represent the absorbance of the sample, the absorbance of the blank, and the absorbance of the control, respectively.

2.5.2 ABTS⁺ · radical scavenging ability

According to a previously reported method^[11], the same volume of ABTS (7.9 mM) and K₂S₂O₈ (2.6 mM) were mixed and held in darkness for 12–16 h to produce ABTS⁺ · radicals. Then 50 µL of the sample solution was added to 200 µL of the ABTS⁺ · working solution and mixed thoroughly. After incubation in the dark for 5 min at room temperature, the absorbance was recorded at 734 nm using a microplate reader.

2.5.3 FRAP assay

The ferric reducing antioxidant power (FRAP) working reagent was freshly prepared prior to analysis by mixing acetate buffer (300 mmol/L, pH 3.6), TPTZ solution (10 mmol/L in 40 mmol/L HCl), and FeCl₃·6H₂O solution (20 mmol/L) at a volume ratio of 10:1:1. For each measurement, 10 µL of sample solution and 30 µL of deionized water were added to 300 µL of FRAP reagent, followed by incubation at room temperature for 5 min. The absorbance was then recorded at 593 nm. FRAP values were calculated based on a standard curve constructed using FeSO₄ solutions and expressed as Fe²⁺ equivalents.

2.5.4 ORAC assay

The ORAC assay followed established protocols^[12] with minor adjustments. The assay utilized black-walled 96-well plates, with each well containing 20 µL of either the sample (diluted in phosphate buffer) or the L-Ascorbic acid standard, along with 200 µL of fluorescein. Plates were kept at 37 °C for 20 min using a Tecan Infinite E Plex microplate reader (Austria). Each well then received 20 µL of 119.4 mM 2,2'-Azodiisobutyramidine dihydrochloride (AAPH) and fluorescence measurements (Ex: 485 nm, Em: 535 nm) were taken with readings recorded over 66 cycles at 2 min intervals. Antioxidant capacity was quantified in µmol L-Ascorbic acid equivalents per gram dry weight (DW).

2.6 α -Glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity of WBDF was determined based on a previously reported method^[13] with minor modifications. α -Glucosidase was dissolved in PBS to prepare the enzyme solution. For the assay, 40 μ L of α -glucosidase solution was mixed with 40 μ L of WBDF sample solution at various concentrations (0–10.0 mg/mL, prepared in PBS), followed by the addition of 50 μ L of PBS. The mixture was pre-incubated at 37 °C for 10 min to allow interaction between the enzyme and the sample. Subsequently, 40 μ L of p-nitrophenyl- α -D-glucopyranoside (pNPG, 1 mM) was added as the substrate, and the reaction was continued for an additional 20 min at 37 °C. The enzymatic reaction was terminated by adding Na₂CO₃ solution, and the absorbance was measured at 405 nm. The α -glucosidase inhibitory activity was calculated using the following equation:

$$\text{Inhibition rate (\%)} = [(A_1 - A_2) - (A_s - A_3)] / (A_1 - A_2) \times 100\%$$

where A_s represents the absorbance of the reaction system containing enzyme, substrate, and sample; A_1 represents the absorbance of the control containing enzyme and substrate without sample; A_2 represents the absorbance of the substrate blank without enzyme and sample; and A_3 represents the absorbance of the sample blank without α -glucosidase.

2.7 Kinetics of α -Glucosidase inhibition

Using Lineweaver-Burk plots, the experiment started by mixing 40 μ L α -Glucosidase, 40 μ L of test sample solution from 0.0 to 10.0 mg/mL and 50 μ L of PBS. After incubating for 10 min, 40 μ L of pNPG with different concentrations (1.0 to 4.0 mM) was added as substrate, then 40 μ L of Na₂CO₃ was added after 20 min.

2.8 Assessment of WBDF on intestinal barrier function

2.8.1 Caco-2/RAW264.7 cell co-culture model on Transwell

Caco-2 and RAW 264.7 cell were both cultured in DMEM medium added with 10% FBS at 37 °C in a moist environment containing 5% CO₂. Caco-2 cells were seeded on Transwell inserts with the medium in both compartments being renewed every day after the seventh day in culture for 21 days^[14].

The working concentrations of WBDF were determined based on preliminary screening experiments. Briefly, a broad concentration range was first evaluated in Caco-2 and RAW264.7 monoculture systems using CCK-8 assays to assess cytotoxicity. An initial anti-inflammatory screening was further conducted in LPS-stimulated RAW264.7 cells. Based on the results and physiological relevance considerations, 25 and 50 μ g/mL were selected for subsequent co-culture experiments. Detailed screening data are provided in the Supplementary Information (Fig. S4).

Cell morphology was observed with an Olympus IX51 inverted optical microscope (Tokyo, Japan) and images were captured using FCSNAP software. Transepithelial electrical resistance (TEER) values ($> 500 \Omega \cdot \text{cm}^2$) of mono-cultured cell layers suggested success in models^[15]. A co-culture model consisting of a Caco-2

cell mono layer in apical side and RAW 264.7 macrophages on the basolateral side was used to simulate the structure of the intestinal barrier. In brief, WBDF was added to the apical side reaching 50 and 100 µg/mL. After 12 h, LPS was added to the basolateral side and the change of ROS content was measured with ROS detection kit 24 h later.

2.8.2 Permeability of Caco-2 mono layer

The permeability of Caco-2 mono layer was determined using previous method^[16] with some modifications. The Transwell inserts were rinsed twice with HBSS, which was added and equilibrated for 30 min. FITC-Dextran (4 kDa) of 0.5 mg/mL was added to the apical chamber (0.5 mL), with fresh HBSS to the basolateral chamber. 100 µL of medium was taken from the basolateral chamber and transferred to a black 96-well plate for fluorescence measurement every 30 min (Ex: 485 nm, Em: 535 nm). After the assay, both chambers were gently washed 3-5 times with PBS and then collected for further experiments.

2.8.3 RNA extraction and q-PCR analysis

Total RNA was extracted using Trizol Reagent (Life Technologies). The cDNA was reverse transcribed using the revert aid first-strand cDNA Synthesis kit (Accurate Biology). Real-time PCR was performed using SYBR Green qPCR Master Mix (Accurate Biology). The primer sequences were exhibited in Table S2. All Q-PCR experiments were performed in triplicate. The relative quantification for the expressed genes was estimated using the $2^{-\Delta\Delta C_t}$ method.

2.9 In vitro fermentation

The method of *in vitro* fermentation was described as follow^[17]. Briefly, fresh fecal samples were obtained from six healthy adult donors (three females and three males) with body mass index (BMI) values ranging from 18.5 to 24.0 kg/m². All donors had no history of antibiotic use for at least three months prior to sample collection. The fecal samples used in the present study were residual materials from a previously approved research project^[18], and their use complied with the scope of the original ethical approval.

