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 **Effect of *Lactobacillus rhamnosus* fermentation on the content of jujube saponins**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
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Completed: student of group BEBT-20
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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty: Chemical and Biopharmaceutical Technologies
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
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Scientific supervisor Ihor Hretskyi, Ph.D., As. prof

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I am familiar with the task:

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SUMMARY

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As a kind of sleep disorder, insomnia has become a common health problem of modern adults. Sleep is an important physiological need of the human body, and good sleep is an indispensable part of maintaining physical and mental health. Jujube seed is a kind of traditional Chinese herbal medicine. Its hypnotic effect on nerves is mainly reflected in that the extract of jujube saponin can shorten the sleep latency of pentobarbital sodium and prolong the sleep time. Jujube seeds contain a variety of alkaloids, in addition to containing fat oil, protein, diol and micro irritating volatile oil, the test can inhibit the central nervous system, showing sedative, hypnotic effect, can be used for neurasthenia, insomnia, dream, night sweat treatment, has the role of tonifying the liver and gallbladder, calm the heart to collect sweat. Currently, jujube seeds are commonly used to treat insomnia and are made into functional foods to improve sleep quality and enhance learning and memory. In China, there is no acid jujube kernel fermentation product which can improve sleep quality by combining probiotics with acid jujube kernel fermentation and using new acid jujube kernel fermentation product and probiotics as main raw materials. The purpose of this study was to study the effect of Lactobacillus rhamnosus fermentation on the content of jujube saponin, including the optimization of temperature, inoculation amount, fermentation time and glucose addition amount during the fermentation process. Finally, the optimal conditions were obtained as fermentation temperature 37℃, inoculation amount 4%, sugar addition amount 4%, fermentation time 48h. The content of jujube saponins increased by 60% under optimal fermentation conditions.

Keywords: Lactobacillus rhamnosus, jujube seed saponins, optimized fermentation

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INTRODUCTION

The purpose of this study was to investigate the effect of Lactobacillus rhamnosus on the biotransformation of Jujuboside A and its effect on the bioactivity. Jujube saponins are a kind of saponins with potential pharmacological effects extracted from jujube kernel, which have been proved to have anti-inflammatory, sedative and hypnotic biological activities. However, its low water solubility and bioavailability limit its application in the field of medicine.

We used *Lactobacillus rhamnosus* as starter culture to ferment jujube saponins in vitro. By optimizing the fermentation conditions, including temperature, pH value, inoculation amount and fermentation time, the efficient transformation of jujube saponin was realized. The fermentation products were analyzed by high performance liquid chromatography (HPLC) and mass spectrometry (MS), and their bioactivity was evaluated by cell experiments and animal models.

The results showed that L. rhamnosus could effectively transform jujube saponins and produce a variety of metabolites, some of which showed higher biological activity than the original saponins. In particular, the fermented products showed significant enhanced effects in improving sleep and anti-anxiety. In addition, the water solubility of the fermentation products is significantly improved, which helps to improve its application potential in preparations.

This study provided a new method for the biotransformation of jujube saponin, and improved its pharmacological properties through bioengineering technology, and provided a scientific basis for the development of new natural drugs and health products.

The relevance of the topic is the effect of *Lactobacillus rhamnosus* on the biotransformation of Jujuboside

The purpose of the study is the provided a scientific basis for the development of new natural drugs and health products.

The objectives of the study improve its application potential in preparations.

The object of the study new natural drugs and health products.

The subject of the study Jujuboside

Research methods Lactobacillus rhamnosus as starter culture to ferment jujube saponins in vitro.

The scientific novelty nobody had done it

The practical significance of the results obtained is health products.

CHAPTER 1
LITERATURE REVIEW

Sleep is defined as a natural and recurring physiological state. All kinds of conscious active behaviors disappear within a certain time every day, and the response to external environmental stimuli is weakened. Its main functions are to maintain the metabolic balance of the body, complete repair, control development and regulate health; Improve learning ability and consolidate memory; Release stress and emotions.

Sleep disorders include insomnia, hypersomnia, and obstructive sleep apnea. Insomnia is one of the most common sleep difficulties or sleep disorders, accompanied by dreamy and easy to wake up, difficult to fall asleep after waking up or seem to sleep, and after waking up, dizziness, fatigue, anxiety and other daytime dysfunctions [1]. Insomnia is a common sleep disorder that can make it difficult to fall asleep or stay asleep. It can also lead to waking up too early and not being able to get back to sleep. You may still feel tired when you wake up. Insomnia can deplete the body's energy levels and affect mood, and it can also affect your health, work performance and quality of life. There are many reasons for this condition, including stress, anxiety, irregular sleep, poor sleep environment, psychiatric or organic disorders. Insomnia is also recognized as a contributing factor to cardiovascular disease, chronic pain syndromes, depression, anxiety, diabetes, obesity and asthma [2].

According to the Chinese Guidelines for the Diagnosis and Treatment of Adult Insomnia, the current drugs for clinical treatment of insomnia mainly include BZRAs, melatonin receptor agonists, orexin receptor antagonists and antidepressants with hypnotic effects. However, these drugs have a single target and single component, which are easy to cause multiple side effects. If they are taken for a long time, they are easy to be tolerated, and are very easy to cause serious damage to the physiological functions of the liver and kidney. Therefore, obtaining a product for insomnia with minimal side effects has become a research hotspot in the current field.

1.1 Sour date palm kernel (Ziziphus jujuba)

1.1.1 Introduction to date palm kernels

Ziziphus jujuba var. spinosa (Bunge) Hu ex H.F.Chow, A perennial shrub or small tree belonging to Rhamnaceae, native to China. The fruits, seeds, leaves, roots, branches, bark and flowers of wild jujube can be used for food, medicine and health products. Among them, the mature seed of wild jujube is a traditional Chinese herbal medicine. According to the Shennong Classic of Materia Medica, wild jujube is mild in nature and sweet and sour in taste. Eating it regularly can invigorate the spleen and appetizer. It has the effect of nourishing the heart, calming the nerves, and astringing sweat. It is mainly used to treat restlessness, excessive sweating due to body deficiency, thirst due to fluid deficiency, and other diseases. The seeds of wild jujube contain a variety of alkaloids, in addition to fatty oil, protein, flutol and a small amount of irritating volatile oil. Through tests, it can inhibit the central nervous system, show sedative and hypnotic effects, and can be used for the treatment of neurasthenia, insomnia, dreaminess, night sweating. It has the effect of tonifying the liver and gallbladder, calming the heart and astringing sweat. At present, jujube kernels are commonly used to treat insomnia and are made into functional food to improve sleep quality and enhance learning and memory ability [3].

1.1.2 Effective components of jujube seed

Sour jujube contains a variety of active ingredients, of which the main chemical components used for sedative-hypnotic, antidepressant, anti-anxiety, antioxidant and memory-improving effects are: sour jujube kernel saponin, sour jujube kernel flavonoids and alkaloids [4].

(1) Ziziphus jujuba saponins: Ziziphus jujuba saponins can inhibit the absorption and synthesis of cholesterol, reduce the levels of LDL (low density lipoprotein) and VLDL (ultra low density lipoprotein), and increase the content of HDL (high density

lipoprotein), which is conducive to the prevention of cardiovascular disease[5]Sour jujube kernel saponin can remove free radicals in the organism and reduce oxidative damage to cells, which has a certain effect on the prevention of tumors and the slowing down of aging; in addition, sour jujube kernel saponin can inhibit excessive inflammatory reaction and reduce the damage caused by inflammation, which is helpful for inflammatory diseases such as periodontitis and arthritis; sour jujube kernel saponin can also improve cognitive function and learning memory, which has a protective effect on the cognitive function of the elderly and on the cerebral vascular disease. It has a protective effect on the cognitive function and cerebrovascular diseases of the elderly [6].

