

# Research Article

# Optimization of the Quality of Chestnut Rose Jiaosu Compound Beverage Based on Probiotic Strains and Fermentation Technology

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Received 29 April 2022; Revised 16 June 2022; Accepted 15 July 2022; Published 11 August 2022

Academic Editor: Bilal Sadiq

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Chestnut rose is an endemic plant in Guizhou Province, China, also known as seedless prickly pear, rich in mineral elements, vitamin C, and flavonoids. With chestnut rose as the main raw material, with grapes, apples, and other fruits as auxiliary ingredients, through the technical process of deastringency, sterilization, fermentation, blending, and other technical processes to obtain the composite beverage, known as a chestnut rose jiaosu compound beverage, it is hereinafter referred to as CRJCB. The CRJCB fermented from chestnut rose juice is nutritious and has a variety of health effects, and the flavor is unique, with good color, aroma, and taste, which is a new type of beverage. However, at present, the fermentation related technology of CRJCB is not perfect, resulting in its low quality, which seriously restricts the industrialization development of CRJCB. In order to improve the quality of the jiaosu compound beverage, probiotics such as Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei, Lactobacillus royale (Lactobacillus reuteri), Lactobacillus acidophilus, and Streptococcus thermophilus, etc., were used. The chestnut rose as the main raw material was used for the fermentation, and grapes, apples, and sugar were used as auxiliary materials to carry out the research on the fermentation of single or mixed probiotics. The key fermentation processes affecting the quality of the jiaosu, such as juice preparation, deastringency of chestnut rose juice, fermentation temperature and time, sugar addition, and taste mixing of the jiaosu, were optimized. The optimized ratio, fermentation process parameters, and strains of probiotics of compound beverage with chestnut rose jiaosu, with high quality, were obtained. The experimental results showed that the SOD enzyme activity value was 167.7 U·ml<sup>-1</sup>, the vitamin C content was 1154.5 mg/100 mL, the soluble solids content was 5.80%, and the nitrite content was 89.8 mg/kg in the compound beverage with chestnut rose jiaosu, obtained by using the optimized ratio and fermentation process parameters proposed in this paper. The development and utilization of chestnut rose resources provide a new idea and is of great significance in promoting the development of the chestnut rose industry.

## 1. Introduction

Chestnut rose (Rosa sterilis) is a variety of prickly pear and belongs to the genus Rosaceae in the family Rosaceae. It was discovered by Shi Shengde in 1985. At present, the chestnut rose is mainly distributed in Anshun and Xingren in Guizhou. It is rich in a variety of amino acids, vitamins, and mineral nutrients. Among them, in the fresh fruit, the vitamin C content is as high as 1133 mg/100 g, 2~11 times higher than that in kiwi, 21 times higher than that in sweet orange, 225 times higher than that in apple, 377 times higher than that in pear; its fresh fruit SOD content of 6000 U/g is over 6 times than that in kiwi, fig, and hawthorn BlackBerry, 12~30 times as that in apple, orange, pear, and grape. In addition, the sugar content of the chestnut rose fruit is 5.04%, 13.3% higher than that of prickly pear, tannin content of chestnut rose is 1.53%, 71.7% lower than that of prickly pear, so the chestnut rose has better taste than prickly pear. In order to promote chestnut rose further, in 2011, Anshun City, Guizhou Province, China, began to try to plant chestnut rose and got very good economic benefits. However, due to the constraints of the fresh fruit preservation technology and processing technology, the further expansion of the chestnut rose market is restricted. Therefore, the development of CRJCB, on the basis of retaining the nutrition of chestnut rose, enriching the production of chestnut rose, can expand the market of chestnut rose effectively.

Jiaosu, also known as fermented fruit and vegetable juices, is increasingly becoming a kind of popular health food [1]. As a kind of jiaosu food rich in vitamin C, amino acids, and other nutrients, CRJCB is receiving more and more attention from experts from all walks of life. However, the research on CRJCB is still in the initial stage, and there are few related reports. At present, the relevant research mainly includes two aspects, on the one hand, the research of food development technology with prickly pear as raw material, and on the other hand, the technology research of jiaosu compound beverage with apple, pear, and other fruits as raw material.

Among them, the research on food development with prickly pear as raw material mainly focuses on important indexes such as antioxidant activity, total phenolic content, antibacterial activity, and stability of the beverage [2–6]. For example, Karabagias used prickly pear juice and its pulp as raw material to develop a biofunctional alcoholic beverage with higher antioxidant activity and total phenolic content than the juice [7]. Garcia-Garcia studied the inhibition of Saccharomyces cerevisiae and Escherichia coli during the storage of prickly pear beverages. The stability of prickly pear beverages was maintained by a pulsed electric field (PEF) technique in combination with other factors [8]. Barba analyzed the effects of traditional and nontraditional processing on prickly pear (Opuntia spp.) and its derivatives [9]. Baccouche studied a whey-based prickly pear beverage. The beverages were found to be physically stabilized by sugar and HM-pectin amount increase using the heat-treated whey [10]. Alvarez used pectin polysaccharides from prickly pear skin to make a functional fermented beverage that remained mechanically and microbiologically stable for up to 42 days at a temperature of 7°C [11].

Research on jiaosu compound beverages using fruits such as apples and pears as raw materials mainly includes studies on fermentation technology, strain screening, probiotic activity, taste, and microbial taxa. For example, Flores analyzed and compared the fermentation characteristics of two low-temperature tolerant yeasts used in cider fermentation [12]. Li et al. studied nonalcoholic fermented apple juice and evaluated its physicochemical parameters, functional composition, aroma, and antioxidant activity at various stages [13]. Velazquez-Quinones et al. analyzed traditional beverages fermented from apple blossom nectar on microbial populations, phenolic substances, antioxidant activity, and digestive enzyme inhibition [14]. Yang et al. conducted a strain screening study for the fermentation of Danshan pear wine [15]. Yang et al. proposed a novel method for the fermentation of pear wine using a mixed inoculum of M. pulcherrima 346 and S. cerevisiae ES488, resulting in higher concentrations of aromatic esters and alcohol than that in pear wine fermented by pure S. cerevisiae [16]. Balthazar et al. developed a new functional fermented beverage using strawberries rich in minerals and proteins

[17]. Fracassetti et al. obtained a fruit beverage with a consumer-friendly flavor by fermenting the juices of grapes, cherries, kiwis, peaches, and strawberries [18]. Kaur et al. prepared a naturally carbonated whey fruit juice based beverage by fermenting a mixture of pineapple juice, strawberry juice, and cheese whey and performed sensory characteristics and shelf life analysis [19, 20]. Mauro et al. studied fermented coconut milk with strawberry pulp [21]. Pereira et al. conducted a study on lactic acid fermentation of unripe tomatoes [22].

