Preliminary Study on Caproic Acid Production by Compound Bacterial Agent of Luzhou-Flavor Liquor Pit Mud

Junwei Li1, *, Chao Zhang1, Qiushuo Xu1, Sun Lin1, Zhanqi Liu2, Xiaoyuan Dong2

1 Yellow Crane Tower Distillery Co., Ltd; Wuhan 430050, China
2 Wuhan Yesbiology Technology Co., Ltd; Wuhan 430000, China

*Corresponding author e-mail: 1614811267@qq.com

Abstract. Pit mud plays an important role in the fermentation of Luzhou-flavor liquor. It is a common problem for Luzhou-flavor liquors when the mud is aging and the fragrance is not prominent. Choose high quality mud, inoculating the mash extract as a mixed bacterial solution in sodium acetate medium, Anaerobic culture method to increase the activity of anaerobic microorganisms, Increase hexanoic acid production; Characterization of hexanoic acid by color reaction of copper sulfate diethyl ether, Determination of hexanoic acid by gas chromatography, Its output is in the range of 1.5 g/L to 3.6 g/L, This is the production of mud maintenance liquid, Technical support is provided to prevent aging of the mud and improve the fragrance of the main body.

1. Introduction
Liquor is a traditional fermented food in China. It is typically classified into several categories based on aroma characteristics: strong aroma, light aroma, soy sauce aroma, rice aroma four basic flavors; Among them, the proportion of Luzhou-flavor liquor is getting bigger and bigger. The representative liquor brands are Wuliangye [1], Yanghe [2], GujingTribute [2], and Yellow Crane Tower Distillery [3] for strong aroma type; Fen [4] for light aroma type; Maotai [5] for soy sauce aroma type and Guilin Sanhua [6] for rice aroma type. The Yellow Crane Tower Distillery with a long history has won not only the 1979 and 1989 two famous Chinese wines, and its Original ecological cave winemaking is also unique.

The pros and cons of Luzhou-flavor liquor are closely related to the mud. The mud is called “Soft gold” and “Solid gold. The mud is rich in microbial resources, among them, anaerobic microorganisms account for a large proportion in the microflora [7]; Using mud as a mixed agent [8], optimized culture conditions, positive regulation of beneficial microbial metabolism, make it a beneficial ingredient such as hexanoic acid, increase the main aroma of liquor, Prevent aging of the fermentation container, do it “take it from the mud, used in mud”, qualitative experiment of hexanoic acid in fermentation broth by color reaction of copper sulfate diethyl ether [9], qualitative identification of hexanoic acid by color reaction, this method is a commonly used detection method at this stage. Gas chromatography (GC) [10] can quantitatively analyze volatile components in fermentation broth, it is an indispensable analytical method in modern research.

Mud is aging and main scent in liquor is not prominent, which is the main problem encountered by Luzhou-flavor liquor. Prevent aging of the fermentation container, optimize mud, promoting the
positive metabolism of microorganisms is an urgent problem to be solved. The use of microbial metabolites as a sludge maintenance solution is a safe and economical method.

2. Materials and Methods

2.1. Materials and equipment

Samples: In this study, pitmud samples were all freshly collected from high quality fermentation container pool bottom mud.

Culture medium selection: Sodium acetate medium: Sodium acetate 5g, Magnesium sulfate 0.2g, ammonium sulphate 0.5g, Yeast extract 1g, dipotassium hydrogen phosphate 0.4g, with final volume as 1L, natural pH, sterilization at 121℃ for 25 min, add 2% absolute ethanol before inoculation and add 3% liquid paraffin seal after inoculation.

Equipment: Desktop electric constant temperature incubator (DHP-9082); Electronic analytical balance (SHP0201147047); Clean bench (JJ-CJ-2FD); Vertical pressure steam sterilizer (LDZX-50FBS); Constant temperature water bath (HH-4); Gas chromatography (7890A (G3440A)).

2.2. Method

2.2.1. Sample preparation. Collect high quality fermentation container, five-point sampling (that is, the bottom corner of the container and the bottom of the container), as shown in Figure 1. Sampling more than three fermentation containers bottom mud, full mixing uniformity, 2g pit mud was inoculated in a conical bottle containing 200ml sterile water, 160 r/min 36℃ constant temperature oscillation for 15 min, put it in a water bath pot at 85℃ for 5 to 10 minutes. In order to remove non-spore microorganisms, it is easy to select microorganisms with spores and strong tolerance. Cool to room temperature, 1ml of pit mud extract was inoculated into 20 ml sodium acetate medium, add 2% absolute ethanol before inoculation and add 3% liquid paraffin seal after inoculation, incubate at 36℃ for 7 days, continuous transfer for 3 cycles.

