MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Construction of lactic acid bacteria fermentation system and analysis of fermentation products of Ruoqiang jujube**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
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APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
___________Olena MOKROUSOVA
«___»_______________2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Fan Jiahao

1. Thesis topic **Construction of lactic acid bacteria fermentation system and analysis of fermentation products of Ruoqiang jujube**
Scientific supervisor Dr. Sc., Prof. Olga Andreyeva

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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

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WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student ____________ Fan JIAHAO

Scientific supervisor ____________ Olga ANDREYEVA

SUMMARY

Fan JIAHAO**. Construction of lactic acid bacteria fermentation system and analysis of fermentation products of Ruoqiang jujube. – Manuscript.** Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

The purpose of this experiment is to establish a lactic acid bacteria fermentation system for *Ruoqiang jujube*, and to investigate the product performance of its new enzyme health food. *Ruoqiang jujube* is nutrient-rich and has strong storage stability, making it widely applicable in the food and pharmaceutical industries. Additionally, lactic acid bacteria are widely used in fermented food processing, capable of improving food quality and regulating the composition of human intestinal microorganisms. The bacteria themselves also exhibit strong functional effects. In this experiment, *Ruoqiang jujube* was selected as the raw material, subjected to pretreatment and sterilization, and then fermented with a mixed lactic acid bacteria culture. Dynamic monitoring was conducted throughout the fermentation process. The results showed that the pH value of the fermentation broth gradually decreased and eventually stabilized at less than 4.0, meeting the indicators for edible plant-derived enzyme products. During the fermentation process, the types of free amino acids increased, and the utilization of reducing sugars and total sugars gradually increased, but the content of vitamin C remained relatively stable. After scoring according to a sensory scoring scale pre-established by a professional panel, the product exhibited excellent sensory characteristics. This study established a fermentation system that enables synergistic interactions between different lactic acid bacteria species and integrates the original bioactive substances of *Ruoqiang jujube* with lactic acid bacteria metabolites, thereby enhancing the bioactivity and nutritional value of the product. This system aligns with the development trend of the modern food industry and provides a corresponding technical foundation for the industrialization of Odor derived enzyme products. It also holds broad prospects for industrialization in functional food products.

Key words: Ruoqiang jujube; lactic acid bacteria fermentation; fermentation system; product analysis; health food

TABLE OF CONTENTS

INTRODUCTION

In recent years, the development of functional fermented foods has gained significant attention due to their potential health benefits. *Ruoqiang jujube*, a nutrient-rich fruit abundant in polysaccharides, vitamins, and bioactive compounds, offers great potential for food and pharmaceutical applications. Lactic acid bacteria fermentation, a traditional biotechnology, has been widely used to enhance food quality, improve nutritional value, and modulate gut microbiota. However, the utilization of *Ruoqiang jujube* in LAB-based fermented products, particularly enzyme-rich health foods (e.g., jujube), remains underexplored.

The relevance of this study addresses this gap by integrating traditional jujube resources with modern fermentation technology, aiming to develop a novel functional food with enhanced bioactivity.

The purpose of the primary objective of this research is to establish a sustainable fermentation system for *Ruoqiang jujube* and analyze the physicochemical and nutritional properties of the fermented products. the study seeks to characterize the dynamic changes in key indices**.**

The objectives of the primary objective of this research are to establish a sustainable LAB fermentation system of *Ruoqiang jujube* and analyze the physicochemical and nutritional properties of the fermented products. Specifically, the study seeks to optimize the fermentation conditions and characterize the dynamic changes in key indices.

The object of the study: *Ruoqiang jujube*.

The subject of the study: Construction of Fermentation System.

Research methods: pH using a pH meter (GB/T 10468). Reducing sugar and total sugar content via the DNS colorimetric method (GB 5009.7). Free amino acids by HPLC (GB 5009.124). Vitamin C content via 2,6-dichloroindophenol titration

(GB 5009.86). Sensory evaluation by a trained panel (n=16) using a standardized scoring system (QB/T 5323-2018).

The scientific novelty: The integration of jujube’s natural bioactive compounds (e.g., vitamin C, polysaccharides) with LAB metabolites (e.g., lactic acid, bacteriocins) to create a functional food with dual nutritional and probiotic benefits.

The practical: The successful establishment of the fermentation system provides a technical foundation for industrial production of *Ruoqiang jujube* enzyme products. Key findings, such as the stable pH (<4.0), increased amino acid diversity, and efficient sugar utilization, demonstrate the feasibility of this approach for developing high-quality fermented foods. Additionally, the study highlights the potential of LAB fermentation to enhance the bioavailability of jujube’s nutrients, offering new opportunities for functional food development and agricultural value-added processing. Further research on scaling up the fermentation process and exploring long-term product stability will support its commercialization, contributing to the growth of the green health industry.

Chapter I

LITERATURE REVIEW

1.1 Introduction to gray jujube

Ziziphus j*ujuba* Mill., belonging to the *Ziziphus* genus of the Rhamnaceae family, is a unique economic fruit tree in China with a cultivation history dating back over 700 years, forming an extremely rich germplasm resource system[1]. As a dominant variety in China's dried fruit industry, jujube trees are widely cultivated nationwide due to their strong adaptability to climate and soil, as well as remarkable stress resistance. In arid and semi-arid regions and saline-alkali lands, jujube trees demonstrate excellent ecological restoration capabilities, while their fruit production injects strong momentum into regional economic development. Take Xinjiang as an example: in recent years, the jujube industry has grown rapidly, with Ruoqiang, Aksu, and the Aral reclamation area around the Tarim Basin becoming major jujube production regions in China[Error! Reference source not found.]. The jujube industry has become a local characteristic pillar industry, playing a key role in increasing farmers' income and promoting rural revitalization[Error! Reference source not found.].

Originating from Xinzheng, Zhongmu, Xihua counties, and the suburban areas of Zhengzhou City in Henan Province, gray jujube has formed a profound planting foundation through centuries of cultivation as a traditional main variety. This variety is renowned for its exceptional stress resistance among jujube species, not only featuring superior drought and sandstorm resistance but also thriving in harsh environments such as severe cold, high salinity-alkalinity, and poor soil, demonstrating strong adaptability to complex ecological conditions. In terms of production performance, gray jujube not only has stable yields but also outstanding quality in dried fruits, with plump fruits rich in sugar and a significantly higher drying rate than most similar varieties, thus becoming a high-quality jujube resource combining ecological and economic values[Error! Reference source not found.].