(2) Nucleotides, nucleobases and amino acids: Nucleotides, nucleobases and amino acids in jujube kernels are closely related to their nutritional benefits. Nucleotides and nucleobases are essential components of biological cells to maintain life activities, and participate in the process of DNA synthesis and metabolism. Amino acids are the basic substances that constitute the protein needed for animal nutrition. It is reported that jujube kernel contains many nucleotides, nucleobases and amino acids, such as proline, tyrosine, tryptophan γ- Aminobutyric acid, cytosine 5 '- monophosphate, guanine 5' - monophosphate and adenosine 5 '- monophosphate [7].

(3) Polysaccharides: Polysaccharides are one of the most common macromolecular compounds in nature, which are made up of the same or different monosaccharides linked by specific glycosidic bonds. In recent years, many studies have shown that polysaccharides play an important role in many biological activities, such as antioxidant and immunomodulation. Some monosaccharides, such as rhamnose, glucose, galactose and xylose, have been reported to be prevalent in the fruits of date plants. Similarly, these monosaccharides have been found to be the major units of polysaccharides in the seeds of Jujube as the main components of Jujube [8]. The polysaccharides in date seeds are mainly acidic heteropolysaccharides consisting of several monosaccharides including glucose (38.59%), arabinose (23.16%), galacturonic

acid (17.64%), galactose (10.44%), glucuronic acid (1.05%), mannose (2.03%), rhamnose (3.74%) and xylose (3.36%).

(4) Fatty oils: The fatty oil compounds of jujube are mainly distributed in the seeds, but are also found in the pulp of jujube. Jujube kernels contain 17 fatty acids, such as oleic acid, linoleic acid, palmitic acid, stearic acid and arachidic acid, etc. Most of these fatty acids are long-chain unsaturated fatty acids. Most of them are long-chain unsaturated fatty acids, and the higher contents are oleic acid and linoleic acid [8]. Unsaturated fatty acid is an important nutrient for human body, and also an important component of all cell membranes. Their content and variety determine the nutritional and health value of wild jujube kernel. In addition to fatty acids and glycerides, the content of unsaponifiable substances in fatty oil is about 1.6%, mainly including vitamin e, squalane β- Sitosterol and carotinol. After frying, the content of unsaturated fatty acids in wild jujube kernel decreased slightly, while the content of saturated fatty acids increased slightly [9].

(5) Flavonoids: At present, there have been a large number of studies on flavonoids in jujube, and a total of 68 compounds have been isolated and characterized, which are mainly distributed in its seeds and leaves [9].The flavonoids in date palm seeds mainly use apigenin as a ligand. Flavonoids in sour date seeds mainly use apigenin as a ligand, while glycosidic bonds are mostly carbon-based glycosides. In contrast, the flavonoids in the leaves of date palm mainly use flavonols (quercetin and caerulein) and flavanes (catechin and epicatechin) as the parent nuclei [10].

(6) Triterpenoids: Up to now, a total of 55 triterpenoids, mainly tetracyclic and pentacyclic triterpenoids, have been isolated and characterized from jujube. The tetracyclic triterpenoid saponins of jujube are mainly distributed in the seeds, and are rarely found in the fruit pulp and leaves. The pentacyclic triterpenoids in the fruit pulp of jujube are more widely distributed than those in the seeds, and most of them belong to ursine-type compounds, including betulin, betulinic acid and xanthic acid. In addition, some oleanolic acid-type, ursolic acid-type and polygalacic acid-type pentacyclic

triterpenoids were also found in the pulp of jujube fruit [11].

(7) Alkaloids: Alkaloids in wild jujube are mainly distributed in seeds, roots and bark. 33 compounds have been isolated and identified. There are two main types of alkaloids in wild jujube seeds, namely cyclic peptide alkaloids and isoquinoline alkaloids. The former includes zizyphulline a, zizyphulline b, zizyphulline d, zizyphulline f, zizyphulline g1, etc. The latter includes zizyphulline e (lotus alkaloid), zizyphulline k (theobromine), etc. Cyclic peptide alkaloids are 13 -, 14 - or 15 membered macrocyclic structures composed of groups and amino acid residues. According to the size of these macrocycles, they are divided into three groups: type i, type ii and type iii. The main cyclic peptide alkaloids in wild jujube belong to type I and type II, and the side chain amino acids are mostly leucine, isoleucine, valine, tryptophan, phenylalanine, alanine and their N-methyl derivatives.

(8) Other components: In addition to those already described, jujube contains many other components. Sterols and cerebrosides isolated from the fruit, six lignans isolated from the root.

1.1.3 Current status of research on date palm kernels

Whether used alone or in combination with other herbs, jujube seed is believed to have sedative and hypnotic effects. The sedative and hypnotic effects of jujube seed are mainly manifested in the fact that the original jujube seed can prolong sleep time. It can shorten the wakefulness period of insomniac rats and prolong the slow-wave sleep time of rats. Its mechanism may be related to the increase of hypothalamic nitric oxide content and the increase of nitric oxide synthase activity. The seeds and leaves of *Ziziphi jujuba* have similar depressant effects on central nervous system functions, while the pulp has a synergistic effect with sodium, prolonging sleep and reducing coordinated movements [11]. In addition, it is reported that the saponins (jujuboside) in Semen *Ziziphi spinosae* have more effective sedative and hypnotic functions than flavonoids, while polysaccharides do not show sedative and hypnotic effects. The hypnotic effect of

jujuboside on normal rats may be affected by the circadian rhythm, and may involve the 5-hydroxytryptamine system. In the daytime, zazipin can significantly increase total sleep and REM sleep, and in the evening, it can significantly increase total sleep and non REM sleep, especially shallow sleep. Jujuboside can significantly enhance the hypnotic effect of sodium, and 5-hydroxytryptamine can enhance this effect. In addition, there are many theories about the molecular mechanism of jujuboside a's sedative and hypnotic effects. Jujuboside a can change GABA in hippocampal neurons α 1、 α 5 and β 2 subunit gene expression, which may be part of the molecular mechanism of its clinical sedative hypnotic effect. Other studies have shown that jujuboside a can not only regulate the expression of GABA receptor subunits, but also affect the cytokine network between neurons in the brain by reducing the secretion of inflammatory cytokines related to the intestinal mucosal system, thus exerting its specific sedative and hypnotic effects, which is similar to the mechanism of melatonin [13]. At present, some scholars believe that the sedative and hypnotic effects of saponins are not saponins themselves, but their metabolites. Jujuboside b and jujuberenol are the main products of saponin hydrolysis, and these metabolites may be the real absorption forms with specific biological activities. In addition, they may also play a sedative role through interaction with GABA-A receptors [14]. Dates are rich in nutrients and have a wide range of health benefits. Rich in nutrients, jujube has a wide range of health benefits, such as antioxidant and hepatoprotective effects, sedative and hypnotic effects, and neuroprotective effects. Jujube kernel is a typical herb used in the treatment of insomnia and has been found to be effective in central nervous system disorders. It has also been found to be beneficial for palpitations, menopausal syndrome and neurasthenia [15]. The pharmacological activity and health benefits of jujube seed have been emphasized over the past decades. In the past decades, many studies have focused on the pharmacological activities and health benefits of jujube saponin, especially its role and mechanisms in the central nervous system. As a natural product, the biological and pharmacological properties of

jujube saponin have received more and more extensive attention. Domestic and foreign studies have shown that jujube saponin has broad application prospects and good development value, and more studies will increase people's knowledge and mastery of jujube saponin.