2.2.2. Qualitative analysis of hexanoic acid. Qualitative analysis of pit mud fermentation broth by copper sulfate ether coloration, take 4ml of fermentation broth, add 2% copper sulfate solution 2ml, anhydrous ether 1ml, set aside after full shaking and mixing, preliminary identification of hexanoic acid production based on color changes in the ether layer of the upper solution, the deeper the blue, the better the effect of producing caproic acid.

2.2.3. Quantitative determination of caproic acid. The caproic acid content in fermentation broth was determined by gas chromatography (GC).

Sample preparation: Samples of 3mL fermentation broth were extracted and centrifuged for 5 minutes at 10000 r/min and 4℃, after filtration through a filter, 1 ml of the filtrate was taken into a 10mL volumetric flask containing 100μL of 2-ethylbutyric acid (1.010 g/L), and make up to 1 degree
of formic acid with a volume fraction of 1%, after mixing uniformly, 1 μL was taken for gas chromatography.
  
  Detector: Hydrogen ion flame detector; chromatographic column DB-Wax (30m×0.25mm×0.25μm).
  
  Temperature program: Keep it at 50 ℃ for 2 minutes; Rise to 230 for 2min at 15 ℃/min; Detector temperature 230 ℃; Carrier gas He at a flow rate of 1 mL/min.

3. Results and Discussion

3.1. Qualitative results of caproic acid
Qualitative experiments were carried out on the content of caproic acid in several fermentation broth samples after multiple transfers of the compound microbial agent, among them, 5 samples had obvious color changes (Sample order from left to right), Copper sulfate diethyl ether color development result is shown in Figure 2, theoretically, the deeper the blue, the higher the hexanoic acid content, It can be seen that the content of caproic acid in the fermentation broth of five pit mud composite microbial agents is significantly higher than that of the blank control.

![Figure 2. Copper sulfate diethyl ether color development result](image)

3.2. Quantitative results of caproic acid
Quantitative maps of caproic acid content in the fermentation broth of the above five pit mud complex microbial agents were determined by GC method as shown in Fig. 3, the content of hexanoic acid in the samples in the above order were as follows: 2.59g/L, 2.62g/L, 3.54g/L, 1.53g/L, 1.81g/L, The content of hexanoic acid is stable from 1.5g/L to 3.6g/L.
Figure 3. Quantitative GC Map of Caproic Acid Content in Pit Mud. Fermentation Broth with Compound Microbial Agent
3.3. Discussion
The main aroma of Luzhou-flavor liquor is ethyl caproate, hexanoic acid is the precursor for the synthesis of ethyl hexanoate. Most liquor-making enterprises are confronted with the problem that the main fragrance is not prominent. Application of microorganisms in high quality pit mud as mixed microbial agents in fermentation, promote the positive regulation of microorganisms, producing beneficial metabolites, more healthy and safer for winemaking; Using high-quality pit mud as compound microbial agent, this method is applied to pit maintenance and pit protection, prevention of pit aging, or directly apply the metabolites to the production of Luzhou-flavor pit mud for technical reserve, However, the experiment of the hexanoic acid produced in the mud is relatively stable, Whether it is still stable in the fermentation containers environment requires a lot of experimental verification.

References
[1] H.S Zhou, L.I Zhang, H.s Cheng. Modern Food Science and Technology, 26 (2010)652-655.
[2] Fan W, Qian, Michael C. Journal of Agricultural and Food Chemistry, 54 (2006) 2695-2704.
[3] J.H Zhou, X.Y Dong, et.al. China Brewing, 44 (2017) 13-18.
[4] Zhang L, Wu C, Ding X, et al. World J Microbiol Biotechnol, 30 (2014) 3055-3063.
[5] Wang C L, Dong-jian Shi, Guo-li Gong. World Journal of Microbiology and Biotechnology, 24 (2008) 2183-2190.
[6] Q.L Tang, J.J Li, L.L Li, et al. Liquor-Making Science and Technology, 9 (2015) 8-11.
[7] H.C, Y.H, Defu Xu, et al. Liquor-Making Science and Technology, 3 (2005)34-38.
[8] Fugui Su. China Brewing, 2 (1994) 22-24.
[9] Fanlin, Yiping Wang. Liquor-Making Science and Technology, 1 (1992) 22-22.
[10] Y.T Chang, Y.J Luo, C.J Qian, et al. Food Safety and Quality Detection Technology, 9 (2018) 144-153.
[11] Xujun, Songyao Tan. Liquor-Making Science and Technology, 5 (2010) 42-43.