Saidah et al. also conducted a study on tomato fermentation and obtained a CMC combination by adding pectin to cheese whey and tomato juice [23]. Liu et al. used Lactobacillus plantarum and Lactobacillus brasiliensis to ferment tomato juice and developed a highly bioactive probiotic beverage [24]. Wang et al. evaluated the effect of tomato juice enriched with the probiotic strain Lactobacillus plantarum ST-III on the flavor and health promoting effects of fermented skim milk [25]. Hashemi et al. used sweet lemon juice fermentation to produce a probiotic juice and found that the ascorbic acid was more stable than that in nonfermented juice [26]. Liu et al. used Issatchenkia terricola for lemon juice fermentation studies and analyzed its dynamic changes in phenolic compounds, organic acids, and antioxidant activity [27]. D'Souza et al. conducted a fermentation study on pomegranate juice and analyzed the free radical scavenging activity, ethanol, and residual sugars of the fermented juice [28]. Escudero-Lopez et al. studied orange juice beverages obtained from alcoholic fermentation and their effect on cardiovascular risk [29, 30]. Mashayekh et al. studied fermentation using a mixture of watermelon, orange, and mango as raw material using Lactobacillus acidophilus [31]. Novelina et al. used pumpkin to make lactic acid bacteria fermented beverages and analyzed the effect of milk powder on the characteristics of pumpkin juice fermented beverages [32]. Ozcan et al. studied the effect of fermentation on the antioxidant activity, total phenols, total flavonoids, and phenolic compounds, and volatile compounds of the essential oil of sea fennel and compared it with fresh samples [33]. Schubertova et al. analyzed the effect of lactic fermentation on the content of organic acids and polyphenols in sea buckthorn juice and explored the role of sea buckthorn fruit and its components in the formulation of new probiotic dairy and nondairy products [34].

In addition, studies have been conducted on red bayberry [35], complex juice fermented smoothie drinks [36], blueberries [37], cantaloupe [38], bacterial microbiota composition [1], and perilla leaves [39].

Through the above research status, we found that the current research on jiaosu mainly focuses on the raw material of conventional fruits such as apple, pear, strawberry, tomato, and lemon, and the research on chestnut rose as raw material is rarely reported.

The research on prickly pear (it is not chestnut rose) beverage is also mainly focused on antioxidant activity, total phenolic content, antibacterial activity, stability, and other important indicators, and the research on its jiaosu compound beverage is also rarely reported. The quality factors such as nutrient content, flavor, and SOD enzyme activity of

Reagent name	Grade	Manufacturers	Reagent name	Grade	Manufacturers
Nutritional agar	Biological	Beijing landbridge	Sodium hydroxide	Analysis	Wuxi yatai united chemical
Nutritional agai	reagents	technology co.	Sourum nyuroxide	pure	со.
Peptones	Biological	Beijing auboxing	Ascorbic acid	Analysis	Sinopharm group chemical
reptones	reagents	biotechnology co.	Ascorbic acid	pure	reagent co.
Yeast paste	Biological	Beijing auboxing	Oxalic acid	Analysis	Sinopharm group chemical
Teast paste	reagents	biotechnology co.	Oxalic acid	pure	reagent co.
Poof posto	Biological	Beijing auboxing	Potassium ferricyanide	Analysis	Sinopharm group chemical
Beef paste	reagents	biotechnology co.	Potassium lenneyamde	pure	reagent co.
Ammonium citrate	A malazzia muna	Tianjin comio chemical	Zin a sulph sta	Analysis	Sinopharm group chemical
Ammomum citrate	Analysis pure	reagent co. Zinc sulphate		pure	reagent co.
Potassium dihydrogen	Amalazzia muna	Cilona asian as as	n-Octanol	Analysis	Tianjin comio chemical
phosphate	Analysis pure	Silong science co. n-Octanol		pure	reagent co.
Manganese sulphate	Analysis pure	Tianjin zhiyuan chemical	o-Benzenetriol	Analysis	Silong acionea co
Manganese suipilate	Analysis pure	reagent co.	0-Belizelleti lõi	pure	Silong science co.
Disodium hydrogen	Analysis pure	Sinopharm group	Tris (hydroxymethyl)	Analysis	Tianjin comio chemical
phosphate	Allalysis pule	chemical reagent co.	aminomethane	pure	reagent co.
Glucose	Analysis pure	Tianjin comio chemical	Cellulase	Analysis	Beijing hong run baoshun
Glucose	Allalysis pule	reagent co.	Cellulase	pure	technology co.
Magnesium sulphate	Analysis pure	Tianjin comio chemical	Pectinase	Analysis	Beijing hong run baoshun
heptahydrate	Analysis pure	reagent co.	Pectillase	pure	technology co.
Sodium chloride	Analusia nura	Tianjin comio chemical			
Soutum chloride	Analysis pure	reagent co.			

TABLE 1: Experimental reagents.

CRJCB are affected by multiple factors such as strains and the fermentation process. Therefore, the strains and fermentation processes of different jiaosu vary greatly. Since no research has been conducted on the strains and fermentation processes suitable for high quality CRJCB, we have explored the fermentation strains and key fermentation processes that affect the quality of CRJCB, aiming to obtain the optimal ratios, fermentation process parameters, and strains that meet the quality requirements of CRJCB.

## 2. Materials and Methods

2.1. Materials and Reagents. The experimental materials are fresh chestnut rose picked from the chestnut rose base in Shuangbao Town, Xixiu District, Anshun City, Guizhou Province. The experimental strains were obtained from China Microbial Strain Collection Management Center, including Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus acidophilus, Streptococcus thermophilus, and other strains. The reagents mainly include nutrient agar, peptone, yeast paste, beef paste, ammonium citrate, potassium dihydrogen phosphate, manganese sulphate, and disodium hydrogen phosphate, etc. See Table 1 for details.

