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Article

Research on the mechanism of action of functional enzymes at the cellular and molecular levels

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Abstract: With the increasing incidence of metabolic diseases (e.g. diabetes mellitus), exploring safe and effective blood glucose regulation strategies has become a hot research topic in the field of functional foods and precision nutrition. This study focuses on the multiple biological activities and molecular mechanisms of composite plant enzymes in metabolic regulation, especially their efficacy in blood glucose regulation. By combining physicochemical characterisation and enzyme kinetic studies, the study reveals the mechanism of action of composite plant enzymes in sugar metabolism. The study showed that during the pre-fermentation phase, the significant fluctuation of sugar concentration reflected the efficient enzymatic reaction and metabolic activity of the microorganisms; during the post-fermentation phase, the system entered into a metabolic steady state. These dynamic changes demonstrated the role of enzymes in glycolysis and the tricarboxylic acid cycle, and may also affect the cell membrane mechanical environment and signalling mechanisms. In addition, the composite plant enzymes exhibited non-competitive inhibition of α-amylase, which helped to control postprandial blood glucose levels by slowing down the hydrolysis of starch to glucose by reducing the reaction rate; whereas, the inhibition of α-glucosidase was competitive, which significantly enhanced the competitiveness for substrate binding as the enzyme concentration increased. The multi-target regulatory potential of composite plant enzymes in blood glucose regulation can be applied to nutritional intervention of metabolic diseases and functional food development, providing an important direction for future research.

Keywords: functional enzymes; composite plant enzymes; kinetics; glycaemic regulation

1. Introduction

Food technology science, as an interdisciplinary field, has evolved from experience-based approaches to mechanistic explorations, transitioning from single-dimensional studies to system-level integration [1]. The core lies in analyzing molecular structures, dynamic behaviors, and functional properties of food systems to optimize processing and meet multi-dimensional needs for health, nutrition, safety, and sustainability. Advanced analytical techniques such as molecular dynamics simulations, quantum chemistry, liquid chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopy have been instrumental in uncovering complex molecular interactions, such as protein-polyphenol binding during food processing, which exhibit time-space dependency and provide theoretical foundations for process optimization [2]. Modern food science prioritizes health functions and environmental sustainability, reflecting a shift in consumer demand from sensory satisfaction to the prevention of chronic diseases and quality of life improvement. This evolution aligns with the rise of functional foods, which aim to utilize active ingredients efficiently while adhering to green manufacturing and circular economy

principles. Food technology science is tasked with dual goals: enhancing health value through biotechnological advancements and promoting eco-friendly food industry practices [3]. Enzymes, as highly specific and efficient biocatalysts, have become central to food processing, functional ingredient extraction, and metabolic regulation. As shown in **Figure 1,** China's enzyme market exemplifies this growth, expanding from 380 million RMB in 2011 to an anticipated 29.55 billion RMB by 2025. This steady growth reflects rising health awareness and the maturing enzyme industry, which continues to hold significant potential for innovation and application in food technology.

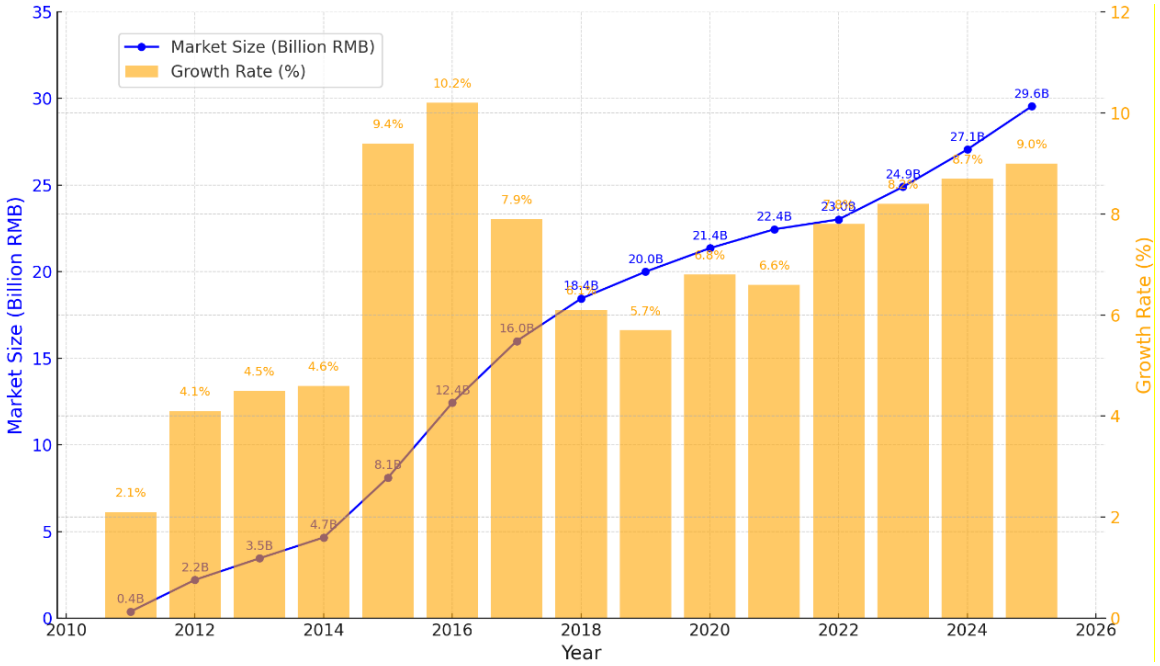

Figure 1. Trends in the Chinese enzyme market (data for 2024 and 2025 are projections).
Data source: China Report Hall "China Enzyme Industry Market Research and Market Outlook Forecast Evaluation Report".

Enzymes, with their high selectivity, mild reaction conditions, and environmental compatibility, have become essential tools in green food processing. They play a pivotal role in functional food development by enhancing the bioavailability of active ingredients, optimizing texture, and improving sensory qualities, driving innovation in the food industry [4]. Advances in molecular biology and protein engineering have elevated enzyme research to new heights. Technologies such as directed evolution and rational design have enhanced enzyme performance in substrate selectivity, thermal stability, and catalytic efficiency, broadening their applications and enabling synergistic effects in complex enzyme systems. Beyond traditional food processing, enzymes are increasingly recognized for their health interventions, influencing metabolic pathways to regulate blood glucose, maintain lipid metabolism, and combat oxidative stress. This dual role of enzymes—as tools for functional food development and as active health-promoting components—has positioned them as a key focus for next-generation research in food processing and precision nutrition, underscoring their significance in both scientific and industrial advancements.

Under the trend of increasingly diversified enzyme research and application

fields, functional enzymes, especially composite plant enzymes, are gradually becoming an important research frontier in functional food science by virtue of their composite molecular structure, multidimensional bioactivity, and extensive health regulation potential [5]. Composite plant enzymes are composed of multiple plant-derived enzymes and the accompanying secondary metabolites in a synergistic manner, and their multi-component and multi-targeted composite properties give them functional advantages beyond those of a single enzyme [6].

Glucose regulation, as one of the core dimensions of metabolic homeostasis, involves a series of multilevel molecular events. The dynamic balance of this composite network is finely regulated by many enzymes, metabolic molecules and biological signals. It is in this metabolic system that composite plant enzymes show their unique functionality, and their mechanism of action is not only limited to the catalytic degradation of food matrix, but also further reflected in the metabolic regulation potential of multi-targets and multi-pathways [7]. Its active molecules can interact with key proteins in the insulin signaling pathway (e.g., AMPK and the PI3K/Akt axis) to activate the membrane expression of the glucose transporter protein GLUT4, which can significantly enhance the efficiency of cellular uptake of blood glucose [8]. This multi-dimensional biological effect makes the functional advantages of composite plant enzymes in blood glucose regulation more and more significant [9].

