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Effect of Gansu starter cultures on the hypoglycemic mechanism of black bean bread

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ABSTRACT: The hypoglycemic mechanism of black bean bread fermented by Gansu Jiaozi (a natural mixed starter cultures) was investigated in this study. Gansu Jiaozi was dominated by *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Saccharomyces cerevisiae*, during the fermentation of which a significant increase in total acidity and peptide content was observed. SEM analysis revealed that black bean addition (UD groups) disrupted the gluten network of bread, while Gansu Jiaozi fermentation (SD groups) reconstructed a uniform porous structure. Notably, the formation of starch-protein/lipid complexes in black bean bread created a physical barrier that masked digestive enzyme binding sites and reduced glucose release during *in vitro* digestion. In diabetic zebrafish, the fermented black bean bread (SD-40 group) significantly decreased the levels of glucose (46.34%), TC (58.28%), TG (34.17%), and LDL-C (53.61%) compared with diabetic model groups (MCG), while restoring insulin and GLP-1 to normal levels. Meanwhile, fermentation-derived metabolites like organic acids and peptides might activated the AMPK and PI3K/Akt signaling pathways, significantly inhibiting the expression of phosphoenolpyruvate carboxykinase (pck-1) and pro-inflammatory factors (GLUT2 expression restored to 70.45%). These findings elucidate that fermented black bean bread exerts hypoglycemic effects through a synergistic mechanism of physical barrier and metabolism, providing novel insights and technical support for developing functional whole grain fermented foods.

Keywords: Black bean bread; Gansu Jiaozi; Fermentation; Hypoglycemic; Glucose metabolism

1. Introduction

Diabetes is a prevalent chronic metabolic disorder characterized primarily by hyperglycemia, unintended weight loss, and elevated insulin secretion ^[1]. According to the 2025 International Diabetes Federation (IDF), the number of diabetes patients aged 20-79 years in the world has reached 589 million in 2025, with projections reaching 853 million by 2050. Type 2 diabetes mellitus (T2DM) is the most common type, which is closely related to obesity, poor dietary habits, and physical inactivity ^[2]. T2DM could lead to severe complications, including cardiovascular diseases, nephropathy, neuropathy, and retinopathy, highlighting the urgent need for effective dietary interventions to manage blood glucose levels.

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Bread is one of the most important staple foods all over the world, which is mainly made of refined wheat flour [3]. However, the high content of rapidly digestible starch in wheat led to a sharp rise in postprandial blood glucose by 40-60%, accompanied by a significant increase in glycated hemoglobin (HbA1c) and insulin resistance index (HOMA-IR) [4]. This type of metabolic damage is not only closely related to decreased insulin sensitivity and impaired beta cell function, but also triggers an imbalance in the gut microbiota [5]. The resulting hyperglycemia and compensatory hyperinsulinemia further form a vicious cycle of metabolic disorders characterized by increased oxidative stress and mild inflammation. These pathological changes highlight the urgent need to develop functional bread substitutes that combine delayed carbohydrate digestion with enhanced glucose regulation.

Studies have addressed these challenges by raw material improvement and process optimization to mitigate the high glycemic index associated with refined wheat flour. The application of whole grain raw materials has become one of the main research directions. The "national whole grain action plan (2024-2035)" launched in China has advocated the use of whole grain flour instead of refined flour to make staple foods. Whole grains not only give natural grain flavor to baked foods, but also contain bioactive ingredients that could effectively reduce food glucose index and improve intestinal health [6]. Dough fermentation has been proved to be another effective technique for reducing the glycemic response of breads [7]. Although the traditional commercial yeast has high fermentation efficiency and strong stability, it has the inherent defect of single strain, which is difficult to endow the product with rich flavor and biological activity. In contrast, Gansu Jiaozi, a characteristic natural starter culture from Northwest China, was widely used in the production of local staple fermented foods such as steamed bread (mantou) in Gansu Province. Different from commercial pure-strain starters, Gansu Jiaozi is prepared by repeated spontaneous fermentation of cereal raw materials like wheat flour or maize flour, without prior sterilization or artificial inoculation of commercial strains [8]. Gansu Jiaozi mainly contains a naturally domesticated complex bacteria of lactic acid bacteria and yeasts, which have co-evolved into a stable symbiotic system adapted to the local arid climate, raw material characteristics, and traditional fermentation processes [9]. It not only achieves efficient dough fermentation but also enhances probiotic properties with diverse metabolites, thereby providing a novel strategy for developing functional breads [10]. However, current research on the hypoglycemic effects of fermented whole grains has primarily focused on commercial pure-strain starters or exogenous probiotics [11, 12]. The unique physiological functions of traditional natural starters, which rely on complex microbial communities and specific metabolites, are often overlooked. As a typical grain and medicine homologous crop, black bean (*Glycine max* var.) is rich in dietary fiber, resistant starch, polyphenolic compounds (such as anthocyanin-3-O-glucoside), and functional proteins [13]. It may potentially synergize with the microbial metabolites generated during the fermentation of Gansu Jiaozi. Nevertheless, there is a lack of systematic research analyzing how these complex microbial consortia interact with the whole grain matrix to regulate glucose metabolism.

Therefore, the primary purpose of this study is to explore the functional enhancement mechanism of black bean addition combined with fermentation by Gansu Jiaozi, and develop functional black bean bread

with glycemic regulatory potential. To achieve this purpose, the systematically analysis of the strain composition and fermentation characteristics of Gansu Jiaozi were firstly analyzed. Then, the effects of black bean addition and Gansu Jiaozi fermentation on the starch structure and digestive properties of bread were investigated by means of SEM, CLSM, and FTIR. Moreover, a zebrafish model with diabetes was constructed to evaluate the regulatory effect of bread under different treatments on the physiological indicators of diabetic zebrafish and the expression of key genes related to glucose metabolism by qRT-PCR and histopathological analysis. The findings of this study could provide theoretical basis and technical support for the high-value resource utilization of Gansu Jiaozi and the development of functional cereal fermented products.

2. Materials and methods

2.1 Materials and chemicals

Commercial whole black beans were purchased from a local market. Gansu Jiaozi (a natural mixed starter cultures) was collected in Gaotai county (Zhangye, Gansu, China). α -amylase (100,000 U/g), pepsin (30,000 U/mg) and pancreatin (4,000 U/mg) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Assay kits for glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were purchased from Suzhou Grace Biotechnology Co., Ltd. (Suzhou, China). Assay kits for insulin (INS) and glucagon-like peptide-1 (GLP-1) were obtained from Ruixin Biotech Co., Ltd. (Quanzhou, Fujian, China). cDNA Synthesis kit was bought from Yeasen Biotechnology Co., Ltd. (Shanghai, China). All other reagents and chemicals used were all analytical grade.

2.2 Preparation of black bean bread

Firstly, the black bean skin was separated from the bean core by soaking for 14 h. Then, 500 g of black bean skin was mixed with a citric acid-sodium citrate buffer solution (pH 5.4) at a ratio of 1:20 (w/v), followed by the addition of 20 mg/g cellulase. The solution was subjected to ultrasonic treatment for 20 min at a power of 350 W and a temperature of 55 °C. After the reaction was completed, the enzyme was inactivated for 5 min and the mixture was dried. The black bean skin powder and black bean core powder were mixed at a ratio of 1:12 (w/w) according to the composition ratio of whole black beans.

To investigate the effects of black bean content and fermentation method on the hypoglycemic properties, seven bread samples groups were prepared: WB (white bread control), UD-20/40/60 (bread with 20%, 40%, or 60% black bean powder added without sourdough fermentation), and SD-20/40/60 (bread with 20%, 40%, or 60% black bean powder added via sourdough fermentation). The sourdough was prepared by soaking Gansu Jiaozi in warm water (1:10 w/v) for 12 h to obtain the starter culture suspension, which was then mixed with black bean powder (1:1 w/w) and fermented at 37 °C for 12 h. Based on the formula in Table 1, different amounts of black bean powder or the prepared sourdough was mixed with high-gluten flour (protein, fat, and carbohydrate contents: 13.2%, 1.4%, and 71.8%), vital wheat gluten and water, followed by the addition of butter and further mixing. The dough was then divided into 50 g portions and proofed for 45 min at 37 °C and

75% humidity. Finally, the prepared black bean dough was baked for 14 min under an upper temperature of 170 °C and a lower temperature of 140 °C.

Table 1. The formulation for the preparation of black bean sourdough and black bean bread *.

Groups		WB	UD-20	UD-40	UD-60	SD-20	SD-40	SD-60
Preparation of black bean sourdough	Black bean powder (g)	0	0	0	0	40	80	120
	Gansu Jiaozi (g)	0	0	0	0	4	8	12
	Water (g)	0	0	0	0	40	80	120
Preparation of black bean bread	Black bean powder (g)	0	40	80	120	0	0	0
	High-gluten flour (g)	200	160	120	80	160	120	80
	Yeast (g)	3	3	3	3	3	3	3
	Vital wheat gluten (g)	0	4.8	9.6	14.4	4.8	9.6	14.4
	Salt (g)	3.2	3.2	3.2	3.2	3.2	3.2	3.2
	Sugar (g)	10	10	10	10	10	10	10
	Water (g)	110	110	115	120	70	30	0
	Butter (g)	16	16	16	16	16	16	16

* WB: White bread (without black bean powder). UD-20, UD-40, UD-60: Breads with 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. SD-20, SD-40, SD-60: Breads with Gansu Jiaozi and 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively.