WBDF and inulin were prepared in the fermentation medium (5 mg/mL), and the blank control was the fermentation medium in absence of WBDF. After the stool was defrosted, PBS was added to the mixed stool sample and later filtered to obtain intestinal bacterial solution (10%, w/v), which was immediately inoculated into the prepared medium and kept in the anaerobic chamber (Thermo Fisher Scientific, USA). Finally, samples with three duplicates were taken every 6 h.

2.9.1 Analysis of pH and SCFAs

A pH meter (FE28, Mettler Toledo Co., Ltd.) was used in the measurement. The SCFAs content was determined using a 6890N Gas Chromatograph (Agilent Technologies, USA) equipped with a hydrogen flame ionization detector (FID) and an HP-INNOWAX column. Chromatographic conditions included: Injector temperature (250 °C), detector temperature (300 °C), 1 µL injection volume, and temperature gradient from 80 °C (2 min) to 220 °C at 10 °C/min within 16 min.

2.9.2 Analysis of gut microbiota

Fecal DNA was extracted by Stool Genome DNA isolation kit (Sangon Biotech Co., Ltd., Shanghai, China) to build PCR system with its corresponding primers. The PCR products were purified and dissolved in Elution Buffer. The HiSeq platform was selected for sequencing by Agilent 2100 Bioanalyzer to test fragment range and concentration results. High-quality Clean data, based on OTU and annotation results, were used for subsequent analysis of microbial complexity and inter-group differences.

2.9.3 Statistical analysis

All experiments have been repeated triply. The data results were analyzed by analysis of variance (ANOVA) and considered statistically significant at $P < 0.05$. Subsequent significance analysis was examined by the Duncan test. The results were expressed as the mean $\pm$ standard deviation (SD).

3. Results and discussion

3.1 Basic properties of WBDF

WBDF was obtained using an alkaline extraction combined with ultrasonication. This combined strategy has been widely applied in recent studies to improve extraction efficiency of plant-derived polysaccharides and dietary fibers without causing severe structural degradation^[19, 20]. Under the optimized conditions used in this study, the extraction yield of WBDF was $5.69\% \pm 0.97\%$.

WBDF extracted by alkaline exhibits distinct characteristics with the total carbohydrate content of $83.86\% \pm 1.89\%$ and the protein content of $9.08\% \pm 0.16\%$ (w/w). The total phenolic content of WBDF was determined to be 0.89 ± 0.09 mg GAE/g (dry weight) (Table S3). The molecular weight of WBDF was 9.35×10^5 Da (Fig. S1), which is higher than traditional water-extracted WBDF^[21]. Molecular weight distribution could be affected by variations in extraction parameters, such as the pH conditions and the temperature disposal during extraction^[22]. Moreover, WBDF is mainly composed of glucose ($12.23 \pm 0.18\%$), xylose ($41.94 \pm 0.28\%$) and arabinose ($35.49 \pm 0.26\%$) (Fig. S2). This compositional profile suggests that arabinoxylans represent the predominant polysaccharide fraction in WBDF, accompanied by minor glucan components, which is consistent with previous reports on wheat bran-derived soluble fibers^[21]. However, as detailed analyses of glycosidic linkages and fine structural features were not performed in the present study, this inference cannot be regarded as a definitive structural confirmation.

3.2 Characteristics of WBDF during simulated digestion in vitro

The human gastrointestinal tract is essential for the processing and uptake of nutrients from ingested foods^[23]. However, natural polysaccharides generally exhibit strong resistance to digestive enzymes, which largely links to their structural features, such as β -glycosidic linkages, high molecular weight, and complex branching patterns^[3]. As shown in Table 1, after simulated digestion in different phases, the total carbohydrate content, reducing sugar content and the total phenolic content (Table S3) of WBDF showed no significant changes, indicating high stability of the WBDF during the digestive process. This phenomenon is likely attributed to structural properties, including a large molecular size and existence of branched arabinoxylan

domains and bound phenolic compounds. Relevant studies indicate that such digestive properties facilitate its relatively intact arrival to the colon, potentially exerting beneficial effects on the gut microbiota^[8].

The molecular weight of WBDF remained essentially stable during the oral and gastric phases, showing only minor fluctuations without a clear decreasing trend. However, a moderate increase in apparent molecular weight was observed during the intestinal phase, followed by a slight decline at the later stage. The observed increase in molecular weight should not be interpreted as true polymer chain elongation. Instead, it is more likely attributable to digestion-induced physicochemical interactions under intestinal conditions. The presence of bile salts, enzymes, and ionic components at near-neutral pH may facilitate intermolecular association or reversible aggregation of WBDF, thereby increasing its hydrodynamic volume and altering its elution behavior during size-exclusion chromatography^[24]. A similar digestion-induced increase in apparent molecular weight has been reported for okra polysaccharides, where salt-related co-precipitation and aggregation effects were proposed as contributing factors^[25].

Table 1 Total carbohydrates, reducing sugars and molecular weight of WBDF during simulated digestion.

	Total carbohydrates (mg/mL)	Reducing sugars (mg/mL)	Molecular weight (Da)
Saliva			
0 min	3.3192±0.4099 ^a	0.7465±0.0738 ^a	9.368×10 ⁵ (±0.388%)
2 min	3.3071±0.4740 ^a	0.7406±0.0406 ^a	9.325×10 ⁵ (±0.372%)
Stomach			
0 h	3.2510±0.5626 ^a	0.6993±0.0028 ^a	9.299×10 ⁵ (±0.326%)
1 h	3.2390±0.0910 ^a	0.6921±0.0114 ^a	9.375×10 ⁵ (±0.376%)
2 h	3.1529±0.3333 ^a	0.6927±0.0418 ^a	9.375×10 ⁵ (±0.376%)
Intestine			
0 h	3.1601±0.4328 ^a	0.7947±0.0043 ^a	9.715×10 ⁵ (±0.383%)
1 h	3.1025±0.1964 ^a	0.8041±0.0824 ^a	9.935×10 ⁵ (±0.376%)
2 h	3.1039±0.3049 ^a	0.8167±0.0270 ^a	9.772×10 ⁵ (±0.407%)

*Different letters at the same phase represent significant difference ($P < 0.05$) among samples.