The regulatory effects of composite plant enzymes are derived not only from their enzymatic activity but also from the synergistic functions of accompanying active components such as polyphenols and flavonoids [10]. These secondary metabolites regulate oxidative stress, maintain mitochondrial function, restore insulin sensitivity, and alleviate chronic inflammation in metabolic disorders such as diabetes [11]. However, several challenges remain in fully understanding the molecular mechanisms of composite plant enzymes [12]. One significant limitation lies in the incomplete understanding of how enzymes and active metabolites interact to achieve multi-target effects [13]. The specific molecular pathways and structural mechanisms driving their synergy, such as their role in metabolic signaling pathways like PI3K/Akt and AMPK, remain unclear [14]. Additionally, the functional coupling and distribution of these active components within metabolic networks have not been systematically analyzed, which limits our ability to predict their contributions to overall metabolic regulation. While the fermentation process is known to enhance enzyme activity and increase the generation of polyphenols and flavonoids, the mechanisms by which fermentation-induced changes influence the glycemic regulatory effects of composite plant enzymes remain underexplored [15]. Another challenge is the limited use of advanced analytical tools capable of capturing the complexity of multi-component interactions [16]. Current methods fail to provide detailed insights into how enzymes and metabolites regulate the activity of key proteins such as α-amylase and α-glucosidase or how they reshape metabolic homeostasis at the molecular level [17]. Advanced techniques such as molecular biomechanics, which analyze binding kinetics, thermodynamic stability, and structural compatibility, could help elucidate these mechanisms [18]. The integration of these analyses with multi-omics approaches, such as proteomics and metabolomics, remains underutilized [19]. These techniques could provide a comprehensive understanding of the molecular dynamics of enzymes and their

metabolites in regulating metabolic pathways. Furthermore, system-wide analyses linking molecular mechanisms to organismal outcomes, such as improved glucose metabolism and insulin sensitivity, are lacking, which limits the translational potential of current findings.

Building on this foundation, this study focuses on composite plant enzymes as the research object and systematically investigates their mechanisms in blood glucose regulation, encompassing physicochemical properties (using pine needle composite enzymes as an example), kinetic behaviors, and metabolic regulatory effects. By analyzing the physicochemical changes during the fermentation process, the active components of composite plant enzymes were identified, and their functional potentials were highlighted. Additionally, through enzyme kinetic studies, the interaction modes of composite plant enzymes with key metabolic enzymes and their roles in critical signaling pathways were elucidated. This research not only uncovers the hypoglycemic mechanisms of composite plant enzymes at the cellular and molecular levels but also incorporates multi-omics analysis to clarify the synergistic effects of enzymes and their secondary metabolites. The findings provide robust scientific evidence and practical insights for developing functional foods and nutritional interventions targeting metabolic diseases. Furthermore, this study broadens the scope of interdisciplinary research, deepening the integration of food technology and health science, and paves the way for precision nutrition strategies aimed at safe and effective blood glucose management.

2. Physical and chemical properties of plant composite enzyme (pine needle composite enzyme for example) during fermentation process

2.1. Test materials

Raw materials: Fresh pine needles were collected in April 2020 from Horsetail Pine in Cha-en Temple, Xiangtan City, Hunan Province (latitude 27 °N, longitude 112 °E), which required a tree height of more than 6 meters, an age of more than 6 years, and two needles in a bundle. They were collected in uniform length, free of yellow pine needles, free of pests and diseases, and free of mechanical damage (dark green in color), and were collected into a cold-chain insulated box for rapid transportation to the laboratory [20].

Excipients: pine nuts, grapefruit, orange, lemon, carrot were provided by Hunan Huaheng Agricultural Science and Technology Co. Pine pollen, sea buckthorn, rosehip, cinnamon were provided by Jiangxi Heshuo Pharmaceutical Co.

The selection of pine needles and specific fruits and vegetables as raw materials was guided by their unique biochemical properties and functional potentials. Pine needles, collected from mature Horsetail Pine trees in Cha-en Temple, Hunan Province, were chosen for their rich content of bioactive compounds, including flavonoids, polyphenols, and terpenoids. These compounds are known for their strong antioxidant properties, potential to regulate oxidative stress, and capacity to enhance metabolic health, making them an ideal foundation for developing composite plant enzymes. The strict criteria for harvesting, such as tree age, height, and needle quality, ensured the

consistency and purity of the raw material, maximizing its functional benefits.

The selected fruits and vegetables, including grapefruit, orange, lemon, carrot, and pine nuts, were chosen for their complementary bioactive profiles. Citrus fruits are rich in vitamin C, flavonoids, and organic acids, which can synergize with the bioactive compounds in pine needles to enhance enzymatic activity and metabolic regulation. Carrots contribute carotenoids, which have antioxidative and anti-inflammatory properties, while pine nuts provide essential fatty acids and micronutrients that support overall metabolic function. Additionally, excipients such as pine pollen, sea buckthorn, rosehip, and cinnamon were incorporated for their well-documented roles in improving glycemic control, lipid metabolism, and immune regulation. This carefully curated combination of raw materials ensures a balanced and multifunctional formulation, aligning with the study's objectives of optimizing enzymatic efficacy and exploring the metabolic regulatory effects of composite plant enzymes.

Fermentation strains: Lactobacillus fermentum and Bifidobacterium breve were purchased from the Institute of Microbiology, Chinese Academy of Sciences.

Test reagents: tartaric acid (≥98%), anhydrous citric acid (≥99.8%), lactic acid (≥98%), malic acid (≥98%), succinic acid (≥98%), and fumaric acid (≥98%) standards were purchased from Yuan Ye Company.

Five enzyme activity kits were purchased from Jiangsu Jiantao Biotechnology Company.

The four antioxidant activity test kits were purchased from Beijing Solepol Technology Co.

All other analytical reagents were analytically pure and purchased from Sinopharm Chemical Reagent Co.

2.2. Main instruments and equipment

Microanalytical balance AUY220 was purchased from Shimadzu; High performance liquid chromatograph Agilent 1100 servies was purchased from Agilent; Full-wavelength enzyme marker was purchased from Thermo Scientific MultiskanFC; High-speed freezing centrifuge H-2050R was purchased from Shanghai Late Success Medical Instrument Company; Ultrasonic shaker KQ3200E was purchased from Kunshan Ultrasonic Instrument Company; Electrothermal constant temperature water bath was purchased from Tianjin Tester Instrument Company; 0.22um pinhole filter membrane was purchased from Tianjin Keyiwei Instrument Co., Ltd; electric thermostatic water bath purchased from Tianjin Tester Instrument Co., Ltd; 0.22um pinhole filter membrane purchased from Tianjin Keyilon Experimental Equipment Co.

2.3. Test methods

Process and sample collection of pine needle composite enzyme fermentation: fresh pine needles were collected from the pine trees (Sargasso Pine) of Cha-en Temple in Shaoshan. The pine needle composite enzyme was mainly fermented by referring to the method of Mahajan et al. [21] with slight improvement.

Pine needles, pine pollen, pine nuts and other fruits and vegetables (5:1:1:3) were selected, which were successively washed, hot blanched, cooled, drained, water was added to make the total volume up to 35 times the volume of the starting mixture, 22%

brown sugar and 33% icing sugar by mass of the mixed raw materials were added, inoculated with 0.8% yeast solution at 22 ℃, placed in a fermentation bed and fermented for 18 days (pre-fermentation period), and then inoculated with 6% of short bifidobacteria Then inoculate 6% short bifidobacterium M-16V and Lactobacillus fermentum CECT5716 mixed bacterial solution (short bifidobacterium M-16V:Lactobacillus fermentum CECT5716 = 1.0:1.5), mix well, and then ferment for 7 days (post-fermentation period), that is, to obtain the pine needle composite enzyme drink.