1.2 Functions of gray jujube

As one of the traditional "Five Fruits" in China, jujube holds an important position in the history of Chinese medicine development. *Shennong's Herbal Classic* lists it as a top-grade herb, recording that it "tastes sweet and mild, mainly dispels pathogenic factors in the heart and abdomen, stabilizes the middle energizer and nourishes the spleen, supports the twelve meridians, regulates stomach qi, unblocks the nine orifices, replenishes qi and body fluids deficiency, and long-term consumption lightens the body and prolongs life"[Error! Reference source not found.]. Over history, jujube has acquired multiple names such as red jujube, dried jujube, and fine jujube, serving both medicinal and edible purposes. As early as in *The Book of Songs*, there is a record of "harvesting jujubes in August and reaping rice in October," vividly depicting the agricultural scene of ancient ancestors harvesting jujubes and reflecting its thousands of years of cultivation and consumption history in China.

1.3 Development and utilization status of gray jujube

Numerous studies show that jujube fruits are not only rich in active components such as polysaccharides, cyclic nucleotides, triterpenoids, and vitamins but also, due to their sweet flavor and "food-medicine homology" attribute, are listed as key herbs for replenishing qi and nourishing blood in classics like Shennong's Herbal Classic and Compendium of Materia Medica. They are recognized as high-quality tonic foods by both traditional Chinese medicine theory and modern nutrition science.

Jujube is also an important symbol of traditional Chinese dietary tonic culture. The folk wisdom "eating three jujubes daily keeps aging at bay" embodies ancient ancestors' empirical understanding of jujube's functional value. This understanding has been authenticated by modern regulatory systems: according to the *List of Items That Are Both Food and Medicine* (Wei Fa Jian Fa [2002] No. 51), jujube is designated as a food-medicine homologous ingredient. Its high vitamin complex content (especially vitamin C up to 243 mg/100g) makes it an optimal carrier for plant-based nutritional supplements[Error! Reference source not found.]. These effects are closely related to jujube's chemical composition, including basic nutrients such as carbohydrates, proteins, and amino acids, as well as bioactive substances like flavonoids, saponins, triterpenoids, and sterols[Error! Reference source not found.].

1.4 Overview of probiotics

1.4.1 Introduction to probiotics

In modern animal health science, probiotics are clearly defined as active microbial communities that can successfully colonize the host intestine at appropriate concentrations and bring positive physiological effects to the host. These microorganisms beneficial to host health mainly include bacteria and fungi, with well-known representative species such as *Bifidobacterium*, *Lactobacillus acidophilus*, and yeasts.

Probiotics colonized in the intestine act like a precisely coordinated health guard team, safeguarding host health through multiple mechanisms: they not only efficiently promote nutrient absorption to help the body intake nutrients but also secrete antibacterial substances and use competitive inhibition to deprive pathogens of living space and nutrients[8], effectively resisting harmful bacteria. Additionally, probiotics play a key role in regulating host immune function, building a solid immune defense for the body.

Notably, most probiotics in the host intestine rely on exogenous intake for supplementation. However, it is challenging for exogenous probiotics to truly exert their effects – they must possess excellent tolerance to withstand the "tests" of gastric acid, bile acids, and gastrointestinal digestive enzymes to successfully reach the colonization areas in the distal small intestine and large intestine. Furthermore, when screening probiotic strains in vitro, their adhesion ability to intestinal epithelial cells is an important evaluation index. Only probiotics with strong adhesiveness can stably colonize and continuously reproduce in the intestine, thereby fully playing their positive roles in resisting pathogens and improving host health [**Error! Reference source not found.**].

1.4.2 Introduction to fermentation strains

Leuconostoc mesenteroides subsp. *cremoris*

Leuconostoc mesenteroides subsp. *cremoris* imparts unique flavors to fermented products through its distinct metabolic processes. When fermenting sucrose, it characteristically produces dextran mucilage alongside fermentation. Its fermentation properties vary with different carbohydrates: glucose fermentation yields lactic acid, ethanol, and carbon dioxide; ribose fermentation produces lactic acid and acetic acid; while fructose fermentation generates lactic acid, acetic acid, carbon dioxide, and mannitol. Studies show that both *Leuconostoc mesenteroides* and its fermentation products exhibit biological effects, significantly enhancing the activity of glutathione peroxidase (GSH—px) and superoxide dismutase (SOD) in animal bodies, effectively reducing serum malondialdehyde (MDA) levels, and maintaining these beneficial effects for a long time to greatly improve the body's antioxidant capacity[10]. Biochemically, *Leuconostoc mesenteroides* is a Gram-positive bacterium that stimulates the growth of normal intestinal flora, regulates gut microecological balance, and antagonizes common pathogens such as *Shigella*, *Salmonella*, and *Staphylococcus aureus*, playing a crucial role in maintaining intestinal health[Error! Reference source not found.].

Lactobacillus helveticus

Lactobacillus helveticus demonstrates significant selectivity in carbohydrate metabolism. While it efficiently metabolizes monosaccharides and disaccharides like fructose and galactose, it cannot ferment 16 types of sugars such as arabinose and sucrose, with its main metabolic end product being DL-lactic acid. Fermented milk by *L. helveticus* is rich in bioactive peptides, which enhance metabolism, regulate immunity, and exhibit antibacterial and lipid-lowering effects after enzymatic hydrolysis and synthesis. Fermented dairy products, abundant in peptide precursors, have the potential to efficiently produce active peptides. When hydrolyzing milk proteins, it can directionally generate angiotensin-converting enzyme inhibitory peptides (ACEIP). These active peptides regulate blood pressure by inhibiting angiotensin-converting enzyme activity, making this strain the most frequently reported lactic acid bacterium in current antihypertensive research. It is a highly sought-after research strain with great development value in functional foods and medicine[Error! Reference source not found.].

Lactobacillus rhamnosus GG (LGG)

As an important resident flora in the human intestine, this strain exhibits intestinal mucosal adhesion and colonization properties due to its unique surface protein and adhesin structures. Studies show it efficiently reduces cholesterol levels. In microecological regulation, it inhibits harmful bacteria growth by secreting bacteriocins, organic acids, and other metabolites, reshaping intestinal flora homeostasis, and has preventive and therapeutic effects on acute and chronic diarrhea[13].

