

Study on the Fermentation Mechanism of Pu'er Tea Fermentation High Temperature Bacteria in the Solid Fermentation Process of Pu'er Tea

Chunhua Zhang¹, Jiye Qiang¹, Hongping Man², Zhilin Jiang¹, Yuanyan Zhao³, Zhiguo Shan^{1,*}

¹Pu'er University, Pu'er, 665000, China

²Pu'er Comprehensive Technical Testing Center, Pu'er, 665000, China;

³Pu'er Tea Science Research Institute, Pu'er, 665000, China

*corresponding author

Keywords: Pu'er Tea, Solid Fermentation, Fermentation Mechanism, Microorganisms

Abstract: Tea is a unique fully fermented tea, its fermentation can not be separated from the role of microorganisms, microorganisms in the process of Pu'er tea fermentation dynamic changes in Pu'er tea quality plays an important role. Therefore, this paper reviews the micro-organisms in the process of pu'er tea one turn, second turn, third turn, four turn and pile, aiming to provide the relevant theoretical basis for the development of Pu'er tea microbial fermentation agent and Pu'er tea industry.

1. Introduction

Pu-erh tea (Pu-erh tea) is a unique kind of reprocessed tea with unique taste and flavor in Yunnan, with blood lipid reduction, blood pressure lowering, blood sugar lowering, regulating gastrointestinal function, antioxidant and other functions, according to the different processing process divided into Pu'er tea (Raw (Tea) (Non-fermented Pu-erh tea) and Pu'er tea (cooked tea) (Pu-erh ripe tea) [1]. (One of the cooked tea, is within the protection of geographical indications of Yunnan large leaf species of green hair tea as raw materials, through moderate tide, microbial solid fermentation and other processes, in micro-organisms, enzymes, enzymes, Under the joint action of wet heat and oxidation, a class of post-fermented tea is processed. Microbial solid enzyme is the key process of the formation of the quality of cooked tea, which is essentially based on , the inclusion of green tea, with the life activity of microorganisms as the main , body, through the extracellular enzymes secreted by microorganisms and the heat generated by breathing, Under the synergy of tea moisture, a variety of substances with tea polyphenols as the main body carried out complex and drastic transformation, and finally formed the characteristics of the unique taste of cooked tea, soup red-brown, Chen Xiang significantly, yellow-brown at the base of the leaf [2]. The fungal community in Pu'er tea is very complex , its main source is the natural breeding and reproduction of the processing process , although so far no cases of , Pu'er tea drinking poisoning have been reported , but the study of micro-biosecurity in the processing process should still attract attention . There have been studies on the separation of dominant fungi and the structure of fungi communities in the process of solid fermentation of cooked tea through traditional culture, molecular biology and other technical means [3].

It has been found that the growth and reproduction of microorganisms and active metabolism play a key role in the quality of Pu'er tea, therefore, the study of Pu'er tea one turn, two turn, three turn, four turn and pile fermentation process of microbial changes and its role in the formation of Pu'er tea quality, the development of Pu'er tea microbial fermentation agent and large-scale production, the development of Pu'er tea industry, promote the consumption of Pu'er tea have a positive significance [4].

2. Microbial Groups During Solid-State Fermentation of Pu'er Tea

According to the current research in China, the microbial flora isolated from the fermentation

process and Pu'er tea includes *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, yeast Yeasts and Bacterium. Many researchers have researched the microorganisms in the fermentation process and Pu'er tea through traditional methods of microbial separation and identification, automatic microbial identification system, and molecular biology, including the following types of microorganisms [5].

(1) *Aspergillus*

Aspergillus niger belongs to the genus Deuteromycotina, Hyphomycetes: Moniliales; Moniliaceae, *Aspergillus*. It is a lower eukaryote, and its safety and edibility are widely recognized in the world. It occupies an important position in industrial production and academic research. Studies have found that *Aspergillus niger* in Pu'er tea accounts for about 80% of the total number of microorganisms. Zhao Longfei et al.'s study on the number of microorganisms during the fermentation of Pu'er tea showed that the number of *Aspergillus niger* compared with the number of other microorganisms was always in a growth advantage both in the early and late stages of fermentation. It can be seen that *Aspergillus niger* occupies an absolute advantage in both the stacking process and the storage period, no matter the traditional process or the new process [6].

In addition, *Aspergillus oryzae*, *Aspergillus oryzae*, *Aspergillus grey* germ, and other microorganisms of the genus *Aspergillus* were isolated during the process of Pu'er tea and Pu'er tea.

(2) *Rhizopus*

Rhizopus is a genus of Zygomycotina from the Mucoraceae family. In addition to bacteria, *Rhizopus* is another important species of L-lactic acid produced by filamentous bacteria so far. Studies such as Zhao Longfei and others showed that the number of *Rhizopus* from the beginning to the third turn of *Rhizopus* always increased and reached the highest point, but the increase was not as large as *Aspergillus niger*, and then decreased.