2.2. Instrumentation. The instruments and equipment mainly used in the experiment include UH-220 electronic balance produced by Shenzhen Ruisheng Technology Co., FA1004 analytical balance produced by Shenzhen Ruisheng Technology Co., HHS-21-6 thermostatic water bath produced by Shanghai Boxun Industrial Co., TC16-WS high-speed centrifuge produced by Shanghai Anting Scientific Instruments Co., UVmini-1240 UV spectrophotometer produced by Shenzhen Ruisheng Technology Co., JYC-

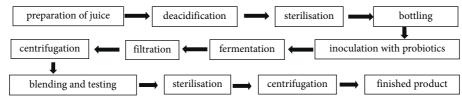
TABLE 2: Single-factor experimental formulation table.

Name of experimental material	Dosage
Chestnut rose juice	500 ml
Sterile water	500 ml
Strain	50 cfu
White granulated sugar	50 g

2HR10 induction cooker produced by Guangdong Midea Life Electric Appliance Manufacturing Co., SW-CJ-2D clean bench produced by Shanghai Yiheng Scientific Instruments Co., DPH-360 biochemical incubator produced by Shanghai Yiheng Scientific Instruments Co., YXQ-LS-50A autoclave produced by Guangzhou Shenhua Biotechnology Co., WAY-2WAJ Refractometer from Shanghai Precision Instrument Science Co., PHS-2F PH meter produced by Shanghai Precision Instrument Science Co., TAISITEWAL-230B electric blast dryer produced by LIChen Instrument Technology Co., and YJL-C022 soybean milk machine produced by Guangdong Midea Life Electric Appliance Manufacturing Co.

#### 2.3. Experimental Methods

2.3.1. Experimental Method for Single-Strain Fermentation. Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus acidophilus, and Streptococcus thermophilus were used for the individual fermentation of chestnut rose juice. The strains were selected using the SOD enzyme activity value and production flavor as the selection index. Firstly, 500 ml of chestnut rose juice, 50 g of sugar, and 50 cfu of the fermentation strain were added to the fermenting container separately. See Table 2 for the recipe. Only one fermenting bacterium was inoculated in





each fermentation flask and 3 parallel experiments were done for each strain. The product was then sealed and fermented at 35°C for 20 days, filtered, and centrifuged, and the product was tested for organoleptic and SOD enzyme activity values. Finally, the results were analyzed according to the average values of SOD enzyme activity calculated for the different fermentation methods.

2.3.2. Experimental Method for Mixed Strain Fermentation. The nutrient content of jiaosu and the type of nutrients varied between probiotic fermentation. Based on the results of the preliminary experiments, the experimental design was two combinations of *Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei,* and *Lactobacillus reuteri* as groups A, B, and C. The fermentation was carried out under the same conditions. The combinations of A, B, and C are as follows.

Group A: Lactobacillus delbrueckii subsp. Bulgaricus and Lactobacillus casei.

Group B: Lactobacillus reuteri and Lactobacillus delbrueckii subsp. bulgaricus

Group C: Lactobacillus reuteri and Lactobacillus casei.

Add 500 ml of chestnut rose juice, 50 g of sugar, and 50 cfu of fermenting bacteria into the fermentation flasks. After fermentation, the product was filtered and centrifuged at  $35^{\circ}$ C for 20 days, and the average value of SOD enzyme activity under different fermentation methods was calculated.

2.3.3. Experimental Method for Optimizing the Production Process of Chestnut Rose Jiaosu. The main steps of this experiment is shown in Figure 1.

Since the preparation of juice, deastringency of chestnut rose juice, fermentation temperature and time, the amount of sugar added, and the blending of jiaosu taste are the key technologies that affect the quality of jiaosu compound beverage, we have done experimental exploration on these technologies, and the related methods are as follows.

(1) Juice preparation method

Preparation of chestnut rose juice: Firstly, the ripe chestnut rose fruit was selected and soaked in 20% salt water for 40 minutes in order to remove some of the bacteria and viruses. The chestnut roses were washed twice in sterile water to remove dust and brine from the surface. The cleaned fruit was cut, the seeds were removed and the mass of the chestnut rose fruit was recorded as  $R_g$ . Then, put into the juicer for breaking while adding  $50\% \times R_g$  of sterile water,  $0.3\% \times R_g$  of cellulase, and  $0.1\% \times R_g$  pectinase. Finally, the juice of the chestnut rose was UV sterilized and sealed in the refrigerator.

Preparation of grape juice: Firstly, the grapes of the Kyoho grape variety were soaked in 20% salt water for 40 minutes to remove some bacteria and viruses. The grapes were washed 2 times to remove dust and brine, and the weight of the grapes was recorded as  $G_g$ . The washed grapes were then placed into a juicer with  $30\% \times G_g$  of sterile water to break. Then, the coarser pieces were filtered out using a 150 mesh sieve. The juice was then placed into a centrifuge and centrifuged at 10,000 rpm twice. Finally, the juice was UV sterilized and placed in the refrigerator.

Preparation of apple juice: Firstly, fresh apple fruit of 70%–80% ripeness and free from pests were selected, peeled, and chopped. The apple pulp mass was recorded as  $A_g$  and then placed in a juicer with 50% ×  $A_g$  sterile water for juicing. The coarser pieces were filtered out through a 150 mesh filter sieve and then repeatedly filtered 3 times with skimmed cotton and gauze. Afterwards, the juice was centrifuged twice at 10,000 rpm in a centrifuge. Finally, the apple juice was UV sterilized and sealed in the refrigerator.

(2) Chestnut rose juice deastringency process optimization experimental method

Fresh chestnut rose fruit has its own sour and astringent flavor, which is mainly derived from the organic acids and small amounts of tannins in the fruit. Pretreatment for chestnut rose juice is to solve the problem of astringency. It is called deastringency. Heating is used for deastringency in this study. The main advantage of chestnut rose jiaosu is that it is rich in vitamin C. Since vitamin C is destroyed in the structure at temperatures above 80°C, this experiment was heated at 74°C, 76°C, 78°C, 80°C, 82°C, and 84°C for 2 min, 3 min, 4 min, 5 min, 6 min, and 7 min, respectively, and its orthogonal experiment is shown in Table 3. The vitamin C content of chestnut rose juice was measured before and after the treatment, and the retention rate of vitamin C under different treatment conditions was calculated.