Lactobacillus plantarum

Lactobacillus plantarum is a homofermentative lactic acid bacterium with an optimal pH of approximately 6.5, suitable for degrading nutrient-rich substances in the early fermentation stage. During reproduction, it produces plantaricin, a biological preservative that effectively inhibits acid odor. This bacterium has multiple health functions: regulating immunity, inhibiting pathogenic bacteria, reducing serum cholesterol to prevent cardiovascular diseases, maintaining intestinal flora balance, promoting nutrient absorption, alleviating lactose intolerance, and inhibiting tumor cell formation[14].

Lactobacillus reuteri

Lactobacillus reuteri is a probiotic with broad host adaptability, widely present in the intestinal microecosystems of vertebrates and mammals. As an important intestinal symbiont, it possesses unique physiological activities in regulating intestinal function and improving allergic constitution, effectively preventing the onset of allergic diseases[Error! Reference source not found.]. Research on its metabolic characteristics has revealed a unique glycerol metabolic pathway, enabling it to synthesize reuterin, a broad-spectrum antibacterial metabolite composed of monomers, hydrates, and cyclic dimers of 3-hydroxypropionaldehyde (3-HPA). Reuterin significantly inhibits various Gram-positive and Gram-negative bacteria as well as fungi[Error! Reference source not found.].

1.4.3 Gray jujube enzymes

Gray jujube puree, with its high sugar content and rich nutrients, provides an ideal substrate for microbial growth and reproduction, making it a high-quality raw material for microbial fermentation to produce gray jujube enzymes. In a modern sense, enzymes refer to fermented products rich in bioactive components formed by microbial fermentation of various raw materials, differing from the traditional concept of pure enzymatic substances.

Fermentation endows enzyme products with unique functional properties. While maximizing the retention of beneficial components from raw materials, it generates multiple new bioactive substances through microbial metabolism. Enzyme products exhibit effects such as hangover relief, liver protection, laxative function, and antioxidation. Rich in various nutrients including enzymes, vitamins, minerals, and secondary metabolites, they play important roles in antibacterial and anti-inflammatory effects, anti-aging, enhancing immunity, and promoting tissue repair[Error! Reference source not found.].

From a compositional analysis, proteases in microbial enzymes efficiently catalyze protein hydrolysis, improving the body's digestion and absorption of proteins; lipases specifically hydrolyze the ester bonds between fatty acids and glycerol in oils, promoting fat breakdown, which has high potential application value in weight management; superoxide dismutase (SOD), a key antioxidant enzyme, effectively scavenges reactive oxygen species (ROS) such as superoxide anions ($O_2\cdot^-$), hydroxyl radicals (OH·), and hydrogen peroxide, playing a core role in maintaining cellular redox balance and resisting oxidative stress damage[Error! Reference source not found.].

1.4.4 Research status of lactic acid bacteria enzymes

In China, lactic acid bacteria research spans multiple dimensions. For fermented foods, microbial diversity studies have been conducted on traditional fermented vegetables such as Sichuan pickles, Northwest Jianghu, and Southern sour bamboo shoots, where *Leuconostoc*, *Lactococcus*, *Weissella*, *Pediococcus*, and *Lactobacillus* are dominant flora[Error! Reference source not found.], providing guidance for enzyme products. In the food industry, probiotics are widely used in various products. In dairy products such as yogurt, fermented milk drinks, and milk powder, common probiotics like lactic acid bacteria and bifidobacteria not only improve product flavor but also regulate intestinal flora, promote digestion and absorption, relieve lactose intolerance, and aid the body's intake of nutrients such as calcium and protein. In fermented meat products, composite starters with lactic acid bacteria as the core, combined with yeasts and micrococci, can decompose proteins and fats, enhance flavor and nutritional value, inhibit harmful bacteria growth, reduce biogenic amine production, enhance product stability, and endow fermented sausages, smoked meats, and other products with health functions[Error! Reference source not found.]. In functional foods, probiotic-fermented fruit and vegetable juices have grown rapidly, enhancing nutritional components, adding antioxidant, antibacterial, hangover-relieving, and liver-protecting effects, improving taste and flavor, and extending shelf life. Additionally, the addition of probiotics to health products, baked goods, candies, etc., has driven the food industry toward nutrition, health, and deliciousness, continuously meeting consumers' pursuit of healthy diets.

1.5 Research and Significance

After years of development, enzyme foods have gained increasing recognition, but research and products combining high-quality *Ruoqiang jujube* with advanced lactic acid bacteria fermentation technology remain scarce. Although the market size of enzyme beverages and other products has grown rapidly in recent years, with an increasing variety of natural enzyme drinks such as fruit enzymes, vegetable enzymes, and grain enzymes, as well as functional enzyme drinks containing Chinese herbal ingredients or collagen, enzyme foods developed from *Ruoqiang jujube* are rarely seen. On one hand, there is a lack of research on its fermentation process, and in-depth exploration is needed on how to precisely regulate fermentation parameters to achieve optimal synergistic effects between the natural active components of gray jujube and lactic acid bacteria metabolites[Error! Reference source not found.]; on the other hand, there are almost no mature *Ruoqiang jujube* enzyme products on the market, making it difficult for consumers to find health foods that perfectly integrate the unique advantages of *Ruoqiang juju be* with the health functions of enzymes.

Based on modern food biotechnology, using traditional tonic *Ruoqiang jujube* as the core raw material and matching with efficient lactic acid bacteria directional fermentation technology, a new type of health food creation technology with both nutritional enhancement and functional characteristics will be constructed in the future. This technology can precisely regulate key parameters such as fermentation temperature, pH, and strain ratio, significantly improving the bioactivity and nutritional value of products. Following the concept of green manufacturing throughout, it avoids the use of chemical synthetic additives, conforming to the development requirements of the modern food industry for "natural, healthy, and safe" products. With the continuous improvement of consumers' health awareness, the gray jujube enzyme series products derived from this technology show broad industrialization prospects in functional foods, nutritional supplements, and other fields, and are expected to become a new growth point in the green health industry.

Summary of the chapter I

1. *Ziziphus jujuba* Mill., a unique economic fruit tree in China with over 700 years of cultivation history, is highly adaptable to diverse climates and soils, thriving in arid, saline-alkali environments. In Xinjiang, regions like Ruoqiang and Aksu have become major jujube production hubs, driving rural economic growth. Gray jujube, originating from Henan, stands out for its drought/sandstorm resistance, high sugar content, and superior drying rate, balancing ecological and economic values.