1.2 Overview of Lactobacillus rhamnosus and current status of research

Lactobacillus rhamnosus GG (abbreviated as *Lactobacillus* GG or LGG), LGG has outstanding performance in resisting gastric acid and bile, can enter human body in vivo, can colonize in human body for up to two weeks, can effectively improve and adjust human gastrointestinal flora, and is very beneficial to human health [16]. Whether it can be colonized in the human body will have a great impact on the physiological function of a probiotic, while most other probiotics cannot be colonized in the human body. After colonization and reproduction in the human intestinal environment, LGG is only attached to the host's intestine Epithelial cells, which can act as a biological barrier to the intestinal mucosa, thereby enhancing the barrier capacity of the host intestinal mucosa [17]. Moreover, LGG can regulate the structure and function of the microbiota community in the host gut, and make it reach a balanced state, so as to improve the function of the host digestive tract system. Relevant research shows that the adhesion rate of LGG to adults is significantly higher than that of children, mainly because the intestinal environment of adults is more mature and suitable for LGG adhesion [18]. Lactic acid is the main fermentation product of lactic acid bacteria, which can be divided into three types: L-lactic acid, D-lactic acid and DL lactic acid. Because there is only L in human body-lactate dehydrogenaseTherefore, it can only metabolize L-lactic acid [19]. WHO regulations, per person intakeD-lactic acidThe amount shall not exceed 100mg/kg, and the food for infants under 3 months old shall not contain D-type and DL type lactic acid. *Lactobacillus rhamnosus* LGG only produces L-lactic acid in the fermentation process, and does not produce other acids that affect the safety and taste of the product. Therefore, fermented foods containing LGG will not cause diarrhea [20].

At present, LGG products sold in the international market are mainly fermented dairy products, in addition to fresh milk, cheese, baby food, fruit juice, fruit juice drinks and drugs, which have been recognized by consumers and medical workers. Lactobacillus rhamnosus is an important probiotics, which has significant effects in regulating the balance of microecology and enhancing intestinal resistance, and is widely used in infant formula food and dietary supplements [21].

Lactobacillus rhamnosus is one of the most studied probiotics, especially among the three probiotics mentioned in the White Paper on the Development of the Intestinal Industry in 2023, LGG, HN001 and GR-1 belong to Lactobacillus rhamnosus. LGG is the most studied and comprehensive probiotic strain at present, with more than 2000 published studies, including more than 400 medical evidence-based related studies.

Treatment of intestinal diseases: Research shows that Lactobacillus rhamnosus can prevent or alleviate inflammatory bowel disease (IBD) through a variety of mechanisms, such as regulating the composition of intestinal microorganisms, protecting the intestinal epithelial cell barrier, etc. For example, diarrhea relief: Lactobacillus rhamnosus has also played an active role in the treatment of diarrhea, especially LGG strain has been widely used in the adjuvant treatment of children's diarrhea, which can shorten the duration of diarrhea and reduce the frequency of diarrhea. Oral health care: *Lactobacillus rhamnosus* LR863 has excellent biological characteristics, which can be used as a candidate probiotic strain of oral health care products, and has antibacterial effect on a variety of oral pathogenic bacteria [22]. Hypoglycemic effect: Some strains of Lactobacillus rhamnosus have potential hypoglycemic effect and can be used as fermentation strains for developing new fermentation products. Allergic disease treatment: clinical research shows that *Lactobacillus rhamnosus* has certain therapeutic effect on allergic rhinitis, atopic dermatitis, asthma, food allergy and other diseases. Diagnosis and treatment of pneumonia caused by COVID-19: During the COVID-19 epidemic, Lactobacillus rhamnosus was also mentioned as a probiotic that may contribute to intestinal health.

Functional characteristics: *Lactobacillus rhamnosus* LGG has the ability to tolerate the digestive tract environment, can colonize in human and animal intestines, and plays a role in regulating intestinal flora [23]. Intervention of type 2 diabetes: *Lactobacillus rhamnosus* GG (ATCC 53103) was registered in clinical trials as a dietary supplement in the intervention/treatment of type 2 diabetes. Effects of lipopolysaccharide (LPS): The new strain of *Lactobacillus rhamnosus* has a alleviating effect on chronic intestinal inflammation caused by lipopolysaccharide in vitro and in vivo. Fermented milk application: *Lactobacillus rhamnosus* has a wide range of applications in fermented milk, which can reduce cholesterol, prevent and treat diarrhea, eliminate toxins, prevent dental caries, etc [24].

1.3 Current status of research on optimization of fermentation conditions

1.3.1 Optimization of the fermentation medium

Culture medium is a mixture of nutrients that can be used for microorganisms to grow and reproduce and produce metabolites on it, which provides microorganisms with carbon, nitrogen, water, inorganic salts and other nutrients needed for growth, in which different group allocation ratios have different effects on microbial growth and development, and also have a great impact on the synthesis and extraction of products.

1.3.2 Optimization of fermentation conditions

Optimization of fermentation conditions plays an important role in microbiology and bioengineering. By adjusting the medium composition, pH value, temperature, oxygen supply and other parameters, the production and quality of products in the process of microbial fermentation can be improved, so as to maximize the benefits of industrial production.

The optimization of fermentation conditions is a key component in improving the yield of the target product, the[25]. The following are a few of the common factors that need to be controlled, the

Time: date nut saponin can be accumulated in large quantities in the early and middle stages of fermentation, but if the fermentation time is too long, it may lead to the exhaustion of the substrate, and the strain will be consumed at this time, which will reduce the final yield.

Temperature: Each microorganism has its own suitable growth temperature range, so we need to set the temperature in the bioreactor according to the characteristics of the production strains. Temperature affects not only cell growth, but also enzyme activity and product synthesis.

PH value: changes in pH value during fermentation will affect the growth of microorganisms and the formation of metabolites. Usually, it needs to be maintained within an optimal pH range, sometimes adjusted by adding acidic or alkaline substances.

Stirring speed: Proper stirring ensures uniform distribution of nutrients in the medium and also affects the efficiency of oxygen transfer.

Nutrient concentration: including carbon, nitrogen and metal ions, which are essential for microbial growth and metabolism. Different production strains have different nutrient requirements and need to be optimized.

Inoculum volume: The size of the inoculum volume affects the start-up rate of the fermentation process and the yield of the final product

In this paper, laboratory fermentation will be optimized for the external environmental conditions of fermentation substrate sugar content, fermentation temperature, inoculum amount and fermentation time. Ultimately the actual yield and quality will be improved.

1.4 Results and significance

By improving the fermentation process of wild jujube kernel by *Lactobacillus rhamnosus*, including the factors such as the content of sugar in the fermentation substrate, fermentation temperature, inoculation amount, fermentation time, etc., the optimal conditions related to fermentation kinetics were obtained as follows:

fermentation temperature 37 ℃, inoculation amount 4%, sugar addition amount 4%, fermentation time 48 h. Under the optimal fermentation conditions, the content of total saponins in wild jujube kernel increased by 60%.