3.3 FT-IR spectroscopy analysis

As shown in Fig. S3, it is suggested the presence of some structural changes in WBDF after digestion. Overall, the spectra of samples at different stages are generally consistent with minor variations. The typical stretching vibration of O-H could be inferred by the broad band at 3400 cm⁻¹. The absorption at 2925 cm⁻¹ indicated the presence of C-H stretching vibration on carbohydrate methylene groups^[26]. In addition, the characteristic absorption at 1630 cm⁻¹ was caused by a free carboxylic carbonyl group asymmetric stretching vibration. The spectra showed several sharp and specific bands in the region of 1200-1000 cm⁻¹, which indicates a pyranose form of sugar^[27]. The contraction vibration of cellulose C-O-C and C-O-H in WBDF caused the absorption peak to be around 1046 cm⁻¹. The characteristic absorption peak at 920 cm⁻¹ was due to glycosidic bond. These results indicate that the simulated digestion process did not change the basic chemical structure of WBDF, as the major characteristic peaks remained present across all samples. However, in the polysaccharide fingerprint region of 1000-1100 cm⁻¹, subtle changes in peak intensity or shape were observed. These variations are likely attributed to the presence of pancreatic and digestive enzymes during the intestinal phase, which may partially hydrolyze some soluble dietary fibers or polysaccharide chains in amorphous

regions. Furthermore, slight changes in the intensity or width of certain peaks among different samples suggest that the effect of simulated digestion on WBDF is not a bulk degradation of the overall structure, but rather involves localized modifications of specific chemical bonds (such as ester bonds) and alterations in physicochemical properties. These results were similar to previous research about WBDF, and some slight differences may be due to different extraction methods^[28].

3.4 Antioxidant activity analysis

3.4.1 DPPH radical scavenging activity

As shown in Fig. 1A, WBDF exhibited strong DPPH radical scavenging activity, which increased in a concentration-dependent manner, reaching $74.95 \pm 0.76\%$ at 2.0 mg/mL. Notably, this antioxidant capacity remained largely unchanged after *in vitro* digestion, indicating that the active structures of WBDF were well preserved despite gastrointestinal treatment. The stability of its radical scavenging activity suggests that WBDF may serve as an effective dietary antioxidant capable of resisting digestive degradation.

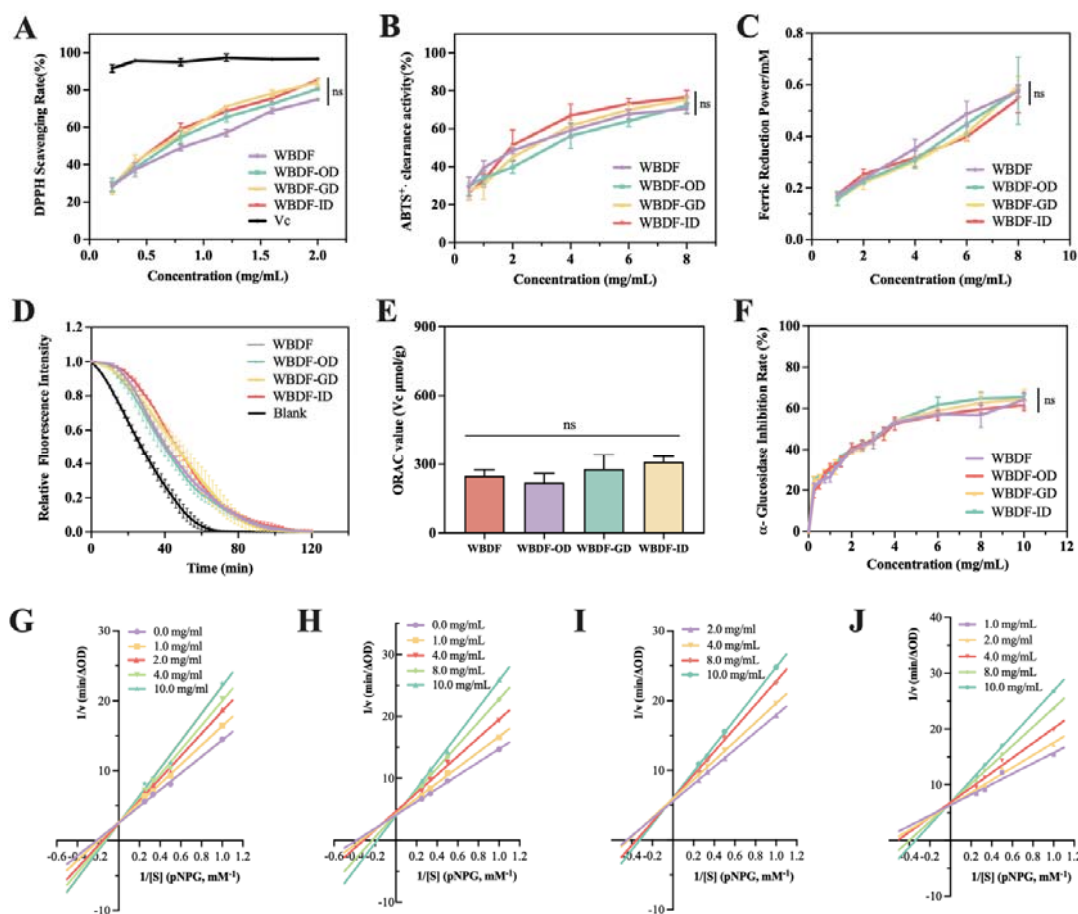


Fig. 1 Characteristics comparison of WBDF, WBDF-OD, WBDF-GD and WBDF-ID. (A) DPPH radical scavenging activity of Vc, WBDF, WBDF-OD, WBDF-GD, and WBDF-ID. (B) $ABTS^{+ \cdot}$ free radicals scavenging activity of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID. (C) Determination of ferric reducing power of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID. (D) The fluorescein decay curves of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID. (E) ORAC values of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID. (F) Inhibitory effect of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID on α -glucosidase activity. (G-J) Inhibition kinetics of WBDF, WBDF-OD, WBDF-GD, and WBDF-ID on α -glucosidase activity. ns: $p > 0.05$.

3.4.2 $ABTS^{+ \cdot}$ radical scavenging activity

In this assay, WBDF demonstrated antioxidant capacity through the hydrogen atom and electron transfer reduction mechanism to quench $\text{ABTS}^+ \cdot$ radicals (Fig. 1B). The $\text{ABTS}^+ \cdot$ clearance activity of WBDF rises with the increase of concentration, from 29.45% at 0.5 mg/mL to 70.60% at 8.0 mg/mL. Furthermore, its radical scavenging capacity remained stable across different digestion phases, suggesting robust retention of antioxidant functionality.

3.4.3 FRAP assay

WBDF exhibited consistent antioxidant activity across multiple assay systems. The Ferric reduction power also demonstrated dose-dependent pattern reaching 0.57 mM at 8.0 mg/mL, with notable stability throughout *in vitro* digestion phases (Fig. 1C) to further support the structural resistance of WBDF to digestive degradation. This retention of antioxidant activity suggests that the functional groups responsible for free radical neutralization remain intact, likely due to the structural resistance of WBDF to enzymatic hydrolysis. The persistent antioxidant profile further highlights its potential as a digestion-resistant bioactive ingredient.