2. Rich in polysaccharides, vitamins (e.g., vitamin C: 243 mg/100g), and bioactive compounds, gray jujube is certified as both food and medicine. Its "food-medicine homology" status and nutritional profile make it a prime candidate for functional supplements and tonic foods.

3. Probiotics are live microorganisms colonizing the gut to confer health benefits, including nutrient absorption, antibacterial secretion, and immune

regulation. Key challenges for exogenous probiotics include resistance to gastric acids and strong adhesion to intestinal epithelia for effective colonization.

4. Despite rising enzyme food demand, *Ruoqiang jujube*-based products remain underexplored. This study aims to develop a green fermentation technology integrating jujube's nutrients with LAB metabolites, addressing technical gaps and offering industrial prospects in functional foods

Chapter II

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental materials and instruments

Materials: Air-dried *Ruoqiang jujube*.

Reagents: Glucose, tryptone, lactose, yeast extract powder, distilled water, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus helveticus*, *Leuconostoc mesenteroides.*

Instruments: Homogenizer, autoclave, incubator, balance, ladle, 1L dispensing bottles, laminar flow cabinet.

2.2 Experimental procedures

2.2.1 Culture media and formulations for viable bacteria detection on gray jujube surface

Five culture media (MRS, M17, LB, SDY, YPD) were prepared to provide a foundation for subsequent plate colony culture and microscopic examination[Error! Reference source not found.]. Detailed formulations (g/L) of each medium for viable bacteria detection are shown in Table 2.1.

Prepare 25 sets of sterile petri dishes (9 cm diameter) and measure 1 L of experimental water, sterilized by moist heat sterilization (121 °C, 15–20 min). Accurately weigh components using an electronic balance according to the formulations in Table 2.2, and prepare 100 mL of each of the five media. After preparation, while the media are still warm, dispense 20 mL of each medium into sterilized petri dishes in a laminar flow cabinet. Allow the media to cool and solidify naturally to obtain solid plate media for viable bacteria detection, ensuring accuracy and consistency of microbial culture conditions.

Table 2.1 **Culture Medium Preparation Formulations**

MRS-Lactobacillus	M17-Streptococcus	LB-Bacteria	SDY-Fungi	YPD-Yeast
MRS 26.2	M17 21.2	Yeast Ext 5	—	Yeast Ext 10
PY 15	PY 15	Peptone 10	Peptone 10	Peptone 20
Glucose 10	Glucose 10	NaCl 10	Glucose 40	Glucose 20
Agar 20	Agar 20	Agar 20	Agar 20	Agar 20

2.2.2 Identification of indigenous bacteria on the surface of *Ruoqiang jujube*

Table 2.2 **Sample Grouping and Preparation for Identification of Indigenous Bacteria on Gray Jujube Surface**

Group	Treatment	Sample Volume
A (Fresh Fruit Pulp)	Washed with sterilized water after pretreatment, homogenized, filtered through double-layer gauze	Spread plating, 100 µL
B (Fresh Fruit Skin)	Washed with sterilized water after pretreatment	Rolled on plate
C (70 °C Sterilized Pulp)	Take 50 mL of Group A, heat in water bath at 70 °C for 20 min, filter	Spread plating, 100 µL
D (80 °C Sterilized Pulp)	Take 50 mL of Group A, heat in water bath at 80 °C for 20 min, filter	Spread plating, 100 µL

2.2.3 Plate numbering and viable bacteria detection by spread plating corresponding to above groups

Table 2.3 **Plate Numbers for Identification of Indigenous Bacteria on Fresh *Ruoqiang jujube* Surface**

MRSA	M17A	LBA	SDYA	YPDA	MRS
MRSB	M17B	LBB	SDYB	YPDB	M17
MRSC	M17C	LBC	SDYC	YPDC	LB
MRSD	M17D	LBD	SDYD	YPDD	YPD

2.2.4 Microbial detection results of fresh *Ruoqiang jujube*

Table 2.4 **Microbial Detection Results of *Ruoqiang jujube* (None of the blank medium controls showed microbial growth)**

Fruit Surface		Fresh Fruit Pulp		70 °C Sterilized Pulp		80 °C Sterilized Pulp	
MRSA		MRSB		MRSC		MRSD	
M17A		M17B		M17C		M17D	
LBA		LBB		LBC		LBD	
SDYA		SDYB		SDYC		SDYD	
YPDA		YPDB		YPDC		YPDD	

2.3 Construction of *Ruoqiang jujube* enzyme fermentation system

2.3.1 Establishment of basic fermentation test conditions based on above results

1. Material Selection: Select 400 g of medium-sized air-dried *Ruoqiang jujube*, add 1,200 mL of water, and boil in water at a material-to-water ratio of 1:3.

Figure 2.1 Boiled *Ruoqiang jujube*

2. Press-crack: After the gray jujubes become soft, press-crack them to expose the pulp and accelerate rehydration.

Figure 2.2 Press-cracked *Ruoqiang jujube*

3. Rehydration: Boil in water for 15 min, then weigh. The partially rehydrated jujubes weigh 500 g. Manually cut the jujubes, add water to reach 2 kg total weight, and continue boiling for 15 min. At this point, 300 g of water evaporates, so replenish 300 g of water[Error! Reference source not found.].