Sour jujube seed is a traditional Chinese herb with the traditional effects of nourishing the heart and nourishing the liver, calming the mind and stopping sweating. It has good curative effect on central nervous system diseases, and is a typical treatment for insomnia. Studies have also found that sour jujube kernel has curative effect on palpitation, menopausal syndrome and neurasthenia. The sedative-hypnotic effect of sour jujube kernel is mainly reflected in the extract of sour jujube saponin can shorten the sleep latency of sodium pentobarbital and prolong the sleep time of sodium pentobarbital. In addition, regulating the composition of intestinal flora may also be an important way to improve insomnia. In the patent literature, there are compositions utilizing ethanol to extract date palm kernel, but there are shortcomings of high cost of extracts, which cannot be used in common food. Since regulating the composition of intestinal flora may also be an important way to improve insomnia in sour jujube kernel, and through the search of patent literature, we have not found any sour jujube kernel fermentation products with improved sleep quality prepared by combining probiotic and sour jujube kernel fermentation and using the new sour jujube kernel fermentation product and probiotic as the main raw materials. The research of this paper can lay a certain foundation for the application of sour date kernel in the field of functional food, and provide a new method for the research and development of new fermented sour date kernel saponin products.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1.1 Strains

Lactobacillus rhamnosus (provided by our laboratory)

2.1.2 Main instruments and materials

Centrifuge, ultrasonic cleaner, ultra-micro pulverizer, electronic balance, thermostatic water bath, thermostatic incubator, grinder, autoclave, ultra-clean bench, ultraviolet spectrophotometer, tabletop high-speed freezing centrifuge, ball mill

Jujube kernel, MRS broth medium, methanol, perchloric acid, glacial acetic acid, vanillin, petroleum ether, brown sugar

2.2 Experimental Procedures

2.2.1 Preparation of raw materials for fermentation and strains

Experimental materials and pretreatment is a very important part of the optimization of fermentation conditions, effective pretreatment and selection of suitable experimental materials can lay the foundation for the smooth development of subsequent experiments. In the experiment of fermentation condition optimization, the selection and pretreatment of experimental materials are crucial links, which directly affect the success or failure of the experiment and the accuracy of the results.

Selection of experimental materials: The selection of suitable experimental materials is the basis for the success of the experiment. For the fermentation production of ginsenoside, we need to choose ginseng materials rich in ginsenoside precursors, such as ginseng root and ginseng beard. At the same time, it is also necessary to consider the freshness of the material, maturity and whether it is affected by pests and diseases. Cleaning of materials: Before the experiment, the selected materials need to be thoroughly cleaned to remove surface impurities and possible microbial contamination

to ensure the cleanliness of the experimental environment. Pretreatment method: Pretreatment is a key step to change material properties and improve fermentation efficiency. Common pretreatment methods include physical crushing, chemical treatment (such as acid, alkali, enzyme treatment), heat treatment, etc. Physical crushing can increase the surface area of the material and promote the contact and metabolism of microorganisms; Chemical treatment can change the structure of the material, making it easier for microorganisms to use; Heat treatment can kill pathogenic microorganisms while activating the activity of certain enzymes. Sterilization treatment: In order to ensure that the fermentation process is not interfered with by foreign microorganisms, it is necessary to sterilize the pre-treated materials. The commonly used sterilization methods are high-pressure steam sterilization, dry heat sterilization and so on. The addition of nutrients: In the fermentation process, in addition to the ginseng material itself, some nutrients need to be added to support the growth and metabolism of microorganisms. These nutrients may include carbon sources, nitrogen sources, vitamins, minerals, etc. pH regulation: Different microorganisms have different adaptations to pH, so the pH needs to be adjusted to the optimal range according to the microbial species used to ensure their growth and metabolic activity. Temperature and humidity control: Temperature and humidity in the fermentation process are also important factors affecting microbial activity. It is necessary to control the temperature and humidity within the most suitable range according to the growth characteristics of the microorganisms. Determination of inoculation amount: The size of inoculation amount directly affects the start-up speed of fermentation and the final yield. It is necessary to determine the appropriate amount of inoculation according to the experimental purpose and the growth characteristics of the microorganisms. Fermentation time arrangement: the length of fermentation time will affect the yield and quality of saponins. The optimum time of fermentation needs to be determined by pre-experiment. Rationality of experimental design: After determining the experimental materials and pretreatment methods, it is necessary to design a reasonable experimental scheme, including the

setting of the experimental group, the arrangement of the control group, and the determination of the number of repeats, so as to ensure the scientific and repeatable experimental results. Through the detailed planning and implementation of the above steps, it can lay a solid foundation for the subsequent fermentation experiment, improve the success rate of the experiment, and provide guarantee for obtaining high-quality ginsenoside products.

(1) Preparation of raw materials for fermentation

The samples were washed and dried, and then put into an ultra-micro pulverizer to obtain the powder of sour date kernel.

(2) Preparation of fermentation medium.

In this experiment, broth culture medium (MRS) was used to cultivate expanded strains.

MRS medium (g/L): Peptone 10.0 g; Beef infusion powder 8.0g; Yeast infusion powder 4.0g; Glucose 20.0 g; Dipotassium hydrogen phosphate 2.0g; Diammonium hydrogen citrate 2.0g; Sodium acetate 5.0g; Magnesium sulfate 0.2g; Manganese sulfate 0.04 g; AGAR 14.0 g; Twain 80 1.0g.

2.2.2 Standard curves

Accurately suck 0.0,0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8 mL of ginsenoside Re standard solution into an evaporating dish, evaporate them at a water bath temperature of 60 ℃, then add 0.4 mL of 5% vanillin glacial acetic acid solution, turn the evaporating dish to dissolve the residue, then add 1.6 mL of perchloric acid, fully shake it, pour it into a 10 mL colorimetric tube, heat it on a 70 ℃ water bath for 20 min, take it out, cool it in an ice bath for 2 min, Accurately add 5.0 mL of glacial acetic acid, shake well, pour into the cuvette and measure its absorbance at the wavelength of 589 nm. Draw standard curve with saponin quality as ordinate and absorbance as abscissa.

The specific operations are as follows: Preparation of control solution: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 mL of ginsenoside control solution are absorbed using a precise

pipette. These control solutions will be used in subsequent experiments to establish standard curves. Drying process: The absorbed control solution is placed in a water bath at 60 ° C for drying, in order to remove the solvent and leave ginsenoside components. Color developer addition: 0.4 mL of 5% vanillin - ice acetic acid is added to the residue after drying, which is a commonly used color developer that can react with ginsenosides to produce color changes. Dissolving and mixing: the residue after color development is dissolved in water to ensure uniform dispersion of ginsenoside components. The addition of perchloric acid: Adding 1.6 mL perchloric acid to the colorimetric dish can further promote the color reaction and enhance the stability of the color. Water bath treatment: The mixed colorimetric dish is heated in a water bath at 70 ° C for 20 minutes, this step helps to complete the color reaction. Cooling treatment: Immediately after removing the colorimetric dish from the water bath, cool it with an ice water bath for 2 minutes to terminate the color reaction. Addition of glacial acetic acid: 5.0 mL of glacial acetic acid is added to the cooled solution, which can stabilize the solution and prevent color change. Shake: Shake the colorimetric dish to ensure that the ice acetic acid is well mixed with the solution. Cupola preparation: The evenly mixed solution is loaded into a cupola with a wavelength of 589 nm, which is the specific wavelength used to measure absorbance. Absorbance measurement: Use a spectrophotometer to measure the absorbance of the solution in the colorimetric dish, which is an important index to measure the ginsenoside content in the solution. Establishment of standard curve: With the content of saponins as the vertical axis and absorbance as the horizontal axis, the standard curve of ginsenosides was established according to the measured absorbance data. Through the above steps, we can get the absorbance values of a series of different concentrations of ginsenoside control solution, and then calculate the content of ginsenoside in unknown samples by using the standard curve. This method is a commonly used quantitative analysis method in analytical chemistry and can provide scientific basis for the quality and control of ginsenosides.

