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# Optimization of Culture Conditions of High-yield Caproic Acid Compound Bacteria and Its Application in Xiaoqu Qingxiangxing Baijiu

**Wei Liao [1], Youhui Deng [2, 3, 4], Pengsen Shen [4], Mindan Zhang [1], Zonghua Ao [1, 2, 3], Caihong Shen [2, 3], Junjie Jia [2, 3], Bin Ni [1, 2, 3, *], Xiaogang Liu [2, 3, *]**

[1] Sichuan University of Science & Engineering, Yibin Sichuan, 644000, China
[2] Luzhou Laojiao Co., Ltd., Luzhou Sichuan, 646000, China
[3] National Engineering Technology Research Center of Solid-State Brewing, Luzhou Sichuan, 646000, China
[4] Meihekou Branch of National Engineering Research Center of Solid-state Brewing, Meihekou, Jilin 135000, China

*** Corresponding author:** Bin Ni and Xiaogang Liu

**Abstract:** In order to determine the best culture conditions of caproic acid-producing compound bacteria liquid, and explore a brewing technology of baijiu with both qingxiangxing and nongxiangxing flavor. In this study, caproic acid-producing compound bacteria solution was used as the experimental object, and the optimum process of caproic acid-producing compound bacteria solution was optimized by single factor, Plackett-Burman and response surface experiments. Then the caproic acid-producing compound bacteria solution was applied to the brewing process of xiaoqu qingxiangxing baijiu. The optimum culture conditions for caproic acid production were as follows: ethanol 2%, culture temperature 37 °C, dipotassium hydrogen phosphate 0.8g /L, anhydrous sodium acetate 5.0g /L, corn steep liquor dry powder 2.0g /L, initial pH value 8, liquid volume 70%. Under these conditions, the caproic acid yield could reach 5.07 g/L. Compared with the alcoholic fermentative material of traditional xiaoqu qingxiangxing baijiu, the alcoholic fermentative material inoculated with caproic acid compound bacteria solution had no significant difference in acidity, significantly increased moisture, and significantly decreased starch and reducing sugar. At the same time, compared with the traditional xiaoqu qingxiangxing alcoholic fermentative material, the content of caproic acid was significantly increased to 3.382 ug/g, the content of acetic acid was significantly decreased to 0.126 ug/g, the content of caproic acid was significantly decreased to 0.126 ug/g, the content of ethyl caproate was significantly increased to 1.593 ug/g, the content of ethyl caproate was significantly increased to 1.593 ug/g, and the content of ethyl acetate was significantly decreased to 0.945 ug/g. This study revealed the effect of caproic acid-producing compound bacteria on the physical and chemical indexes and flavor substances of traditional xiaoqu qingxiangxing alcoholic fermentative material, and significantly increased the content of ethyl caproate, which had guiding significance for the production of qingxiangxing and nongxiangxing baijiu.

**Keywords:** Compound Bacterial Liquid of Caproic Acid; Caproic Acid; Xiaoqu Qingxiangxing Baijiu; Flavor Substances.

## 1. Introduction

Xiaoqu qingxiangxing baijiu is made of sorghum, wheat, etc. as raw materials. After steaming grain and drying, it is produced by a process of solid-state fungus cultivation and saccharification in a tank, then fermentation in a tank, and finally distillation. This kind of wine is characterized by a soft and sweet taste, with Xiaoqu unique fragrance and bad aroma. Its technical characteristics include the use of whole grain raw materials, through soaking grain, braising grain, steaming grain, drying, adding xiaoqu saccharification, mixing grains into the tank for fermentation, solid distillation, with high wine yield, short fermentation period characteristics [1], its disadvantage is that the wine body fullness is not enough, short after taste.

The nongxiangxing baijiu, one of the four fundamental aroma types in China, enjoys widespread popularity among the public. It is characterized by its rich fragrance, mellow and sweet taste, and harmonious aroma [2]. The brewing process of nongxiangxing baijiu is fundamentally a process of microbial interaction [3]. The use of mud pits for solid-state fermentation is a significant feature in the production of nongxiangxing baijiu. The saying "a thousand-year-old pit produces ten-thousand-year-old residue" underscores the close relationship between the quality of nongxiangxing baijiu and its fermentation pit and residue. A key factor determining the aromatic characteristics of this type of baijiu lies in its undergoing at least 1-2 months of solid-state fermentation within a mud pit [4].

The fermentation mud serves as the primary carrier for microorganisms during the fermentation process and is rich in a variety of functional microbes [5]. The diverse microbial metabolism within the fermentation mud leads to the production of organic acids such as caproic acid, lactic acid, acetic acid, and butyric acid. These organic acids act as precursors for ester compounds, with ethyl caproate being the predominant aromatic ester in nongxiangxing baijiu. Consequently, the concentration of organic acids significantly influences the quality of nongxiangxing baijiu [6,7]. Among these functional microbes, caproic acid bacteria are notable for their ability to produce caproic acid; this metabolite undergoes esterification with ethanol to form ethyl caproate, which constitutes a major aromatic component in nongxiangxing baijiu [8]. Currently, a significant number of researchers are identifying caproic acid bacterial strains through isolation and purification, and conducting fermentation characteristic studies on different purified strains. However, there is relatively limited research on the composite microbial liquid from pit mud [9-11]. Research findings indicate that the application of composite functional microbial liquids is significantly superior to that of pure functional bacterial fermentations [12,13]. Therefore, it is essential to conduct further cultivation of the stable production of caproic acid composite microbial liquid and to optimize its culture conditions and components through experimental research. The nutrients and fermentation conditions (including the culture medium) required for the growth of microbial strains or microbial liquids significantly influence the level of microbial growth. Therefore, each type of microbial fermentation product has its optimal formulation of culture medium and specific growth and fermentation conditions [14].

This study focuses on the production of caproic acid using a composite bacterial liquid. Through single-factor experiments, the optimal components and cultivation conditions of the medium were determined. Subsequently, Plackett-Burman experiments and response surface methodology were employed to identify the best fermentation conditions and component concentrations for the caproic acid-producing composite bacterial liquid. As a result, the optimal cultivation conditions for this composite bacterial liquid were established. At the same time, the production of caproic acid bacterial liquid was inoculated into the alcoholic fermentative material of xiaoqu qingxiangxing baijiu. The physicochemical properties and flavor components of the alcoholic fermentative material were studied to provide a reference for the production of baijiu that combines both qingxiangxing and nongxiang styles.

# 2. Materials and Methods

## 2.1. Materials and Reagents

### 2.1.1. Reagents and Materials

Raw materials: xiaoqu qingxiangxing alcoholic fermentative material and caproic acid composite bacterial liquid, sourced from a certain distillery in Sichuan.

Reagents: Anhydrous sodium acetate, ammonium sulfate, potassium ferrocyanide, zinc sulfate, phenolphthalein, yeast extract, corn syrup dry powder: Fuchen (Tianjin) Chemical Reagent Co., Ltd.; Hydrochloric acid: Chongqing Chuandong Chemical (Group) Co., Ltd; Sodium hydroxide, anhydrous glucose, methylene blue, dipotassium hydrogen phosphate, pentahydrate copper sulfate, potassium sodium tartrate, heptahydrate magnesium sulfate, and anhydrous ethanol: Chengdu Cologne Chemical Co., Ltd. The reagents used in the above experiments are all of analytical grade. The hexanoic acid, phosphoric acid, ethanol, and methanol were obtained from Shenggong Biological (Shanghai) Co., Ltd., and are all of chromatographic purity.

### 2.1.2. Culture medium

Ethanol-Sodium Acetate Medium (ES Medium) [15]: Yeast extract 1.0 g/L, $CH_3COONa$ 5.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, $K_2HPO_4$ 0.4 g/L, $(NH_4)_2SO_4$ 0.5 g/L. After sterilization at 121 °C for 20 minutes, sterile operation is performed to add anhydrous ethanol to a final concentration of 2%.

## 2.2. Instruments and Equipment

SX-300 Fully Automatic High-Pressure Steam Sterilizer: Shanghai Boxun Medical Biological Instrument Co., Ltd.;DHG-9420A Electric Heating Forced Air Drying Oven: Shanghai Yiheng Scientific Instrument Co., Ltd; TGL-20B High-Speed Refrigerated Centrifuge: Shanghai Anting Scientific Instrument Factory;HCB-1300V Clean Workbench: Qingdao Haier Biomedical Co., Ltd.;BSA224S Electronic Balance: Sidoscience Instruments (Beijing) Co., Ltd; SB25-12DTD Ultrasonic Cleaning Machine: SCIENTZ New Zhi"; GCMS-QP2020NX Gas Chromatograph-Mass Spectrometer, LC-20AT Liquid Chromatograph, HP-INNOWax Capillary Column (60 m × 0.25 mm × 0.25 μm): Shimadzu Corporation, Japan; C18 chromatography column (2.1 mm × 100 mm × 1.7 μm): Waters Corporation, USA; Multiskan SkyHigh full-wavelength microplate reader: Thermo Fisher Scientific.