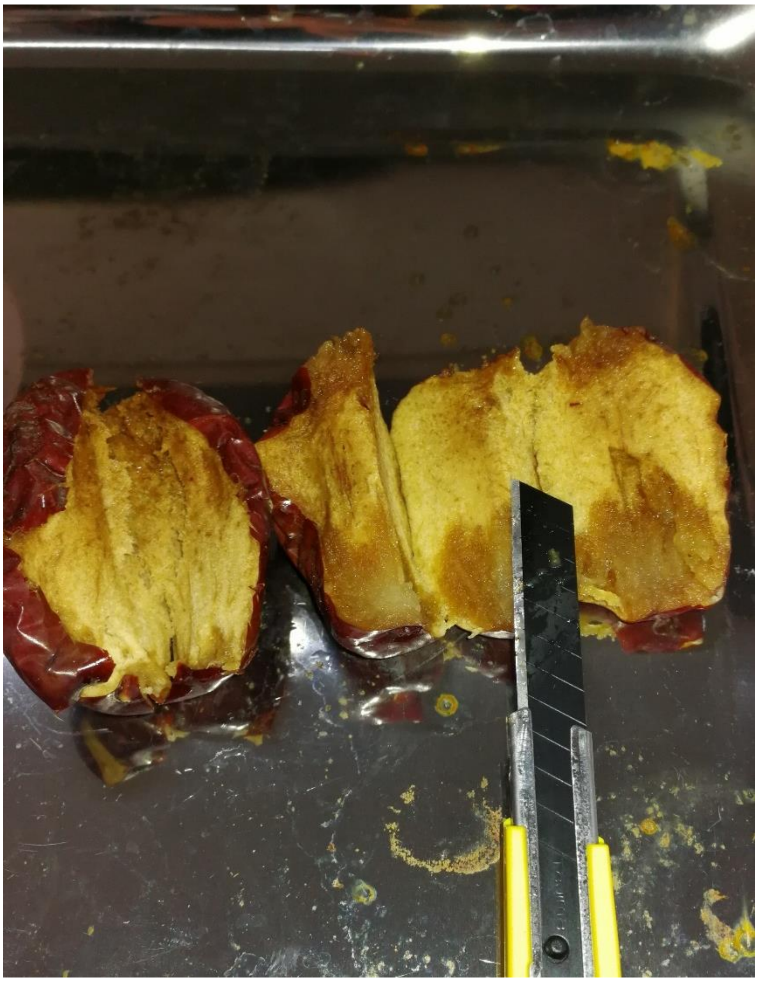

Figure 2.3 Sliced Pulp of Gray Jujubes

4. Homogenization: Homogenize at the lowest speed for 1 min, pour out the liquid, and retain the jujube cores (weight: 60 g per 400 g dried jujubes). Add water to reach 3 kg total weight, then homogenize from low to high speed for 2 min to obtain a thick jujube puree. Add 600 g of water to reach a total weight of 3,600 g (approximately 3.6 L in volume).

Figure 2.4 High-speed Homogenization of Jujube Pulp

5. Dispensing: Evenly dispense the filtered juice into four 1-L blue-cap bottles (approximately 400 mL per bottle). After dispensing, secure a breathable membrane over the bottle mouths with a rubber band—do not tighten the lids.

6. Fermentation system preparation and sterilization: Add fermentation agents to the filtered juice: 10% glucose (70 g), 2% tryptone (14 g), and 2% yeast extract powder (14 g). After preparing the fermentation system, ensure the bottle lids are not tightened. Place the bottles in an autoclave, set the temperature to 105 °C, sterilize for 15 min, and then remove them.

Table 2.5 **Construction of *Ruoqiang jujube* Pulp Fermentation System (400 mL)**

	A	B	C	D
Original Pulp Concentration	25%	50%	75%	100%
Original Pulp Volume	100mL	200mL	300mL	400mL
Glucose	10g	10g	10g	10g
Peptone	1g	1g	1g	1g

Note: Prepare fermentation components as shown in the table, sterilize at 110 °C for 15 minutes; meanwhile, take 15 mL of original pulp per tube, prepare 10 tubes, and store them frozen for later inspection. Both the surface and pulp of *Ruoqiang jujube* contain heterotrophic bacteria but no yeasts; prepare fermentation broths with pulp concentrations set at gradient levels of 25%, 50%, 75%, and 100%; add 2% glucose and 0.1% tryptone for initial bacterial enrichment; sterilize the pulp at 110 °C for 15 minutes.

2.3.2 Fermentation method: probiotic inoculation

Based on preliminary experimental results, mixed-strain fermentation is more conducive to high-quality production of enzyme products than single-strain fermentation; commercial strains are superior to endogenous strains in terms of safety and fermentation efficiency. Therefore, we selected mixed commercial strain fermentation to prepare *Ruoqiang jujube* enzymes. First, transfer the sterilized jujube pulp, measure appropriate amounts of activated probiotic strains on an ultraclean workbench, mix them into the jujube pulp; add the activated probiotic-inoculated jujube pulp, seal with a breathable membrane (do not tighten the bottle caps) to allow beneficial bacteria in the air to enter for fermentation; finally, incubate in a constant-temperature incubator at 37–38 °C.

The composite strain consists of six strains (Bac1-Bac6). After activation in enrichment medium, adjust the bacterial concentration to 1.5×10^6 CFU/mL by dilution or concentration. Inoculate them into the jujube pulp simultaneously at a volume ratio of 1% for each strain, and conduct closed fermentation at a constant temperature of 37–38 °C to ensure the stability and controllability of the fermentation process.

2.3.3 Fermentation process parameters and technical route

Based on practical experience, combined with laboratory equipment, the inherent characteristics of *Ruoqiang jujube*, and the biological properties of fermenting probiotics, the core fermentation parameters for *Ruoqiang jujube* enzymes were determined as follows: fermentation temperature at 38 °C, initial fermentation time set at 30 days. During fermentation, evaluate the fermentation status by real-time monitoring of physicochemical indicators such as pH of the fermentation broth and dynamic parameter evaluation values calculated from scores by an expert sensory evaluation panel, and continuously adjust various process parameters involved in the fermentation process.