(3) Experimental method for fermentation temperature optimization

Temperature is one of the main factors affecting microbial activity, and either too high or too low a

TABLE 3: Orthogonal experiments on temperature and heating time of deastringency.

Level	Factor	s
Level	Temperature (°C)	Time (min)
1	74	2
2	76	3
3	78	4
4	80	5
5	82	6
6	84	7

temperature will affect the quality of jiaosu. In order to find the most suitable fermentation temperature, this paper used the temperature single-factor experiment to explore the best fermentation temperature. The experiment was conducted with 500 ml of chestnut rose juice and 50 g of sugar. The fermentation temperatures were controlled to be 28°C, 30°C, 32°C, 34°C, and 36°C, respectively, and *Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei* were used at constant fermentation temperatures. After 20 days of fermentation, the vitamin C content and flavor of the ferment were used as a selection indicator.

(4) Experimental method for fermentation time optimization

Fermentation time is one of the key factors in determining the quality of jiaosu. If the fermentation time is too short, jiaosu will not mature, resulting in poor taste and flavor. If the fermentation time is too long, the long-term accumulation of fermentation probiotics metabolites will lead to an increase in the total acid content of jiaosu. At the same time, too long a fermentation time will also lead to the fruit pomace becoming smaller and dissolving in jiaosu, thus increasing the difficulty of processing the jiaosu at a later stage. As chestnut rose juice undergoes microbial fermentation, the metabolism of microorganisms will produce nitrite. Therefore, the experimental fermentation termination time is based on the content of nitrite in jiaosu. Using 500 ml chestnut rose juice, 50 g sugar, choosing Lactobacillus delbrueckii subsp. Bulgaricus and Lactobacillus casei were mixed for fermentation. The fermentation temperature was controlled at 32°C. The fermentation was carried out on day 7, day 9, day 11, day 13, day 15, day 17, and day 19 after fermentation, and the nitrite and vitamin C content in chestnut rose jiaosu were used as reference indicators.

(5) Experimental method for optimizing the amount of sugar added

The production process of jiaosu is to use the metabolism of microorganisms to transform the organic matter in raw materials *into* the substances we need. Microorganisms need energy to grow and reproduce and metabolize, so a certain amount of sugar needs to be added to the chestnut rose juice as

an energy source for the microorganisms. The amount of sugar added will affect the quality of jiaosu. In order to explore the best sugar ratio, we designed five different groups of white granulated sugar mass ratios for fermentation under the same other conditions. Experiments were conducted using 500 ml of chestnut rose juice, different masses of white granulated sugar, and Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei at 32°C for 15 days fermentation. The ratio of chestnut rose juice to white sugar was 10:1 (group A), 10:1.5 (group B), 10:2 (group C), 10:2.5 (group D), and 10:3 (group E). The soluble solids content and sensory evaluation of the fermented juice were carried out to establish the relationship between the amount of white granulated sugar added and the quality of chestnut rose jiaosu.

(6) Optimization of jiaosu taste blending method

*Using* only chestnut rose as a raw material will result in a single flavor, making it difficult to achieve consumer satisfaction. Grape jiaosu has antioxidant properties and can remove excess free radicals from the body to reduce damage to the organism. Apple jiaosu has good antioxidant properties and can prevent various diseases, slow down the aging process, and prevent constipation. Therefore, grape and apple were chosen to adjust the taste and flavor of the product, and the two solutions were used to study the taste and flavor of chestnut rose jiaosu.

The first option is an orthogonal experiment with mixed fermentation of fruit juice. Chestnut rose juice, apple juice, grape juice are mixed together for fermentation. The amount of grape juice was 10 ml, 15 ml, and 20 ml, the amount of apple juice was 20 ml, 25 ml, and 30 ml, and the amount of chestnut rose juice was 50 ml, 70 ml, and 90 ml, as shown in Table 4.

The second option was a mixed juice blend experiment. The experiment was conducted with apple juice and grape juice to blend chestnut rose jiaosu. The amount of grape juice was 10 ml, 15 ml, and 20 ml, the amount of apple juice was 20 ml, 25 ml, and 30 ml, and the amount of chestnut rose jiaosu was 50 ml, 70 ml, and 90 ml, as shown in Table 5.

2.3.4. Measurement Parameters. This experiment mainly focuses on parameters such as SOD enzyme activity force value, vitamin C content, nitrite content, and *E. coli* count. The test method is mainly based on the relevant requirements of the national mandatory standards of the People's Republic of China.

In addition, an organoleptic evaluation was conducted. The organoleptic evaluation of the compound beverage with chestnut rose jiaosu included color, odor, aroma, taste, uniformity, and consistency. The main scoring was done by 100 tasters (50 male, 50 female) who scored the jiaosu according to the requirements of the scoring rules, and the scoring table is shown in Table 6.

Level	Grape juice	B apple juice	C Chestnut rose juice
	(ml)	(ml)	(ml)
1	10	20	50
2	15	25	70
3	20	30	90

TABLE 4: Orthogonal experiments for the fermentation of complex juices.

#### 3. Results and Analysis

#### 3.1. Results of Single-Strain Fermentation Experiments

3.1.1. Sensory Performance of Jiaosu under Fermentation with Different Strains of Bacteria. As can be seen from Table 7, jiaosu from individual fermentation of chestnut rose juice using Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei and Lactobacillus reuteri have good taste and color performance separately. The taste was mainly characterized by a strong aroma of chestnut rose; without astringency and slightly sour, the color was mainly yellow. However, it became darker after fermentation with Lactobacillus acidophilus, and it tastes slightly bitter after fermentation with Streptococcus thermophilus.

3.1.2. Effect of Fermentation with Different Strains of Probiotics on the SOD Enzyme Activity Values of Jiaosu. From Figure 2, it can be seen that under the same fermentation conditions, the order of SOD enzyme activity force values in the fermented chestnut rose jiaosu was group using Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus casei, Lactobacillus royale (Lactobacillus reuteri), Lactobacillus acidophilus, and Streptococcus thermophilus. The SOD enzyme activity of all five species was 120 U·ml<sup>-1</sup> after 20 days of constant fermentation at 35°C, and the highest SOD enzyme activity was found in the jiaosu fermented by Lactobacillus delbrueckii subsp. Bulgaricus.