## 2.3. Experimental Methodology

### 2.3.1. Culture of Strain

The caprylic acid composite microbial solution was inoculated into the ES culture medium at a 10% inoculation rate, with a liquid volume of 90%. The mixture was then incubated at 37 °C for 7 days [15].

### 2.3.2. Optimization of Cultivation Conditions for High-Yield Caproic Acid: A Single-Factor Experiment

In the context of ES culture medium [16, 17], we conducted a study on various types of carbon sources (soluble starch, sodium acetate anhydrous, glucose, and corn starch) were added at a concentration of 5.0 g/L. The types of nitrogen sources (ammonium sulfate, corn steep powder, yeast extract, and peptone) were added at a concentration of 1.0 g/L. The addition levels of Anhydrous sodium acetate (0.0 g/L, 1.0 g/L, 3.0 g/L, 5.0 g/L, 7.0 g/L, and 9.0 g/L). The addition levels of corn syrup dry powder (0.0 g/L, 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, and 2.5 g/L). The addition levels of dipotassium hydrogen phosphate (0.0 g/L, 0.4 g/L, 0.8 g/L, 1.2 g/L, 1.6 g/L, and 2.0 g/L). Ethanol addition levels (0%, 1%, 2%, 3%, 4%, and 5%). Initial pH levels (5, 6, 7, 8, 9, and 10). Cultivation temperatures (28 °C, 31 °C, 34 °C, 37 °C, 40 °C, and 43 °C) and liquid volume (50%, 60%, 70%, 80%, 90%, and 100%) on the production of caproic acid by composite bacterial cultures were investigated.

### 2.3.3. Plackett-Burman Experiment

In this study, based on single-factor experiments and using caproic acid yield as the evaluation criterion, we selected ethanol (*A*), anhydrous sodium acetate (*B*), dipotassium hydrogen phosphate (*C*), corn steep powder (*D*), liquid volume (*E*), cultivation temperature (*F*), and initial pH value (*G*) as the factors for assessment. A Plackett-Burman (PB) experiment was conducted to evaluate these factors. The design of the PB experiment, including its factors and levels, is presented in Table 1.

**Table 1.** Plackett-Burman experimental design factors and levels

| Factors | Levels | |
|---|---|---|
| | -1 | 1 |
| *A* (initial pH) | 7 | 9 |
| *B* (culture temperature) / ° C | 34 | 40 |
| *C* (liquid volume) /% | 60 | 80 |
| *D* (ethanol) /% | 1 | 3 |
| *E* (anhydrous sodium acetate)/(g/L) | 3 | 7 |
| *F* (corn syrup dry powder)/(g/L) | 1.5 | 2.5 |
| *G* ($K_2HPO_4$)/(g/L) | 0.4 | 1.2 |

### 2.3.4. Response Surface Methodology Optimization

According to the PB experiment, significant factors were identified, with ethanol (*D*), temperature (*B*), and dipotassium hydrogen phosphate (*G*) determined as the three primary influencing factors. Using these three factors as independent variables and caprylic acid yield (*Y*) as the response variable, a Box-Behnken response surface experiment was designed employing Design-Expert software. The experimental factors and their levels are presented in Table 2.

**Table 2.** Factors and Levels of response surface tests for optimization of fermentation conditions

| Factors | Levels | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| *A* (ethanol) /% | 1 | 2 | 3 |
| *B* (culture temperature) / ° C | 34 | 37 | 40 |
| *C* ($K_2HPO_4$)/(g/L) | 0.4 | 0.8 | 1.2 |

### 2.3.5. Application of Caproic Acid Composite Bacterial Liquid in the Production Process of Xiaoqu Qingxiangxing Baijiu

The process flow is illustrated in Figure 1. Select plump grains of japonica sorghum that are uniform in color, free from mold and off-odors. Soak the selected grains in hot water at a temperature range of 74 to 78°C for 22 to 24 hours. After draining, perform an initial steaming under normal pressure for 40 minutes. Subsequently, steam the grains in hot water at approximately 80 to 85°C for an additional 120 minutes. After draining again, conduct a second steaming under normal pressure for another 30 minutes. The cooked grains should meet the standards of being free from raw centers internally and not sticky externally. Finally, spread the cooked grains out to cool down to a temperature range of 22 to 26°C before use. The xiaoqu (starter culture) was mixed uniformly with the sun-dried sorghum. After a saccharification period of 24 hours, the grains and the alcoholic fermentative material were blended in a ratio of 1:3 until homogeneous. The mixture was then spread out to cool at temperatures ranging from 20 to 24 °C before being transferred into glass jars for preliminary fermentation [18]. Following six days of preliminary fermentation, a compound bacteria solution producing caproic acid was added for subsequent fermentation, which lasted for 40 days before concluding the fermentation process [19]. The physicochemical indicators and flavor compounds of the alcoholic fermentative material were analyzed upon completion. Additionally, a control sample consisting of normally fermented xiaoqu qingxiangxing alcoholic fermentative material from within the workshop was utilized for comparison.

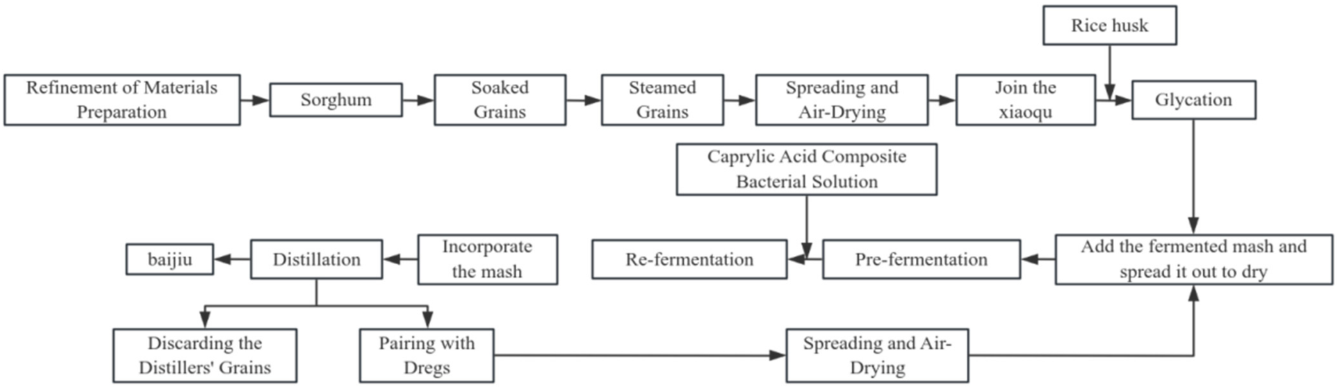

**Fig 1.** process flow diagram

### 2.3.6. Determination of Caproic Acid in Fermentation Broth

The caproic acid in the fermentation broth was determined using GC-MS. The sample pretreatment and measurement conditions were referenced from the method described by Guo Ying et al. [20].

### 2.3.7. Determination of Physicochemical Indicators and Volatile Components in Alcoholic Fermentative Material

The determination of acidity, reducing sugars, moisture content, and starch in the alcoholic fermentative material refers to DB 34/T 2264—2014. The volatile components in the alcoholic fermentative material are analyzed using GC-MS, with sample analysis conditions based on the methods outlined by Pu Lingping et al. [21]. The measurement of lactic acid in the alcoholic fermentative material is conducted via liquid chromatography, with sample pretreatment and analytical conditions referencing the methodology established by Yu Songbai et al. [22].

### 2.3.8. Data Analysis

The data organization was conducted using Excel 2021, while the statistical analysis of experimental data was performed with SPSS version 27.0. A one-way ANOVA procedure was employed for independent t-tests, and graphical representations were created utilizing Origin 2024 and SIMCA 14.1 software.