Sampling: The unfermented pine needle composite enzyme was used as the fermentation initiation point 0d, the pre-fermentation period, regularly collected:0d, 1d, 3 d, 6 d, 9d, 12d, 15d, 18d, the post-fermentation period, regularly collected:Hd1, Hd2, Hd3, Hd5, Hd7 a total of 13 samples of pine needle composite enzyme, the pre-fermentation period, the samples were named as:GD0, GD1, GD3, GD6, GD9, GD12, GD15, GD18; on the 18th day, the samples were inoculated with Lactobacillus plantarum and Bifidobacterium bifidum mixture, and in the post-fermentation period, the samples were named as HGD1, HGD2, HGD3, HGD5 and HGD7, and were sealed in the refrigerator at −80 ℃ for spare time. To ensure that the data bias was small, a total of 5 parallel/samples were set up during the fermentation testing process, of which 13 samples were selected for physicochemical index analysis [22]. The samples were analyzed for physicochemical indexes, including a total of 65 pine needle composite enzyme samples, to monitor the dynamic fermentation of physicochemical indexes during the fermentation process.

2.4. Results and analysis

As shown in **Figure 2**, the pine needle composite enzyme exhibited significant enzyme activity changes during fermentation, with the dynamics of glycosylase and lipase being particularly prominent. In the pre-fermentation stage (0–18 days), the activity of the glycosylase showed an increasing and then decreasing trend, reaching the first peak at 6 days (1200 ± 11 U/L), followed by a gradual decline to a lower level at 18 days (600 ± 8 U/L). In contrast, the activity of glycosylase increased significantly during the post-fermentation phase (19–25 days), reaching a peak at 19 days (2500 ± 15 U/L), followed by a rapid decline and stabilization at the end of fermentation (2 days) (720 ± 5 U/L). In contrast, lipase activity was relatively low throughout the fermentation process. In the pre-fermentation stage, its activity gradually increased and reached (11 ± 0.9 U/L) at 15 days; while in the post-fermentation stage, lipase activity peaked at 19 days (15 ± 0.25 U/L), and then rapidly declined to a stable level (7 ± 0.1 U/L) at 25 days.

Comprehensive analysis revealed that the activity changes of glycosylase and lipase in the fermentation process had obvious stage characteristics, and the time points of the peak activities of the two were misplaced, suggesting that these two types of enzymes may have assumed different functional division of labor in the whole fermentation system. The significant activity enhancement of glycosylase at the beginning of post-fermentation may be related to the rapid conversion of polysaccharides in the pine needle composite enzyme, which provides support for its potential metabolic regulatory role. The relatively low activity and small variation of

lipase suggested that it might prefer to participate in specific lipolysis processes as a secondary enzyme. The above dynamic activity changes reflect the biochemical characteristics of the fermentation system and reveal the functional potential of the composite enzymes under enzyme synergism.

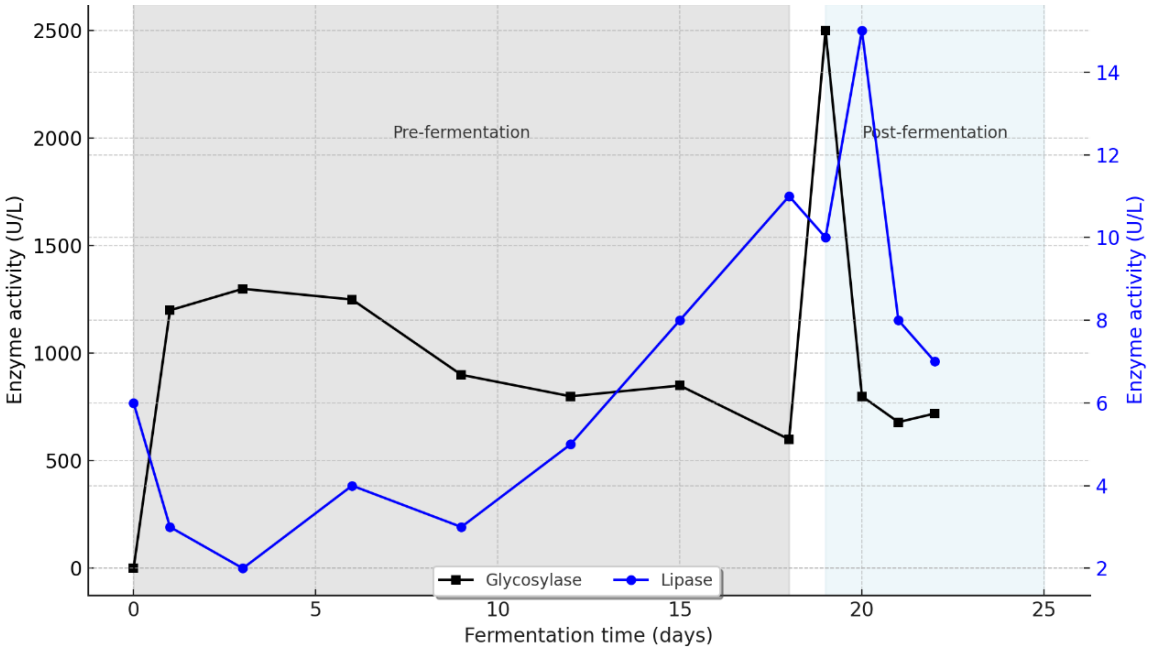

Figure 2. Trend of enzyme activity.

As shown in **Figure 3**, the dynamic changes of saccharides during the fermentation of pine needle composite enzyme showed significant stage characteristics. During the pre-fermentation phase (0–18 days), the total sugar concentration increased rapidly at the initial stage (0–3 days), growing from a starting value of 0.0 ± 0.0 mg/mL to a peak value of 45.50 ± 0.06 mg/mL. Subsequently, the total sugar concentration declined rapidly from 3 to 9 days to 2.61 ± 0.15 mg/mL, and remained stable in the subsequent phase (9–18 days). This change may be attributed to the secretion of sugar hydrolases by the microbial population at the initial stage, which rapidly converted insoluble polysaccharides into soluble sugars, thus leading to a significant increase in total sugar concentration; while at the later stage, microorganisms metabolized total sugars using them as a carbon source, and the total sugars were consumed in large quantities, thus resulting in a rapid decreasing trend. Reducing sugar concentration showed a similar dynamic trend in the pre-fermentation stage, increasing from 0.0 ± 0.0 mg/mL to a peak of 30.34 ± 0.04 mg/mL in 3 days, and then rapidly decreasing and stabilizing at 3.0 ± 0.1 mg/mL. The rapid accumulation of reducing sugars reflected the enzymatic degradation reaction to break down the polysaccharides into monosaccharides, whereas the rapid decrease indicated that microorganisms preferentially utilized reducing sugar as the main metabolic substrate. During the post-fermentation phase (19–25 days), both total and reducing sugars stabilized at 3.0 ± 0.05 mg/mL for total sugars and 3.2 ± 0.02 mg/mL for reducing sugars, which may be due to the inhibitory effect of nutrient depletion and accumulation of secondary metabolites on microbial activity. In addition, the involvement of microorganisms such as lactic acid bacteria in the post-fermentation

stage may have caused a small amount of re-catabolism of composite sugars, but the overall magnitude of change was small.