2.3 Strains identification

Total microbial genomic DNA was extracted using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.). The content and concentration of DNA were determined by 1.0% agarose gel electrophoresis and NanoDrop 2000 spectrophotometer (Thermo Scientific, United States). The products of polymerase chain reaction (PCR), which were extracted from 2% agarose gel, were purified by a PCR Clean-Up kit and quantified using Qubit 4.0 (Thermo Fisher Scientific, USA). Functional prediction of the 16S rRNA gene was conducted using PICRUSt2 (version 2.2.0). The Pearson correlation analysis was used to analyze the relationship between *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus panis* and amino acid metabolism, carbohydrate metabolism and lipid metabolism.

2.4 Characterization of black bean sourdough

2.4.1 Determination of PH and total acid in black bean sourdough

During the dough fermentation process, samples of each group was taken every 2 h. The dough samples were diluted in sterile water at a 10% (w/v) ratio and homogenized for 30 minutes. The system was titrated with 0.1 M NaOH to a final pH of 8.6, and the NaOH consumption (mL) was recorded as the total acidity (TTA).

2.4.2 Determination of peptides in black bean sourdough

The dough samples were diluted in sterile water at a 10% (w/v) ratio and homogenized for 30 min. The mixture was centrifuged at 8,000 g for 10 min at 4°C. Subsequently, an equal volume of 20% trichloroacetic acid (TCA) was added to the supernatant to precipitate high-molecular-weight proteins, followed by another centrifugation under the same conditions. The peptides content in the supernatant was determined by the method of Lowry^[14]. The standard curve was drawn with bovine serum protein standard.

2.5 Characterization of Bread structure

2.5.1 Confocal laser scanning microscopy (CLSM)

The network structure of dough was observed using CLSM (FV1200, Olympus, Japan) according to the method described by Jia et al.^[15]. The dough samples were pre-frozen at -40 °C for 4 h, then lyophilized at -50 °C, 4 mbar for 48 h. The freeze-dried dough was stained with 0.2% (w/v) fluorescein 5-isothiocyanate (FITC) and 0.025% (w/v) rhodamine B solution in a ratio of 10:1. FITC preferentially stains starch and rhodamine B preferentially dyes protein. Samples were incubated at room temperature overnight in the dark to ensure the complete diffusion of the dye. The protein and starch structures were observed using a 40x eyepiece, and the excitation/emission wavelengths were 488nm and 543nm.

2.5.2 Scanning electron microscopy (SEM)

The microstructure of bread was observed using scanning electron microscopy (SEM) according to the method described by Xin et al. with some modifications^[16]. The surface of freeze-dried bread slices was exposed to gold sputtering. Images were captured at an acceleration voltage of 1.0 kV with magnifications of 30× and 1000×.

2.5.3 FT-IR spectroscopy

The molecular structure of the samples was analyzed using a Fourier Transform Infrared (FT-IR) spectrometer. Air was used as the blank for background correction. The spectra were recorded in the wavelength range of 4000-400 cm⁻¹ with a resolution of 4 cm⁻¹, and each sample was scanned 32 times.

2.6 In vitro digestion

The starch digestibility of bread was determined based on the study of Yang et al.^[17]. Briefly, 1 g of freeze-dried bread powder was mixed with 3 mL of phosphate buffer and 1 mL of preheated α -amylase (37 °C) for 15 s. Then, 6 mL of pepsin-guar gum solution (containing 0.05 g pepsin) was added, and the mixture was stirred at pH 1.5 and 37 °C for 30 min. The pH was adjusted to 6.9, followed by the addition of 200 μ L MgCl₂-CaCl₂ solution, 400 μ L glucoamylase and 125 μ L pancreatin solution. The final volume was adjusted to 50 mL, and the mixture was magnetically stirred at 37 °C for 180 min. At specific time intervals, 1 mL of the digestion solution was taken and mixed with 4 mL of anhydrous ethanol (preheated to 60 °C). After enzyme deactivation in a boiling water bath, the samples were centrifuged at 8000 rpm for 10 min, and the supernatant was collected for subsequent experiments. The reducing sugar content was determined by DNS method. The equivalent release of glucose was calculated as follows:

$$\text{glucose release (mg/g)} = \frac{\text{reducing sugar content (mg)}}{\text{sample total weight (g)}} \quad (1)$$

2.7 Evaluation of hypoglycemic effect

2.7.1 Establishment of diabetes zebrafish model

Three-month-old zebrafish (AB strain) were purchased from Hunter Biotechnology Co., Ltd. located in Hangzhou, Zhejiang, China. Zebrafish were raised under standard conditions with a 14/10 light-dark cycle

and constant temperature (26 ± 1 °C). After 1 week of adaptive feeding, the zebrafish model of diabetes was established. During the model establishment period (7 days), the zebrafish were immersed in a 2% glucose solution for 1 h at 9:00 AM daily, followed by feeding at 11:00 AM. The purified water in the culture system was replaced with a 0.15% egg yolk powder solution for 1 h at 1:00 PM, followed by a second feeding at 4:00 PM. The above processes were all carried out at a constant temperature (26 ± 1 °C). The zebrafish were randomly divided into six groups (n=25): Normal groups (NCG); Model groups (MCG); Positive groups (PCG); WB; UD-40 and SD-40. The experimental conditions for each group were detailed in Table 2. The freeze-dried bread samples were processed into granules using a fish pellet machine to obtain particles with a diameter of approximately 0.5 mm, which were stored at 4 °C until use. Zebrafish were fed twice daily (at 11:00 AM and 4:00 PM). Based on the principle that food should be fully consumed within 5-15 minutes, the feeding amount was optimized through pre-experiments to three granules per fish. The experimental intervention period was 7 days, and zebrafish were fasted for 12 h before the indicator detection. All of the experimental procedures followed the Guide for the Care and Use of Laboratory Animals: Eighth Edition (ISBN-10: 0-309-15401-4), and the animal experiments were approved by the animal ethics committee (SCXK (Zhe) 2022-0003).

Table 2. Groups of zebrafish experiments.

Groups	Experimental conditions
Normal groups (NCG)	Normal zebrafish fed with normal feed
Model groups (MCG)	Diabetes zebrafish fed normal feed
Positive groups (PCG)	Diabetes zebrafish fed normal feed and 0.02 mg/mL metformin hydrochloride
WB	Diabetes zebrafish fed WB
UD-40	Diabetes zebrafish fed UD-40
SD-40	Diabetes zebrafish fed SD-40

2.7.2 Determination of body mass index (BMI)

Zebrafish were euthanized on ice and surface moisture was blotted dry. The body weight of each zebrafish was precisely measured, and the distance from mouth to the most distal end of the tail was recorded as the body length. The body mass index (BMI) was calculated using the following formula:

$$\text{BMI} = \frac{\text{body weight (kg)}}{\text{body length (m)}^2} \quad (2)$$

2.7.3 Detection of physiological indicators

Zebrafish were accurately weighed and homogenized in sterile water at a ratio of 1:9 (w/v) under low-temperature conditions. Subsequently, the homogenate was centrifuged at 2500 rpm for 10 min at 4 °C, and the supernatant was collected as the test sample. The levels of Glu, TG, TC, HDL-C, LDL-C, INS, and GLP-1 in zebrafish tissues were determined using corresponding assay kits.

2.7.4 qRT-PCR Assay

Total RNAs were purified from zebrafish liver tissues using the Trizol reagent, followed by reverse transcription with cDNA Synthesis kit. qRT-PCR primers for key genes, including *TNF α* , *IFN γ* , *IL-1 β* , *GLUT2*, *Insulin*, and *pck-1*, were designed using the NCBI Primer-BLAST tool and synthesized by Genesoul

Technology Co., Ltd (Heilongjiang, China). The qRT-PCR reactions were performed using SYBR Green method, and the relative expression levels of target genes were quantified by applying the $2^{-\Delta\Delta C_t}$ method. The sequences of all qRT-PCR primers used in this study were provided in Table S1.

2.7.5 Immunohistochemical stain

Immunohistochemical (IHC) staining was performed on paraffin-embedded liver tissue sections to analyze the gene expression of IL-1 β , Insulin, and pck-1. The sections were incubated with buffer for antigen repair, followed by overnight incubation with primary antibodies at 4 °C. After incubation with corresponding secondary antibodies, the sections were stained with DAB. Subsequently, the sections were counterstained with hematoxylin, dehydrated through a graded alcohol series and xylene, and observed under a microscope.

2.7.6 Hematoxylin and eosin (H&E) stain

After fixation and dehydration, zebrafish were embedded in paraffin tissue blocks. Sections of 5 μ m thickness were cut using a microtome and mounted onto glass slides. After drying at 60 °C for 1 hour, the slices were deparaffinized in xylene and anhydrous ethanol. Hematoxylin and eosin staining was then performed. Following dehydration and mounting, the sections were observed under a microscope, and images were captured at 40 $\times$ magnification.