# 3. Results and Discussion

## 3.1. The Impact of Medium Components on The Production Yield of Caproic Acid by Mixed Bacterial Cultures.

### 3.1.1. The Impact of Carbon Source Types and Optimal Carbon Source Addition Amount on Caproic Acid Production

The carbon sources used in the culture medium included soluble starch, glucose, sodium acetate anhydrous, and corn starch. The other components were identical to those of the ES culture medium. This study investigated the impact of different carbon sources on the production yield of caproic acid by a mixed bacterial culture capable of producing caproic acid, as illustrated in Figure 2a. All four carbon sources were utilized by the caproic acid-producing mixed bacterial culture; however, the yields of caproic acid varied with different carbon sources, indicating varying degrees of dependence on these substrates. When sodium acetate anhydrous was employed as a carbon source, the maximum yield of caproic acid reached 3.67 g/L, which was significantly higher than that obtained from other carbon sources ($P < 0.05$). Therefore, sodium acetate anhydrous is identified as the optimal carbon source for this mixed bacterial culture. Additionally, we examined how varying amounts of sodium acetate anhydrous affected caproic acid production; results are presented in

Figure 2b. As the concentration gradient of sodium acetate anhydrous increased, there was initially a rise followed by a decline in caproic acid yield. Within a range of 0 to 5.0 g/L for sodium acetate anhydrous concentration, caproic acid levels increased with rising concentrations until reaching a peak at 5.0 g/L with a maximum yield of 3.70 g/L before gradually decreasing thereafter. In conclusion, we recommend using sodium acetate anhydrous as the optimal carbon source at a concentration level of 5.0 g/L for enhanced production efficiency.

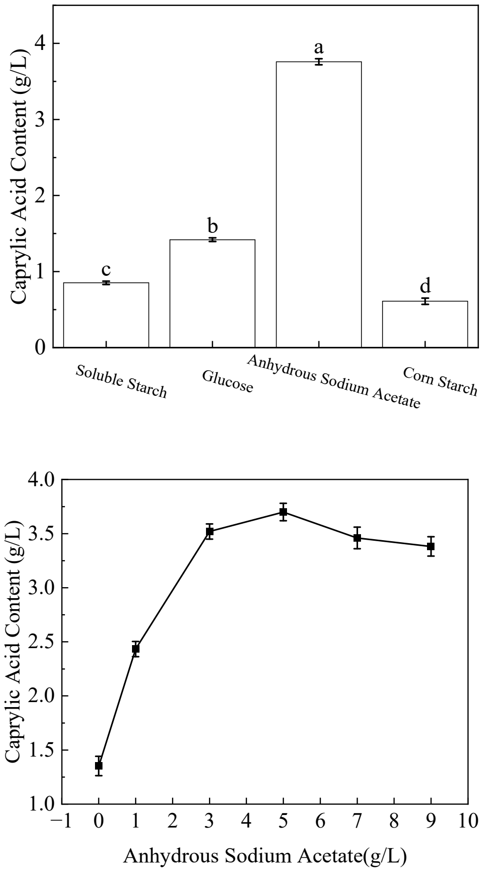

**Fig 2.** a. Effects of carbon sources on caproic acid production b. Effects of anhydrous sodium acetate addition on caproic acid production

Note: Different lowercase letters indicate significant differences ($P < 0.05$) (and similarly hereafter)

### 3.1.2. The Impact of Different Types of Organic Nitrogen Sources and Optimal Addition Amounts on Caproic Acid Production

The organic nitrogen sources used in the culture medium included peptone, yeast extract, ammonium sulfate, and corn steep powder. The other components were identical to those of the ES medium. This study investigated the effects of different organic nitrogen sources on the production yield of caproic acid by a mixed bacterial culture. The results are presented in Figure 3a. Under conditions where only the organic nitrogen source was altered, corn steep powder yielded the highest caproic acid production at 3.76 g/L, followed closely by yeast extract at 3.69 g/L. In contrast, ammonium sulfate and peptone resulted in lower yields of caproic acid. Thus, corn steep powder is identified as the optimal organic nitrogen source for this mixed bacterial culture aimed at producing caproic acid. Additionally, we examined how varying amounts of corn steep powder affected caproic acid yield; these findings are illustrated in Figure 3b. The addition of corn steep powder significantly influenced caproic acid production. As the amount of corn steep powder increased incrementally, there was an initial rise in caproic acid yield followed by a decline thereafter. When the concentration reached 2.0 g/L, maximum production was achieved at 3.88 g/L before rapidly decreasing. in conclusion, we recommend using corn steep powder as the most suitable organic nitrogen source with an optimal addition level set at 2.0 g/L for enhanced caproic acid production.

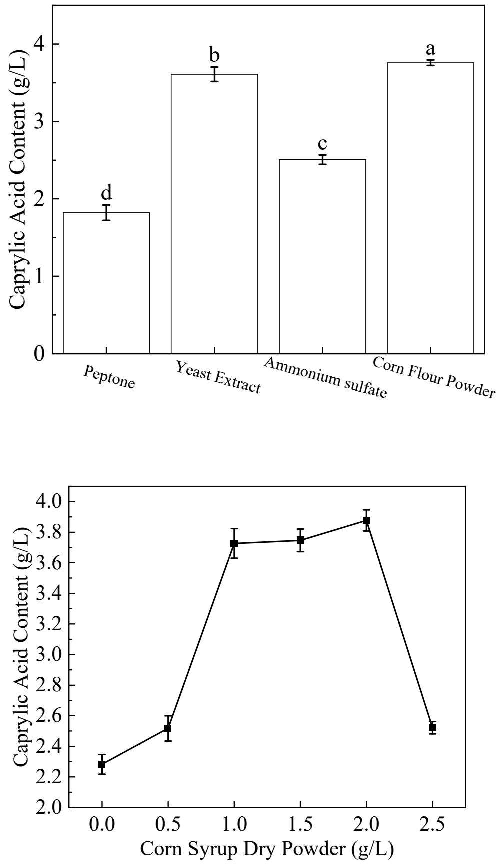

**Fig 3.** a. Effect of nitrogen source type on caproic acid yield b. Effect of corn pulp dry powder addition amount on caproic acid yield

### 3.1.3. The Impact of Dipotassium Hydrogen Phosphate Addition on Caprylic Acid Yield

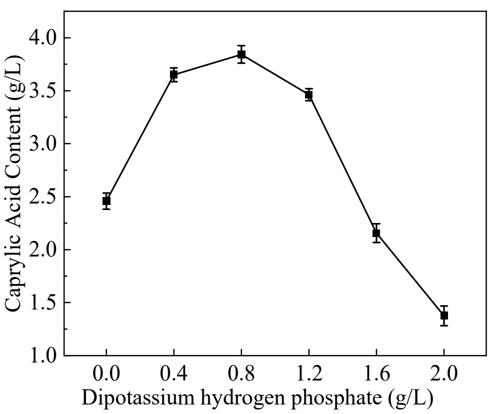

**Fig 4.** Effect of dipotassium hydrogen phosphate supplemental level on caproic acid yield

Potassium hydrogen phosphate, as a trace element in the culture medium for the production of caproic acid by composite microbial liquid, has a significant impact on enhancing caproic acid yield. The effect of varying amounts of potassium hydrogen phosphate on caproic acid production is illustrated in Figure 4. As the amount of potassium hydrogen phosphate increases, the yield of caproic acid initially rises and then declines. The maximum yield of 3.84 g/L is achieved when potassium hydrogen phosphate is added at a concentration of 0.8 g/L. Therefore, the optimal addition level of potassium hydrogen phosphate for producing caproic acid using composite microbial liquid is determined to be 0.8 g/L.

#### 3.1.4. The Impact of Ethanol Addition on the Yield of Caproic Acid

Ethanol plays a significant role in the growth of caproic acid-producing bacteria. As illustrated in Figure 5, with an increasing gradient of ethanol addition, the yield of caproic acid initially rises and then declines. At ethanol concentrations of 0% and 1%, the caproic acid yields are at their lowest, measuring only 0.31 g/L and 0.44 g/L, respectively. When the ethanol concentration reaches 2%, there is a rapid increase in caproic acid production, peaking at a value of 3.58 g/L. However, as the ethanol concentration continues to rise beyond this point, the yield of caproic acid gradually decreases again. This decline may be attributed to the inherent toxicity of ethanol; excessive concentrations can inhibit both the growth and metabolism of caproic acid bacteria [23]. Therefore, it can be concluded that the optimal addition level of ethanol for producing caproic acid using mixed bacterial cultures is 2%.