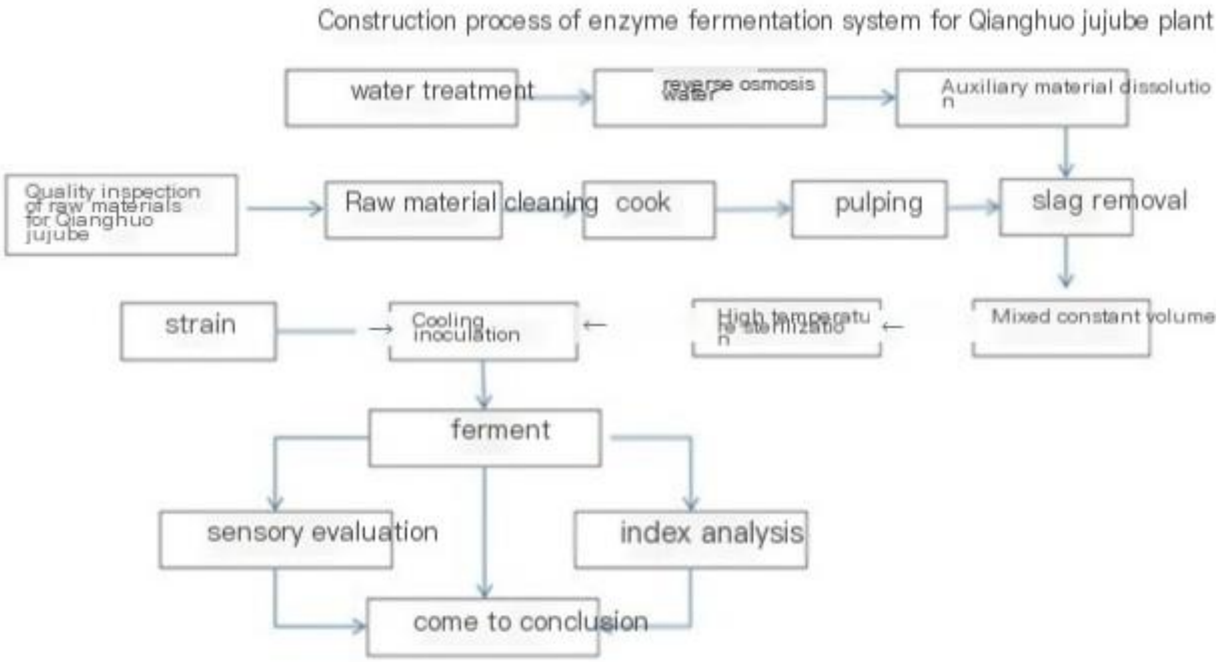

Figure 2.5 Fermentation Route of *Ruoqiang jujube*

Summary of chapter II

1. This study utilized air-dried *Ruoqiang jujube* as the primary material, supplemented with reagents and instruments. Five culture media were prepared for microbial detection on jujube surfaces.

2. *Ruoqiang jujube* enzyme fermentation employed mixed commercial probiotics at 37–38 °C for 30 days. Core parameters: fermentation temperature, initial time, monitored via pH changes and sensory scoring. The process included sterilization of pulp-nutrient mixtures, aseptic inoculation in breathable-sealed bottles, and dynamic parameter adjustment based on real-time physicochemical/sensory data. Composite strains were synchronized at 1% volume ratio each, ensuring stability. This approach leveraged microbial detection insights to design a probiotic-driven fermentation, optimizing enzyme quality through controlled temperature, nutrient gradients, and sensory-guided process refinement.

Chapter III
EXPERIMENTAL PART

3.1 Microbial identification on the surface of *Ruoqiang jujube*

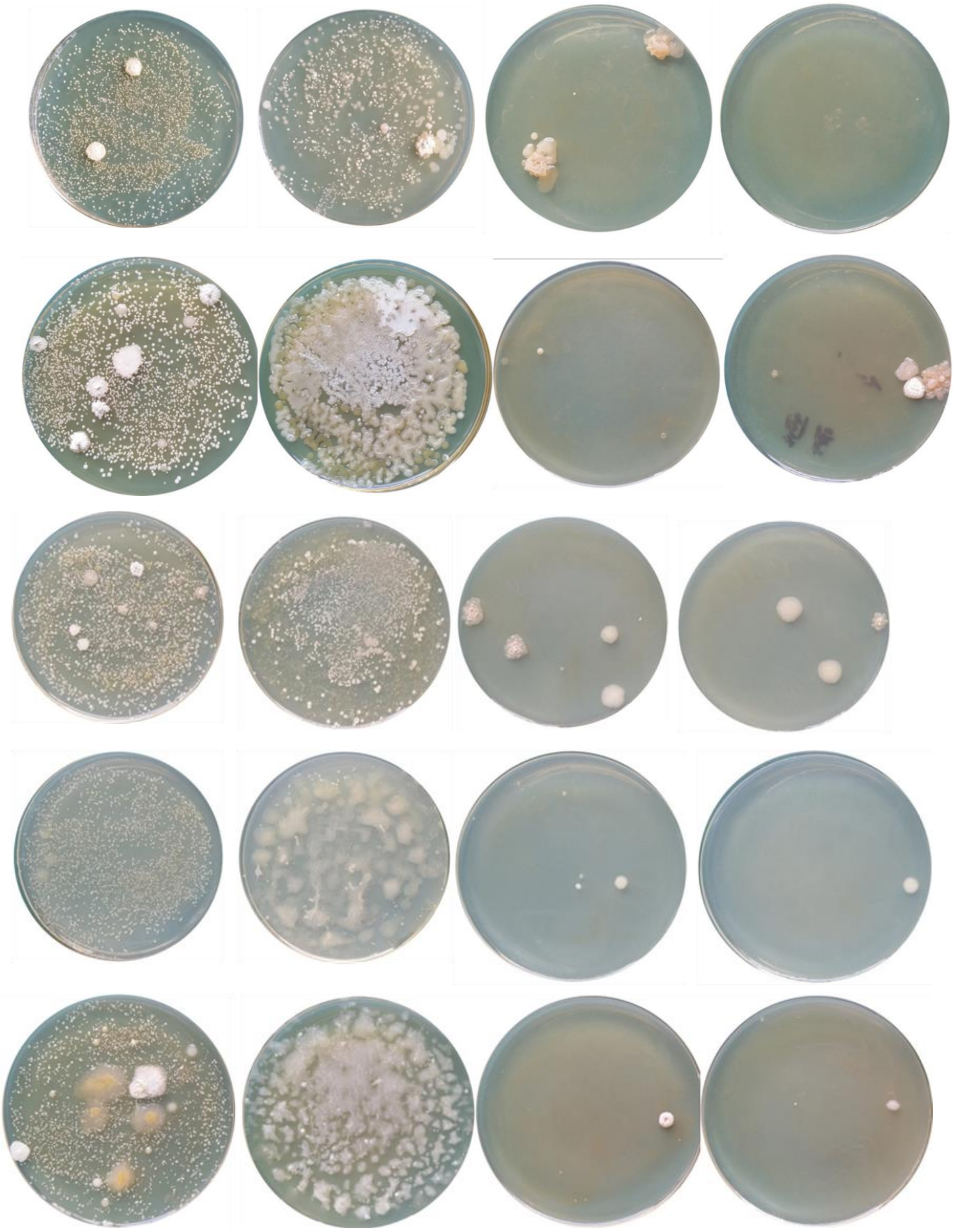

Figure 3.1 Indigenous Bacteria Identification of *Ruoqiang jujube*

3.2 Fermentation status and index detection of *Ruoqiang jujube* enzymes

Mainly monitored changes in pH, total sugar content, amino acid content, etc. of *Ruoqiang jujube* enzymes.