2.3 Optimization of fermentation conditions

2.3.1 Effect of different media sugar levels on yield

Select a conical flask with proper capacity, wash it with pure water, add 140 mL of pure water and 20 g of sour jujube kernel powder in the ratio of pure water (V): sour jujube kernel powder (m) 7:1, ultrasonic at 40 ℃ for 30 min, add different amounts of brown sugar, prepare fermentation substrate with sugar content of 2%, 4% and 6%, seal the bottle mouth with sealing film, put it into a sterilization pot for sterilization at 115 ℃ for 30 min, take it out and cool it to room temperature, After Lactobacillus rhamnosus was activated by liquid MRS medium, the saponin content was determined after fermentation under the condition that the inoculation amount, fermentation time and fermentation temperature were kept constant, and the optimal medium sugar content was determined. At the same time, the non inoculation control group was set.

Table 2.1-**One-factor experiments on culture medium brix**

Experiment number	**Medium Sugar Levels**	**Inoculum**	**Fermentation time**	**fermentation temperature**
1	2%	4%	24h	37℃
2	4%	4%	24h	37℃
3	6%	4%	24h	37℃
4	2%	0%	24h	37℃
5	4%	0%	24h	37℃
6	6%	0%	24h	37℃

2.3.2 Effect of different inoculum levels on yiel

Select a conical flask with proper capacity, wash it with pure water, add 140 mL of pure water and 20 g of sour jujube kernel powder in the ratio of pure water (V): sour jujube kernel powder (m) of 7:1, ultrasonic at 40 ℃ for 30 minutes, seal the bottle mouth with sealing film, put it into a sterilization pot for sterilization at 115 ℃ for 30 minutes, take it out and cool it to room temperature. Lactobacillus rhamnosus was activated by liquid MRS medium, and then inoculated into the fermentation medium of Semen Ziziphi spinosae according to different inoculation amount (2%, 4%, 6%, by volume fraction) to ensure that other fermentation conditions remain unchanged. After fermentation, the saponin content was determined to determine the optimal fermentation inoculation amount. At the same time, the non inoculation control group was set.

Table 2.2-**One-factor experiment on inoculum size**

Experiment number	**Medium Sugar Levels**	**Inoculum**	**Fermentation time**	**fermentation temperature**
1	4%	0%	24h	37℃
2	4%	2%	24h	37℃
3	4%	4%	24h	37℃
4	4%	6%	24h	37℃

2.3.3 Effect of different temperatures on yield

Select a conical flask with proper capacity, wash it with pure water, add 140 mL of pure water and 20 g of sour jujube kernel powder in the ratio of pure water (V): sour jujube kernel powder (m) of 7:1, ultrasonic at 40 ℃ for 30 minutes, seal the bottle mouth with sealing film, put it into a sterilization pot for sterilization at 115 ℃ for 30 minutes,

take it out and cool it to room temperature. Lactobacillus rhamnosus was activated by liquid MRS medium, and then inoculated into the fermentation substrate of Semen Ziziphi spinosae at different fermentation temperatures (35 ℃, 37 ℃, 39 ℃) to ensure that other fermentation conditions remain unchanged. After fermentation, the saponin content was determined to determine the optimal fermentation inoculation amount. At the same time, the non inoculation control group was set.

Table 2.3-**Fermentation temperature one-factor experiment**

Experiment number	**fermentation temperature**	**Inoculum**	**Fermentation time**	**Medium Sugar Levels**
1	35℃	4%	24h	4%
2	37℃	4%	24h	4%
3	39℃	4%	24h	4%
4	35℃	0%	24h	4%
5	37℃	0%	24h	4%
6	39℃	0%	24h	4%

2.3.4 Effect of different fermentation times on yield

Select a conical flask with proper capacity, wash it with pure water, add 140 mL of pure water and 20 g of sour jujube kernel powder in the ratio of pure water (V): sour jujube kernel powder (m) of 7:1, ultrasonic at 40 ℃ for 30 minutes, seal the bottle mouth with sealing film, put it into a sterilization pot for sterilization at 115 ℃ for 30 minutes, take it out and cool it to room temperature. After *Lactobacillus rhamnosus* was activated by liquid MRS medium, according to different fermentation time (36h, 48h, 60h), to ensure that other fermentation conditions remain unchanged, determine the saponin content after fermentation to determine the optimal fermentation inoculation amount. At the same time, set up a control group without inoculation.

Table 2.4-**Fermentation time one-way experiment**

Experiment number	**Fermentation time**	**Inoculum**	**fermentation temperature**	**Medium Sugar Levels**
1	36h	4%	37℃	4%
2	48h	4%	37℃	4%
3	60h	4%	37℃	4%
4	36h	0%	37℃	4%
5	48h	0%	37℃	4%
6	60h	0%	37℃	4%

2.4 Sample saponin content assay

Sample determination: evaporate and concentrate the fermented liquid with a rotary evaporator, and then dry it. After the water has completely evaporated, carefully scrape the crystals, collect them, grind them into powder, and put them into a sealing tape for storage. Take precisely weighed and dry to 1g of constant weight jujube kernel test sample, add precisely measured 5mL methanol, shake for 1min, centrifuge, draw 2.0mL into a 5mL volumetric flask, add methanol to dilute to the scale, and shake well. Accurately measure 0.10 mL of the solution into a 20 mL tube with a stopper, evaporate the solvent in a water bath, then add 0.4 mL of 5% vanillin glacial acetic acid solution, rotate the evaporating dish to dissolve the residue, then add 1.6 mL of perchloric acid, fully shake it up, pour it into a 10 mL colorimetric tube, heat it in a 70 ℃ water bath for 20 min, take it out, cool it in an ice bath for 2 min, accurately add 5.0 mL of glacial acetic acid, shake it up, Pour into the cuvette and measure its absorbance at the wavelength of 589 nm.

2.5 Integrated experiments

After obtaining the above values, the optimum conditions were synthesized and

the fermentation experiment was carried out again with a control group without inoculation, and the total saponin content was determined after obtaining the products to obtain a conclusion.

Detailed steps: Data analysis: First, we need to analyze the absorbance value obtained in the experiment, and calculate the ginsenoside content in each control solution through the standard curve. This step is crucial because it will help us understand ginsenoside production under current conditions. Condition optimization: According to the experimental results, we can identify the key factors affecting the saponin yield, such as fermentation time, temperature, pH value, nutrient concentration, etc. Then, we need to adjust these conditions to find the most suitable fermentation conditions. Design experiment: After determining the optimal conditions, we need to design a new fermentation experiment. This experiment should include different treatment groups, each of which applies different fermentation conditions to test their effect on saponin yield. Setting up control group: In order to ensure the accuracy of the experimental results, we need to set up a control group without vaccination. This control group will help us distinguish between saponins produced during fermentation and saponins from other sources that may be present. Fermentation experiments: After determining the most suitable conditions, fermentation experiments are carried out according to these conditions. Ensure that all experimental groups are fermented according to the same method and time for easy comparison. Product collection and treatment: After fermentation, fermentation products are collected and treated appropriately, such as centrifugation, filtration, etc., in order to facilitate subsequent saponin extraction and determination. Saponins extraction: The use of appropriate solvents and methods to extract saponins from fermentation products. This step requires the extraction efficiency and purity of saponins. Total saponin content determination: The absorbance determination method established before was used to determine the total saponin content extracted. This step will directly reflect the effect of optimization conditions on saponin yield. Results Comparison: The total saponin content after

optimization was compared with the control group and the original experimental condition, and the specific influence of different conditions on the saponin production was analyzed. Conclusion Conclusion: According to the experimental results, the most suitable fermentation conditions were summarized and the conclusion was drawn. If the total saponin content is significantly increased under the optimized conditions, it indicates that our condition optimization is successful. Further research: After the initial conclusions are reached, we may need to conduct more experiments to verify the stability and reproducibility of these results. In addition, other factors that may affect the production of saponins, such as the selection of microbial strains and oxygen supply during fermentation, can be explored. Through this series of experimental steps, we can not only optimize the fermentation conditions of ginsenosides, but also provide scientific basis and technical support for the industrial production of ginsenosides.