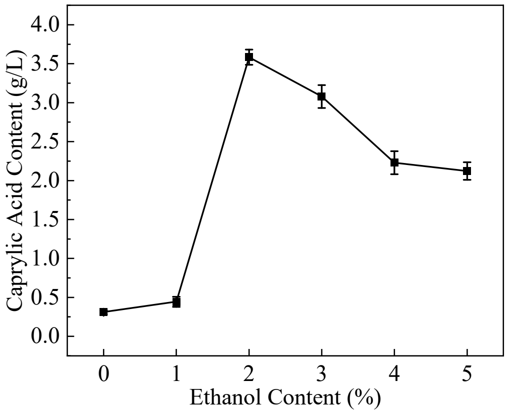

**Fig 5.** Effect of ethanol addition on caproic acid yield

### 3.2. The Influence of Cultivation Conditions on the Production Yield of Caproic Acid by Composite Bacterial Liquid

#### 3.2.1. The Impact of Initial pH on Bacterial Density and Caproic Acid Production

As shown in Figure 6, with the increase of the initial pH value ranging from 5 to 10, the bacterial density exhibits a trend of rapid increase followed by stabilization and eventual decline. When the initial pH is set at 5, the bacterial density is at its lowest. At an initial pH of 6, there is a swift rise in bacterial density to a relatively high level. The maximum bacterial density occurs when the initial pH values are between 7 and 8. However, as the initial pH reaches values between 9 and 10, the optical density (OD) begins to decrease, indicating that a near-neutral environment is conducive to the growth of caproic acid-producing microbial consortia. Simultaneously, as the gradient of initial pH increases, caproic acid production initially rises before experiencing a sharp decline. The maximum caproic acid yield was 3.81 g/L at the initial pH value of 8, which was higher than that at other initial pH values. Therefore, it can be concluded that an optimal initial pH value for producing caproic acid using this microbial consortium is determined to be 8.

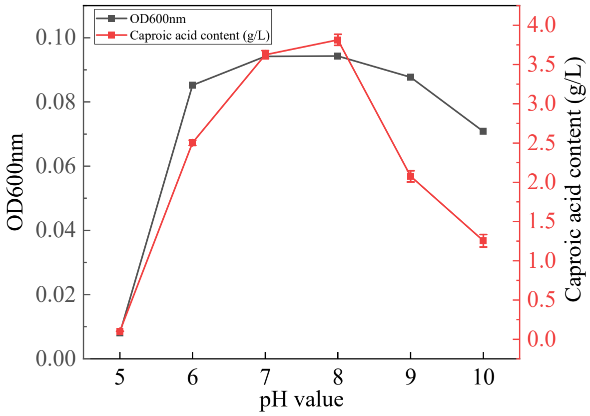

**Fig 6.** Effect of initial pH on cell density and caproic acid production

#### 3.2.2. The Impact of Cultivation Temperature on Microbial Density and Caproic Acid Production

As shown in Figure 7, with the increase of cultivation temperature within the range of 28 to 43 °C, the microbial density exhibits a trend of initially rising and then declining. The maximum microbial density is reached at a cultivation temperature of 40 °C. When the cultivation temperature exceeds 40 °C, the microbial density begins to decrease. Concurrently, as the cultivation temperature gradient increases, the concentration of caproic acid also shows an initial rise followed by a decline. The highest yield of caproic acid occurs at a cultivation temperature of 37 °C, reaching 3.66 g/L. Therefore, the optimal cultivation temperature for producing caproic acid using mixed bacterial cultures is determined to be 37 °C.

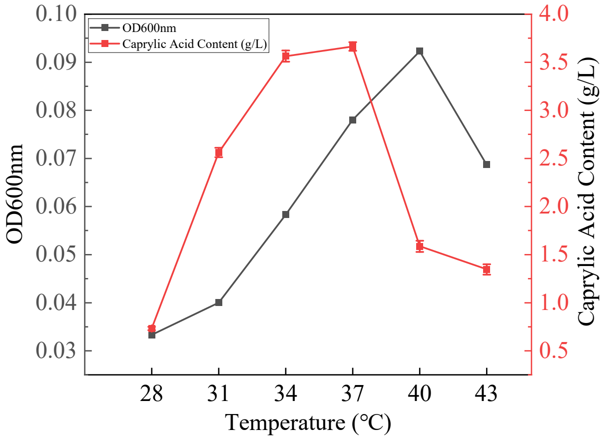

**Fig 7.** Effect of culture temperature on cell density and caproic acid production

#### 3.2.3. The Impact of Liquid Volume on Bacterial Density and Caproic Acid Production

As shown in Figure 8, with the increase of liquid volume within the range of 50% to 100%, both microbial density and caproic acid content initially rise and then decline. This indicates that either excessively high or low oxygen levels are detrimental to the growth and metabolic acid production of caproic acid bacteria. When the liquid volume reaches 70%, both microbial density and caproic acid content peak, resulting in a maximum caproic acid yield of 3.80 g/L.

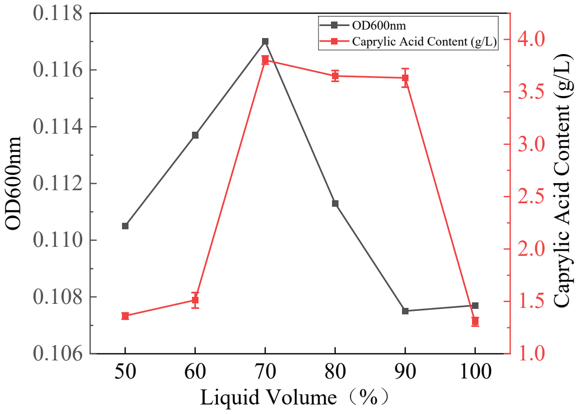

**Fig 8.** Effects of liquid volume on bacterial density and caproic acid production

However, as the liquid volume continues to increase, there is a noticeable downward trend in both microbial density and caproic acid content. This may be attributed to the gradual decrease in oxygen concentration within the fermentation environment, which adversely affects the metabolic activity of caproic acid bacteria [11]. Notably, at a liquid volume of 100%, the yield of caproic acid drops to its lowest point at only 1.30 g/L. Therefore, it can be concluded that the optimal liquid volume for producing mixed cultures capable of synthesizing caproic acid is 70%.

## 3.3. Optimization of Caproic Acid Production by Composite Bacterial Liquid Using Plackett-Burman Experimental Design

In the context of single-factor experiments, a PB experimental design was conducted to investigate the effects of several factors on caprylic acid production. The factors considered include initial pH value (*A*), cultivation temperature (*B*), liquid volume (*C*), ethanol (*D*), anhydrous sodium acetate (*E*), corn slurry dry powder (*F*), and dipotassium hydrogen phosphate (*G*). The PB experimental design and results are presented in Table 3, while the variance analysis is detailed in Table 4.

**Table 3.** Plackett-Burman experimental design and results

| Experiment Number | A | B | C | D | E | F | G | Caprylic Acid Content (g/L) |
|---|---|---|---|---|---|---|---|---|
| 1 | 7 | 34 | 80 | 1 | 0.7 | 0.25 | 0.04 | 2.12 |
| 2 | 9 | 40 | 60 | 3 | 0.7 | 0.25 | 0.04 | 1.81 |
| 3 | 9 | 34 | 80 | 3 | 0.7 | 0.15 | 0.04 | 2.60 |
| 4 | 9 | 34 | 60 | 1 | 0.7 | 0.15 | 0.12 | 2.39 |
| 5 | 7 | 34 | 60 | 1 | 0.3 | 0.15 | 0.04 | 2.13 |
| 6 | 9 | 34 | 80 | 3 | 0.3 | 0.25 | 0.12 | 3.35 |
| 7 | 7 | 34 | 60 | 3 | 0.3 | 0.25 | 0.12 | 3.52 |
| 8 | 9 | 40 | 60 | 1 | 0.3 | 0.25 | 0.04 | 1.56 |
| 9 | 9 | 40 | 80 | 1 | 0.3 | 0.15 | 0.12 | 1.88 |
| 10 | 7 | 40 | 60 | 3 | 0.7 | 0.15 | 0.12 | 2.59 |
| 11 | 7 | 40 | 80 | 1 | 0.7 | 0.25 | 0.12 | 2.18 |
| 12 | 7 | 40 | 80 | 3 | 0.3 | 0.15 | 0.04 | 2.70 |

**Table 4.** Analysis of variance for Plackett-Burman test results

| Sources | Sum of Squared Deviations | Degrees of Freedom | Mean square | F value | P value | Significance |
|---|---|---|---|---|---|---|
| Model | 3.72 | 7 | 0.53 | 16.15 | 0.009 | ** |
| A | 0.23 | 1 | 0.23 | 6.90 | 0.058 | |
| B | 0.96 | 1 | 0.96 | 29.15 | 0.006 | ** |
| C | 0.06 | 1 | 0.06 | 1.75 | 0.260 | |
| D | 1.55 | 1 | 1.55 | 47.11 | 0.002 | ** |
| E | 0.18 | 1 | 0.18 | 5.33 | 0.082 | |
| F | 0.00 | 1 | 0.00 | 0.16 | 0.710 | |
| G | 0.75 | 1 | 0.75 | 22.67 | 0.009 | ** |
| Residual error | 0.13 | 4 | 0.03 | | | |
| Total | 3.85 | 11 | | | | |

Note: "*" indicates a significant impact on the results ($P < 0.05$); "**" denotes an extremely significant impact on the results ($P < 0.01$). The same applies hereafter.