Figure 3.2 Status of Fermentation Broth at Different Concentrations of *Ruoqiang jujube*

3.3 pH changes in *Ruoqiang jujube* enzymes

The pH changes in *Ruoqiang jujube* enzymes during fermentation were determined according to the method specified in GB/T 10468. See the figure below.

As shown in the above charts and data, during the one-month fermentation process, the pH value of the fermentation broth exhibited a continuous downward trend and finally stabilized below 4.0. Specifically, by the 10th day of fermentation, the pH had dropped below 4.5.

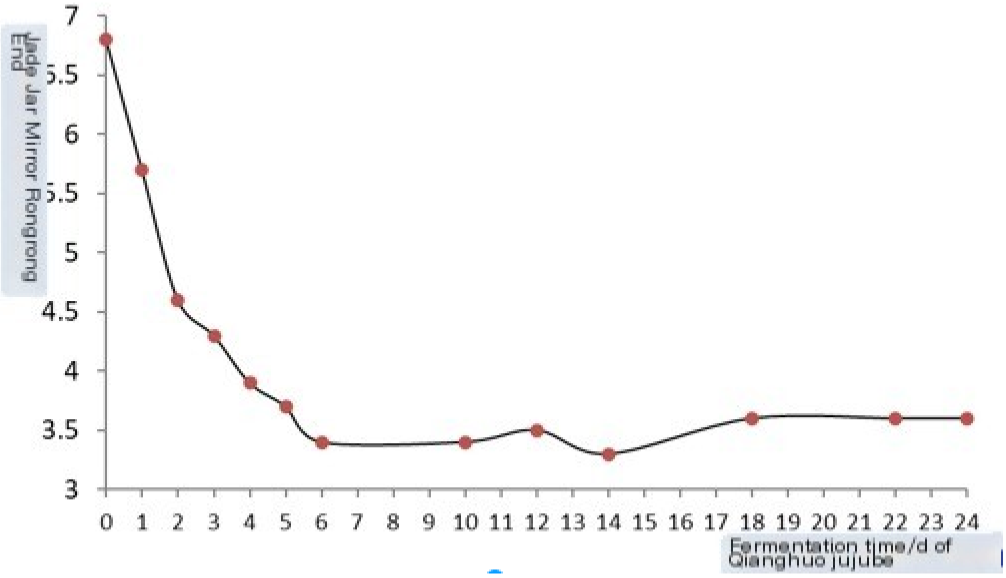

Figure 3.3 pH Changes in *Ruoqiang jujube* during Fermentation

The final pH of this fermented product meets the relevant pH specifications in the physicochemical indicators for edible plant enzymes, indicating that its acidity falls within the acceptable range for edible enzyme products.

3.4 Amino acid content and changes during fermentation of *Ruoqiang jujube*

The determination of amino acid content and changes during the fermentation of *Elaeagnus crispa Thunb*s referred to the method specified in GB 5009.124.

Analysis of the table data shows that during the fermentation of *Ruoqiang jujube*, the types of free amino acids gradually increased, with many amino acids undetectable at the start of fermentation but appearing and gradually increasing thereafter.

Table 3.1 **Amino Acid Content and Changes during Fermentation of *Ruoqiang jujube***

Amino Acid	Original Pulp	Fermentation			50% Group Fermentation6d
		2d	4d	6d	
Asp	370.9	352.7	346.1	444.7	230.9
Thr	1318.0	1214.5	2135.1	2121.8	1011.6
Ser	3917.7	3204.5	2611.5	1943.2	700.4
Glu	0.0	282.4	246.9	836.4	163.5
Gly	0.0	107.6	111.2	165.3	127.5
Ala	1433.9	1162.1	1015.2	1115.9	528.6
Cys	40.9	43.3	34.7	48.6	28.3
Val	230.1	192.9	164.3	207.3	113.5
Met	0.0	6.6	3.3	24.5	15.2
Ile	61.3	38.3	32.0	62.0	36.6
Leu	280.6	215.1	178.6	251.1	127.4
Tyr	41.9	79.9	66.3	134.2	47.3
Phe	86.7	69.3	52.5	1286.5	638.5
Lys	103.3	88.6	82.5	136.9	91.2
His	305.1	233.9	205.3	251.8	139.9
Arg	1329.3	759.6	43.7	28.4	28.4
Pro	5507.6	4773.0	4179.8	4006.8	1889.2

3.5 Reducing sugar content and changes in fermentation broth

Reducing sugar and total sugar contents are key indicators for evaluating the quality of *Ruoqiang jujube* enzyme fermentation broth. Among various detection methods, the 3,5-dinitrosalicylic acid (DNS) colorimetric method**Error! Reference source not found.** and redox titration are the most widely used.

Principle of reducing sugar determination: In an alkaline environment, DNS reacts with reducing sugars through a redox reaction upon heating. DNS is reduced to form reddish-brown 3-amino-5-nitrosalicylic acid, while reducing sugars are oxidized to sugar acids. The absorbance of this reddish-brown substance is measured at 540 nm using a visible spectrophotometer (where it exhibits maximum absorption), and the reducing sugar content is calculated by comparing with a standard curve.

The reducing sugar content in fresh jujube pulp was 13.9%. Utilization rates: 2d – 10.9%, 4d – 34%, 6d ~46% (Table 3.2).

Table 3.2 **Results of reducing sugar determination**

Sample (content)	Original Pulp	Fermentation			50% Group 4d	50% Group 6d
		2d	4d	6d		
Reducing sugar（mg/mL）	146	129	94.5	77.5	28	25

3.6 Total sugar content determination in fermentation broth

The total sugar content is calculated by measuring the total reducing sugar before and after hydrolysis using the DNS colorimetric method, subtracting the pre-hydrolysis value from the post-hydrolysis value, and multiplying by a conversion factor.

It has been experimentally established that the total sugar content of fresh jujube pulp is 15.9%; utilization factors: 2d – 6.0%, 4d – 36.5%, 6d ~37% (Table 3.3).