Table 2.5-**Comprehensive experiments**

Experiment number	**Inoculum**	**Fermentation time**	**fermentation temperature**	**the sugar content of the medium**
1	0	48h	37℃	4%
2	4%	48h	37℃	4%

CHAPTER 3
EXPERIMENTAL PART

3.1 Standard Curve for Saponin Content

Measure the absorbance at the wavelength of 589 nm, take the content of ginsenoside Re as the ordinate and the absorbance as the abscissa, and draw the standard curve as shown in Figure 3.1. The regression equation is y=1.451x-0.002720, and the correlation coefficient R2=0.99889. Results There was a good linear relationship between the absorbance and the absorbance in the range of 0.00~0.10 mg/g.

In conducting experiments to determine ginsenoside Re content, we used spectrophotometry, a commonly used quantitative analysis method that determines the concentration of substances in a sample by measuring absorbance at a specific wavelength. Specific steps are as follows：

1. Preparation of the experiment: First, we need to prepare a series of standard solutions of ginsenoside Re at known concentrations, which will be used to draw the standard curve.
2. Absorbance determination: Using a spectrophotometer, the absorbance of each standard solution is measured at a specific wavelength of 589 nm. This wavelength is the maximum absorption wavelength of ginsenoside Re and therefore provides the most accurate measurement results.
3. Data logging: Record the absorbance values of each standard solution and ensure repeatability and accuracy during measurement.
4. Drawing standard curve: The absorbance value is taken as the horizontal coordinate, and the ginsenoside Re concentration is taken as the vertical coordinate to draw the standard curve. Usually, this will be a linear plot showing the linear relationship between absorbance and concentration.
5. Regression analysis: Using statistical software or mathematical methods, regression analysis is performed on absorbance and concentration data, and regression equations are obtained. This equation will be used to convert the

absorbance value of an unknown sample to the concentration of ginsenoside Re.

6. Linear range determination: According to the experimental results, the linear range between ginsenoside Re content and absorbance was determined. In this range, the relationship between absorbance and concentration is linear and can be used to accurately determine ginsenoside Re content in unknown samples.
7. Experimental results: The experimental results showed that there was a good linear relationship between ginsenoside Re content and absorbance in the range of 0.00 ~ 0.10 mg/g. This means that within this concentration range, we can accurately calculate ginsenoside Re content by measuring absorbance.
8. Applying standard curve: The absorbance value of unknown sample was substituted into the regression equation to calculate the actual ginsenoside Re content in the sample.
9. Experimental verification: In order to verify the accuracy and reliability of the standard curve, some verification experiments can be carried out, such as the determination of ginsenoside Re solution with known concentration to compare the difference between the actual value and the predicted value.
10. Experimental report: At last, the experimental process, results and conclusions were sorted into a detailed experimental report, including standard curve graph (Figure 3-1), regression equation, linear range, experimental data and analysis, etc.

Through the above steps, we can not only accurately determine the content of ginsenoside Re, but also provide a scientific basis for the quality and control of ginseng and its related products. This method has the advantages of simple operation, rapid and accurate, and is widely used in the analysis and research of natural products.

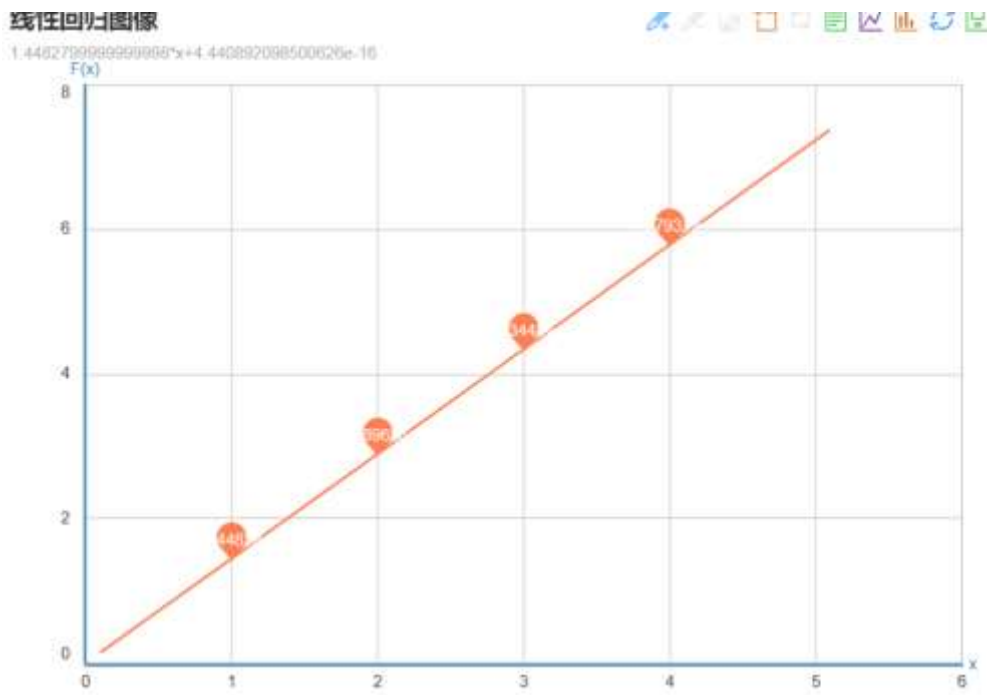

Figure 3.1-**Standard curve for saponin standards**

3.2 Lactobacillus rhamnosus fermentation experiment one-factor condition optimization experimental results

3.2.1 Results of one-way experiments with different media sugars

The experimental results are shown in Fig. 3.2, under the condition of other factors are the same, with the increase of medium sugar, the saponin content in fermentation products appeared to increase firstly and then decrease, and the peak value appeared in the condition of 4%. Therefore, it can be concluded that the optimum medium sugar level is 4%.