#### 3.2. Results of Fermentation Experiments with Mixed Strains

3.2.1. Effect of Mixed Strain Fermentation on Enzyme SOD Activity Values in Jiaosu. As can be seen from Figure 3, under the same fermentation conditions, the SOD enzyme activity force values in jiaosu were in the order of Group A (Lactobacillus delbrueckii subsp. Bulgaricus) and Lactobacillus casei), Group C (Lactobacillus royale (Lactobacillus reuteri) and Lactobacillus casei), Group B (Lactobacillus reuteri and Lactobacillus delbrueckii subsp. Bulgaricus)). After 10 days of constant fermentation at 35°C, the SOD enzyme activity of all three combinations was 140 U·ml<sup>-1</sup>, while the jiaosu was fermented by Lactobacillus delbrueckii subsp. Bulgaricus, and Lactobacillus casei had the highest SOD enzyme activity values. The highest SOD enzyme activity values were found in the jiaosu fermented by Lactobacillus delbrueckii subsp. Group A is the optimal strain combination.

3.3. Chestnut Rose Juice Deastringency Process Optimization Results

3.3.1. Effection of Different Heating Temperatures and times on the Retention of Vitamin C. As can be seen from Figure 4, when the temperature was at 74°C and 76°C, the retention rate of vitamin C showed a decreasing trend after heating for 7 min, but it still remained above 80%; at 78°C, the retention rate of vitamin C was already below 70% after heating for 7 min; when the temperature reached 80°C, the retention rate of vitamin C obviously showed a significant decrease in the time period of 0~4 min, and when heated to 3 min the retention rate of vitamin C was less than 50%. When the temperature was 82°C, the retention rate of vitamin C was lower than that at 80°C during the same heating time, and the retention rate of vitamin C was less than 30% when the temperature was 82°C heated for 3 min. Therefore, when heated at 74°C and 76°C for 7 min, the retention rate of vitamin C in the process of deastringency of chestnut rose juice was higher than 80%.

3.3.2. Effect of Different Heating Temperature and Time on Sensory Evaluation of Chestnut Rose Juice. Overall sensory scores increased with increasing heating temperature for the same heating time, i.e.,  $82^{\circ}C > 80^{\circ}C > 78^{\circ}C > 76^{\circ}C > 74^{\circ}C > 72^{\circ}C$ . Sensory scores increased with a longer heating time at the same heating temperature. The sensory scores were all above 80 when heated to 7 min at 74°C, 76°C, 78°C, 80°C, 82°C, and 84°C, as shown in Figure 5.

#### 3.4. Process Results for Fermentation Temperature Optimization

3.4.1. Effect of Different Temperature Fermentation Conditions on Vitamin C in Jiaosu. The fermentation temperature was 28°C, 30°C, 32°C, 34°C, and 36°C, and the highest vitamin C content was 1240 mg/100 mL at 34°C, while the lowest was 1191 mg/100 mL at 30°C (Figure 6). The overall effect of fermentation temperature on the vitamin C content of the jiaosu was not significant, fermented at 34°C with a relatively high vitamin C content.

3.4.2. Effect of Jiaosu Flavor under Different Temperature Fermentation Conditions. As can be seen from Table 8, the study examined fermentation at different temperatures:  $28^{\circ}$ C,  $30^{\circ}$ C,  $32^{\circ}$ C,  $34^{\circ}$ C, and  $36^{\circ}$ C, with other conditions being the same, and the results were that there was a significant difference in the taste of the jiaosu as the temperature increased, with the  $32^{\circ}$ C group having the best taste. In terms of color, there was no significant change at the beginning of the temperature increase, and when the temperature reached  $32^{\circ}$ C and beyond, its color began to change significantly. It can be seen that the fermentation at  $32^{\circ}$ C resulted in a better flavor of the jiaosu.

Level		Factors	
Level	A grape juice (ml)	B apple juice (ml)	C Chestnut rose jiaosu (ml)
1	10	20	50
2	15	25	70
3	20	30	90

#### TABLE 5: Juice blending orthogonal experiment.

#### TABLE 6: Sensory rating scale.

Projects	Standard	Score
Color and lustra (20 nointe)	Pale red	16-17
Color and lustre (20 points)	Orange	17 - 20
	With a rich aroma of chestnut rose	18-20
$\Lambda$ normal (20 noints)	Clearly fragrant	16-17
Aromas (20 points)	Light fragrance	14-15
	No aroma or very lightly scented	<14
	Compound beverage with chestnut rose jiaosu has a good flavor and is pleasant to the palate	36-40
Tests (40 points)	Compound beverage has a distinctive flavor and no irritation in the mouth	32-35
Taste (40 points)	Compound beverage with a pronounced and unpleasant taste in the mouth	28-31
	No distinctive drink characteristics, no sensation in the mouth	<28
	Uniform and transparent, no precipitation	18-20
Product appearance (20 points)	Homogeneous, poor clarity, no sedimentation	16-17
	Splitting occurs, with sedimentation after standing	14-15
	Significant stratification, large amounts of sediment present	<14

#### TABLE 7: Sensory performance of jiaosu under fermentation with different strains of bacteria.

Strain	Lactobacillus delbrueckii subsp. Bulgaricus	Lactobacillus casei	Lactobacillus reuteri	Lactobacillus Acidophilus	Streptococcus thermophilus
Taste	With a mild scent of chestnut rose, nonastringent but slightly sour	With a mild scent of chestnut rose, nonastringent but slightly sour	With a lighter scent of chestnut rose, nonastringent but slightly sour	With a lighter scent of chestnut rose, nonastringent but slightly sour	With a lighter scent of chestnut rose, nonastringent but slightly bitter
Color	Yellow	Pale yellow	Yellow	Dark yellow	Yellow

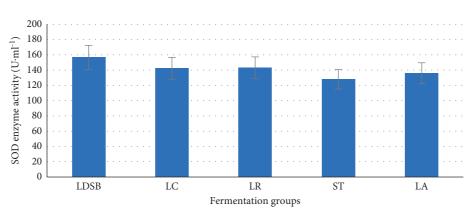


FIGURE 2: Effect of fermentation with different strains of bacteria on SOD enzyme activity force (P < 0.05).