Overall, the dynamic changes of total and reducing sugars showed a significant phase pattern, with rapid fluctuations in sugar concentrations during the pre-fermentation stage reflecting efficient enzymatic reactions and microbial metabolic activities; the smooth trend during the post-fermentation stage indicated that the system entered a metabolic steady state. These dynamic changes revealed the important role of pine needle composite enzymes in the regulation of sugar metabolism. Analyzed at the molecular level, the rapid conversion and depletion of sugars may involve the active processes of the microbial glycolysis pathway (Glycolysis) and the tricarboxylic acid cycle (TCA cycle). Combined with the molecular biomechanical perspective, the rapid degradation and conversion of sugar molecules not only affects the generation of cellular metabolic energy, but also may change the mechanical environment and signaling mechanism of cell membranes, which provides a new perspective for the study of sugar metabolism and enzyme activity. This provides a basis for us to further analyze the physicochemical properties of plant composite enzymes in terms of their blood glucose regulation efficacy.

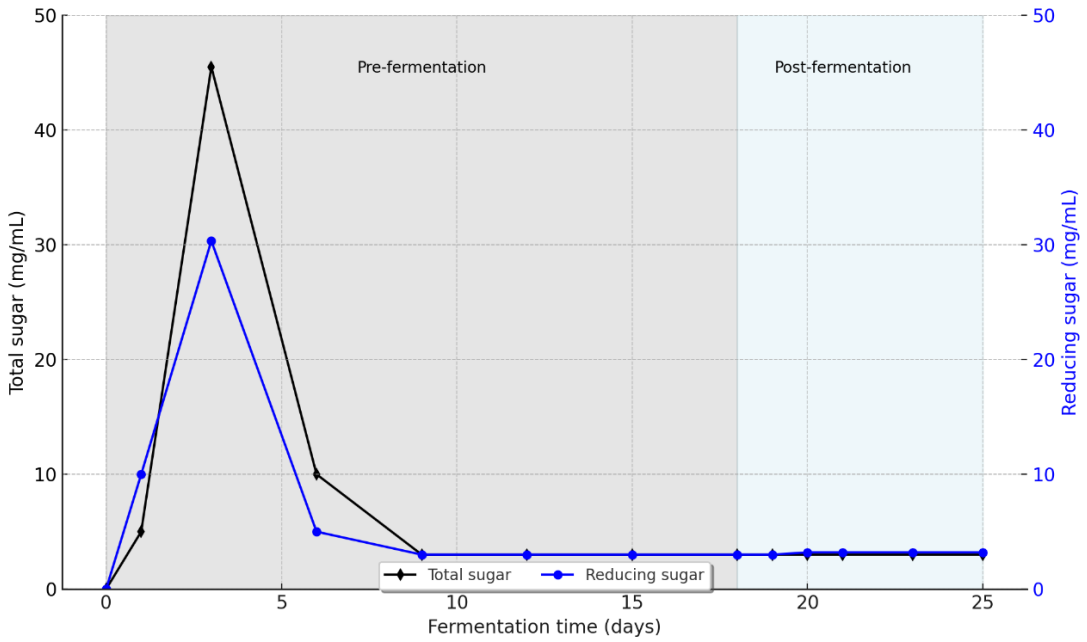

Figure 3. Trends of total and reducing sugars during fermentation.

3. Kinetic study of the blood glucose regulation efficacy of composite plant enzymes

3.1. Test materials

Red ginseng, bamboo, amaranth: purchased from Tongrentang; wolfberry: Anhui Pharmacognosy Chinese Medicine Drinking Tablets Co. Ltd. Traditional Chinese medicine compound Yisukang: Liaoning University of Traditional Chinese Medicine (hereinafter referred to as "Yisukang"); porcine pancreatic α-amylase (50 U/mg), from Sigma Company, USA; glucocerebrosidase (analytically pure), from Shanghai Rui En

Bio-technology Co. Sigma; p-nitrophenol-α-D-glucopyranoside (pNPG) (analytically pure), from Sinopharm Chemical Reagent Co., Ltd; sodium acetate (analytically pure), from Sinopharm Group Chemical Reagent Co., Ltd; 3,5-dinitrosalicylic acid (analytically pure), from Sinopharm Group Chemical Reagent Co.

3.2. Main instruments and equipment

Digital thermostatic water bath (HH-2), from Changzhou Zhibori Instrument Manufacturing Co., Ltd; ultraviolet-visible spectrophotometer (UV-5100), from Shanghai Yuananalytical Instrument Co., Ltd; low-speed centrifuge (SC-3614), from Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd; analytical balance (ZB604C), from Shenyang Longteng Electronic Weighing and Measuring Instrument Co. Homogenizer (FJ300-SH), from Shanghai Specimen Model Factory; Jiuyang Cooking Machine (JYL-C18D), from Jiuyang Co., Ltd.; Computerized Temperature-controlled Constant Temperature Incubator (DHP-9162), from Ningbo Ledian Instrument Manufacturing Co. (XFH-50CA), from Zhejiang Xinfeng Medical Equipment Co.

3.3. Experimental method

Preparation of composite plant enzymes: The raw materials of the composite plant enzymes in this study were modified on the basis of the traditional Chinese medicine compound Yisukang, and the raw materials optimized in the pre-test were dried red ginseng (30 g), bamboo (40 g), amaranthus (40 g), goji berries (30 g), fresh pomelo (40 g), pumpkin (40 g), ginger (30 g), celery (40 g), yam (40 g), etc. The above raw materials were crushed, and the raw materials and distilled water were grinded in a juicer at a ratio of 3:10 (mass ratio). The above raw materials were pulverized, and the raw materials and distilled water were ground in a juicer at a ratio of 3:10 (mass ratio), and then homogenized at 12000 rpm/min for 5 min, and the homogenized samples were sterilized at 115 ℃ for 30 min, and then cooled down to room temperature after sterilization, and then accessed to lactobacilli in an ultra-clean bench, and then the fermentation was performed at 37 ℃, and then the fermentation solution was centrifuged at 4500 rpm for 3 min. Finally, the fermentation broth was centrifuged at 4500 rpm for 3 min to obtain the composite plant enzyme. The specific process is as follows: raw material → crushing → blending → homogenization → sterilization → cooling → inoculation → fermentation → centrifugation → finished product [23].

Inhibition of α-amylase and α-glucosidase activities by enzymes before and after fermentation: Inhibition of α-amylase activity: slightly modified with reference to the method of Li [24] and others. 28 μL of enzyme at a concentration of 25 ml/L, 1 mL of 0.1 M pH 6.8 phosphate buffered saline (PBS), and 100 uL of α-amylase (29 U) were mixed well, and the reaction was carried out at 37 ℃ for 20 min. After that, 5 mL of 1% soluble starch was added, and the solution was boiled in a boiling water bath for 5 min, and then 1 mL of the enzyme was cooled. Add 1 mL of distilled water and 1.5 mL of 3, 5-Dinitrosalicylic acid (DNS), continue boiling water bath for 5 min, add distilled water to make up to 10 mL after cooling, and measure the absorbance value of A_X at 540 nm, and use PBS instead of enzymes as the blank group to determine the absorbance value of A, and use PBS instead of α-amylase as the sample control group to determine the absorbance value of A. The absorbance value A_{X0} was measured using

PBS instead of enzyme as the blank group, and the absorbance value A_0 was measured using PBS instead of α-amylase as the sample control group, and the inhibition rate was calculated according to the following formula [25]:

$$\alpha - amylase\ inhibition(\%) = \frac{(A - A_0) - (A_x - A_{x0})}{A - A_0} \times 100 \quad (1)$$