2.8 Statistics analysis

Except for the twenty-five tests of the zebrafish experiment, the rest experiments were carried out in triplicate. The values were expressed as mean $\pm$ standard deviation (SD). Graphpad Prism version 9.5 was used for statistical analysis and plotting. Significant differences were identified through ANOVA and Tukey test. P-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1 Characterization of fermentation properties

3.1.1 Strains composition of Gansu Jiaozi

The results of strains composition analysis of Gansu Jiaozi were presented in Fig. 1A. The Gansu Jiaozi exhibited a predominant microbial community consisting of lactic acid bacteria and saccharomyces, where *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Saccharomyces cerevisiae* were identified as the dominant species. The abundance of *Lactobacillus plantarum* and *Lactobacillus brevis* accounted for 17.80% and 14.40% of the bacterial community, respectively. The abundance of *Saccharomyces cerevisiae* accounted for 91.20% of the fungal community. *Lactobacillus plantarum* and *Lactobacillus brevis* could produce active substances such as organic acids and polysaccharides during metabolism [18]. These substances not only improved fermentation characteristics by promoting gluten network reconstruction, but also delayed starch digestion by inhibiting alpha amylase activity [19, 20]. *Saccharomyces cerevisiae* could efficiently metabolize sugars to produce CO₂, which was contributed to bread fluffiness [21]. Meanwhile, its metabolic process formed a synergistic effect with lactic acid bacteria, further optimizing the stability of the fermentation system [22]. These advantageous strains and metabolites could significantly enhance the fermentation performance of black bean

dough, laying the foundation for improving the processing quality and hypoglycemic function of black bean bread.

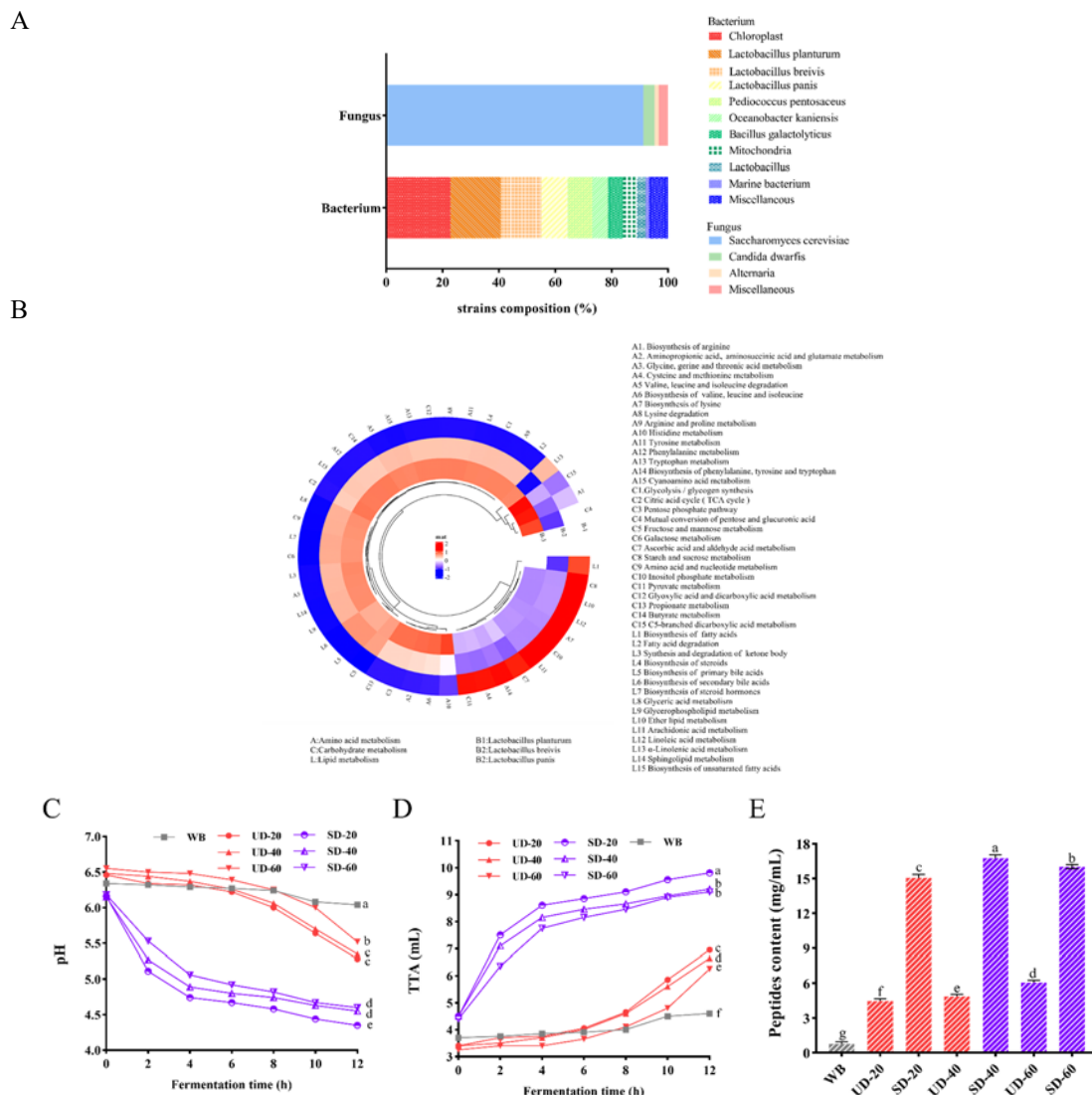


Figure 1. Strains composition (A) and functional prediction (B) of Gansu Jiaozi; Changes in pH (C), total acidity (D) and peptides content (E) during fermentation. WB: White bread (without black bean powder). UD-20, UD-40, UD-60: Breads with 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. SD-20, SD-40, SD-60: Breads with Gansu Jiaozi and 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. Values with different letters are significantly different at $P < 0.05$.

3.1.2 Functional prediction of Gansu Jiaozi

Pearson correlation analysis revealed significant associations between *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus panis* and amino acid metabolism, carbohydrate metabolism, and lipid metabolism, as shown in Fig. 1B. Notably, the strong positive correlation between *Lactobacillus plantarum* and starch and sucrose metabolism suggested a potential role in modulating starch degradation and availability within the dough matrix, which may indirectly influence the postprandial blood glucose response [23]. *Lactobacillus brevis* showed a positive correlation with both primary and secondary bile acid biosynthesis. These bile acids have been demonstrated to enhance GLP-1 production and insulin secretion by inhibiting intestinal FXR signaling, thereby contributing to glucose homeostasis regulation [24]. Furthermore, the direct modulation of glucose metabolism was attributed to the secretion of specific bioactive metabolites.

Specifically, *Lactobacillus plantarum* was positively correlated with fatty acid biosynthesis, indicating the potential production of short-chain fatty acids (SCFAs). SCFAs stimulate GLP-1 secretion by activating intestinal GPR41/GPR43 receptors, subsequently enhancing insulin sensitivity and suppressing hepatic gluconeogenesis [25]. In addition, *Lactobacillus brevis* was positively correlated with the TCA cycle. The key intermediates of TCA cycle such as α -ketoglutarate could be converted to L-glutamate via glutamate dehydrogenase (GDH) and subsequently synthesized into a large amount of γ -aminobutyric acid (GABA) via the glutamate decarboxylase (GAD) pathway. GABA effectively alleviated islet inflammation by inhibiting the NF- κ B pathway in macrophages and reducing the release of inflammatory factors such as TNF- α and IL-6 [26]. Moreover, *Lactobacillus brevis* and *Lactobacillus panis* could metabolize tryptophan into bioactive indoles like indole-3-lactic acid (ILA) and indole-3-propionic acid (IPA), which mitigated metabolic inflammation by activating the aryl hydrocarbon receptor (AHR) [27]. Collectively, the microbial strains in Gansu Jiaozi exhibit significant hypoglycemic potential through the multi-target regulation of these specific metabolic pathways. Overall, functional predictions offered a hypothesis regarding the potential mechanisms underlying the hypoglycemic of fermented black bean bread, providing a theoretical basis for interpreting the subsequent results.

3.1.3 Changes in pH and TTA of sourdough

The changes in pH and total titratable acidity (TTA) during the fermentation of each group were shown in Fig. 1C-D. During 12-hour fermentation period, WB group maintained relatively stable pH (5.82-5.91) and TTA levels (3.2-3.5 mL), which limited fermentation activity. Compared with WB group, the pH value of UD groups significantly decreased to the range of 5.28-5.52, while SD groups showed a more pronounced acidification trend with a pH value dropping to 4.35-4.60 ($P < 0.05$). Correspondingly, the SD groups showed significantly higher TTA values (9.1-9.8 mL) compared to other groups ($P < 0.05$). This significant acidification phenomenon was mainly attributed to the accumulation of organic acids produced by the metabolism of lactic acid bacteria (LAB). Cai et al. confirmed that *Lactobacillus plantarum* significantly promotes the production of lactic acid in jujube juice [28]. Cizeikiene et al. also observed a similar acidification phenomenon when fermenting whole wheat sourdough with *Lactobacillus delbrueckii* subsp. *bulgaricus* [29]. The Gansu Jiaozi used in black bean bread was rich in *Lactobacillus plantarum* and *Lactobacillus brevis*, thus producing more significant acidification effects.