3.4.4 ORAC assay

The ORAC assay quantifies antioxidant level by measuring the ability of antioxidants to delay the fluorescence decay of a probe (such as fluorescein) under oxidative stress induced by free radicals (e.g., AAPH), thereby reflecting radical scavenging capacity^[30]. As shown in Fig. 1D and 1E, WBDF from different digestion phases exhibited similar trends in relative fluorescence intensity over time, and quantitative ORAC values showed no significant differences among them. The ORAC value of WBDF is 247.55 Vc $\mu\text{mol/g}$, meaning the antioxidant capacity of one gram of WBDF is equivalent to that of 247.55 micromoles of Vc. It is speculated that the reducing hydroxyl groups of the fiber plays a crucial role in enhancing its ORAC value. During *in vitro* digestion, the sources of these hydrogen donors may remain largely unchanged, which could explain the consistent ORAC values observed across different digestive phases.

3.4.5 Structural basis of the antioxidant behavior of WBDF

Natural polysaccharides are known to exhibit antioxidant activity through multiple structure-dependent pathways^[9]. Based on the structural features characterized above, the polysaccharide matrix of WBDF is expected to contain abundant hydroxyl groups along the backbone. These functional groups may play a key role in its antioxidant behavior by participating in hydrogen donation and electron transfer processes^[9, 26]. The macromolecular features of WBDF, including its high molecular weight and polymeric architecture, are also likely to influence its antioxidant behavior by providing multiple reactive sites along the chain^[24].

Furthermore, phenolic compounds associated with the fiber matrix may exert a synergistic effect, enhancing radical scavenging activity^[29]. Importantly, the phenolic content in WBDF remained stable across different stages of *in vitro* digestion (Table S3), with no significant changes observed in either the phenolic concentration or the overall antioxidant capacity. This suggests that the antioxidant properties of WBDF are primarily driven by the structural features of the polysaccharides, and that these properties remain unaffected by gastrointestinal conditions, supporting its potential as a digestion-resistant antioxidant. Collectively, the

antioxidant activity of WBDF appears to arise from the combined contribution of its polysaccharide structure and associated components. Further structural elucidation will be required to clarify the specific structure-activity relationships involved.

3.5 α -Glucosidase inhibitory activity assay

As a hydrolytic enzyme on the brush border of small intestinal epithelium, α -glucosidase facilitates the cleavage of terminal α -1,4-glycosidic linkages in substrates, thereby releasing glucose and promoting carbohydrate absorption^[31]. WBDF and its digested fractions demonstrated notable α -glucosidase inhibitory activity (Fig. 1F). Specifically, WBDF exhibited a dose-dependent inhibition, with the highest inhibition rate reaching $64.25\% \pm 1.71$ at elevated concentrations.

The inhibitory activity of WBDF may be attributed to its structural and compositional features. WBDF is enriched in arabinose and xylose, which have been reported to interact with the active site of α -glucosidase through hydrogen bonding and steric hindrance, thereby limiting substrate accessibility^[26]. In addition, residual bound phenolic compounds associated with WBDF may further contribute to enzyme inhibition via direct interactions with catalytic residues or by altering enzyme conformation^[32]. These combined effects likely underlie the observed inhibitory potency of WBDF.

Additionally, the inhibitory effect of WBDF remained largely stable after simulated digestion, suggesting its potential to reach the small intestine intact and exert its inhibitory action on carbohydrate absorption, indicating a promising role in modulating carbohydrate digestion and lowering the glycemic index of foods.

The type of α -glucosidase inhibition by WBDF and its digested samples was further characterized using Lineweaver–Burk double-reciprocal plots. All plots exhibited intersection at the y-axis, with unchanged $V_{\max}$ and increasing K_m values as WBDF concentration increased (Fig. 1G–1J), indicating a competitive mode of inhibition^[33]. Moreover, the inhibition type remained consistent across different digestion stages. This consistency suggests that the active inhibitory components in WBDF are structurally stable and retain their functional specificity throughout the digestive process, underscoring their potential for effective glycemia management in physiological conditions.

3.6 Assessment of WBDF on intestinal barrier function

3.6.1 Changes of TEER level and permeability

The above results demonstrated that WBDF could resist upper gastrointestinal digestion and reach the colon with its structure largely preserved. Therefore, its effects on intestinal barrier function were subsequently investigated. We established an LPS-induced co-culture model of Caco-2 cells and RAW 264.7 cells to mimic the intestinal environment under inflammation activated by lipopolysaccharide (LPS). Following a 24 h induction of the Caco-2 monolayer with LPS, the TEER showed about 25.91% reduction compared with NC group, while treatment with WBDF (100 $\mu\text{g/mL}$) notably repaired the TEER level of LPS group by 125.19% (Fig. 2A). The TEER of the cell monolayer model to a certain extent reflects its integrity^[15].

The reduction in TEER following LPS stimulation reflects a disruption of intercellular tight junction integrity, whereas treatment with WBDF effectively mitigated this impairment.