## 3.4. Design and Results of the Box-Behnken Experimental Method

### 3.4.1. Results of Response Surface Experiments and Variance Analysis

Based on the single-factor experiments and PB tests, ethanol (*A*), temperature (*B*), and dipotassium hydrogen phosphate (*C*) were identified as the three primary influencing factors. These three factors were selected for further investigation, with caprylic acid yield (*Y*) serving as the response variable. A Box-Behnken response surface experiment was designed using Design-Expert software, incorporating three factors at three levels. The experimental design and results are presented in Table 5, while the analysis of variance is detailed in Table 6.

The data presented in Table 6 was subjected to multiple quadratic regression analysis using Design-Expert software. The resulting quadratic polynomial regression equation is as follows:

$$Y = -156.59369 + 7.92289A + 8.64100B + 193.04583C - 0.072500AB - 2.02083AC - 0.93750BC - 0.10419A^2 - 1.39275B^2 - 704.84375C^2.$$

**Table 5.** Response surface test design and results

| Experiment Number | A | B | C | Caprylic Acid Content (g/L) |
|---|---|---|---|---|
| 1 | 3 | 40 | 0.08 | 2.53 |
| 2 | 2 | 37 | 0.08 | 4.79 |
| 3 | 2 | 37 | 0.08 | 4.7 |
| 4 | 2 | 40 | 0.12 | 2.74 |
| 5 | 2 | 40 | 0.04 | 2.82 |
| 6 | 2 | 37 | 0.08 | 5.32 |
| 7 | 2 | 34 | 0.12 | 3.65 |
| 8 | 2 | 34 | 0.04 | 2.76 |
| 9 | 3 | 37 | 0.12 | 2.84 |
| 10 | 1 | 40 | 0.08 | 2.22 |
| 11 | 1 | 37 | 0.12 | 2.41 |
| 12 | 1 | 37 | 0.04 | 2.16 |
| 13 | 2 | 37 | 0.08 | 5.27 |
| 14 | 3 | 34 | 0.08 | 3.67 |
| 15 | 1 | 34 | 0.08 | 2.49 |
| 16 | 2 | 37 | 0.08 | 5.21 |
| 17 | 3 | 37 | 0.04 | 2.74 |

**Table 6.** Variance analysis of regression model

| source | Sum of Squared Deviations | Degrees of Freedom | Mean square | F value | P value | Significance |
|---|---|---|---|---|---|---|
| Model | 21.20 | 9 | 2.36 | 38.24 | < 0.0001 | ** |
| A | 0.64 | 1 | 0.64 | 10.37 | 0.0147 | * |
| B | 0.78 | 1 | 0.78 | 12.68 | 0.0092 | ** |
| C | 0.17 | 1 | 0.17 | 2.73 | 0.1424 | |
| AB | 0.19 | 1 | 0.19 | 3.07 | 0.1231 | |
| AC | 0.24 | 1 | 0.24 | 3.82 | 0.0916 | |
| BC | 0.01 | 1 | 0.01 | 0.091 | 0.7713 | |
| $A^2$ | 3.70 | 1 | 3.70 | 60.12 | 0.0001 | ** |
| $B^2$ | 8.17 | 1 | 8.17 | 132.61 | < 0.0001 | ** |
| $C^2$ | 5.36 | 1 | 5.36 | 86.95 | < 0.0001 | ** |
| Residual | 0.43 | 7 | 0.062 | | | |
| Missing Item | 0.094 | 3 | 0.031 | 0.37 | 0.7773 | |
| Absolute Error | 0.34 | 4 | 0.084 | | | |
| Total | 21.63 | 16 | | | | |

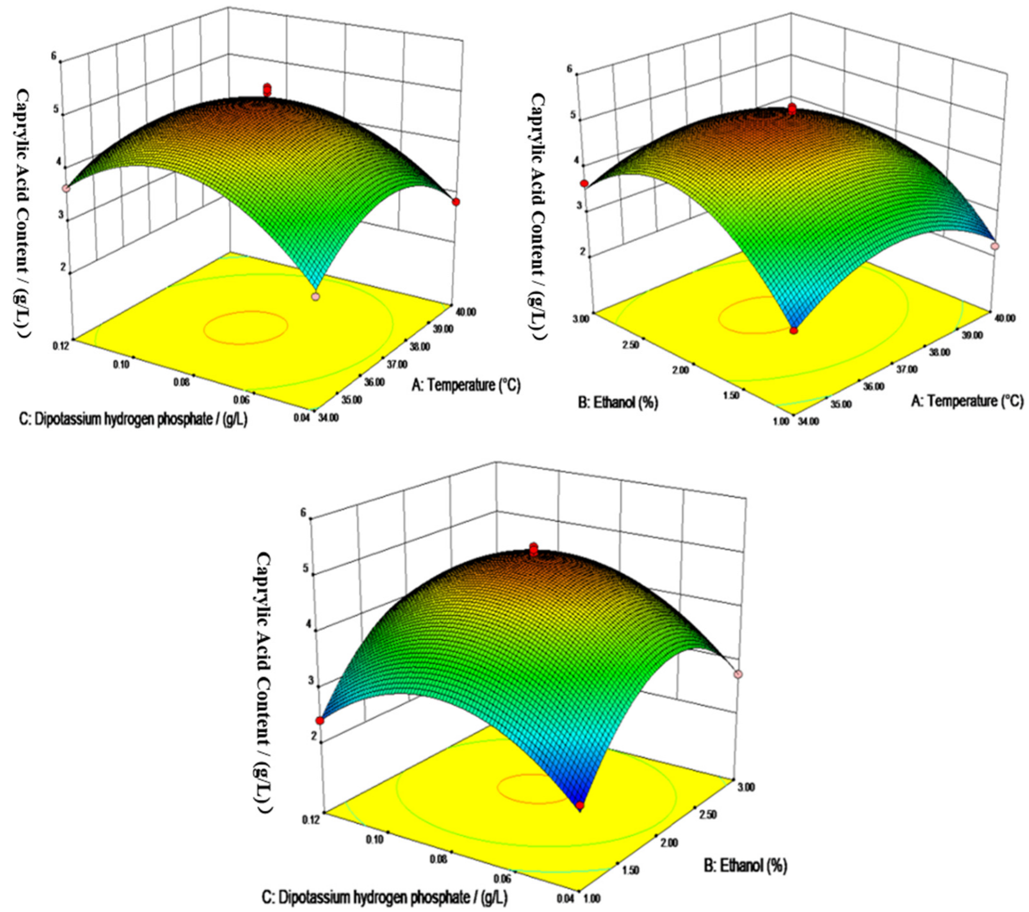

**Fig 9.** Response surface diagram of interaction between factors

The results presented in Table 6 indicate that the model is highly significant with a $P$ value of less than 0.0001. The lack-of-fit term has a $P$ value of 0.094, which exceeds the threshold of 0.05, suggesting that it is not significant and thereby confirming the reliability of the model. The coefficient of variation (CV) is relatively low at 7.23%, further indicating a high level of reliability for the model. The determination coefficient $R^2$ equals 0.9801, while the adjusted determination coefficient $R^2_{adj}$ stands at 0.9544, demonstrating that this equation fits well to the experimental data; additionally, the predictive determination coefficient $R^2_{pre}$ is recorded as 0.9058. The difference between the adjusted and predictive coefficients being less than 0.2 signifies high accuracy and minimal error within this model. Furthermore, Table 6 reveals that linear term A significantly affects the outcome ($P < 0.05$), while linear term $B$ and quadratic terms $A^2$, $B^2$, $C^2$ exhibit extremely significant effects ($P < 0.01$). Other terms do not show significant influence on outcomes ($P > 0.05$). The response surface model visually illustrates how various factors impact caproic acid yield, as depicted in Figure 9. From Figure 9, it can be observed that all response surfaces are convex and possess maximum values; specifically, contour lines for interaction terms $AB$ and $AC$ are elliptical in shape whereas those for interaction term $BC$ are circular—indicating that interactions $AB$ and $AC$ have a more substantial effect on caproic acid yield compared to interaction $BC$—consistent with findings from variance analysis shown in Table 6.

### 3.4.2. Determination and Validation of Optimal Fermentation Conditions

The Design-Expert software was utilized to perform a secondary multiple regression analysis, resulting in the optimal fermentation conditions being identified as follows: ethanol concentration of 2.13%, temperature of 36.47 °C, and dipotassium hydrogen phosphate concentration of 0.8 g/L. Under these conditions, the model predicted a caproic acid yield of 5.11 g/L. For practical implementation, the optimal fermentation parameters were revised to an ethanol concentration of 2%, a temperature of 37 °C, and a dipotassium hydrogen phosphate concentration of 0.8 g/L. Three repeated validation experiments were conducted under these revised conditions, yielding an actual caproic acid production value of 5.07 g/L. This result indicates that the established model is well-fitted and reliable, demonstrating significant optimization effects.