3.1.4 Changes in peptides content of sourdough

The changes in peptides content during the fermentation revealed significant variations among different groups (Fig. 1E). WB group exhibited a small molecular peptides content of 0.76 mg/mL. In UD groups, reaching to 4.66, 4.88, and 6.04 mg/mL, peptides content showed a dose-dependent increase with black bean supplementation. Notably, SD groups demonstrated a significant enhancement in peptides content (15.05, 16.75, and 16.03 mg/mL) with increasing black bean incorporation ($P < 0.05$). This could be attributed to the synergistic enhancement of protein hydrolysis activity by *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Saccharomyces cerevisiae* presented in the Gansu Jiaozi. During the fermentation process, *Lactobacillus*

plantarum secreted extracellular proteases (such as PrtP, PepO) to hydrolyze black bean globulins into low molecular weight peptides [30]. Meanwhile, *Saccharomyces cerevisiae* metabolism produced redox agents (such as glutathione, etc.) which reduced disulfide bonds and further promoting protein breakdown [31]. In addition, WB and UD groups were unable to induce protein denaturation under normal pH conditions, while the acidic environment generated during fermentation effectively promoted protein conformational changes and exposed more hydrolysis sites, resulting in a significant increase in peptides content of SD groups.

3.2 Microstructure of bread core

The microstructure of bread core was presented in Fig. 2. Compared with WB group, the addition of black beans in UD groups resulted in decreased bread volume and tighter crumb structure. With the increase of black bean addition, the number of pores in bread crumb significantly reduced while the thickness of air chamber walls increased. The dispersion degree of pore distribution in UD groups was significantly higher than that in WB group, which was due to the fact that the fibers in black beans weakened the continuity of the gluten network through physical hindrance [32]. SD groups treated with sourdough fermentation exhibited a looser crumb structure with more regular pore distribution and larger pore size. This phenomenon was primarily attributed to the hydrogen bond-mediated cross-linking between gluten and exopolysaccharides produced by *Lactobacillus plantarum* and *Lactobacillus brevis* [33]. Meanwhile, the CO₂ generated during fermentation also promoted directional expansion of gluten network to form a stable porous architecture. Furthermore, the gluten network in UD groups appeared coarse and discontinuous, with numerous starch granules exposed on the surface. By contrast, SD groups exhibited well-embedded starch granules within the gluten matrix which resulted in a smooth and continuous network structure. Sourdough treatment facilitated the interaction of intramolecular hydrophobic amino acids, so that promoting a more homogeneous and integrated microstructure [34]. However, although sourdough fermentation effectively alleviated the loss of gluten network continuity caused by black bean addition, SD groups bread still exhibited a clear structural gradient change with the increase of black bean content. The degree of internal structure densification and surface roughness increased synchronously, indicating that the amount of black bean addition still affected the optimal construction of the fermentation network to a certain extent.

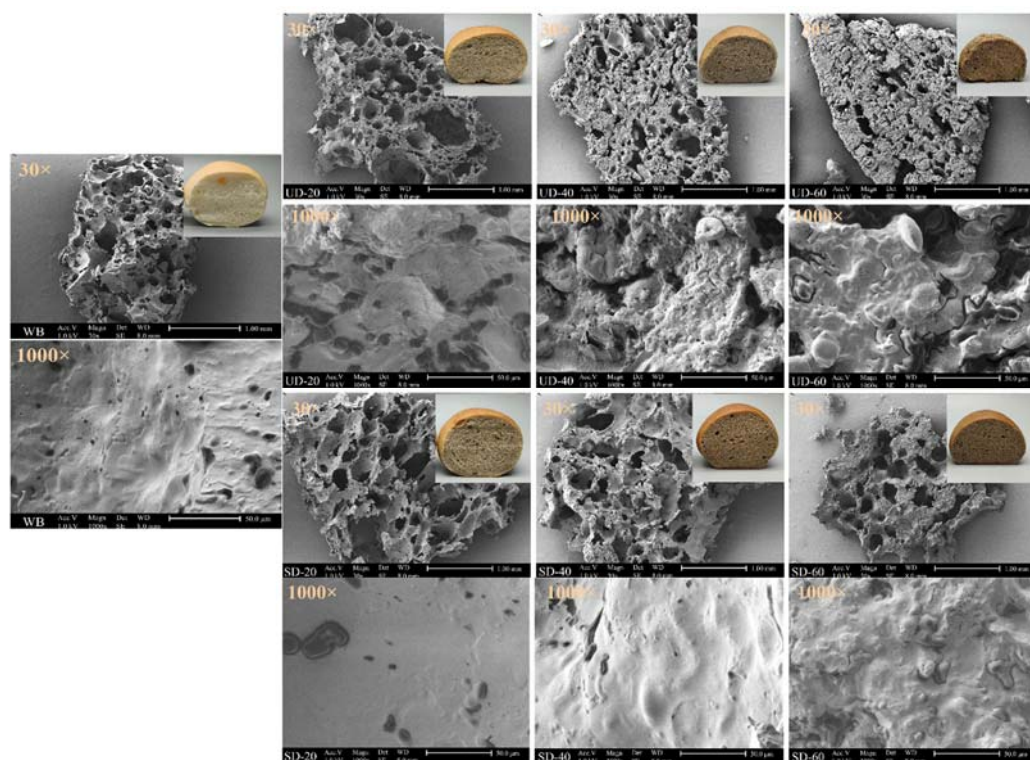


Figure 2. Scanning electron microscope images of bread. WB: White bread (without black bean powder). UD-20, UD-40, UD-60: Breads with 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. SD-20, SD-40, SD-60: Breads with Gansu Jiaozi and 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively.

3.3 Complex formation of bread

3.3.1 CLSM

The CLSM results of dough were shown in Fig. 3A. The gluten protein (red) in WB dough formed a continuous and uniform network structure, completely enveloping starch granules (green). As the amount of black bean added increases from 20% to 60%, black bean protein interacted with wheat gluten protein. This competitive binding interfered with the normal cross-linking of gluten proteins, causing the gluten network to break and form irregular block aggregates [35]. It was worth noting that although the starch granules were partially separated from the gluten, the yellow complex area increased significantly, black bean protein and dietary fiber promoted the formation of starch-protein complex. After sourdough treatment, the structure of gluten network was significantly improved, which was mainly due to the organic acids produced by the metabolism of *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus panis*. Organic acids could promote the deacylation reaction of protein glutamine, and enhance the hydrogen bond and hydrophobic interaction between molecules, thereby realized the recombinant cross-linking of gluten protein [36]. The addition of black bean mainly destroyed the gluten network through protein competitive binding and fiber physical cutting, while sourdough treatment realized the remodeling of the network structure through the synergistic effect of pH regulation and microbial metabolites.

3.3.2 FT-IR

The FT-IR results of bread were presented in Fig. 3B. Compared with WB group, the peak of UD and SD groups at 2916 cm^{-1} , 2848 cm^{-1} , 1740 cm^{-1} and 1022 cm^{-1} were changed. Specifically, the peak intensities at

1740 cm^{-1} and 2848 cm^{-1} of UD and SD groups gradually increased, which were attributed to the asymmetric stretching vibration of methylene ($-\text{CH}_2-$) and carbonyl ($\text{C}=\text{O}$) groups. These were likely originated from the formation of starch-lipid complex, which represented a predominant form of resistant starch [37]. The peak at 1022 cm^{-1} represented the amorphous structure of starch, and the absorbance ratio of 1047/1022 cm^{-1} could characterize the short-range ordered structure of starch-lipid complex [38]. The peak intensity at 1022 cm^{-1} of UD and SD groups significantly decreased compared to WB group, indicating a reduction in the amorphous proportion of starch and enhanced short-range molecular order, which suggested the increased formation of starch-lipid complex. Moreover, the peaks at 2916 cm^{-1} became progressively more pronounced with increasing black bean addition, suggesting enhanced ordering of lipid acyl chains that could stabilize linear starch complex [39].