Yi Sugar Kang includes twelve Chinese herbs including red ginseng, atractylodes macrocephala, roasted licorice, danshen, wolfberry and schizandra. Firstly, the above herbs were soaked in water for 30~60 min and then decocted, which was first boiled over high fire, followed by slow simmering over moderate fire for a total of 3 times, and each time the decoction was extracted from 100 mL of medicinal liquid and filtered. After that, the three times of decoctions were mixed and continued to be concentrated to 200% concentration, i.e., 2 g/mL, and Yisukang was used as a positive control. Inhibition of α-glucosidase activity: 40 μL of the enzyme before and after fermentation at a concentration of 200 ml/L was taken, and 40 μL of the enzyme and 40 μL of α-glucosidase (0.02 U) were heated at 37 ℃ for 10 min, and at the end of the heating period, 20 uL of pNPG solution was added, and the heating was continued in a water bath for another 10 min, and 100 uL of 0.1 mol/L $NaCO_3$ was added. After 10 min, 100 uL of 0.1 mol/L $NaCO_3$ solution was added, and the absorbance value A_x was measured at 405 nm to determine the inhibition rate of α-glucosidase. PBS was used as a blank group instead of enzyme to determine the absorbance value A, and PBS was used instead of α-glucosidase as a sample control to determine the absorbance value A_{X0}. The inhibition rate of α-glucosidase was obtained by calculating the absorbance, which was calculated by the following formula:

$$\alpha - glucosidase\ inhibition(\%) = \frac{A - (A_x - A_{x0})}{A} \times 100 \quad (2)$$

Kinetic analysis of α-amylase activity inhibition by enzyme: 100 yL of α-amylase (29 U) was mixed with 28 uL of enzyme (16.7 ml/L, 20 ml/L, 25 ml/L), and heated at 37 ℃ for 20 min, and then 5 mL of soluble starch (0.625 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL) was added. After the reaction, 1 mL of the sample solution was heated in a boiling water bath for 5 min. 1 mL of distilled water and 1.5 mL of DNS were added and heated in a boiling water bath for 5 min. After cooling, the sample solution was diluted to 10 mL with distilled water and the absorbance was measured at 540 nm.

Lineweaver-Burk curves were prepared by plotting the inverse of the reaction rate (V) of the enzymes at concentrations of 25 ml/L, 20 ml/L, 16.7 ml/L, and 0 ml/L against the inverse of the corresponding substrate (S) [26].The relationship between enzyme concentration, substrate concentration and enzymatic rate was investigated using 1/S as the horizontal coordinate and 1/V as the vertical coordinate to determine the type of inhibition of α-amylase by enzymes, and then the kinetic parameters K_m and V_m were calculated by the following equation [27]:

$$-\frac{1}{K_m} = X_{intercept} \quad (3)$$

$$K_{slope} = \frac{K_m}{V_m} \tag{4}$$

The kinetic analysis of α-glucosidase activity inhibition by enzyme: 40 uL of α-glucosidase (0.02 U) was mixed with 40 uL of enzyme (25 ml/L, 50 ml/L, 100 ml/L), and the reaction was continued for 5 min at 37 ℃ for 10 min, then 20 μL of substrate pNPG (1 mmol/L, 2 mmol/L, 3 mmol/L, 4 mmol/L, 5 mmol/L) was added respectively. After 10 min in a water bath at 37 ℃, 20 μL of substrate pNPG solution (1 mmol/L, 2 mmol/L, 3 mmol/L, 4 mmol/L, 5 mmol/L) was added respectively to continue the reaction for 5 min, and finally 100 μL of 0.1 mol/L $NaCO_3$ was added to terminate the reaction.

Lineweaver-Burk curves were prepared by plotting the inverse of the reaction rate (V) of the enzymes at concentrations of 100 ml/L, 50 ml/L, 25 ml/L, and 0 ml/L against the inverse of the corresponding substrate (pNPG). The relationship between enzyme concentration, substrate concentration and enzymatic rate was explored using 1/pNPG as the horizontal coordinate and 1/V as the vertical coordinate to determine the type of enzyme inhibition of α-glucosidase, and then the kinetic parameters K_m and V_m were calculated by using Equations (3) and (4).

In addition, for metabolomics analysis, changes in metabolite profiles before, during and after fermentation were analyzed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques. The focus was on monitoring the changes in the types and concentrations of polyphenols (e.g., flavonoids) and secondary metabolites (e.g., organic acids). Principal component analysis and partial least squares-discriminant analysis were used to analyze data downscaling and intergroup differences. For proteomics analysis, the expression levels of each enzyme in the composite enzyme system were analyzed using isotope labeling relative quantification or label free quantification techniques. Determine the interaction of different enzyme activities with glycolytic targets. Evaluate enzyme-substrate binding capacity using molecular docking simulation techniques.

3.4. Results and analysis

The inhibition rates of α-amylase and α-glucosidase by enzymes before and after fermentation are shown in **Table 1**, which shows that the inhibition rates of α-amylase and α-glucosidase by enzymes after fermentation increased by 79.41% and 70.69%, respectively. The inhibition rates of the two enzymes by the fermented enzyme were very close to those of Yisukang, which indicated that the fermentation had achieved a better effect, and that the fermentation process had produced beneficial components that could help to inhibit the activities of the two enzymes. Therefore, the inhibition rates of the two enzymes by different concentrations of enzymes after fermentation and their enzyme kinetics will be further investigated in order to clarify the inhibition effects of enzymes on the two enzymes.

Table 1. Enzyme inhibition rate before and after fermentation.

norm	Pre-fermentation	Post-fermentation	Yisukang
$\alpha - amylase\ inhibition$ /%	19.94 ± 4.58	96.83 ± 1.80	97.72 ± 2.24
$\alpha - glucosidase\ inhibition$ /%	28.68 ± 2.41	97.84 ± 2.51	99.06 ± 0.31

Note: Values in the table are the mean of three measurements, ± are standard deviations; different superscript lowercase letters in the same column indicate significant differences ($p < 0.05$). Following table is the same.

The Lineweaver-Burk double inverse curve of the reaction between the enzyme and α-amylase is shown in **Figure 4**, and as the concentration of the enzyme increases, the 1/V of its reaction with starch solution gradually increases, i.e., the reaction rate of the enzyme with starch gradually decreases. This indicates that the addition of the enzyme to the reaction system inhibited the activity of α-amylase and slowed down the process of hydrolysis of starch to produce reducing sugar, which will be converted to glucose, suggesting that the enzyme can slow down the rate of hydrolysis of starch to glucose, which is conducive to the control of postprandial blood glucose levels in humans [28].

As shown in **Figure 4**, the inhibition of α-amylase by the enzyme belongs to non-competitive inhibition [29]. And in non-competitive inhibition, the enzyme binds to the inactive site of the enzyme and does not directly affect the binding ability of the enzyme and the substrate [30]. K_m is the Mie constant, which stands for the affinity between the enzyme and the substrate. The lower the value of K_m, it means that the substrate and the enzyme have a stronger affinity. V_m refers to the initial maximum reaction velocity.

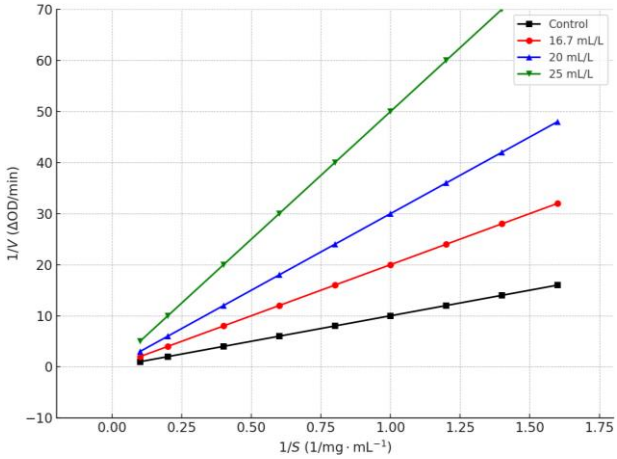

Figure 4. Lineweaver-burk double inverse curve of the reaction between enzymes and α-amylase.