## 3.5. Application of Caproic Acid Composite Bacterial Liquid in Fragrant Type Baijiu

### 3.5.1. The Impact of Inoculating Caprylic Acid Composite Bacterial Solution on Physicochemical Indicators

As shown in Table 7, a comparison of the physicochemical indicators such as acidity, starch, moisture content, and reducing sugars between the alcoholic fermentative material with added caproic acid-producing mixed bacterial solution and Traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material reveals that the addition of the bacterial solution significantly increased the moisture content of the alcoholic fermentative material. There was no significant difference in acidity; however, both starch and reducing sugars were markedly reduced. This may be attributed to the enrichment of acid-producing microbial populations and their quantities during fermentation due to the addition of caproic acid-producing mixed bacteria. A greater number of microorganisms hydrolyze starch into reducing sugars, which are subsequently converted into alcohols, acids, esters, and other organic compounds [24].

**Table 7.** Physicochemical indexes of xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with caproic acid-producing compound bacteria solution and traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material

| Category | Physicochemical Indicators | | | |
|---|---|---|---|---|
| | Acidity / (mmol per 10 g) | Starch /% | Moisture Content /% | Reducing Sugar /% |
| Traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material | 1.65$^a$ | 6.71$^a$ | 68.37$^b$ | 0.71$^a$ |
| Xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with Caproic acid bacteria liquid compound bacteria solution | 1.80$^a$ | 5.88$^b$ | 83.16$^a$ | 0.22$^b$ |

### 3.5.2. The Impact of Inoculating Caprylic Acid Composite Bacterial Liquid on the Flavor Compound Content in alcoholic fermentative material

The main flavor compounds in the alcoholic fermentative material from two different processes were analyzed using GC-MS, and the results are presented in Table 8. A total of 63 flavor compounds were detected, including 26 esters, 11 alcohols, 12 acids, 5 phenols, and 9 other substances. Among these, esters emerged as the predominant flavor components in the beverage, accounting for 41% of all identified flavor compounds. This was followed by alcohols at 18%, acids at 19%, and phenols at 8%.

The ethyl ester compounds contribute to the pleasant fruity and floral aromas in white wine. Among these, ethyl acetate (which imparts a light fruity aroma) is considered the primary aromatic substance in fragrant-type white wines. Ethyl caprylate (associated with brandy-like notes) is regarded as the flavor compound with the highest intensity in fragrant-type white wines, while ethyl hexanoate (providing fruity notes) is identified as the main flavor component in nongxiangxing baijiu [26,27]. In addition, ethyl benzoate (with grassy and leather notes), ethyl pentanoate (apple aroma), ethyl decanoate (coconut scent), and diethyl succinate (fruity fragrance) exhibit distinct aromatic characteristics. Compared to the traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material, the xiaoqu qingxiang alcoholic fermentative material inoculated with a compound culture of caproic acid-producing bacteria shows an increase in ester compounds such as ethyl butyrate and ethyl stearate. Furthermore, the content of ethyl caproate is significantly elevated.

**Table 8.** The main flavor substances of xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with caproic acid-producing compound bacteria and traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material

| Category | Volatile Substances | Concentration of quality (μg/g fresh alcoholic fermentative material) | |
|---|---|---|---|
| | | Xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with Caproic acid bacteria liquid compound | Traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material |
| Esters | Ethyl butyrate | 0.061±0.009 | - |
| | Succinic acid, monomethyl ester | - | 0.112±0.011 |
| | 3-methyl-1-butanol acetate | 0.031±0.001$^{b}$ | 0.122±0.015$^{a}$ |
| | Ethyl caproate | 1.593±0.084$^{a}$ | 0.319±0.076$^{b}$ |
| | Ethyl enanthate | 0.005±0.001$^{b}$ | 0.045±0.004$^{a}$ |
| | Ethyl lactate | 0.249±0.028$^{b}$ | 6.738±0.364$^{a}$ |
| | Isobutyl caproate | 0.007±0.002 | - |
| | Ethyl caprylate | 0.078±0.003$^{b}$ | 1.044±0.138$^{a}$ |
| | 2, 3-butanediol diacetate | - | 0.073±0.007 |
| | Isobutyl lactate | 0.006±0.001 | - |
| | Ethyl pelanoate | 0.008±0.002$^{b}$ | 0.195±0.003$^{a}$ |
| | Dl-2-hydroxycaproate ethyl ester | 0.043±0.007$^{b}$ | 0.512±0.042$^{a}$ |
| | Ethyl (E) -2-octenate ester | - | 0.042±0.019 |
| | isovalerate | 0.038±0.012$^{b}$ | 0.461±0.050$^{a}$ |
| | Ethyl caprate | - | 0.826±0.034 |
| | Diethyl succinate | - | 0.708±0.107 |
| | Ethyl phenylacetate | 0.006±0.001$^{b}$ | 0.053±0.004$^{a}$ |
| | Phenyl ethyl acetate | 0.069±0.007$^{b}$ | 0.751±0.069$^{a}$ |
| | Ethyl tetradecate | 0.008±0.001$^{b}$ | 0.073±0.011$^{a}$ |
| | Tridecyl acetate | - | 0.101±0.003 |
| | Ethyl hexadecanoate | 0.168±0.024$^{b}$ | 1.604±0.190$^{a}$ |
| | Ethyl 9-hexadecanoate | 0.007±0.002$^{b}$ | 0.093±0.006$^{a}$ |
| | Monoethyl succinate | - | 0.077±0.011 |
| | Ethyl stearate | - | 0.035±0.007 |
| | Ethyl elaionate | 0.043±0.006$^{b}$ | 0.394±0.027$^{a}$ |
| | Ethyl acetate | 0.945±0.094$^{b}$ | 2.563±0.267$^{a}$ |
| Alcohols | 2-methyl-1-propanol | 0.043±0.007$^{b}$ | 0.203±0.012$^{a}$ |
| | Isoamyl alcohol | 0.517±0.017$^{b}$ | 2.798±0.127$^{a}$ |
| | 4-ethylcyclohexanol | - | 0.028±0.003 |
| | Acetylmethylcarbinol | - | 0.069±0.012 |
| | N-hexyl alcohol | 0.171±0.010$^{a}$ | 0.164±0.035$^{a}$ |
| | linalool | 0.010±0.002 | - |
| | N-octyl alcohol | 0.022±0.004$^{b}$ | 0.315±0.023$^{a}$ |
| | 1-nonyl alcohol | 0.012±0.001 | - |
| | 3-methylthiopropyl alcohol | - | 0.107±0.012 |
| | Benzyl alcohol | - | 0.077±0.001 |
| | Benzyl alcohol | 1.001±0.055$^{b}$ | 5.914±0.409$^{a}$ |
| Acids | Acetic acid | 0.126±0.004$^{a}$ | 0.998±0.096$^{b}$ |
| | Butyric acid | 0.236±0.019 | - |
| | Isobutyric acid | - | 0.127±0.010 |
| | Valeric acid | 0.015±0.002 | - |
| | Atolac acid | 0.008±0.001 | - |
| | Caproic acid | 3.382±0.336$^{a}$ | 0.357±0.083$^{b}$ |
| | Caprylic acid | 0.074±0.007$^{a}$ | 0.118±0.020$^{a}$ |
| | Cetanoic acid | - | 0.036±0.032 |
| | Pelanoic acid | - | 0.028±0.004 |
| | Decanoic acid | 0.009±0.001$^{b}$ | 0.036±0.003$^{a}$ |
| | Benzoic acid | - | 0.032±0.028 |
| | Lactic acid | 0.532±0.664$^{b}$ | 2.35±0.046$^{a}$ |
| Phenols | 4-methylguaiacol | 0.140±0.022$^{a}$ | 0.271±0.036$^{a}$ |
| | Phenol | 0.010±0.001$^{b}$ | 0.038±0.003$^{a}$ |
| | p-methylphenol | 0.007±0.001$^{a}$ | 0.007±0.012$^{a}$ |
| | 4-ethylphenol | - | 0.059±0.006 |
| | 2, 4-di-tert-butylphenol | 0.010±0.001$^{a}$ | 0.025±0.006$^{a}$ |
| Other Classes | Octamethylcyclotetrasiloxane | 0.037±0.003$^{b}$ | 0.151±0.011$^{a}$ |
| | Cyclopentamethylene dimethylsiloxane | 0.058±0.004$^{b}$ | 0.187±0.019$^{a}$ |
| | 2,2,11, 11-tetramethyl-dodecane | - | 0.028±0.003 |
| | Cyclohexyl hexamethylsiloxane | 0.055±0.010$^{b}$ | 0.179±0.010$^{a}$ |
| | tetradecane | - | 0.044±0.004 |
| | 2-methylhexacosane | - | 0.015±0.013 |
| | cetylcyclooctasiloxane | - | 0.032±0.004 |
| | 3-methyl-4-nononone | - | 0.017±0.015 |
| | phenylbutanone | - | 0.030±0.026 |

Note: The symbol “-” indicates that the measurement was not obtained.