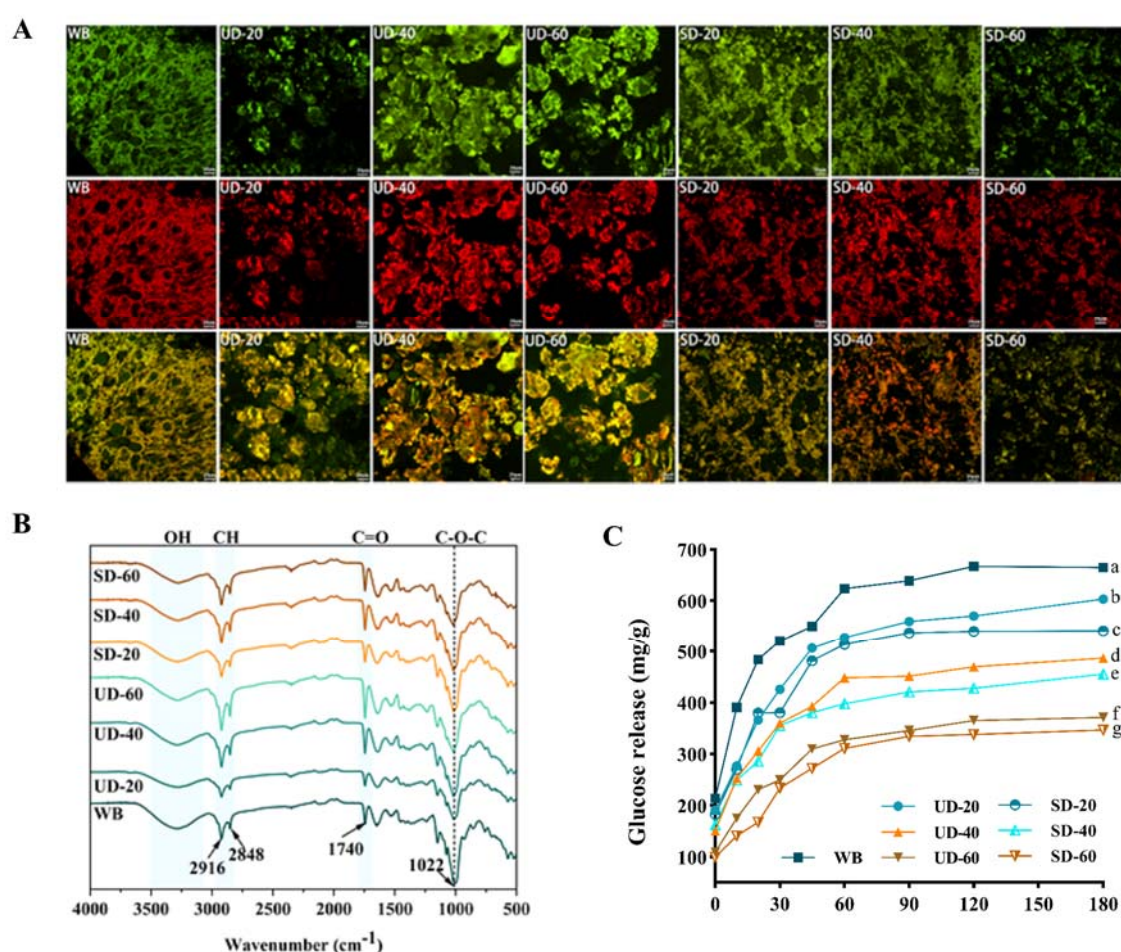


Figure 3. Confocal laser scanning microscope of sourdough (A); FT-IR spectra of bread (B); Glucose release of bread during *in vitro* digestion (C). WB: White bread (without black bean powder). UD-20, UD-40, UD-60: Breads with 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. SD-20, SD-40, SD-60: Breads with Gansu Jiaozi and 20%, 40%, and 60% (w/w of total flour) black bean powder added, respectively. Values with different letters are significantly different at $P < 0.05$.

3.4 Digestion properties of black bean bread

The glucose release of black bean bread during *in vitro* digestion was presented in Fig. 3D. A significant reduction in glucose release equivalents was observed with increasing black bean addition in UD groups ($P < 0.05$). This inhibitory effect might stem from the formation of the starch-protein complex, which might mask

accessible sites of digestive enzymes and delay starch digestion. Meanwhile, the black beans promoted the formation of linear starch-lipid complex (V-shaped crystals). This type of resistant starch had stronger resistance to enzymatic hydrolysis, further reducing the proportion of digestible starch^[40]. Compared with UD groups, the glucose release in SD groups were further reduced, which may be closely related to the effects induced by sourdough fermentation ($P < 0.05$). The organic acids produced during the fermentation process placed the bread substrate in an acidic environment, thereby partially inhibiting enzyme activity and slowing down its catalytic efficiency^[41]. Additionally, the acidic environment might activate endogenous proteases derived from grains or microorganisms, which hydrolyzed black bean protein to release bioactive peptides exhibiting α -amylase inhibitory activity^[42]. These mechanisms collectively contributed to the reduction in glucose release in SD groups.

3.5 Hypoglycemic effect evaluation

3.5.1 Physiological index evaluation

The physiological effects of black bean bread in zebrafish models were demonstrated in Fig. 4. Compared with the normal group (NCG), the model group (MCG) exhibited a highly significant increase in BMI, Glu, TC, TG, and LDL-C levels, but a decrease in HDL-C, INS, and GLP-1 levels ($P < 0.05$). This trend was highly consistent with the pathological characteristics of patients with type 2 diabetes. After intervention with WB, UD-40, the physiological indicators of zebrafish showed varying degrees of improvement. By contrast, SD-40 intervention showed better therapeutic effects with physiological indicators returning to near complete normal levels. Specifically, the Glu levels of zebrafish in SD-40 group decreased by 46.34% compared to MCG ($P < 0.0001$). The TC, TG, and LDL-C levels decreased to near NCG levels, with reductions of 58.28%, 34.17%, and 53.61%, respectively. In addition, the INS and GLP-1 levels of zebrafish in the SD-40 group increased to 101.65% and 108.74% of the NCG, respectively. This may be related to metabolites produced during sourdough fermentation. Studies have shown that organic acids (such as propionic acid and lactic acid) produced by *Lactobacillus plantarum* and *Saccharomyces cerevisiae* during fermentation could improve glucose and lipid metabolism through multiple mechanisms. On the one hand, organic acids reduced the intestinal cholesterol reabsorption by chelating bile acids, and inhibited the activity of HMG-CoA reductase, thereby decreasing the levels of TC and LDL-C^[43]; On the other hand, it promoted the conversion of bound polyphenols in black beans to free form, which reduced lipid accumulation through the PPAR γ signal pathway^[44]. Furthermore, small molecular peptides derived from black bean protein during fermentation might enhance glucose-stimulated insulin secretion via a GLP-1 receptor-dependent pathway^[45]. This multi-target regulatory network collectively improved the glucose and lipid metabolism disorders in zebrafish.

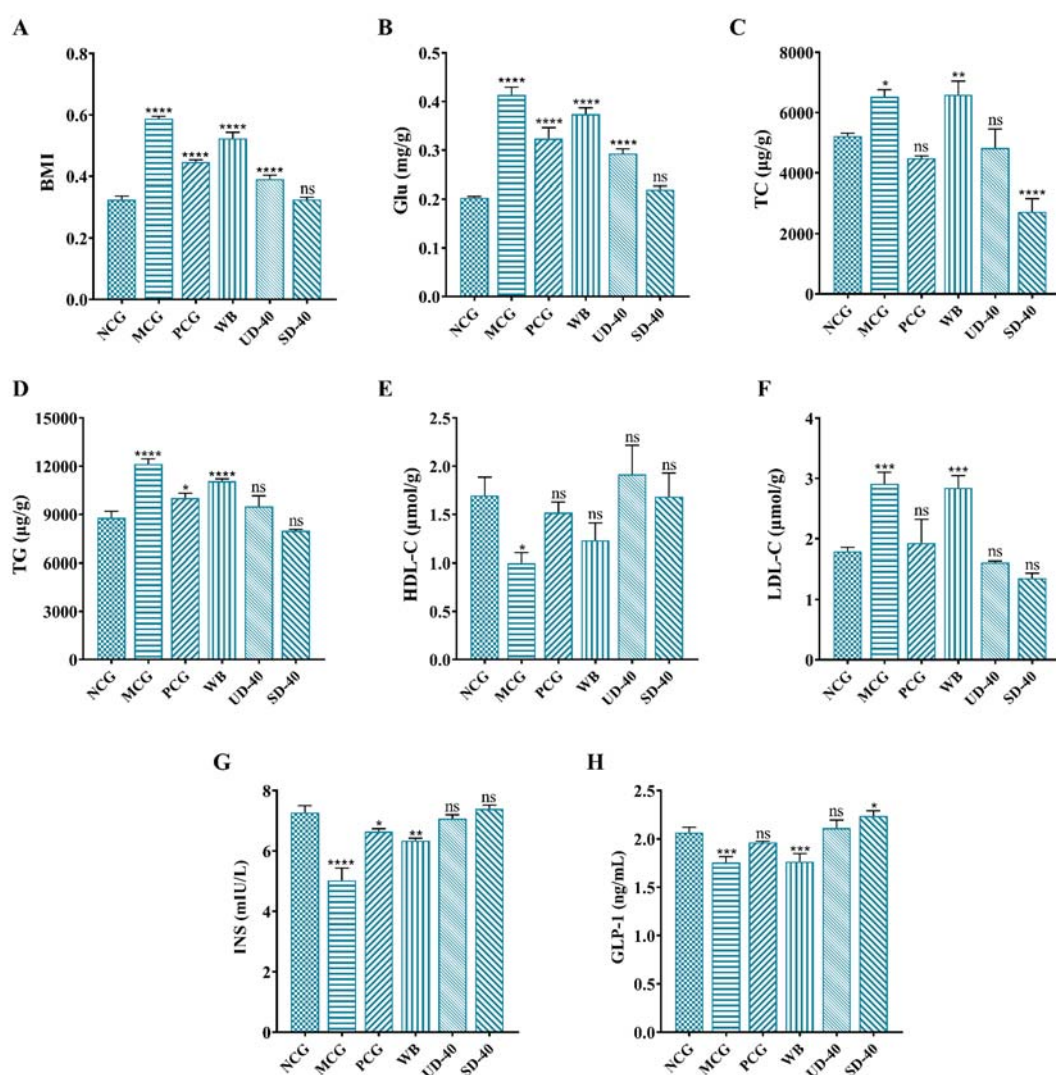


Figure 4. Effect of black bean bread on physiological indexes of zebrafish, including body mass index (A), the content of glucose (B), total cholesterol (C), triglyceride (D), high-density lipoprotein cholesterol (E), low-density lipoprotein cholesterol (F), the level of insulin (G) and glucagon-like peptide-1 (H). NCG: Normal groups; MCG: Model groups; PCG: Positive groups; WB: WB intervened groups; UD-40: UD-40 intervened groups; SD-40: SD-40 intervened groups. Asterisks (*) denote the significance levels of group differences. ns: no significance; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, **** $P < 0.0001$.