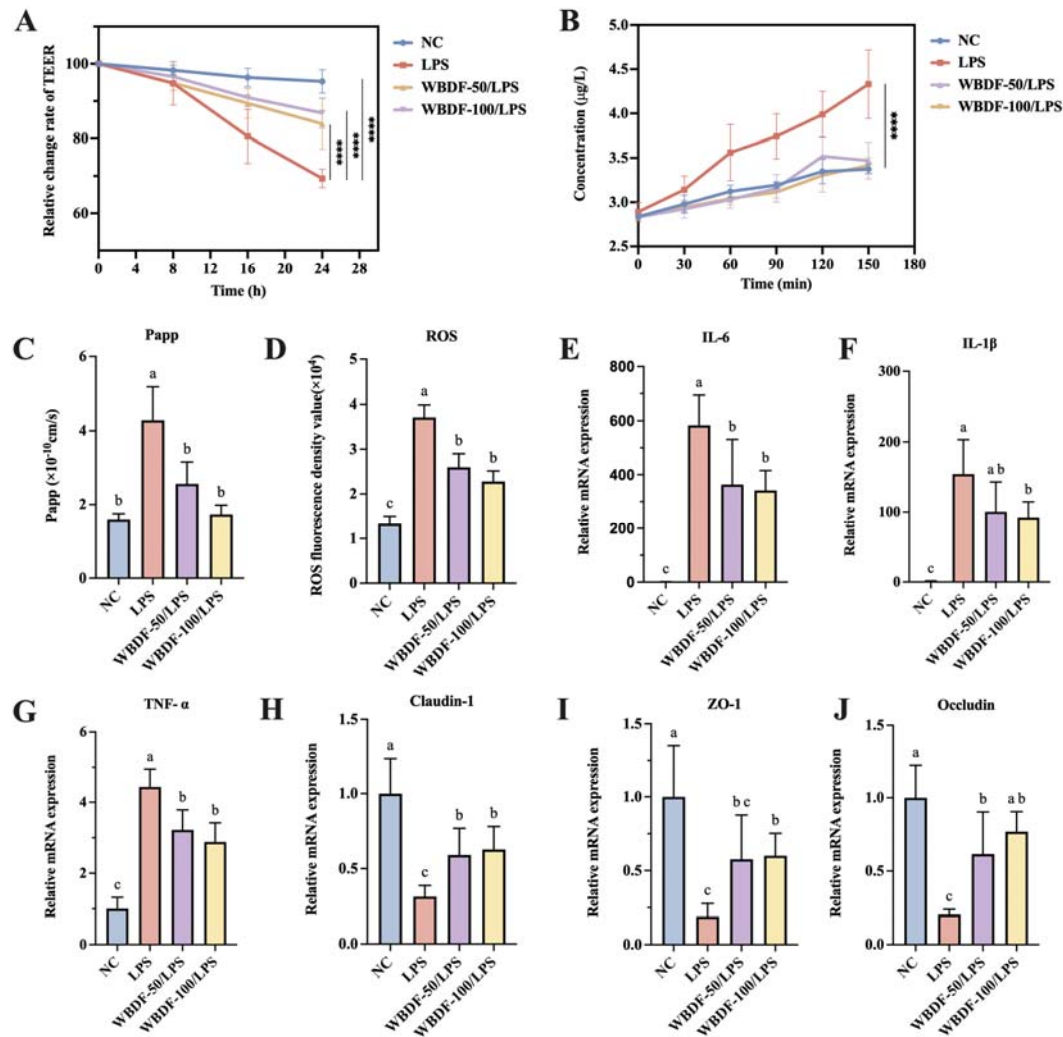


Fig. 2 Effect of WBDF on the co-culture Caco-2/RAW264.7 model. (A) Cell membrane resistance of Caco-2/RAW264.7 cells induced by LPS under different concentrations of WBDF. Normalized by the TEER value at day 21. $P < 0.0001$ ****, compared with LPS group. (B-C) FITC-dextran permeability of LPS-induced Caco-2 monolayer cell model under the effect of different concentrations of WBDF and the Papp value of FITC-dextran at 150 min of incubation. $P < 0.0001$ ****, compared with LPS group. (D) Effect of WBDF on LPS-induced ROS secretion. (E-G) Effects of WBDF on LPS-induced secretion of IL-6, TNF- α and IL-1 β of RAW264.7 cells in Caco-2/RAW264.7 model. (H-J) Effects of WBDF on LPS-induced secretion of Claudin-1, ZO-1 and Occludin of Caco-2 cells in Caco-2/RAW264.7 model. Different letters indicate a significant difference ($P < 0.05$) among different groups.

When the integrity of a monolayer cell model is compromised, molecules such as FITC-dextran can leak through intercellular gaps. The FITC-dextran osmotic concentration increased over time in the LPS-treated group reaching 4.33 $\mu\text{g/L}$ after 150 min, whereas the 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ WBDF-treated group maintained a low permeability level at 3.46 $\mu\text{g/L}$ and 3.41 $\mu\text{g/L}$ comparable to the NC group of 3.37 $\mu\text{g/L}$ after 150 min (Fig. 2B-2C). Intuitively, the Papp values in the LPS-treated group were higher than the NC group by 2.68-fold ($P < 0.05$), indicating a severe loss of barrier integrity. In contrast, treatment with WBDF reduced the LPS-induced increase in permeability to 1.60-fold and 1.09-fold of the NC group ($P < 0.05$), suggesting a protective effect on monolayer integrity. These findings indicate that LPS treatment severely impairs monolayer integrity, while WBDF incubation markedly alleviates this disruption.

3.6.2 ROS levels

Macrophages are highly plastic immune cells that respond to diverse stimuli by shifting their morphology and function. Upon LPS stimulation, polarization toward the M1 phenotype comes along with a sharp increase in ROS generation, reflecting oxidative stress within the cells^[34]. LPS markedly elevated ROS production by 2.8-fold compared to the NC group, whereas WBDF treatment attenuated this increase, reducing ROS levels to 1.95-fold and 1.72-fold of the NC group at the concentrations of 50 µg/mL and 100 µg/mL respectively (Fig. 2D). This reduction in ROS production can be attributed to the antioxidant capacity of WBDF, which is likely due to its hydroxyl-rich polysaccharide backbone and potential bound phenolic compounds. These structural features may interact with redox-sensitive pathways and neutralize free radicals, providing protective effects against oxidative damage. The observed results suggest that the antioxidant activity of WBDF is primarily driven by its polysaccharide structure, and that this activity is relevant in reducing oxidative stress in inflammatory macrophages.

3.6.3 Results in gene expression

To further investigate the mechanism of WBDF on the intestinal-like model under inflammation, gene expression analysis of RAW264.7 cells and Caco-2 cells was performed by q-PCR measurements. Since cytokine levels reflect both the degree of inflammatory damage and the efficacy of anti-inflammatory agents^[35], we quantified the expression of IL-6, IL-1 β , and TNF- α . LPS stimulation upregulates inflammatory cytokines including IL-6 by 583.74-fold (Fig. 2E), IL-1 β by 154.20-fold (Fig. 2F) and TNF- α by 4.43-fold (Fig. 2G). The WBDF treatment at the concentrations of 50 µg/mL and 100 µg/mL exhibited a relative expression level of 363.59 and 341.80 (IL-6), 100.50 and 92.24 (IL-1 β), 3.22 and 2.88 (TNF- α) compared to the NC group respectively, showing an overall trend of downregulation compared to the LPS group.

As for the gene expression patterns of TJ proteins including Claudin-1, ZO-1, and Occludin are shown in Fig. 2H-2J. LPS treatment significantly suppressed the expression of Claudin-1 (relative expression of 0.31), whereas both 50 µg/mL WBDF (relative expression of 0.59) and 100 µg/mL WBDF (relative expression of 0.63) markedly up-regulated its expression compared to the LPS group. ZO-1 relative expression was downregulated by LPS treatment to 0.19, which was reversed by WBDF (0.58 at 50 µg/mL and 0.60 at 100 µg/mL). Similarly, Occludin expression, which was suppressed by LPS to 0.20, also exhibited a recovery upon WBDF treatment, reaching 0.62 and 0.77 at the same concentrations.

These results indicate an activated inflammatory response and compromised barrier integrity was taken place in the model after LPS stimulation. These changes reflect a shift toward epithelial dysfunction and increased permeability under inflammatory stress. Therefore, we hypothesized that WBDF can mitigate these effects by restoring barrier function and suppressing inflammatory activation.