The presence of alcohol compounds not only contributes to the fullness and richness of the baijiu's body, as well as its diverse aromas, but also serves as precursor substances for ester synthesis, playing a significant role in constructing the overall flavor profile of baijiu [28]. In xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with caproic acid-producing microbial liquid, the content of alcohol compounds was found to decrease significantly, predominantly consisting of higher alcohols. This reduction can effectively alleviate symptoms such as headaches and nausea experienced by consumers after drinking. Additionally, terpenoid compounds like linalool emerged during fermentation; this compound is characterized by floral notes reminiscent of lily or lily-of-the-valley [29]. Acidic compounds represent an important category of flavor substances primarily characterized by their sour taste. The main acids identified include acetic acid, caproic acid, butyric acid, and lactic acid; these acids play a supportive and buffering role in enhancing the primary aroma [30,31]. Notably, there was an increase in various acidic substances such as butyric and pentanoic acids while caproic acid levels rose significantly. Conversely, both lactic and acetic acids exhibited marked decreases in concentration; this may be attributed to their conversion into caproic acid [32]. Furthermore, acidic substances can react with alcohols to form esters which subsequently influence the content of ester compounds. Moreover, other types and quantities of substances were notably elevated following the addition of caproic-acid-producing microbial liquid. This indicates that such supplementation has a pronounced effect on enriching both the variety and quantity of flavor components present in xiaoqu qingxiangxing baijiu alcoholic fermentative material.

### 3.5.3. Analysis of the Variability in Flavor Compounds of Caproic Acid Fermentation Broth

As a supervised multivariate statistical analysis method, OPLS-DA is employed to further explore data information by setting predefined classification variables. This approach quantifies the degree of difference between treatment groups, thereby mitigating the influence of uncontrolled variables on the data. OPLS-DA maximally showcases the differences among various groups. To gain deeper insights into the flavor differences of xiaoqu qingxiangxing baijiu from different origins, an OPLS-DA analysis was conducted on the main flavor compounds of two distinct production process alcoholic fermentative material types, as illustrated in Figure 10a. The xiaoqu qingxiangxing alcoholic fermentative material inoculated with a compound culture of caproic acid and traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material were categorized into two separate groups, demonstrating that OPLS-DA effectively distinguishes between these two processes. As shown in Figure 10b, the model fitting results are acceptable. Furthermore, results from 200 permutation tests indicate that there is no overfitting present in the model.

The volatile flavor compounds with variable importance in the project (VIP) > 1, $P < 0.05$, and relative percentage content greater than 1% are defined as differential flavor substances, as illustrated in Figure 10c. A total of 14 volatile flavor compounds were identified to have significant differences between the two types of alcoholic fermentative material, including ethyl lactate, phenethyl alcohol, caproic acid, isoamyl alcohol, lactic acid, ethyl acetate, hexadecanoic acid ethyl ester, caprylic acid ethyl ester, acetic acid decanoate, butanedioic diethyl ester, phenethyl acetate, and DL-2-hydroxycapric acid ethyl ester. Compared to Traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material, the addition of a compound yeast solution that produces caproic acid significantly increased the contents of caproic acid and its ethyl ester while decreasing those of ethyl lactate and ethyl acetate. The incorporation of this compound yeast solution resulted in alterations in both the microbial community composition and metabolic activities within the alcoholic fermentative material. Consequently, these changes led to variations in flavor compounds between traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material and that xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with caproic acid bacteria liquid compound.

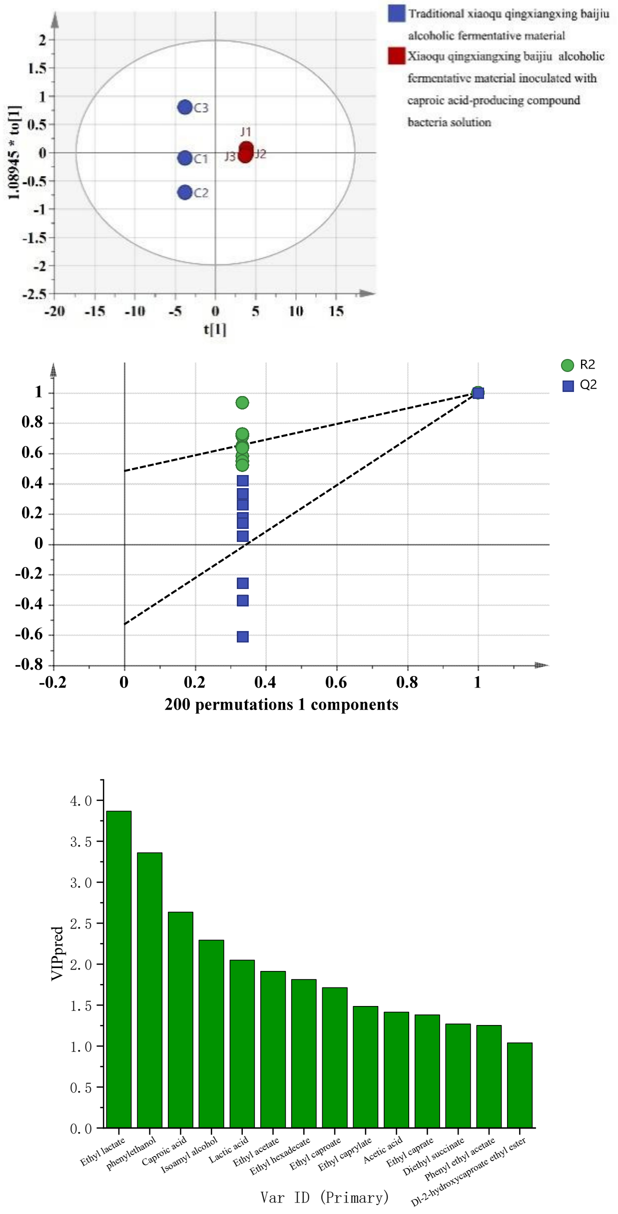

**Fig 10.** a. Partial least squares discriminant analysis of volatile flavor substances in alcoholic fermentative material b. Replacement test c. Partial least squares discriminant analysis of VIP value distribution map

## 4. Conclusion

This study focuses on the composite bacterial liquid for caproic acid production as the experimental subject, with caproic acid yield serving as the evaluation index. A preliminary optimization was conducted through single-factor experiments to assess the effects of various factors, including the addition amounts of ethanol, anhydrous sodium acetate, dipotassium hydrogen phosphate, and corn steep powder; as well as liquid volume, cultivation temperature, and initial pH value. Subsequently, Plackett-Burman design and response surface methodology were employed to determine the optimal cultivation conditions for caproic acid production from the composite bacterial liquid. The optimal parameters identified are: ethanol addition at 2%, a cultivation temperature of 37 °C, dipotassium hydrogen phosphate at 0.8 g/L, anhydrous sodium acetate at 5.0 g/L, corn steep powder at 2.0 g/L, an initial pH value of 8, and a liquid volume ratio of 70%. Under these conditions, it was found that the most significant factors influencing caproic acid yield in the composite bacterial liquid are ethanol addition amount, cultivation temperature, and dipotassium hydrogen phosphate concentration. The maximum yield achieved was recorded at 5.07 g/L.

The application of caproic acid composite microbial liquid in the fermentation process of traditional xiaoqu qingxiangxing baijiu aims to produce a spirit that embodies both qingxiang and nongxiang styles. The results indicate that, in terms of physicochemical indicators, there is no significant difference in acidity between the xiaoqu qingxiangxing baijiu alcoholic fermentative material inoculated with caproic acid bacteria liquid compound and that of traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material; however, moisture content significantly increased while starch and reducing sugars decreased markedly. Among the primary flavor components, 14 distinct flavor substances were identified between the two types, including ethyl lactate, phenethyl alcohol, caproic acid, isoamyl alcohol, lactic acid, ethyl acetate, hexadecanoate ethyl ester, caprylate ethyl ester, octanoate ethyl ester, acetic acid, decanoate ethyl ester, diethyl succinate, phenethyl acetate and DL-2-hydroxycaprate ethyl ester. Overall analysis reveals that when inoculated with caproic acid composite microbial liquid compared to traditional xiaoqu qingxiangxing baijiu alcoholic fermentative material: the contents of phenethyl alcohol and isoamyl alcohol within alcoholic substances are significantly reduced; conversely, the concentration of caproate esters has notably increased while those of acetate esters and lactates have declined significantly. Additionally, in terms of acidic compounds, caproic acid levels have risen substantially whereas lactic and acetic acids have decreased markedly. These findings align with previous research conducted by Wang Jiangbo et al, providing insights into how caproic acid composite microbial liquid influences both physicochemical properties and flavor profiles in xiaoqu qingxiangxing baijiu alcoholic fermentative material—offering guidance for producing baijiu that harmonizes both qingxiang and nongxiang characteristics.