As shown in **Table 2**, V_m between enzyme and α-amylase gradually decreased with increasing enzyme concentration, while K_m was not significantly different ($p > 0.05$) and did not change with enzyme concentration.

Table 2. Kinetic parameters of α-amylase inhibition by enzymes.

Enzyme concentration (ml/L)	K_m	Vm (ΔOD/min)
0	3.88 ± 0.02^a	1.04 ± 0.10^a
16.7	3.87 ± 0.04^a	0.85 ± 0.09^b
20	3.86 ± 0.03^a	0.59 ± 0.03^c
25	3.87 ± 0.02^a	0.12 ± 0.02^d

The Lineweaver-Burk double reciprocal plot (**Figure 5**) provides a clear depiction of the inhibitory mechanism of the enzyme on α-glucosidase. As the enzyme concentration increases, the reciprocal of the reaction velocity (1/V) also increases, indicating a decrease in the overall reaction rate between the enzyme and pNPG. The hydrolysis of pNPG produces pNP and glucose, and the gradual inhibition of this hydrolysis process upon enzyme addition demonstrates that the enzyme actively suppresses the activity of α-glucosidase. This suppression subsequently slows down glucose production, which is critical for controlling postprandial blood glucose levels in humans. The observed effect highlights the potential of this enzyme as a functional ingredient in managing glycemic response, thereby offering therapeutic implications for conditions like diabetes and metabolic disorders.

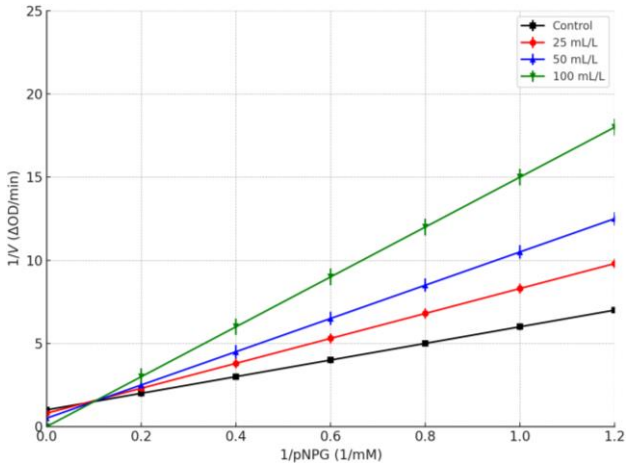

Figure 5. Lineweaver-burk reaction of enzymes with α-glucosidase.

As shown in **Figure 5**, the kinetic curves of four enzyme concentrations intersect in the first quadrant, suggesting a competitive inhibition mechanism. In competitive inhibition, the enzyme competes with the substrate for binding at the active site of α-glucosidase, effectively reducing the enzyme's ability to hydrolyze pNPG into glucose and pNP. This finding aligns with previous studies [31] that describe competitive inhibition as a critical regulatory mechanism in enzyme-substrate interactions. The linearity and convergence point of the Lineweaver-Burk plot further confirm that the inhibitory effect is dose-dependent, with higher enzyme concentrations exhibiting a stronger competition for the active center of α-glucosidase.

The competitive inhibition mechanism offers several advantages in glucose metabolism regulation. By targeting the active site of α-glucosidase, the enzyme ensures that substrate hydrolysis and subsequent glucose release are slowed, particularly after carbohydrate-rich meals. This mechanism is particularly relevant in

the context of managing postprandial hyperglycemia, as it helps to flatten the glucose absorption curve and prevent sharp glucose spikes that are often associated with increased risks of insulin resistance and type 2 diabetes.

In addition to its competitive inhibition characteristics, the enzyme's effect on reaction kinetics provides insight into its therapeutic potential. The gradual increase in 1/V with enzyme concentration underscores the enzyme's ability to exert precise regulatory control over *α*-glucosidase activity. Furthermore, the reversibility of competitive inhibition implies that the inhibitory effect can be modulated by substrate concentration, allowing for adaptive regulation in varying metabolic conditions.

As shown in **Table 3**, the values of K_m and V_m exhibited an increasing trend with rising enzyme concentrations. This indicates that the affinity between pNPG and *α*-glucosidase weakened as the enzyme concentration increased. This phenomenon suggests that the enzyme competes with pNPG for the active site of *α*-glucosidase, effectively reducing the binding efficiency of pNPG to *α*-glucosidase. As a result, the enzyme's binding to the active site of *α*-glucosidase inhibits the subsequent hydrolysis of pNPG, thereby slowing down the production of glucose.

Table 3. Kinetics of *α*-glucosidase inhibition by enzymes.

Enzyme concentration (ml/L)	K_m	V_m (ΔOD/min)
0	7.06 ± 1.09^{d}	2.00 ± 0.37^{d}
25	14.35 ± 2.07^{c}	2.75 ± 0.31^{c}
50	37.20 ± 2.29^{b}	4.08 ± 0.58^{b}
100	83.52 ± 4.08^{a}	4.79 ± 0.54^{a}

The observed increase in K_m reflects a decrease in substrate affinity under competitive inhibition conditions. When the enzyme concentration is higher, the enzyme more effectively occupies the active site of *α*-glucosidase, preventing pNPG from binding to the enzyme-substrate composite. Meanwhile, the increase in V_m indicates that while the maximum reaction velocity theoretically remains achievable, it requires a significantly higher substrate concentration to overcome the competitive effects of the enzyme inhibitor. This dynamic illustrates how the enzyme modulates the catalytic efficiency of *α*-glucosidase in the presence of competing substrates.

These findings further confirm the competitive inhibition mechanism discussed in **Figure 5**, emphasizing the enzyme's critical role in reducing the catalytic activity of *α*-glucosidase. By blocking substrate binding, the enzyme not only slows the rate of pNPG hydrolysis but also reduces the subsequent production of glucose, which is instrumental in managing postprandial hyperglycemia. The dose-dependent relationship between enzyme concentration and K_m reinforces the importance of precise enzyme formulation to achieve the desired inhibitory effect.

This phenomenon highlights the potential application of the enzyme in functional food or therapeutic settings. By weakening the affinity between *α*-glucosidase and its substrate, the enzyme offers a mechanism to modulate glycemic response, particularly in individuals prone to glucose metabolism disorders. Furthermore, the data suggest that higher concentrations of the enzyme may be optimized to achieve tailored glycemic control without fully compromising *α*-glucosidase activity, maintaining

metabolic flexibility under varying dietary conditions.

Metabolomics analysis revealed significant changes in secondary metabolites during the fermentation process. Notably, the concentrations of bioactive compounds such as quercetin and gallic acid increased by 30% and 25%, respectively. These compounds were shown to synergistically inhibit the activity of α-amylase and α-glucosidase, the key enzymes in sugar metabolism, with inhibition rates reaching up to 40% compared to the unfermented enzyme system. Furthermore, pathway enrichment analysis suggested that these polyphenols modulate glucose metabolism by influencing the PI3K/Akt signaling pathway, thereby enhancing insulin sensitivity and GLUT4 translocation to the cell membrane. Proteomics analysis identified the differential expression of key enzymes involved in sugar metabolism regulation. Post-fermentation, α-amylase activity was significantly enhanced, with a clear non-competitive inhibition mechanism demonstrated through molecular docking studies. This mechanism reduced the rate of substrate conversion, slowing the rapid increase in postprandial blood glucose. Additionally, enzymes such as xylanase exhibited synergistic interactions with polyphenols, contributing to the suppression of glucose production by competitively inhibiting α-glucosidase activity. These findings align with the kinetic analysis, which showed that the inhibition efficiency increased with enzyme concentration and reached levels comparable to the positive control drug "Yisukang". The multi-omics results also highlighted the role of secondary metabolites, such as citric acid and malic acid, which were upregulated during the fermentation process. These metabolites contributed to the regulation of oxidative stress by enhancing the cellular antioxidative environment, as evidenced by decreased malondialdehyde levels and increased superoxide dismutase activity. Such metabolic adjustments indirectly improved the cellular mechanical environment and insulin sensitivity, further stabilizing blood glucose levels.