3.7 Properties of WBDF in vitro fermentation

3.7.1 Changes of WBDF

Following confirmation of its barrier-protective capability, *in vitro* fermentation experiments were conducted to explore how WBDF interacts with gut microbiota. Based on variations in sugar content (Fig. 3A), molecular weight (Fig. 3B), and monosaccharide composition (Fig. 3C-3D), it was observed that WBDF

was gradually metabolized by the gut microbiota at different fermentation time points. The fermentation rate was relatively rapid between 0 and 12 h, during which the majority of WBDF was utilized. From 18 to 24 h, the rate of metabolism became more moderate. The glucose portion of WBDF was preferentially consumed and was almost completely depleted by 12 h. In contrast, the metabolism of arabinose and xylose occurred at a slower rate, continuing over 24 h. This may be related to the highly polymerized and structurally complex arabinoxylans composed of arabinose and xylose in WBDF^[36].

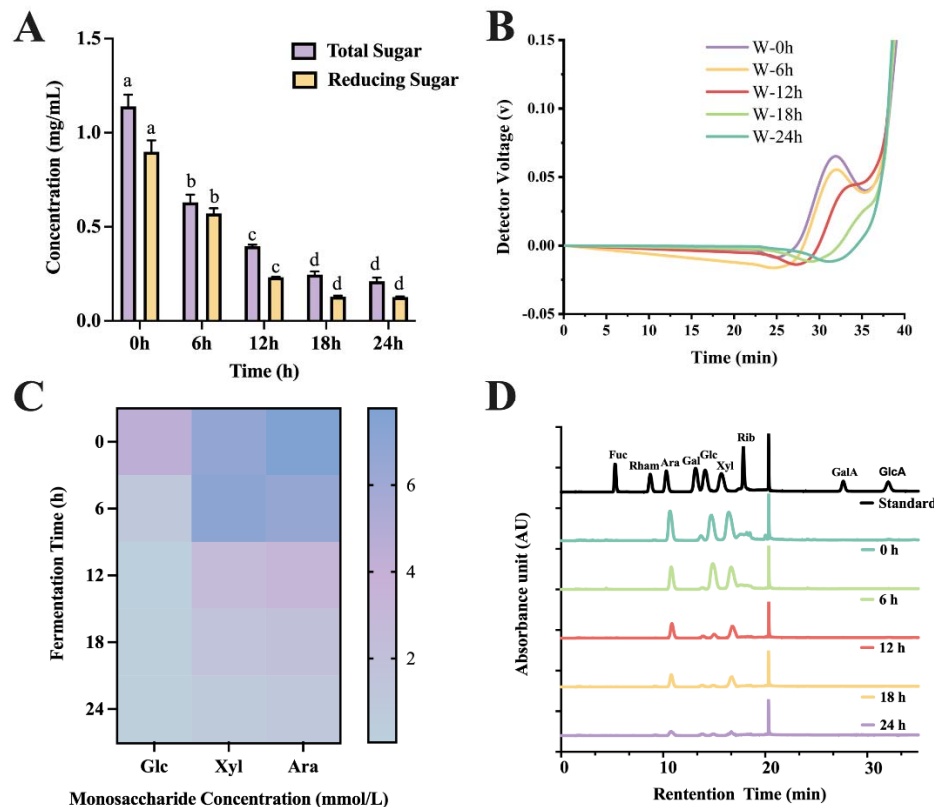


Fig. 3 Changes of WBDF during fermentation. (A) Changes of total sugar and reducing sugar of WBDF during fermentation. (B) Changes of HPSEC. (C) Heat map of monosaccharide composition of WBDF *in vitro* fermentation at different time point. (D) HPLC profiles of monosaccharide compositions. Different letters indicate a significant difference ($P < 0.05$) among different groups.

3.7.2 Variations of pH and productions of SCFAs

As a major carbon source, dietary fibers are anaerobically utilized by gut microbiota into SCFAs, which lower the intestinal pH, modulate host immunity, maintain intestinal barrier function, and serve as an energy source^[37]. To facilitate the interpretation of fermentation outcomes, inulin was used as a comparison substrate in this study. Inulin has been recognized by international authorities, including FAO/WHO, as a representative prebiotic fiber and is widely applied in *in vitro* fermentation models. Owing to its well-defined fermentability and reproducible SCFA production profile, inulin provides a suitable reference for evaluating the microbial fermentation characteristics of WBDF. The pH of the blank group remained nearly unchanged throughout the fermentation period, whereas WBDF led to a decrease in pH from 7.00 to 5.14 over 24 h (Fig. 4A). At the same time, inulin lowered the pH even further to 4.22. These results indicated that gut microbiota utilized WBDF and inulin (INL) during separate fermentations, leading to the generation of acidic metabolites. The

corresponding drop in pH reflects the acidogenic bacteria activity and the efficiency of substrate utilization within the fermentation system.