#### 3.5. Process Results for Fermentation Time Optimization

3.5.1. Effect of Fermentation Time on the Vitamin C Content of the Jiaosu. From Figure 7, it can be seen that the vitamin C content in the chestnut rose jiaosu tends to decrease with the fermentation time, but the decrease is relatively small.

The vitamin C content in jiaosu remained above 1200 mg/100 mL from day 7 to 19.

3.5.2. Effect of Fermentation Time on the Nitrite Content of Jiaosu. From Figure 8, it can be seen that under the same

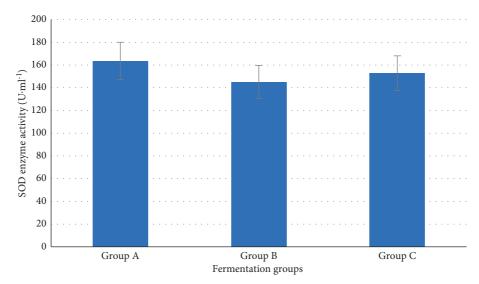
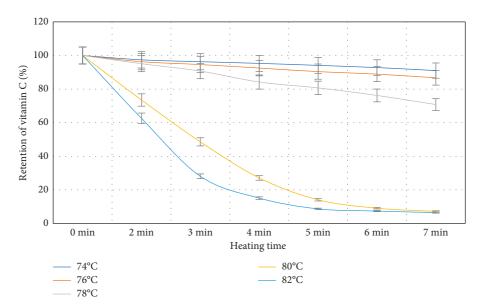


FIGURE 3: SOD enzyme activity values under mixed strain fermentation conditions (P < 0.05).



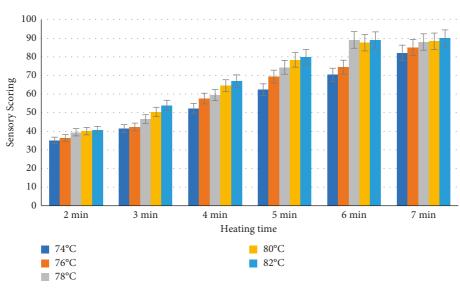


FIGURE 4: Variation of vitamin C retention with time at different heating temperatures.

FIGURE 5: Variation in sensory scores of chestnut rose juice with heating time for different heating temperatures and times (P < 0.05).

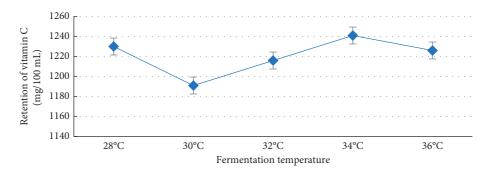


FIGURE 6: Effect of different heating temperatures on the vitamin C content of chestnut rose jiaosu.

Taste	Color
With a mild scent of chestnut rose, astringent flavor	Pale yellow
With a lighter scent of chestnut rose and less astringency	Pale yellow
With a strong scent of chestnut rose, nonastringent but slightly sour	Pale yellow
With a lighter scent of chestnut rose and slightly sour, pungent acidity	Yellow
With a lighter scent of chestnut rose and more acidic, pungent taste	Dark yellow
	With a mild scent of chestnut rose, astringent flavor With a lighter scent of chestnut rose and less astringency With a strong scent of chestnut rose, nonastringent but slightly sour With a lighter scent of chestnut rose and slightly sour, pungent acidity

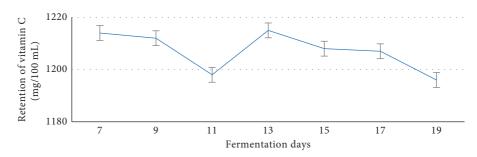


FIGURE 7: Effect of different fermentation times on the vitamin C content of chestnut rose jiaosu.

fermentation conditions, the content of nitrite in chestnut rose jiaosu changes with time as that: it increases first and then decreases. At day 9, the nitrite content reaches a peak of 1643 mg/kg. From day 9, the nitrite content starts to decrease. From day 9 to 15, the nitrite content appears to decrease significantly. After day 15 the nitrite content is stable at 94 mg/kg.

3.5.3. Effect of Different Fermentation times on the Flavor of Jiaosu. As can be seen from Table 9, the results of the study are that there is a significant difference in the taste of the jiaosu as the fermentation time becomes longer, with the best taste being achieved on days 13–15 under the same conditions and with different fermentation times: 7, 9, 11, 13, 15, 17, and 19 days. There is no significant change in color at the beginning of the fermentation time, but after the 11th day of fermentation, there is a significant change in color.

#### 3.6. Process Results for Optimizing the Amount of Sugar Added

3.6.1. Effect of Sugar Addition on Soluble Solids Content of Jiaosu. The overall soluble solids content increased with the

addition of white granulated sugar under the same fermentation conditions, gradually increasing from 5.2% for a volume of chestnut rose juice to white granulated sugar mass ratio of 10:1 to 7.6% for a volume of chestnut rose juice to white sugar mass ratio of 10:3, as shown in Figure 9.

3.6.2. Effect of Sugar Addition on the Sensory Evaluation of the Jiaosu. From the overall view of the sensory score in the same fermentation conditions with the increase in the amount of sugar added appeared to first increase and then decrease phenomenon, when the volume of chestnut rose juice to white sugar mass ratio at 10:1.5, the sensory score reached the highest 87 points, chestnut rose juice volume to white sugar mass ratio greater than 10:1.5, sensory score gradually decreased, the lowest value in the chestnut rose juice volume to white sugar mass ratio of 10:3 was 70 points, as shown in Figure 10.

#### 3.7. Results of the Optimization Process for Jiaosu Taste and Flavor Blending

3.7.1. Results of Orthogonal Experiments on Mixed Fermentation of Fruit Juices. The greater the extreme difference

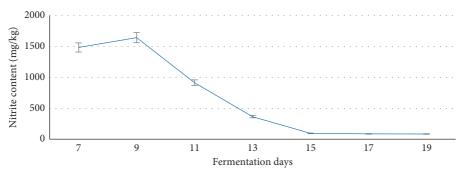


FIGURE 8: Variation of nitrite content with fermentation time.

TABLE 9: Effect of different fermentation times on the taste and flavor of the jioasu.