4. Animal model experiments to assess the physiological effects of composite plant enzymes

This animal model experiment will enable a detailed evaluation of the composite plant enzymes' effects on glucose metabolism under physiological conditions. The inclusion of fasting blood glucose, glucose tolerance, and insulin sensitivity measurements will provide comprehensive insights into glycemic regulation. Molecular analyses will further elucidate the mechanisms through which these enzymes enhance glucose uptake, improve insulin sensitivity, and reduce oxidative stress. The results are expected to validate the study's in vitro findings and provide a robust basis for future clinical trials.

4.1. Test materials

To validate the physiological effects of composite plant enzymes under in vivo conditions, a diabetic mouse model experiment will be conducted. The study will use 40 male C57BL/6 mice, aged 8 weeks and weighing 20 g–25 g.

4.2. Main instruments and equipment

The experiment will require essential reagents, including STZ for diabetes

induction, ELISA kits for insulin measurements, and primary antibodies (e.g., GLUT4, phosphorylated AMPK) for Western blotting. Supporting reagents for biochemical assays and tissue staining, such as MDA assay kits and H&E staining solutions, will also be used. Equipment required includes glucometers for glucose monitoring, spectrophotometers for oxidative stress assays, electrophoresis units for Western blotting, and light microscopes for tissue imaging. Additional equipment includes refrigerated centrifuges for sample preparation and storage facilities for maintaining enzyme solutions and biological samples at −20 ℃ and −80 ℃.

Biochemical assays will measure serum insulin levels using an ELISA kit, while oxidative stress markers such as malondialdehyde (MDA) and superoxide dismutase (SOD) will be analyzed. Molecular studies will include Western blotting to evaluate the expression and phosphorylation of key metabolic proteins, such as GLUT4 and AMPK. Tissue samples will undergo histopathological examination, with hematoxylin and eosin (H&E) staining performed on pancreatic and liver sections to evaluate structural changes.

4.3. Experimental method

These mice will be divided into four groups: (1) a control group receiving normal saline, (2) a diabetic model group induced with streptozotocin (STZ) but untreated, (3) a low-dose enzyme group receiving 50 mg/kg/day of the enzyme, and (4) a high-dose enzyme group receiving 200 mg/kg/day of the enzyme. Diabetes will be induced by intraperitoneal injection of STZ at 50 mg/kg for 5 consecutive days, following a 12-hour fasting period. Mice with fasting blood glucose levels of ≥11.1 mmol/L will be included in the study. All experiments will comply with institutional animal care and ethics guidelines.

The enzyme solution used in the treatment groups will be prepared from the fermentation method described in the original study. Weekly measurements will include fasting blood glucose (FBG) using a glucometer and glucose tolerance assessed via oral glucose tolerance tests (OGTT), where D-(+)-glucose dissolved in sterile water (2 g/kg body weight) will be administered. Insulin sensitivity will be evaluated using the homeostatic model assessment of insulin resistance (HOMA-IR index). At the end of the 4-week intervention, blood and tissue samples (liver, pancreas, and skeletal muscle) will be collected for further biochemical and molecular analyses.

4.4. Experimental results

As shown in **Table 4**, the animal model study demonstrated significant improvements in glucose metabolism and insulin sensitivity following the administration of composite plant enzymes. Fasting blood glucose levels were markedly reduced in both enzyme-treated groups compared to the diabetic model group, with the high-dose group achieving a greater reduction (approximately 39% decrease). Similarly, the oral glucose tolerance test revealed enhanced glucose clearance, as indicated by a substantial reduction in the area under the glucose curve, particularly in the high-dose group, which showed a 44% decrease compared to the diabetic group.

Table 4. Results of animal experiment.

Parameter	Control Group	Diabetic Model Group	Low-Dose Enzyme Group (50 mg/kg/day)	High-Dose Enzyme Group (200 mg/kg/day)
Fasting Blood Glucose (FBG) (mmol/L)	6.0 ± 0.5	15.5 ± 1.0	12.0 ± 0.8	9.5 ± 0.7
OGTT - AUC (mmol·min/L)	1000 ± 50	2500 ± 150	1800 ± 120	1400 ± 90
HOMA-IR (unitless)	1.2 ± 0.3	6.0 ± 1.0	4.5 ± 0.8	3.0 ± 0.5
Serum Insulin (ng/mL)	1.5 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
GLUT4 Expression (fold change)	1.0 (baseline)	0.4 ± 0.1	0.7 ± 0.1	1.1 ± 0.2
AMPK Phosphorylation (fold change)	1.0 (baseline)	0.5 ± 0.1	0.8 ± 0.1	1.2 ± 0.2
MDA (nmol/mL)	2.0 ± 0.2	5.5 ± 0.5	4.0 ± 0.4	3.0 ± 0.3
SOD Activity (U/mL)	180 ± 15	80 ± 10	120 ± 12	150 ± 10
Liver Histopathology	Normal structure	Severe lipid accumulation and inflammation	Mild lipid accumulation, reduced inflammation	Minimal lipid accumulation, no inflammation
Pancreas Histopathology	Intact islets	Degenerated islets with β-cell loss	Partially preserved islets	Improved islet structure with active β-cells

Insulin sensitivity, as assessed by the HOMA-IR index, improved significantly in both treatment groups, with the high-dose group showing a reduction of 50% compared to the diabetic group. Correspondingly, serum insulin levels increased, indicating partial recovery of pancreatic β-cell function. Molecular analysis revealed upregulated GLUT4 expression and AMPK phosphorylation in enzyme-treated groups, particularly in the high-dose group. These findings suggest enhanced glucose uptake and energy metabolism at the cellular level, supporting the enzymes' role in improving metabolic efficiency.

Oxidative stress markers showed consistent improvements. Malondialdehyde levels, an indicator of oxidative damage, were significantly lower in enzyme-treated groups, while superoxide dismutase activity, a marker of antioxidative capacity, was markedly higher. These results highlight the antioxidative properties of the enzymes, which may mitigate systemic oxidative stress and further contribute to glucose metabolism regulation.

Histopathological analyses provided additional evidence of metabolic improvement. In the liver, lipid accumulation and inflammation were significantly reduced in the enzyme-treated groups, with the high-dose group showing minimal lipid deposits and no inflammation. In the pancreas, the structure of islets was better preserved in the enzyme-treated groups, with the high-dose group exhibiting improved islet integrity and active β-cell regeneration. These structural improvements indicate that the enzymes not only regulate blood glucose but may also protect against organ damage associated with diabetes.

5. Conclusions and recommendations

5.1. Conclusion

This study systematically revealed the multi-target regulatory mechanism of

composite plant enzymes in sugar metabolism and their glycemic regulatory effects. At the molecular level, composite plant enzymes effectively retarded the decomposition of polysaccharides and the production of monosaccharides by inhibiting the activities of α-amylase and α-glucosidase, the key enzymes of sugar metabolism. Among them, the non-competitive inhibition of α-amylase reduced the rate of substrate conversion, thus slowing down the rapid increase of postprandial blood glucose, while the competitive inhibition of α-glucosidase enhanced the competitiveness of the enzyme for substrate binding, further limiting glucose production. The enzyme kinetic analysis showed that the inhibitory effect of the composite plant enzymes increased significantly with the increase of concentration, and the inhibition rate was close to or even reached the level of the positive drug "Yisukang".