In the in-depth study of fermentation process, we paid special attention to the influence of the sugar degree of the medium on the saponin content, because the sugar degree is one of the key factors affecting the growth and metabolic activities of microorganisms. Through a series of well-designed experiments, we found that the adjustment of the sugar content of the medium has a significant effect on the saponin content of the fermentation products under the condition that other conditions remain unchanged. At the beginning of the experiment, we set up a number of media with different sugar levels, gradually increasing from low to high, to observe the specific

effect of the change of sugar level on the saponin yield. At the initial stage, with the increase of sugar content, we observed that the saponin content of fermentation products increased correspondingly. This phenomenon can be attributed to the fact that microorganisms obtain more energy and carbon sources in the medium with higher sugar content, thus promoting their growth and metabolic activities, increasing the synthesis and accumulation of saponins. However, when the sugar degree continues to increase and reaches a certain point, we find that the saponin content no longer rises, but instead begins to decline. This trend indicates that excessive sugar content may inhibit the growth of microorganisms, or change the metabolic pathway of microorganisms, resulting in the synthesis of saponins. This phenomenon is particularly pronounced in high concentrations of sugar and is known as the "hyperglycemic effect". Among all the sugar levels tested, we particularly found that the saponin content in fermentation products reached the highest value when the medium sugar content was 4%. This discovery revealed that under the conditions tested, a sugar content of 4% is the optimal point to obtain the optimal saponin yield. Therefore, we can conclude that the optimal medium sugar content is 4%, which provides an important parameter for the optimization of fermentation process. This conclusion is not only based on direct observation of experimental data, but also verified by scientific statistical analysis. We ensure the accuracy and reliability of the conclusion through regression analysis and other methods. In addition, this discovery also helps us to understand the growth characteristics and metabolic mechanism of microorganisms in different sugar content environments, and provides a theoretical basis for further optimizing fermentation conditions and increasing saponin yield. In future studies, we plan to combine this finding with other fermentation parameters, such as inoculation volume, temperature, pH, etc., for comprehensive optimization, with a view to achieving more efficient and stable saponin production. At the same time, we will continue to explore the performance of different types of microorganisms under different sugar conditions, in order to find better production strains, and further improve the efficiency of fermentation process and market competitiveness of products.

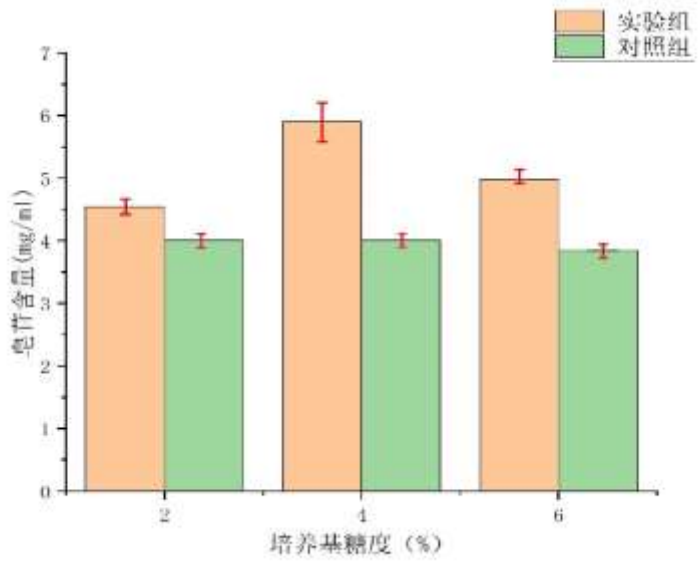

Figure 3.2-**One-factor experiment on media sugar levels**

3.2.2 Results of one-factor experiments with different inoculum levels

The experimental results are shown in Figure 3-3, with the increase of the inoculum amount, the saponin content in the fermentation products appeared to increase and then decrease, and peaked at the inoculum amount of 4%, which shows that the optimal inoculum amount of *Lactobacillus rhamnosus* is at 4%.

With the increase of inoculation amount, the saponin content in fermentation products went through a process of first increasing and then decreasing. This finding revealed the dynamic relationship between the amount of inoculation and the production of saponins, and showed that the production of saponins could reach the maximum under a certain amount of inoculation. Specifically, the experimental data showed that when the amount of inoculation was increased from a lower level, the saponin content in the fermentation products also increased. This may be because with the increase of inoculation amount, the number of microorganisms involved in the fermentation process increased, thus improving the conversion efficiency of ginsenoside precursor substances, resulting in the increase of saponin yield. However, when the amount of inoculation continued to increase to a certain extent, we observed that the saponin content began to decrease. This may be due to the increased

competition among microorganisms caused by excessive inoculation amount, or the relative deficiency of nutrients, which affected the growth and metabolic activities of microorganisms, and then reduced the production of saponins. In particular, it is worth noting that the saponin content in the fermentation products reaches a peak when the inoculated amount is 4%. The results showed that 4% inoculation rate was the best inoculation rate in Lactobacillus rhamnosus fermentation process, which could achieve the highest saponin yield. This means that in actual fermentation production, the selection of 4% inoculated volume can maximize the conversion capacity of microorganisms, resulting in more saponin products. This finding has important guiding significance for optimizing fermentation process. By precisely controlling the amount of inoculation, we can obtain higher quality fermentation products while maintaining fermentation efficiency. In addition, the results also provide experimental basis for further research on the dynamic mechanism of microbial fermentation process, and help to further understand the relationship between microbial growth, metabolism and product formation. In future studies, based on this finding, we can further explore the effects of different inoculations on other aspects of the fermentation process, such as the growth rate of microorganisms and the types and proportions of metabolites. At the same time, it can also be combined with other fermentation conditions, such as temperature, pH value, nutrient supply, etc., to achieve a more efficient and stable saponin production process.

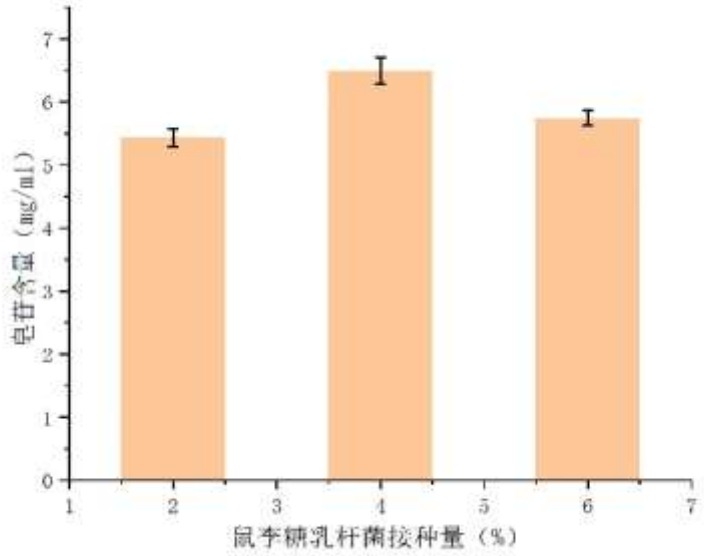

Figure 3.3-**one-way experiments with different inoculum levels**

3.2.3 Results of one-factor experiments at different temperature

The experimental results are shown in Figs. 3.4, with the increase of temperature, the saponin content in the fermentation products appeared to increase and then decrease, and the peak value appeared at 37°C. The optimum fermentation temperature was 37°C, which was consistent with the optimum growth temperature of lactic acid bacteria.