# References

[1] Liu Xiao, Deng Jie, Wu Ling, et al. Analysis of flavor substances Difference of Sichuan Xiaoqu Baijiu brewed from japonica Sorghum and glutinous sorghum[J]. Journal of Food Safety and Quality Inspection, 2024, 15(07): 20-27.

[2] Jing Xiangyi, Ling Li, Mao Hao, et al. Investigation into the Screening and Fermentation Characteristics of Caproic Acid Producing Bacteria[J]. Brewing Technology, 2021, (10): 106-111.

[3] Zhang Qianying, Yuan Yuju, Luo Wen, et al. Characterization of Prokaryotic Community Diversity in New and Aged Pit Muds from Chinese Luzhou-flavor Baijiu Distillery[J]. Food Science and Technology Research, 2017, 23(2): 213-220.

[4] Huang Zho, Pu Ling-Ping, Luo Hui-Bo, et al. Process optimization of ester production by esterifying bacteria of Luzhou-flavor Baijiu[J]. Modern Food Science and Technology, 201, 37(02): 64-71+146.

[5] Zou Wei, Zhao Changqing, Luo Huibo. Diversity and Function of Microbial Community in Chinese Luzhou-Flavor Baijiu Ecosystem: A Review[J]. Frontiers in microbiology, 2018, 9: 671.

[6] Gao Zhanzheng, Wu Zhengyun, Zhang Wenxue. Effect of Pit Mud on Bacterial Community and Aroma Components in Yellow Water and Their Changes during the Fermentation of Chinese Luzhou-Flavor baijiu[J]. Foods, 2020, 9(3): 372.

[7] Zheng Qi, Lin Bairong, Wang Yibin, et al. Proteomic and high-throughput analysis of protein expression and microbial diversity of microbes from 30- and 300-year pit muds of Chinese Luzhou-flavor Baijiu[J]. Food research international (Ottawa, Ont.), 2015, 75: 305-314.

[8] Wei Yang, Zou Wei, Shen Caihong, et al. Basic flavor types and component characteristics of Chinese traditional Baijiu: A review[J]. Journal of food science, 2020, 85(12): 4096-4107.

[9] Yuan Huawei, Tan Li, Chen Hao, et al. Vinegar production from post-distillation slurry deriving from rice shochu production with the addition of caproic acid-producing bacteria consortium and lactic acid bacterium[J]. Journal of Bioscience and Bioengineering, 2017, 124(6): 653-659.

[10] Zhang Huifang, Zhang Wanying, Zhou Suo, et al. Isolation and screening of caproic acid-producing bacteria from Shedian Laojiu cellar and acid production conditions [J]. Modern Food Science and Technology, 2023, 39(02): 188-196.

[11] Zou Wei, Ye Guangbin, Zhang Kaizheng. Diversity, Function, and Application of Clostridium in Chinese Luzhou-flavor Baijiu Ecosystem: A Review[J]. Journal of food science, 2018, 83(5): 1193-1199.

[12] Wei Chunhui, Zhang Llan, Luo Huibo, et al. Optimization of cooperative fermentation conditions of acid-producing bacteria in Luzhou-flavor Baijiu pit mud[J]. Modern Food Science and Technology, 2021, 37(01): 54-64.

[13] Yang Jiang, Xu Jingzheng, Zhang Shunhe, et al. Preparation and optimization of culture conditions of high yield caproic acid bacteria nutrient solution in cellar[J]. Wine Technology, 2023(07): 17-21.

[14] Zhao Changqing, Yan Xueling, Yang Shiting, et al. Screening of Bacillus strains from Luzhou-flavor Baijiu making for high-yield ethyl hexanoate and low-yield propanol[J]. LWT-Food Science and Technology, 2017, 77: 60-66

[15] Hu Zhihui, Chen Bingxu, Ren Xue, et al. Optimization of fermentation conditions and medium composition of caproic acid fermentation broth for Baijiu production[J]. China Brewing, 2018, 37(4): 16-22.

[16] Deng Xiachan, Huang Duo, Tao Yong, et al. Screening and identification of high-yielding ethyl acetate yeast and optimization of fermentation conditions[J]. China Brewing, 2019, 43(10): 134-141.

[17] Huang Zhijiu, Xue Ruiqi, Zhang Yu, et al. Effect of different process parameters on the quality of Xiaoqu Qingxiangxing Baijiu[J]. Food and Fermentation Technology, 2022, 58(04): 32-38.

[18] Ren Xue, Wei Xiaoqing, Han Yuchen, et al. Production of Luzhou-flavor Baijiu by fermentation without mud[J]. Food and Fermentation Industry, 2019, 46(07): 105-109.

[19] Guo Ying. Research on Main functional bacteria isolation and preparation of cellar mud curing solution[D]. Zhengzhou: Henan University of Technology, 2021.

[20] Pu Lingping, Huang Zhiguo, Rao Jiaquan, et al. Spatial and temporal differences of flavor substances in fermented grains of Luzhou-flavor Baijiu in central Sichuan[J]. Modern Food Science and Technology, 2023, 39(12): 262-269.

[21] Yu Songbai, Zhao Xingrong, Shao Yan, et al. Spatial and temporal distribution of 30 organic acids in the fermentation

process of Luzhou-flavor Baijiu[J]. Food and Fermentation Industry, 2019, 50(14): 297-307.

[22] Zhu Xiaoyu, Tao Yong, Liang Cheng, et al. The synthesis of n-caproate from lactate: a new efficient process for medium-chain carboxylates production[J]. Scientific Reports, 2015, 5(1): 14370.

[23] Yang Niutian. Study on microbial community structure and its correlation with physicochemical parameters and flavor substances in brewing of Qingxiang Baijiu[D]. Jinzhong: Shanxi Agricultural University, 2022.

[24] Zhang Weijian. Study on characteristic flavor of Luzhou-flavor Baijiu in Yimeng Old area, Shandong Province[D]. Wuxi: Jiangnan University, 2019.

[25] Wu Chenglin. Study on characteristic flavor of Sichuan Xiaoqu Baijiu[D]. Chongqing: Southwest University, 2022.

[26] Zhou Jinhu, Wei Haolin, Zhang Mingchun, et al. Mutagenesis breeding of a Bacillus licheniformis with high yield of ethyl caprylate[J]. Wine-making, 2017, 44(03): 20-24.

[27] Jhony Willian Vargas-Solórzano, Carlos Wanderlei Piler Carvalho, Cristina Yoshie Takeiti, et al. Physicochemical properties of expanded extrudates from colored sorghum genotypes [J]. Food Research International, 2014, 55: 37-44.

[28] Ma Li, Zhang Fengyuan, Xiang Fu, et al. Determination of linalool in honeysuckle dew by gas chromatography[J]. China Brewing, 2018, 37(04): 191-195.

[29] Xu Youqiang, Zhao Jingrong, Liu Xia, et al. Flavor mystery of Chinese traditional fermented Baijiu: The great contribution of ester compounds[J]. Food Chemistry, 2022, 369: 130920.

[30] Jin Xiangyi, Wang Hua, Tian Huixue, et al. Caproiciproducens converts lactic acid into caproic acid during Chinese Luzhou-flavor Baijiu brewing[J]. International Journal of Food Microbiology, 2025, 426: 110931.

[31] Wang Ruiming, Peng Yin, Zhang Guangzheng. Liquor Blending technology [M]. Chemical Industry Press: 202310. 414.

[32] Leos Uttl, Kamila Hurkova, Vladimir Kocourek, et al. Metabolomics-based authentication of wines according to grape variety[J]. Czech Journal of Food Sciences, 2019, 37: 239-245.

[33] Wang Jiangbo, Zhu Jialu, Jin Weijun, et al. Study on fermentation process of Luzhou-flavor Baijiu without mud based on microcapsules of caproic acid bacteria [J/OL]. Food and fermentation industry, 1-13 [2025-01-03].