Fermentation time (days)	Taste	Color
7	With a mild scent of chestnut rose, astringent flavor, sweeter and less sour	Pale yellow
9	With a mild scent of chestnut rose, astringent flavor, sweeter and less sour	Pale yellow
11	With a mild scent of chestnut rose, astringent flavor, sweet and less sour	Pale yellow
13	With a mild scent of chestnut rose, less astringent flavor, less sweeter and mild sour	Yellow
15	With a mild scent of chestnut rose, less astringent flavor, less sweeter and mild sour	Yellow
17	With a mild scent of chestnut rose, less astringent flavor, less sweeter and more sour	Yellow
19	With a mild scent of chestnut rose, less astringent flavor, no sweet and more sour	Dark yellow

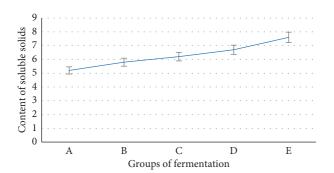


FIGURE 9: Variation of soluble solids with sugar addition.

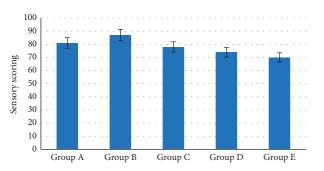


FIGURE 10: Variation of sensory scores with the amount of sugar added (P < 0.05).

(*R*) value of the factors, the more obvious the results from Table 10 can be seen C > A > B, indicating that the amount of chestnut rose has the greatest impact on the sensory evaluation of the product, so the amount of chestnut rose jiaosu must be appropriate. The extreme difference of factor *A* is greater than factor *B*, which means that the amount of grape juice has a greater influence on the sensory evaluation of the

product than the amount of apple juice. From Table 10, it can be seen that the best combination of grape, apple, and chestnut rose composite jiaosu is  $A_2 B_3 C_{1,}$  that is, grape juice 15 ml, apple juice 30 ml, and chestnut rose jiaosu 50 ml.

3.7.2. Results of Orthogonal Experiments on the Dosage of Compound Juice Ratios. The greater the *R* values, the more obvious the effect of the factor on the results. From Table 11, we can see that C > A > B, which means that the amount of chestnut rose jiaosu has the greatest effect on the sensory evaluation of the product, so it must be appropriate. The *R* of factor *A* is greater than factor *B*, which means that the amount of grape juice has a greater influence on the sensory evaluation of the product than that of apple juice. From Table 11, it can be seen that the best combination of grape, apple, and chestnut rose jiaosu is  $A_2 B_1 C_2$ , that is, grape juice 10 ml, apple juice 20 ml, and chestnut rose jiaosu 70 ml.

#### 3.8. Production Process Validation Results

3.8.1. Test Results for SOD Enzyme Activity, Vitamin C Content, Soluble Solids, and Nitrite Content. Six parallel tests were carried out for SOD enzyme activity, vitamin C content, soluble solids content, and nitrite content, respectively, in the validation experiment. The results showed that the average value of SOD enzyme activity was  $167.7 \text{ U} \cdot \text{ml}^{-1}$  with an error of  $\pm 3.1\%$ ; the average content of vitamin C was 1154.5 mg/100 mL with an error of  $\pm 1.7\%$ ; the average content of soluble solids was 5.80% with an error of  $\pm 1.3\%$ ; the average content of nitrite was 89.8 mg/kg with an error of  $\pm 3.15\%$ . Its nitrite content is lower than the national regulation in beverage food: 0.15 g/kg, which is in line with the national standard. The relevant data are shown in Table 12.

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Even online out a such ou	Factor				
Experiment number	Α	В	С	Average score for sensory evaluation	
1	1	1	1	3.5	
2	1	2	2	3.9	
3	1	3	3	3.8	
4	2	1	2	4.0	
5	2	2	3	3.8	
6	2	3	1	4.6	
7	3	1	3	3.9	
8	3	2	1	4.5	
9	3	3	2	3.7	
<i>K</i> <sub>1</sub>	11.2	11.4	12.6		
K <sub>2</sub>	12.4	12.2	11.6		
$\overline{K_3}$	12.1	12.1	11.5		
k <sub>1</sub>	3.73	3.80	4.20		
$k_2$	4.13	4.07	3.87		
$\tilde{k_3}$	4.03	4.03	3.83		
R	0.30	0.27	0.37		

TABLE 10: Results of orthogonal experiments on the fermentation dosage of compound juice in scheme I.

TABLE 11: Results of orthogonal experiments on the dosage of compound juice ratios in scheme II.

Ever anim ant assembles	Factor				
Experiment number	Α	В	С	Average score for sensory evaluation	
1	1	1	1	3.6	
2	1	2	2	3.9	
3	1	3	3	4.0	
4	2	1	2	4.3	
5	2	2	3	3.5	
6	2	3	1	4.1	
7	3	1	3	3.9	
8	3	2	1	4.2	
9	3	3	2	3.8	
$K_1$	11.5	11.8	11.9		
$K_2$	11.9	11.6	12.0		
$\tilde{K_3}$	11.9	11.9	11.4		
$k_1$	3.83	3.93	3.97		
$k_2$	3.97	3.87	4.00		
$\tilde{k_3}$	3.97	3.97	3.80		
Ř	0.14	0.10	0.20		

*3.8.2. E. coli Count Results.* The test results showed that the coliforms did not exceed 3 MPN/100 g, which is in line with the national standard, as shown in Table 13.

3.8.3. Electronic Nose Test Results. According to the preliminary experiment and pilot experiment to arrive at the best production process, through the final test experiment to produce composite beverage samples, the samples will be tested by an electronic nose, the smaller the point dispersion on the graph, indicating that the more similar the active ingredients of the composite juice, the better the taste and flavor. It was finally concluded that the best jiaosu odor was obtained by fermenting chestnut rose juice alone first, then using grape juice 10 ml, apple juice 20 ml, and chestnut rose juice 70 ml for blending, as shown in Figure 11.