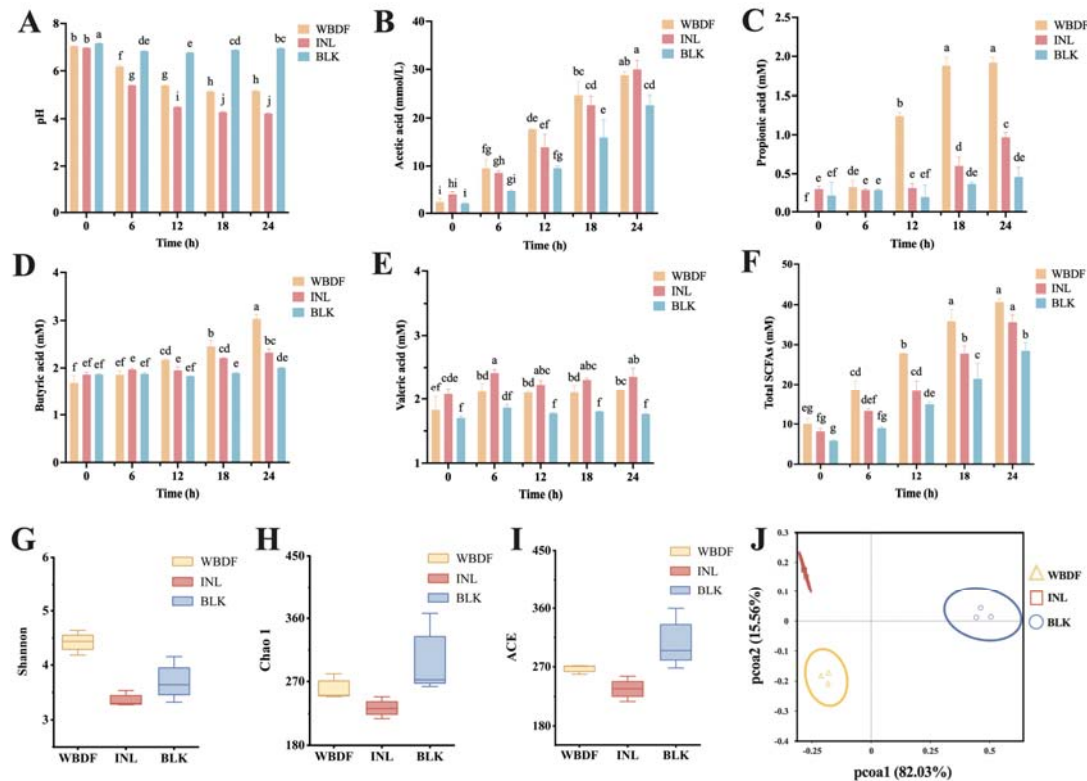


Fig. 4 Changes of short-chain fatty acids and pH value during fermentation, and analyses of comparison of overall bacterial composition between each group. (A) Changes in pH value during fermentation. (B) Changes of acetate. (C) Changes of propionate. (D) Changes of butyrate. (E) Changes of valerate. (F) Changes of total SCFAs. Different letters indicate a significant difference ($P < 0.05$) among different groups. (G) Shannon index. (H) Chao 1 index. (I) ACE index. (J) PCoA based on Bray-Curtis. Different letters indicate a significant difference ($P < 0.05$) among different groups.

SCFAs, defined as fatty acids with fewer than six carbon atoms, are primary fermentation products of dietary fibers by gut microbiota. They serve as energy sources for the host, modulate immune responses, maintain intestinal barrier integrity, and participate in glucose and lipid metabolism^[2]. SCFA concentration is also a key indicator of the prebiotic potential of dietary fibers. Therefore, we quantified SCFA levels at 0, 6, 12, 18, and 24 h during fermentation. After 24 h, the concentrations of acetic acid (Fig. 4B), propionic acid (Fig. 4C), butyric acid (Fig. 4D), and valeric acid (Fig. 4E) in the WBDF group were all higher than the blank group. Specifically, total SCFA concentration (Fig. 4F) in the WBDF group was 3.05-fold higher than that of the blank group (40.67 to 13.31 mM), with acetic acid contributing most substantially to this elevation. Inulin, used as a recognized prebiotic, also significantly promoted SCFA production. However, unlike WBDF, inulin caused a more pronounced drop in pH, while yielding a slightly lower total SCFA concentration. This could be explained by the fact that inulin is fermented more rapidly, leading to an immediate surge in acid-producing bacterial activity and proton (H^+) release. Some of the acids may have subsequently been converted into other metabolites such as gases, thereby reducing the net accumulation of SCFAs. Previous studies have shown that the fermentation of glucose, arabinose, and xylose mainly drives the production of acetic and propionic acids^[36]. Consistent with this, our results showed a prominent increase in these two SCFAs, suggesting that

their elevated levels are largely attributed to microbial utilization of WBDF components containing arabinose, xylose, and glucose.

3.7.3 Impacts of WBDF fermentation on fecal microbiota

The above results demonstrated that during *in vitro* fermentation, the structural properties of WBDF, environmental pH, and SCFA concentrations underwent significant changes, indicating that its degradation process was accompanied by dynamic shifts in both microbial community composition and metabolic functions. To further investigate these microbial changes, we employed high-throughput 16S rRNA sequencing to evaluate the effects of WBDF and INL on the richness and composition of the gut microbiota (Fig. 5).

We assessed gut microbial diversity with different groups using four α -diversity indices: Shannon (Fig. 4G), Chao1 (Fig. 4H), and ACE (Fig. 4I). According to the results, there was no significant difference in community richness among the three groups. The initial inoculum from the fecal microbiota could have possessed inherently high diversity, thereby limiting the observable impact of the interventions. Moreover, the selective fermentation of WBDF and inulin may have altered the relative abundance of specific taxa without markedly changing the overall diversity metrics. Beta diversity reflects differences in species composition or the rate of species turnover across distinct habitats. Principal coordinate analysis (PCoA) revealed a clear statistical separation among the three groups (Fig. 4J), indicating that WBDF and inulin treatments each induced distinct shifts in gut microbiota composition.

The 16S rRNA sequencing revealed significant compositional alterations in the microbial consortium across three experimental groups. At the phylum level, the relative abundances of the dominant taxa (*Firmicutes*, *Fusobacteriota*, *Actinobacteriota*, *Bacteroidota*, and *Proteobacteria*) varied significantly depending on the substrate provided (Fig. 5A). Notably, the relative abundance of *Fusobacteriota*, which has been linked to systemic disorders such as colorectal cancer and rheumatoid arthritis^[38], markedly declined in response to both WBDF and inulin interventions. In contrast, the abundances of *Actinobacteriota* and *Firmicutes*, phyla generally considered beneficial to health, exhibited opposite trends in the WBDF and inulin groups. To further elucidate the microbial shifts, genus-level analyses were conducted (Fig. 5B). *Bifidobacterium* was the dominant strain grown under inulin intervention, in agreement with previous research findings^[39]. WBDF increased the relative abundances of *Bifidobacterium*, *Prevotella_9*, *Blautia*, and *Enterococcus*. Within these genera, *Bifidobacterium* strains are potentially known for their roles in strengthening the intestinal barrier^[40]. Moreover, the WBDF group exhibited a decreased relative abundance of *Sutterella* and *Fusobacterium*. Some *Sutterella* species, in particular, has been implicated in conditions such as compromised gut barrier integrity, autism spectrum disorders and down syndrome^[41].