3.5.2 Gene regulation evaluation

The regulation of black bean bread on liver glucose metabolism and inflammation related genes in zebrafish with diabetes were illustrated in Fig. 5. It could be seen that compared with NCG, MCG exhibited significantly decreased expression levels of Insulin and GLUT2, while showing markedly upregulated expression of inflammatory factors ($\text{TNF}\alpha$, $\text{IFN}\gamma$, $\text{IL-1}\beta$) and pck-1 . These results indicated the presence of insulin resistance, impaired glucose uptake, and chronic inflammation in the diabetic model. After WB intervention, the expression levels of Insulin and GLUT2 only recovered to 34.45% and 18.28% of NCG, and the decrease in inflammatory factors and pck-1 was not significant. UD-40 and SD-40 interventions significantly improved the above situation, with SD-40 achieving a level closer to that of the NCG. The expression levels of Insulin and GLUT2 in SD-40 group reached 88.84% and 70.45% of NCG, while $\text{TNF-}\alpha$,

IFN- γ , IL-1 β , and pck-1 were downregulated by 82.03%, 59.80%, 65.27%, and 82.92% relative to MCG levels ($P < 0.01$).

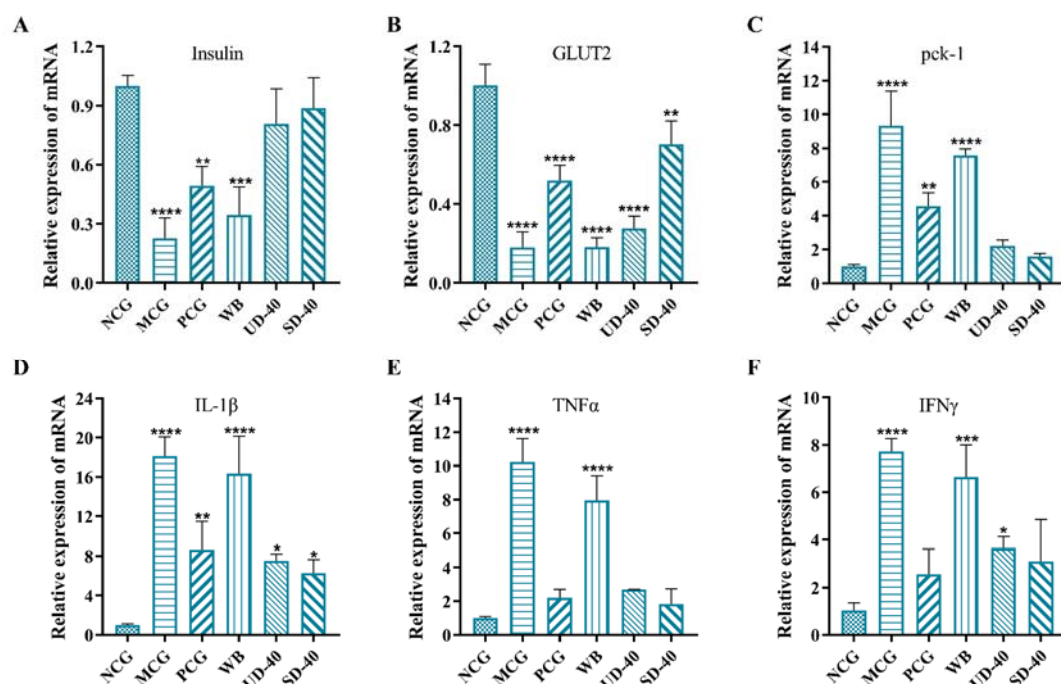


Figure 5. Changes in glucometabolic related genes of zebrafish. NCG: Normal groups; MCG: Model groups; PCG: Positive groups; WB: WB intervened groups; UD-40: UD-40 intervened groups; SD-40: SD-40 intervened groups. Asterisks (*) denote the significance levels of group differences. ns: no significance; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, **** $P < 0.0001$.

The representative genes Insulin, pck-1, and IL-1 β were selected for immunohistochemical staining analysis in terms of activating the insulin pathway, inhibiting gluconeogenesis, and anti-inflammatory effects. With yellow staining indicating cell expression and blue representing nucleus, Fig. 6 illustrated the antibodies distribution of Insulin, pck-1, and IL-1 β in zebrafish hepatic tissues. It could be observed that NCG exhibited uniformly arranged hepatocytes with homogeneous staining and no nuclear accumulation. By contrast, MCG exhibited significant nuclear accumulation and uneven staining of the cell membrane cytoplasm, which manifested as liver cell swelling and loose edema. WB intervention displayed continuous yellow staining patterns characteristic of cytoplasmic disruption and necrosis. UD-40 intervention restored cellular alignment but confluent yellow regions suggesting compromised membrane integrity. Notably, SD-40 intervention achieved optimal recovery, with well-organized cellular architecture, uniform staining, and structural integrity comparable to NCG. The low expression of IL-1 β further indicated that SD-40 helped downregulate the expression of inflammatory cytokines in cells. These histopathological observations were consistent with the PCR data in Fig. 5.

In the process of regulating glucose metabolism mentioned above, peptides and organic acids produced by the metabolism of Gansu Jiaozi played a vital role. Studies have confirmed that the small molecular peptides could regulate the balance of Th1/Th2 and alleviate chronic inflammation [46]. Also, it could improve insulin receptor sensitivity by activating PI3K/Akt pathway and promoting IRS-1 tyrosine phosphorylation, or directly stimulate the proliferation of pancreatic β cells and up regulate insulin synthesis [45, 47]. At the same

time, the peptides could inhibit the NF- κ B and MAPK pathways, thereby reducing the expression levels of pro-inflammatory factors such as TNF- α and IL-1 β [48]. Furthermore, organic acids could directly activate AMPK, thereby suppressing the expression of key hepatic gluconeogenic enzymes (e.g., PEPCK/pck-1) and reducing hepatic glucose output. These multi-level regulatory mechanisms induced that black bean fermented dough effectively improved insulin resistance and metabolic inflammation of diabetes zebrafish.

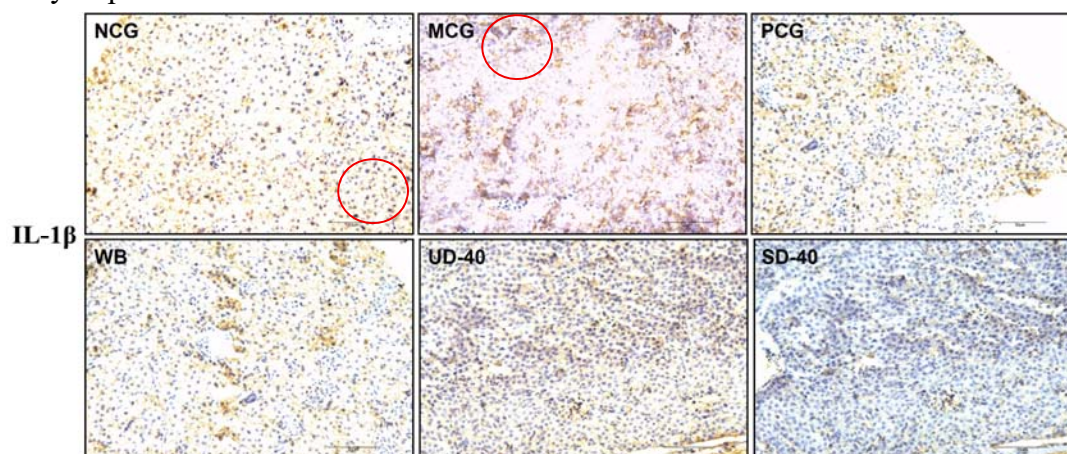


Figure 6. Immunohistochemical staining of insulin (A), pck-1 (B) and IL-1 β (C) in zebrafish liver. NCG: Normal groups; MCG: Model groups; PCG: Positive groups; WB: WB intervened groups; UD-40: UD-40 intervened groups; SD-40: SD-40 intervened groups.

3.5.3 Histopathological analysis

The histopathological analysis of zebrafish was shown in Fig. 7. The circled areas denote liver tissue sections, with arrows indicating pathological changes in hepatocytes. The results indicated that MCG exhibited significant hepatic damage characteristics, including extensive hepatocellular necrosis, fibrous septum formation, and pseudolobule structure. In PCG, the green arrows marked white areas representing central veins, where only minor nodular formations were observed surrounding the veins while the majority of hepatic architecture remained intact. WB intervention exhibited exacerbated hepatocellular damage and fibrosis, likely attributed to its high glycemic index and fat content. UD-40 intervention significantly improved hepatic structure. This may be related to the regulation of gut microbiota diversity by SDF in black beans, which reduced the entry of lipopolysaccharides (LPS) into the bloodstream and inhibited the NF- κ B inflammatory pathway suppression [49]. SD-40 showed superior therapeutic efficacy with only minimal necrotic areas remaining. This significant effect was attributed to the SCFAs during the fermentation process, which activated FFAR2 receptors to inhibit adipocyte differentiation and promoted browning of white adipose tissue [50]. Additionally, small molecule peptides generated during fermentation attenuated NF- κ B phosphorylation and downregulated pro-inflammatory cytokines (TNF α and IL-1 β), promoting hepatocyte proliferation and tissue repair [47].