Table 3.3 **Results of Total Sugar Determination**

Sample (content)	Original Pulp	Fermentation			50% Group 4d	50% Group 6d
		2d	4d	6d		
Total Sugar （mg/mL）	149.63	140.20	93.44	92.25	54.62	49.09

3.7 Vitamin C content determination in fermentation broth

As an essential nutrient for humans, vitamin C (ascorbic acid) is widely present in fresh fruits and vegetables. This study used the classic 2,6-dichloroindophenol titration method for determination:

Principle: In an acidic environment, the oxidizing 2,6-dichloroindophenol solution is rapidly reduced to colorless by vitamin C. Once vitamin C is completely oxidized, excess dye turns the solution pale pink. The titration endpoint is reached when the pale pink color persists for 15 seconds[Error! Reference source not found.].

Calculation formula:

$$H = \frac{(V_1 - V_2) \times A}{W} \times \frac{a}{b} \times 100 \qquad (3.1)$$

where H – represents the vitamin C content (mg/100 mL); $V1$ – represents the volume of titration solution consumed (mL); $V2$ – represents the volume of titration solution consumed in the blank titration (mL); A – represents the vitamin C content corresponding to 1 mL of dichloroindophenol (mg/mL); B – represents the volume of the fermented sample solution taken (mL); b – represents the total volume of the diluted fermented sample solution (mL); a – represents the mass of the sample (g).

According to the measured vitamin C content *Vc* in the fermentation broth, the vitamin C content in *Ruoqiang jujube* was approximately 12.6 mg/kg. The content of vitamin C remained stable and was basically unaffected by fermentation.

Table 3.4 **Determination results of vitamin C**

Index (content)	Original Pulp	Fermentation			50% Group 4d	50% Group 6d
		2d	4d	6d		
Vc （μg/mL）	12.77	10.21	6.73	5.04	3.86	0.79

3.8 Sensory evaluation

In accordance with the industry standard QB/T 5323-2018 Sensory Evaluation Requirements for Plant Enzymes and relevant literature[Error! Reference source not found.], the sensory scoring criteria for *Ruoqiang jujube* enzymes were established as shown in the Table 3.8 below.

Table 3.8 **Sensory evaluation scorecard for *Ruoqiang jujube* enzymes**

Item	Evaluation Criteria	Rating
1	*2*	*3*
Odor	Rich aroma of *Ruoqiang jujube* with a fragrant aroma of plant enzyme fermentation.	Excellent
	Gradually intensifying jujube aroma with a mild alcoholic odor from fermentation.	Good
	Insufficient jujube aroma with a strong alcoholic odor.	Average

Continuation of Table 3.8

1	2	3
Odor	No jujube aroma, with a pungent or foul smell.	Poor
Taste	Moderate sweet-sour balance, dense and smooth texture without gritty sensation.	Excellent
	Imbalanced sweet-sour ratio (slightly too sour or sweet) with a slightly astringent taste.	Good
	Excessively sour or sweet with a harsh astringency.	Average
	Strong bitter taste and extremely astringent texture.	Poor
Impurities	No visible impurities.	Excellent
	Minor impurities present.	Good
	Moderate amount of impurities.	Average
	Abundant impurities.	Poor

To ensure the scientific validity and accuracy of the evaluation, a quantitative scoring system was adopted, dividing ratings into four levels: Excellent, Good, Average, and Poor. Panel Composition and Evaluation Protocol Panel Selection: Through a rigorous screening process, 16 individuals highly sensitive to sensory indicators (odor, taste, color, and impurity morphology) were selected from numerous candidates to form the enzyme sensory evaluation panel.

Objectivity Controls: Emotional states of panelists were closely monitored before evaluations, with prompt intervention to mitigate any subjective biases affecting fairness. For taste evaluations, panelists were required to thoroughly rinse their mouths with plain water to eliminate residual food debris and ensure unbiased gustatory assessments. Results 8 panelists rated the product as Excellent, 5 as Good, and only 3 as Average. No Poor ratings were recorded, indicating overall high acceptability of the fermented *Ruoqiang jujube* enzymes in sensory attributes.

Summary of chapter III

1. The fermentation broth pH continuously decreased and stabilized below 4.0, meeting edible enzyme quality standards for acidity.

2. Fermentation promoted an increase in free amino acid types, with previously undetected amino acids emerging and accumulating over time.

3. Reducing sugar and total sugar contents were analyzed using the DNS colorimetric method, showing gradual utilization throughout fermentation.

4. Vitamin C content remained stable during fermentation, indicating minimal impact from fermentation conditions.

CONCLUSIONS

This experiment successfully established a lactic acid bacteria fermentation system for *Ruoqiang jujube*, conducting multi-faceted analyses of fermentation products. While the results are of significant significance and value, several limitations also exist.

Regarding the construction of the fermentation system, effective control over the fermentation process was achieved through pretreatment of *Ruoqiang jujube*, determination of fermentation conditions, and selection of composite lactic acid bacteria strains. The pH value of the fermentation broth stabilized below 4.0, meeting the physicochemical indicators for edible plant enzymes, which demonstrates the stable operation of the fermentation system and the appropriate acidity of the final product. During fermentation, the types of free amino acids gradually increased, and the content of certain amino acids significantly rose, reflecting the metabolic transformation of nutrients in gray jujubes by lactic acid bacteria.

In terms of product composition analysis, the utilization rates of reducing sugar and total sugar gradually increased with extended fermentation time, indicating that the carbohydrate substances in gray jujubes were fully utilized by lactic acid bacteria. This not only reflects the good fermentation efficiency of the established system but also reveals a series of beneficial biochemical changes occurring in the product during fermentation. Notably, the vitamin C content remained basically stable throughout fermentation, preserving the original nutritional characteristics of gray jujubes and demonstrating the product's stable nutritional profile.

From an overall research perspective, future studies could be deepened in areas such as the synergistic and inhibitory relationships between lactic acid bacteria during fermentation, interactions between metabolites, and the long-term stability of the product. Additionally, as this research was primarily conducted at the laboratory scale, challenges in actual industrial production—such as precise control of fermentation conditions, equipment selection, and cost control—warrant further investigation. Translating these findings into industrial applications is expected to foster a new growth point for the green and healthy food industry.

Finally, the rapid development of biotechnology has injected new vitality into traditional agriculture. The optimized fermentation research on *Ruoqiang jujube* with composite probiotics demonstrates the powerful potential of modern biotechnology in enhancing food added value, extending shelf life, and improving taste and nutritional composition. This is of great significance for enhancing the market competitiveness of agricultural products and achieving sustainable agricultural development.

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