#### 4. Discussion

Through the techniques in the paper, the CRJCB obtained has the characteristics of strong aroma, good taste, pleasant in the mouth, and uniform color. However, we found that the juice is prone to browning in the process of deastringency. After analysis, we believe that the main reason for it is caused by enzymatic oxidation. In view of this, increasing the temperature of the deastringency treatment so that the enzyme inactivation or reduce the activity, which is an effective means to prevent browning. However, we need to effectively remove the tannins in chestnut rose while preserving the flavor as much as possible and to obtain a good sensory score and vitamin C retention. Therefore, the temperature of deastringency is not suitable for high temperature. So, the deastringency temperature needs to be controlled in a lower range.

TABLE 12: Test results for SOD enzyme activity, vitamin C content, soluble solids content, and nitrite.

Serial number	Irrigation no. 1		Irrigation no. 2		Irrigation no. 3		A	Error
	1	2	1	2	1	2	Average	EIIOI
SOD enzyme activity force value U·ml <sup>-1</sup>	169.5	172.4	168.2	162.4	168.6	164.9	167.7	±3.1%
Vitamin C content (mg/100 mL)	1174	1134.5	1162.8	1157.1	1154.8	1143.9	1154.5	$\pm 1.7\%$
Soluble solids %	5.72	5.86	5.81	5.74	5.87	5.81	5.8	±1.3%
Nitrite content mg/kg	91	87	90	92	89	90	89.8	±3.15%

TABLE 13: Final test results for E. coli.

Serial number		Original e	Blank controls	
		Dilute 100 times	Dilute 1000 times	Diank controls
Irrigation no. 1	1	165	37	0 colonies
	2	197	41	0 colonies
Irrigation no. 2	1	215	51	0 colonies
	2	148	48	0 colonies
Irrigation no. 3	1	172	36	0 colonies
	2	167	43	0 colonies

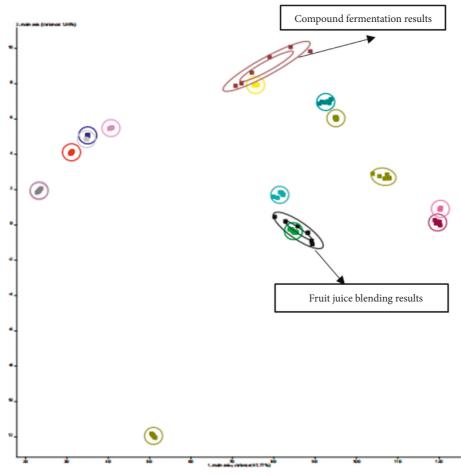


FIGURE 11: Electronic nose measurement results.

In our fermentation experiments, we found that the quality of the jiaosu obtained by mixed fermentation with *Lactobacillus deutschenbachia Bulgarian subspecies* and *Lactobacillus casei* was better than other strain combinations

or single-strain fermentation. We believe that the main reason for this is that the different strains in the mixed strains collaborate with each other and influence each other to enhance their physiological advantages through physical and biochemical activities to achieve complementary optimization of metabolic flow nodes. Therefore, in the subsequent experiments, we will further investigate the differences in the composition and content of jiaosu flavor substances between single-strain fermentation and mixed fermentation so as to explore the changes in the metabolic flow of mixed strains.

As people pay more attention to nutrition, health, and safety, low-calorie flavoring agents are increasingly popular. Therefore, we used apple juice and grape juice as flavoring agents to maintain low sugar in the composite beverage while not only satisfying consumers' demand for taste but also reducing the intake of calories. Through sensory evaluation experiments, we found that the use of apple juice and grape juice for flavoring can significantly improve the taste and sweetness of golden prickly pear enzyme complex beverage, and the sweetness is crisp without leaving an aftertaste, so we choose both as auxiliary flavoring agents at the same time, and further optimize the formulation of golden prickly pear enzyme complex beverage on the basis of the test, which has achieved better results. From the perspective of the market and social demand, the sensory and nutrients of golden prickly pear enzyme complex beverage are an important index for consumers to accept the product, and subsequent research can be carried out on the aroma composition, nutrient content, and stability of golden prickly pear enzyme complex beverage to further improve the taste, stability, and storage quality of golden prickly pear enzyme complex beverage.

# **5.** Conclusion

In this paper, chestnut rose fresh fruit was used as the raw material and used single-factor experiment and orthogonal experiment to study the deastringency of chestnut rose, the screening of fermentation strain, fermentation temperature, fermentation time, the amount of sugar added, and the blending ratio of compound beverages. It finally produced a pale yellow, chestnut rose aroma, good taste with a uniform color of the jiaosu. This paper is mainly on the chestnut rose to deastringency treatment, fermentation strain screening, fermentation temperature, time, the amount of sugar added, juice proportioning relationship to optimize the research and achieve certain results. It can be used as a theoretical basis for the production of chestnut rose and compound beverages.

- (1) Chestnut *rose* juice deastringency technology: the organic acid and a small amount of tannin in the fresh chestnut rose fruit lead to a sour and astringent taste, which can be treated by using high temperature soaking. Experiments show that the chestnut rose juice to  $76^{\circ}$ C in the constant temperature heating 7 min, so as to achieve the chestnut rose to deastringency treatment and can obtain a better vitamin C retention rate and sensory.
- (2) Chestnut rose juice fermentation technology: temperature, bacteria, and fermentation time are important factors affecting the microbial activity and

jiaosu quality. Experiments showed that a mixture of *Lactobacillus deutschenbachia Bulgarian subspecies* and *Lactobacillus casei* was used to obtain a high SOD enzyme activity by fermentation at the temperature of  $32^{\circ}$ C. The fermentation time was determined to be 15 d based on the nitrate and vitamin C content, with a nitrite content of 94 mg/kg and a vitamin C content of 1208 mg/100 mL.

(3) Optimization of flavoring agents and auxiliary ratios: in order to obtain good organoleptic scores and soluble solids *content*, a ratio of 10:1.5 of chestnut rose juice volume to sugar mass was used based on taste and electronic nose test results, and chestnut rose juice was fermented alone firstly and then blended with 10 ml of grape juice, 20 ml of apple juice, and 70 ml of chestnut rose jiaosu, resulting in the best aroma of the compound beverage with chestnut rose jiaosu.

# **Data Availability**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

# **Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

# Acknowledgments

This study was a phase result of the Guizhou Provincial Science and Technology Plan Project (project no. Qiankehe Foundation [2019] 1152) and the Guizhou Institute of Technology High-Level Talent Initiation Project (project no. XJGC20190927).

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