When discussing the effect of temperature on saponin content in fermentation process, we found a very interesting phenomenon: with the gradual increase of temperature, the saponin content in fermentation products showed a trend of first increasing and then decreasing. This observation provides us with important insights into the effect of temperature on the microbial fermentation process. At the beginning of the experiment, we conducted fermentation experiments under a range of different temperature conditions with the aim of determining how temperature affects saponin synthesis. At lower temperatures, we noticed that the saponin content was relatively low. With the gradual increase of temperature, the saponin content began to increase significantly, which may be because the increase of temperature promoted the metabolic activity of microorganisms, thus accelerating the biosynthesis process of saponin. However, when the temperature continues to rise and reaches a certain point, we observe

that the saponin content no longer increases, but instead begins to decline. This trend suggests that excessive temperatures may have an adverse effect on microbial growth and metabolic activity, resulting in a decrease in the efficiency of saponin synthesis. This may be because high temperature may lead to the reduction of enzyme activity or the destruction of protein structure, thus affecting the normal physiological function of microorganisms. In particular, it is worth noting that the saponin content of fermentation products reaches the highest value at 37℃. This finding indicates that 37℃ is the best temperature to obtain the optimal saponin yield. This conclusion is not only based on the analysis of experimental data, but also consistent with the optimal growth temperature of lactic acid bacteria, which further validates our experimental results. This conclusion has important guiding significance for the optimization of fermentation process. By precisely controlling the temperature during the fermentation process, we can obtain more saponin products while ensuring the normal growth and metabolic activities of microorganisms. In addition, this discovery also contributes to our in-depth understanding of the growth characteristics and metabolic mechanisms of microorganisms under different temperature conditions, and provides a theoretical basis for further optimization of fermentation conditions and improvement of saponin yield. In future studies, we plan to combine this conclusion with other fermentation parameters, such as culture medium sugar content, pH value, inoculation amount, etc., for comprehensive optimization in order to achieve more efficient and stable saponin production. At the same time, we will continue to explore the performance of different types of microorganisms under different temperature conditions, in order to find better production strains, and further improve the efficiency of fermentation process and market competitiveness of products. In addition, we will study the effects of temperature changes on other metabolites to fully understand the role of temperature in the microbial fermentation process.

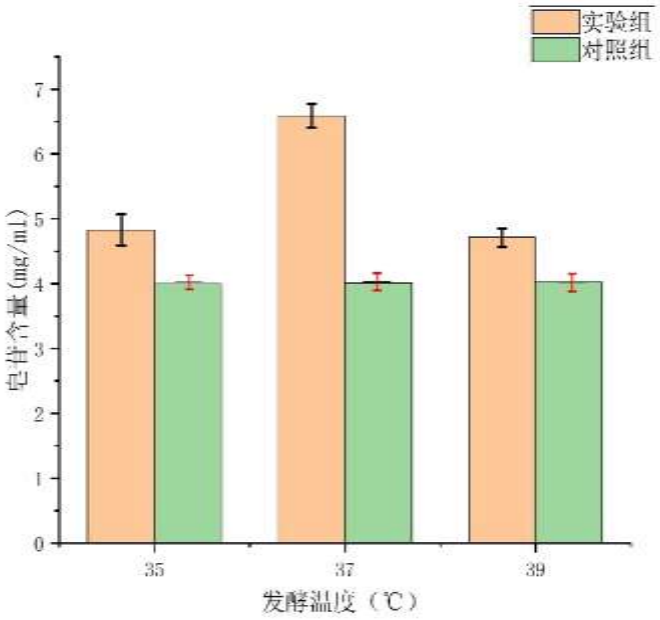

Figure 3.4-**one-way experiments with different fermentation temperatures**

3.2.4 One-way experiments with different fermentation times

The experimental results are shown in Figure 3-5. With the increase of fermentation time, the content of saponins in the fermentation products increases first and then decreases, and the peak value appears at 48h. Therefore, the optimal fermentation time should be 48 h.

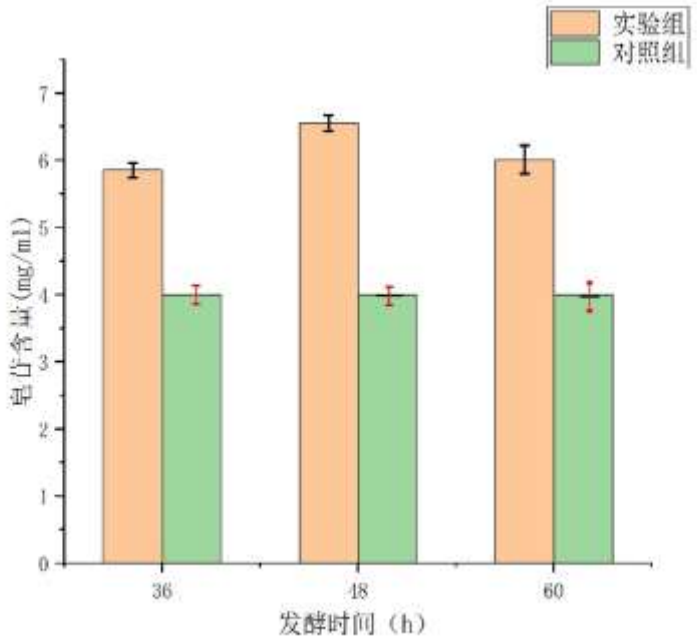

Figure 3.5-**one-way experiments with different fermentation times**

3.3 Integrated experiments

On the premise of obtaining the above optimal fermentation conditions, the fermentation experiment was conducted again under the conditions of 37 ℃ fermentation temperature, 4% addition of medium sugar, 4% inoculation of Lactobacillus rhamnosus strain, and 48 h fermentation time. The experimental results are shown in Figure 3-6. The measured saponin content in the product increased by 60% compared with the control group.

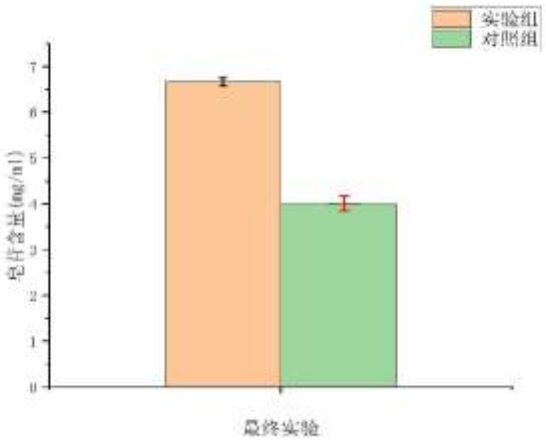

Figure 3.6-**Comprehensive experiment**

CONCLUSIONS

The experimental results of this study showed that the content of saponins in Semen Ziziphi spinosae was higher than that in the blank group under different fermentation conditions. The results showed that Lactobacillus rhamnosus fermentation could effectively promote the increase of saponin content in Semen Ziziphi spinosae, the principle of which was that lactic acid and other acidic substances were secreted during the fermentation process of lactic acid bacteria, which could dissolve the cell wall of Semen Ziziphi spinosae, so that saponins and other effective substances were released outside the cells. By optimizing the fermentation conditions of Lactobacillus rhamnosus, we found that the content of jujuboside in the product was the highest under the conditions of 4% of the fermentation substrate sugar, 37 ℃, 2% of the inoculation amount, and 48 h of fermentation time in the anaerobic and closed environment, which increased by 60% compared with that before fermentation. This study provides an experimental basis for the research and development of new fermented jujuboside products.

Directions and perspectives for follow-up research

Based on the study on the effect of Lactobacillus rhamnosus fermentation on the saponin content of sour date palm kernel, the following are the directions and prospects for further research.

(1) Explore more influencing factors: further study the factors that affect the fermentation of Lactobacillus rhamnosus in wild jujube kernel, such as the pH of fermentation broth, stirring rate, nutrient content, etc., in order to obtain a more appropriate initial program design of Lactobacillus rhamnosus fermentation.

(2) To use bioinformatics to explore the mechanism and law of Lactobacillus rhamnosus fermentation at the basic level, and to design corresponding bioreactors to replace Lactobacillus rhamnosus to realize higher efficiency and lower cost of fermentation production.

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