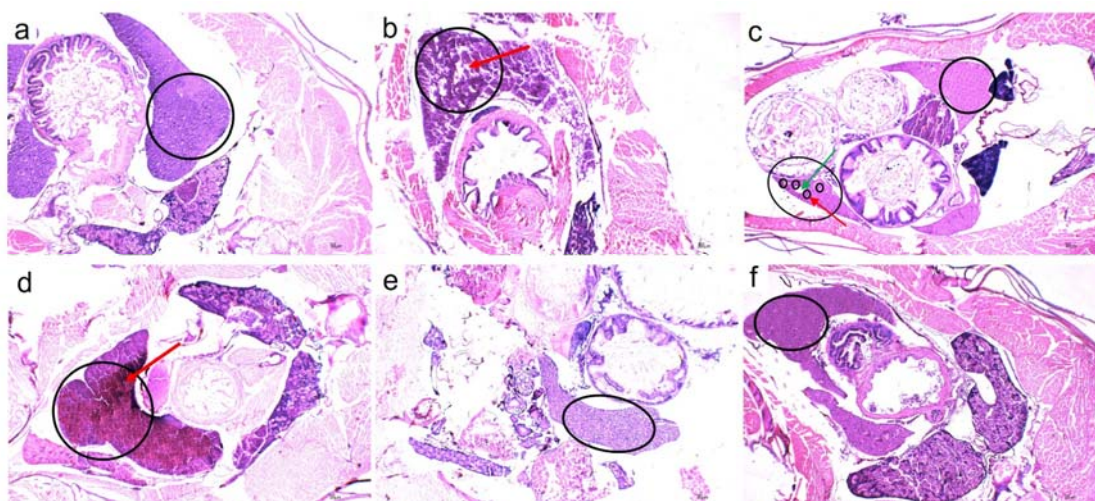


Figure 7. H&E staining analysis of zebrafish liver tissue. a: NCG (Normal groups); b: MCG (Model groups); c: PCG (Positive groups); d: WB (B intervened groups); e: UD-40 (UD-40 intervened groups); f: SD-40 (SD-40 intervened groups).

4. Conclusion

This research systematically revealed the multi-mechanism synergistic regulation of glucose metabolism in black bean bread fermented by Gansu jiaozi. Sourdough fermentation and the addition of black beans not only reorganized the gluten starch network structure, but also promoted the formation of starch-lipid/protein complex. By constructing physical barriers and increasing the proportion of resistant starch, the glucose release of black bean bread during digestion was significantly reduced. Moreover, the bioactive components released by a variety of microbial strains in Gansu Jiaozi effectively improve lipid metabolism disorder and inflammatory response by activating key gluconeogenesis inhibitory enzymes and down regulating pro-inflammatory cytokines. This study elucidated the hypoglycemic mechanism of black bean bread fermented with Gansu sourdough from the perspectives of food matrix structure and microbial metabolic systems, identifying the SD-40 formulation as the optimal process parameters for balancing product texture and metabolic regulation efficacy. However, as these conclusions are primarily derived from zebrafish models, their relevance to humans requires further validation through clinical trials. Furthermore, while the overall functional contribution of the dominant microbial community has been clarified, the specific roles and interactions of individual strains remain to be investigated.

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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References

- [1] L. Liang, F. Zhang, Q. Li, et al. Characterization of squash polysaccharide and the anti-diabetic effect on type 2 diabetic rat revealed by urine metabolomics analysis, *Food Science and Human Wellness*. 13 (2024) 2937-2946. <https://doi.org/10.26599/FSHW.2022.9250237>.
- [2] A.F.R. Silva, R. Silva-Reis, R. Ferreira, et al. The impact of resveratrol-enriched bread on cardiac remodeling in a preclinical model of diabetes, *Antioxidants*. 12 (2023) 1066. <https://doi.org/10.3390/antiox12051066>.
- [3] X. Wang, X. Lao, Y. Bao, et al. Effect of whole quinoa flour substitution on the texture and in vitro starch digestibility of wheat bread, *Food Hydrocolloids*. 119 (2021) 106840. <https://doi.org/10.1016/j.foodhyd.2021.106840>.
- [4] M. Bohl, S. Gregersen, Y. Zhong, et al. Beneficial glycaemic effects of high-amylose barley bread compared to wheat bread in type 2 diabetes, *European journal of clinical nutrition*. 78 (2024) 243-250. <https://doi.org/10.1038/s41430-023-01364-x>.
- [5] J. Yang, L.J. Zhang, F. Wang, et al. Molecular imaging of diabetes and diabetic complications: Beyond pancreatic β -cell targeting, *Advanced Drug Delivery Reviews*. 139 (2019) 32-50. <https://doi.org/10.1016/j.addr.2018.11.007>.
- [6] M. Fall, K.S.S. Hlaing, M. Jiang, et al. Synergistic impact on postprandial glycemic response to whole grain fonio flour (*Digitaria iburua*) by its particle size and released phenolic compounds, *Journal of Cereal Science*. 125 (2025) 104250. <https://doi.org/10.1016/j.jcs.2025.104250>.
- [7] H. Demirkesen-Bicak, M. Arici, M. Yaman, et al. Effect of different fermentation condition on estimated glycemic index, *in vitro* starch digestibility, and textural and sensory properties of sourdough bread, *Foods*. 10 (2021) 514. <https://doi.org/10.3390/foods10030514>.
- [8] X. Sun, Z. Ye, M. Xiao, et al. The formation mechanism of volatile and nonvolatile flavor substances in sourdough based on genomics and metabolomics, *Food Chemistry*. 476 (2025) 143283. <https://doi.org/10.1016/j.foodchem.2025.143283>.
- [9] H. Li, Y. Lv, Y. Zhang, et al. Fermentation properties and functional stability of dough starter Jiaozi and Laomian after frozen storage, *Frontiers in Microbiology*. 15 (2024) 1379484. <https://doi.org/10.3389/fmicb.2024.1379484>.
- [10] X. Sun, L. Yu, M. Xiao, et al. Exploring Core fermentation microorganisms, flavor compounds, and metabolic pathways in fermented Rice and wheat foods, *Food Chemistry*. 463 (2025) 141019. <https://doi.org/10.1016/j.foodchem.2024.141019>.
- [11] A. J. Valdés-Alvarado, E. D. Castañeda-Reyes & E. G. Mejia. Optimized fermentation conditions of pulses increase scavenging capacity and markers of anti-diabetic properties, *Antioxidants*. 14 (2025) 523. <https://doi.org/10.3390/antiox14050523>.
- [12] M. Ayyash, S. K. Johnson, S. Q. Liu, et al. Cytotoxicity, antihypertensive, antidiabetic and antioxidant activities of solid-state fermented lupin, quinoa and wheat by *Bifidobacterium* species: *In-vitro* investigations, *LWT-Food Science and Technology*. 95 (2018) 295-302. <https://doi.org/10.1016/j.lwt.2018.04.099>.
- [13] Q. Yang, C. Gu, X. Zhang, et al. The research on metabolomics mechanism of calcium ion-induced whole black bean polyphenols and biological activities, *LWT-Food Science and Technology*. 195 (2024) 115851. <https://doi.org/10.1016/j.lwt.2024.115851>.
- [14] O.H. Lowry. Protein measurement with the Folin phenol reagents, *Journal of Biological Chemistry*. 193 (1951) 265-275.
- [15] F. Jia, Z. Ma, X. Wang, et al. Effect of kansui addition on dough rheology and quality characteristics of chickpea-wheat composite flour-based noodles and the underlying mechanism, *Food Chemistry*. 298 (2019) 125081. <https://doi.org/10.1016/j.foodchem.2019.125081>.
- [16] T. Xin, S. Tang, T. Su, et al. Impact of replacing wheat flour with lychee juice by-products on bread quality characteristics and microstructure, *LWT-Food Science and Technology*. 165 (2022) 113696. <https://doi.org/10.1016/j.lwt.2022.113696>.
- [17] Q. Yang, X. Zhang, C. Gu, et al. The mediation mechanism of calcium ions on black bean type 3 resistant starch: Metabolomics, structure characteristics and digestibility, *Food Chemistry*. 446 (2024) 138883. <https://doi.org/10.1016/j.foodchem.2024.138883>.
- [18] L. Pan, C.J. Zhang, Z. Bai, et al. Effects of different strains fermentation on nutritional functional components and flavor