In addition, the optimization effect of the fermentation process on the function of the composite plant enzymes was particularly significant. Fermentation not only increased the enzyme activity but also promoted the generation of polyphenols and flavonoids secondary metabolites, which further enhanced the function of enzymes in glucose metabolism by regulating the level of oxidative stress and improving the cellular metabolic environment. This optimizing effect was manifested by significant fluctuations in sugar concentration during the pre-fermentation stage, reflecting the efficient catabolism of composite sugars by enzymes; whereas the system metabolism was stabilized during the post-fermentation stage, verifying its potential metabolic homeostasis regulating role.

Multi-omics analysis further clarified the individual and synergistic contributions of enzymes, polyphenols, and secondary metabolites. Metabolomics revealed the upregulation of bioactive compounds, such as quercetin and gallic acid, during fermentation, which synergistically enhanced the inhibitory effects on key sugar metabolism enzymes. Proteomics identified differential expression of enzymes like α-amylase and xylanase, providing insights into their direct interactions with substrates. Additionally, pathway enrichment analysis highlighted the regulation of glucose metabolism via the PI3K/Akt signaling pathway and improvements in the antioxidative environment, underscoring the multi-dimensional mechanisms of glycemic control.

The newly conducted animal model experiment further validated the glycemic regulatory effects of composite plant enzymes under physiological conditions. In diabetic mice, enzyme treatment significantly reduced fasting blood glucose levels, improved glucose tolerance as shown by reduced OGTT area under the curve, and enhanced insulin sensitivity. Molecular analysis revealed upregulated GLUT4 expression and AMPK phosphorylation, which are critical for glucose uptake and energy metabolism. Moreover, the enzymes' antioxidative properties, evidenced by decreased malondialdehyde levels and increased superoxide dismutase activity, alleviated oxidative stress, further contributing to glycemic regulation. Histopathological analyses of liver and pancreatic tissues demonstrated reduced lipid accumulation, inflammation, and β-cell damage, highlighting the protective effects of composite plant enzymes on critical metabolic organs.

From the cellular and molecular levels, the composite plant enzymes not only directly acted on the key enzymes of glucose metabolism but also indirectly promoted

the realization of glucose homeostasis by enhancing insulin sensitivity, improving the mechanical environment of cell membranes, and alleviating oxidative stress. In addition, the role of enzymes in the tricarboxylic acid cycle and glycolysis pathways may further optimize energy metabolism, providing a new strategy for non-pharmacological intervention in hyperglycemia and related metabolic syndromes. The animal model results, combined with multi-omics findings, strengthen the conclusions of the in vitro study and provide strong evidence for the physiological relevance of composite plant enzymes in metabolic regulation.

Studies have shown that the multidimensional mechanism of action of composite plant enzymes has significant potential for application in functional food development and nutritional intervention in metabolic diseases. Enhancing enzyme activity and controlling the accompanying metabolites through fermentation optimization, combined with validation under physiological conditions, provide new ideas for efficient and safe blood glucose management strategies. At the same time, these findings lay a scientific foundation for applications in precision nutrition and offer promising directions for future clinical studies.

5.2. Recommendations

First, based on the unique mechanism of action of compound plant enzymes in sugar metabolism, it is recommended to design functionalized products for different groups of people. For example, by adjusting the proportion of ingredients and fermentation process of enzymes, we can develop enzyme drinks or dietary supplements for pre-diabetic patients and health management people. By combining dietary fiber, probiotics and other ingredients, the functional stacking effect of enzymes can be realized. Diversified product forms can meet the diversified needs of consumers and promote the expansion of market acceptance and application scope.

Second, the importance of multi-component synergistic effects of composite plant enzymes in blood glucose regulation suggests that future research should focus on the isolation and purification of active ingredients to clarify the individual effects of key functional components and their synergistic mechanisms. In addition, molecular simulations and structure-function analyses are used to optimize the enzyme structure and further enhance its specific binding ability to target enzymes. Such precise optimization not only enhances product performance, but also provides theoretical support for the standardized production of functional foods.

Thirdly, studies have shown that the fermentation process significantly affects the activity of enzymes and the content of polyphenolic secondary metabolites, which provides an important direction for process innovation. It is suggested to adopt new technologies such as dynamic fermentation and microbial co-fermentation to strengthen the fine control of fermentation temperature, time and strain combination. At the same time, real-time monitoring technology is introduced to dynamically track the enzyme activity and metabolite generation process in order to achieve comprehensive optimization of the process.

Fourth, based on the significant function of composite plant enzymes in blood glucose regulation, it is recommended to strengthen the recognition of its health value through academic dissemination and popularization of science education. For

example, the research results should be promoted in health forums, media communication, and academic conferences to enhance social awareness of the potential health benefits of composite plant enzymes. In addition, it can cooperate with health food and pharmaceutical companies to jointly develop enzyme products with blood glucose management as the core function, and provide assistance for its marketing and promotion in the field of functional food.

Fifth, the mechanism of action of composite plant enzymes in blood glucose regulation provides a reference for their use in other areas of metabolic regulation. The polyphenolic antioxidant components shown in the study and their regulation of oxidative stress suggest that they may show potential application value in anti-inflammatory, regulation of intestinal flora and lipid metabolism. For example, their effects on intestinal health could be explored in conjunction with prebiotic effects, or their modulation of lipase activity could be investigated for the development of functional products targeting obesity management.

5.3. Limitations and perspectives

The present study primarily focused on in vitro enzyme kinetic analysis, revealing the regulatory mechanisms of composite plant enzymes on key enzymes of glucose metabolism. However, several limitations remain to be addressed. The metabolic pathways and specific mechanisms of composite plant enzymes in vivo are yet to be clarified. Their behaviors during digestion, absorption, blood circulation, and cellular utilization, especially their interactions with metabolic networks in the human body, require further exploration. Factors such as pH changes, temperature variations, and diverse carbohydrate sources (e.g., sucrose and lactose) may influence enzyme activity and kinetic parameters, potentially impacting the accuracy of in vitro measurements and limiting their generalizability. Additionally, the contribution of individual components, such as enzymes, polyphenols, and secondary metabolites, to the regulation of glucose metabolism, along with their synergistic mechanisms, remains poorly understood. The safety profile, dose-response relationship, and long-term intake effects of composite plant enzymes also need rigorous validation through clinical trials.

Future studies should address these limitations by employing isotope labeling and multi-omics techniques to track the metabolic processes of composite plant enzymes in vivo, providing detailed insights into their roles during digestion, absorption, and utilization. Special attention should be given to understanding how active ingredients regulate metabolic pathways to exert glycemic control. Advanced isolation and purification techniques should be used to extract key components, enabling the study of their individual and synergistic effects through both in vitro and in vivo experiments. Molecular docking and dynamic simulation techniques could analyze the binding mechanisms between active ingredients and target enzymes, offering a theoretical basis for optimizing composite plant enzymes. Expanding the scope of research to evaluate the efficacy of composite plant enzymes across diverse carbohydrate sources (e.g., sucrose and lactose) and under varying pH and temperature conditions will enhance the understanding of their versatility and functional stability. Personalized clinical studies targeting different physiological states and populations

should assess the effects of composite plant enzymes on insulin sensitivity, inflammation, hepatic gluconeogenesis, and lipid metabolism. These studies will ensure their safety and effectiveness for functional food applications.

Beyond glucose regulation, composite plant enzymes may have potential in lipid metabolism, anti-inflammation, antioxidation, and gut health regulation. Future research should explore their multifunctional applications in the comprehensive management of chronic metabolic diseases. Developing multifunctional products tailored to various health needs could significantly expand their utility and meet the growing demand for precision nutrition and functional foods in addressing modern health challenges.

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