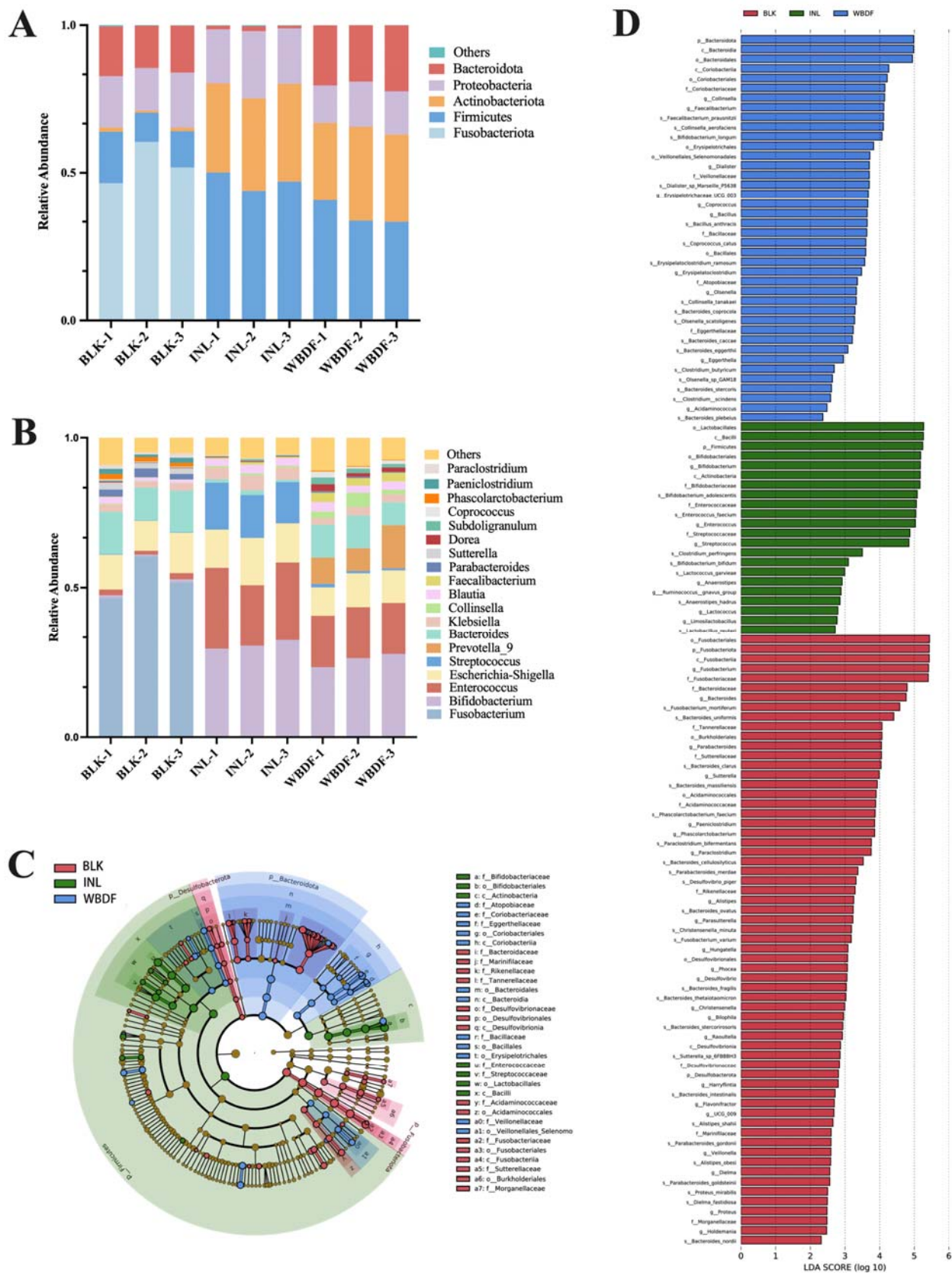


Fig. 5 (A) The relative abundance of bacterial community at the phylum level. (B) The relative abundance of bacterial community at the genus level. (C) Comparison of microbiota among BLK, INL, and WBDF groups based on linear discriminant analysis effect size (LEfSe), the clustering diagram. (D) Comparison of microbiota among BLK, INL, and WBDF groups based on linear discriminant analysis effect size (LEfSe), the LDA diagram.

It is well established that *Bifidobacterium* metabolizes glucose (or dietary fiber-derived monosaccharides) via a unique bifid shunt pathway. This route does not directly yield propionate or butyrate

but instead generates acetate and lactate via the fructose-6-phosphate phosphoketolase pathway, along with ATP synthesis^[42]. Species of *Prevotella* possess membrane-bound electron transport complexes that facilitate propionate production via the succinate pathway^[43]. Furthermore, members of the genus *Faecalibacterium* generate butyrate directly through the acetyl-CoA transferase pathway. Compared to the INL-treated group, the WBDF group showed lower relative abundance of *Bifidobacterium*, but higher levels of *Prevotella* and *Faecalibacterium*, a profile consistent with the observed SCFA distribution.

In addition, current research confirms the potential of arabinoxylans and β -glucans found in cereals for enriching beneficial microbes, like *Prevotella* and *Bifidobacterium*. Among systematic tests, the 3:1 arabinoxylan and β -glucan mixture from wheat exhibited maximal prebiotic efficacy, showing significantly bifidogenic activity and increased concentrations of SCFAs^[44]. Bai et al.^[45] revealed *Prevotella_9* exhibited proliferative response to β -glucan and arabinoxylan, with increased glycosidase activity (β -glucosidase, β -mannosidase) and lactate and acetate production.

To identify key bacterial populations associated with each treatment, linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted. As shown in Fig. 5D, 40, 22, and 63 OTUs with significant differences (LDA score > 2) were identified in the WBDF, INL, and blank (BLK) groups, respectively. The majority of OTUs in the WBDF group had LDA scores between 3 and 5, suggesting a more consistent microbial response to WBDF intervention. Furthermore, phylogenetic clustering of dominant taxa (Fig. 5C) indicated that the *Bacteroidota* phylum dominated the purple region of the WBDF group, whereas *Firmicutes* dominated in INL group. These results suggest that *Bacteroides* is likely to be a distinguishing bacterial signature of WBDF treatment, setting it apart from the INL group.

4. Conclusion

This study explored the protective effects of WBDF on gut health and its underlying mechanisms. WBDF showed stable structural features in FT-IR analysis and remained resistant to gastrointestinal digestion. Both WBDF and its digested samples displayed strong DPPH, ABTS⁺ ·, ORAC radical scavenging activity, ferric reduction power, and α -glucosidase inhibitory effects. In the LPS-induced Caco-2/RAW 264.7 co-culture model, WBDF improved epithelial barrier function. It increased TEER values and reduced FITC-dextran permeability, which were closely related to the enhanced expression of tight junction proteins such as ZO-1, Claudin-1 and Occludin, as well as the suppression of ROS generation and the reduced secretion of inflammatory cytokines including TNF- α , IL-6 and IL-1 β . In the *in vitro* fermentation model, WBDF was efficiently utilized by gut microbiota. It lowered the pH of the fermentation system and increased the production of total SCFAs, especially acetic, butyrate and propionate. WBDF also altered the microbial community by increasing the abundance of beneficial bacteria and shifting overall diversity. Taken together, WBDF is a promising functional food ingredient. It supports gut health by protecting the intestinal barrier while lowering inflammation and promoting the production of SCFAs through prebiotic activity. However, the *in vitro* design may not fully reflect *in vivo* conditions, and future studies should validate these effects in animal models.

Supporting information

Primer sequences for qRT-PCR (Table S1); HPLC chromatogram of molecular weight of WBDF (Fig. S1); HPLC chromatogram of monosaccharide composition of WBDF (Fig. S2); FT-IR spectrum comparison of WBDF, WBDF-OD, WBDF-GD and WBDF-ID (Fig. S3).

Declaration of competing interest

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

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