- p>compounds of sweet potato slurry,
- Frontiers in Nutrition*
- . 10 (2023) 1241580.
- <https://doi.org/10.3389/fnut.2023.1241580>
- .
- [19] Q. Yue, Z. Wang, F. Yu, et al. Changes in metabolite profiles and antioxidant and hypoglycemic activities of *Laminaria japonica* after fermentation, *LWT-Food Science and Technology*. 158 (2022) 113122. <https://doi.org/10.1016/j.lwt.2022.113122>.
- [20] F. Tang, B. Wei, C. Qin, et al. Enhancing the inhibitory activities of polyphenols in passion fruit peel on α -Amylase and α -Glucosidase via β -Glucosidase-producing *Lactobacillus* fermentation, *Food Bioscience*. 62 (2024) 105005. <https://doi.org/10.1016/j.fbio.2024.105005>.
- [21] S. Vandenbosch, Y.D. Bondt, J. Steensels, et al. The thermotolerance of industrial yeast strains in a dough matrix determines their impact on the oven rise during bread baking, *Food Chemistry*. 491 (2025) 145151. <https://doi.org/10.1016/j.foodchem.2025.145151>.
- [22] D. Xu, Y. Zhang, K. Tang, et al. Effect of mixed cultures of yeast and lactobacilli on the quality of wheat sourdough bread, *Frontiers in Microbiology*. 10 (2019) 2113. <https://doi.org/10.3389/fmicb.2019.02113>.
- [23] Z.X. Mu, Z.Z. Li, B.X. Liu, et al. A hypoglycemic peptide from *pinus pumila* nut oil meal improves glycolipid metabolism via multi-dimensional regulation in type 2 diabetic mice, *Nutrients*. 17 (2025) 2903. <https://doi.org/10.3390/nu17172903>.
- [24] Y. Yan, Z. Niu, C. Sun, et al. Hepatic thyroid hormone signalling modulates glucose homeostasis through the regulation of GLP-1 production via bile acid-mediated FXR antagonism, *Nature Communications*. 13 (2022) 6408. <https://doi.org/10.1038/s41467-022-34258-w>.
- [25] T. Sharma, P. Ranawat, A. Garg, et al. Short-chain fatty acids as a novel intervention for high-fat diet-induced metabolic syndrome, *Molecular and Cellular Biochemistry*. 480 (2025) 3169-3184. <https://doi.org/10.1007/s11010-024-05185-9>.
- [26] A. Zhang, X. Jiang, Y. Ge, et al. The effects of GABA-rich adzuki beans on glycolipid metabolism, as well as intestinal flora, in type 2 diabetic mice, *Frontiers in Nutrition*. 9 (2022) 849529. <https://doi.org/10.3389/fnut.2022.849529>.
- [27] Y. Wu, T. Li, B. Chen, et al. Tryptophan indole derivatives: key players in type 2 diabetes mellitus, *Diabetes, Metabolic Syndrome Obesity: Targets Therapy*. 18 (2025) 1563-1574. <https://doi.org/10.2147/dms.s511068>.
- [28] W. Cai, L. Fei, D. Zhang, et al. Impact of *Lactobacillus plantarum* fermentation on jujube juice: Insights from intelligent sensory evaluation, tolerance index, and acidogenic potential, *LWT-Food Science & Technology*. 217 (2025) 117185. <https://doi.org/10.1016/j.lwt.2024.117185>.
- [29] D. Cizeikiene, J. Jagelaviciute, M. Stankevicius, et al. Thermophilic lactic acid bacteria affect the characteristics of sourdough and whole-grain wheat bread, *Food Bioscience*. 38 (2020) 100791. <https://doi.org/10.1016/j.fbio.2020.100791>.
- [30] M. Kieliszek, K. Pobiega, K. Piwowarek, et al. Characteristics of the proteolytic enzymes produced by Lactic acid bacteria, *Molecules*. 26 (2021) 1858. <https://doi.org/10.3390/molecules26071858>.
- [31] L. Zhao, X. Liu, S. Wang, et al. Research progress on fermentation-produced plant-derived bioactive peptides, *Frontiers in Pharmacology*. 15 (2024) 1438947. <https://doi.org/10.3389/fphar.2024.1438947>.
- [32] X. Li, L. Wang, P. Jiang, et al. The effect of wheat bran dietary fibre and raw wheat bran on the flour and dough properties: A comparative study, *LWT-Food Science and Technology*. 173 (2023) 114304. <https://doi.org/10.1016/j.lwt.2022.114304>.
- [33] E. Korcz, L. Varga. Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry, *Trends in Food Science & Technology*. 110 (2021) 375-384. <https://doi.org/10.1016/j.tifs.2021.02.014>.
- [34] B. Yan, H. Yang, Y. Wu, et al. Quality enhancement mechanism of alkalifree chinese northern steamed bread by sourdough acidification, *Molecules*. 25 (2020) 726. <https://doi.org/10.3390/molecules25030726>.
- [35] Y. Wang, P. Sorvali, A. Laitila, et al. Dextran produced in situ as a tool to improve the quality of wheat-faba bean composite bread, *Food Hydrocolloids*. 84 (2018) 396-405. <https://doi.org/10.1016/j.foodhyd.2018.05.042>.
- [36] M.G. Gänzle, J. Zheng. Lifestyles of sourdough lactobacilli-Do they matter for microbial ecology and bread quality?, *International Journal of Food Microbiology*. 302 (2019) 15-23. <https://doi.org/10.1016/j.ijfoodmicro.2018.08.019>.
- [37] J. Wang, S. Zhao, G. Min, et al. Starch-protein interplay varies the multi-scale structures of starch undergoing thermal processing, *International Journal of Biological Macromolecules*. 175 (2021) 179-187. <https://doi.org/10.1016/j.ijbiomac.2021.02.020>.
- [38] P. Liu, Y. Fang, X. Zhang, et al. Effects of multienzyme treatment on the physicochemical properties of maize starch-lauric acid complex, *Food Hydrocolloids*. 107 (2020) 105941. <https://doi.org/10.1016/j.foodhyd.2020.105941>.

-
- [39] S. Turksoy, M. Guzel, N. Guzel. Effect of sourdough addition on gluten-free sorghum bread fortified with plant-based protein and dietary fiber: Functional, textural, and structural properties, *Cereal Chemistry*. 101 (2024) 518-529. <https://doi.org/10.1002/cche.10752>.
- [40] S. Sun, Y. Hong, Z. Gu, et al. Different starch varieties influence the complexing state and digestibility of the resulting starch-lipid complexes, *Food Hydrocolloids*. 141 (2023) 108679. <https://doi.org/10.1016/j.foodhyd.2023.108679>.
- [41] Y. Wang, X. Tian, Z. Zhang, et al. Investigation of the potential mechanisms of α -amylase and glucoamylase through ultrasound intensification, *LWT-Food Science and Technology*. 198 (2024) 115979. <https://doi.org/10.1016/j.lwt.2024.115979>.
- [42] S. Yu, W. Wang, T. Bu, et al. Digestion, absorption, and transport properties of soy-fermented douchi hypoglycemic peptides VY and SFLLR under simulated gastrointestinal digestion and Caco-2 cell monolayers, *Food Research International*. 164 (2023) 112340. <https://doi.org/10.1016/j.foodres.2022.112340>.
- [43] Y. Liu, Y. Gao, F. Ma, et al. The ameliorative effect of *Lactobacillus plantarum* Y44 oral administration on inflammation and lipid metabolism in obese mice fed with a high fat diet, *Food & Function*. 11 (2020) 5024-5039. <https://doi.org/10.1039/d0fo00439a>.
- [44] T. Nishijima, Y. Yamashita, H. Ashida, et al. Black soybean seed coat polyphenols have different effects on glucose and lipid metabolism in growing and young adult mice, *Food & Function*. 15 (2024) 1004-1020. <https://doi.org/10.1039/d3fo04269c>.
- [45] L. Mojica, A.S. Ramos-Lopez, O.A. Sánchez-Velázquez, et al. Black bean (*Phaseolus vulgaris* L.) protein hydrolysates reduce acute postprandial glucose levels in adults with prediabetes and normal glucose tolerance, *Journal of Functional Foods*. 112 (2024) 105927. <https://doi.org/10.1016/j.jff.2023.105927>.
- [46] K. Pakaew, P. Chonpathompikunlert, N. Wongmanee, et al. *Lactobacillus reuteri* TISTR 2736 alleviates type 2 diabetes in rats via the hepatic IRS1/PI3K/AKT signaling pathway by mitigating oxidative stress and inflammatory mediators, *European Journal of Nutrition*. 64 (2025) 27. <https://doi.org/10.1007/s00394-024-03529-1>.
- [47] H. Yang, F. Wang, P. Zhao, et al. Black soybean peptide mediates the AMPK/SIRT1/NF- κ B signaling pathway to alleviate Alzheimer's-related neuroinflammation in lead-exposed HT22 cells, *International Journal of Biological Macromolecules*. 286 (2025) 138404. <https://doi.org/10.1016/j.ijbiomac.2024.138404>.
- [48] R.P. Rahayu, B. Sumaryono, N.F. Sandi. The potential of *Lactobacillus casei* on TNF- α and IL-1 β levels type 2 diabetes mellitus, *Research Journal of Pharmacy and Technology*. 16 (2023) 107-110. <https://doi.org/10.52711/0974-360x.2023.00019>.
- [49] T. Xu, X. Wu, J. Liu, et al. The regulatory roles of dietary fibers on host health via gut microbiota-derived short chain fatty acids, *Current Opinion in Pharmacology*. 62 (2022) 36-42. <https://doi.org/10.1016/j.coph.2021.11.001>.
- [50] F. Li, L. Tai, X. Sun, et al. Molecular recognition and activation mechanism of short-chain fatty acid receptors FFAR2/3, *Cell Research*. 34 (2024) 323-326. <https://doi.org/10.1038/s41422-023-00914-z>.