(3) *Penicillium*

Penicillium Most species of *Penicillium* have only asexual stages in life history, belonging to Deuteromycotina; Moniliaceae, only a few species have sexual stages. Bi Tingju et al. Found that *Penicillium* is a common bacterium in the fermentation process, often accompanied by *Aspergillus*. *Penicillium* was detected in sun-green tea, moist tea, and fermented tea samples. Another study found that in the fermentation process of Wodui, the amount of *penicillium* reached the highest point in the second turn, and it fell to the lowest point in the third turn. *Penicillium* is slightly higher [7].

3. Microbial Effects in Solid Fermentation of Pu'er Tea

Microbes are essential to the formation of the unique flavor quality of Pu'er tea. Among them, black clostridium can produce more than 20 kinds of hydrolase, can break down pectin, natural fiber, insoluble compounds and other organic matter. Zhou Caibi and other advantages of the strains of black clostridium to Pu'er tea pure-seed fermentation test, found that the tea-like aroma is pure, soup color is black-brown, taste more peaceful, tea pigment changes are more profound, and mycotoxin content is lower, indicating that black clostridium can be used as fermentation bacteria, suitable for Pu'er tea stack fermentation, fermentation tea-like safety. In addition to its own rich in human-friendly nutrients and rich enzyme systems and physiologically active substances, yeast can also metabolize the production of vitamin B1, B2, C and other components [8]. Kang Yanshan and so on to the tanning green hair tea as the material, in the pu'er tea fermentation process added several exogenous enzymes and exogenous yeast. The results showed that the exogenous enzyme could significantly increase the content of water-soluble total sugar and free amino acids in Pu'er tea, and the transactive yeast could significantly promote the conversion of tea polyphenols. Chen cocoa and so on to use the advantages of separation from Pu'er tea fermentation reactor identification of the advantages of the fungus, vaccination test and specialty bacteria fermentation production test. The results suggest that the real bacteria can control the fermentation process and change the sensory characteristics of tea. The types and combinations of fungus sage have a significant effect on the composition and content of tea polyphenols in Pu'er cooked tea, as well as the content of characteristic components such as no edible acid, lyapline and caffeine. Jeng et al. isolated from Pu'er tea to identify two strains of streptomycin gray and pink cocci, and further studied its effects

on inclusions (tea polyphenols, statins, GABA)) during tea fermentation. The results showed that the content of inclusions in Pu'er tea, which was sterilized and fermented for a short period of time, the color of tea soup was significantly deepened, and the free radical removal capacity was enhanced, indicating that streptomycin was gray and pink micrococcal to shorten the fermentation time of Pu'er tea. Chen Xiuyan and so on will be separated from Pu'er cooked tea into the advantage of bacteria — gray-green clostridium and green mold back to Pu'er cake tea and loose tea, found that its taste and aroma can effectively improve the quality of Pu'er tea, speed up the aging speed [9].

With the development of the times, people pay attention to health and nutrition, Pu'er tea with its very high health effect, by the vast number of consumers love, Pu'er tea industry has been greatly developed. In order to further improve the quality of Pu'er tea, the study of the population, quantity and growth law of microorganisms in the fermentation process of Pu'er tea, the study of its biological characteristics, the selection of the best culture conditions, the formation of secondary metabolites accumulation law and the main enzyme system, the domestication and cultivation of wild bacteria, the cultivation of special strains suitable for industrial production, the development of more in line with factory production, can improve the quality of Pu'er tea special fermentation bacteria, is particularly important [10].

4. Conclusions

Previous research on fungi in cooked tea has focused on pure fungal technology to isolate fungi during fermentation, and then use molecular methods to identify them. For example, Yang Ruijuan and others applied traditional culture combined with ITS sequencing to believe that *Aspergillus*, *Mucor*, *Rhizopus*, and *Penicillium* fungi are mainly present in the solid-state fermentation of ripe tea. Factors affecting its growth and metabolism include nutrition, temperature, humidity, and light. Because only 0.1% to 10% of the environmental microorganisms in nature can be purely cultured, some low-level or even microscopic flora will automatically disappear during the cultivation process, so this method cannot fully and accurately reflect the fungal community Distribution. With the deep development of gene sequencing technology, the application of culture-free technology to study the microorganisms in solid fermentation of Pu'er tea is rising. For example, Zhao Ming et al. Used the 454 pyrosequencing technique to study the ITS region of the fungal 18S rRNA gene, and considered that the fungi involved in the fermentation of cooked tea were 5 genera, 49 families, and 73 genera. Zhang Yang et al. Used PCR-DGGE technology to analyze the fungal 18S rDNA, and concluded that although the dominant fungi dominate the solid-state fermentation of cooked tea, the species are quite concentrated, including *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Photosporein Mold (Penicillium glabrum)*. Some research results are the conclusions obtained by directly sequencing the fungi, which can reflect the fungi in the solid-state fermentation of cooked tea to a certain extent, but the amount of collected data is relatively small, and some results are still controversial. At present, no research has been conducted on the diversity and changes of fungal communities between layers in the solid-state fermentation process of cooked tea. The results of this study show that the dynamics of the fungal community between different layers of ripe tea, but the specific role of fungi and its secreted extracellular enzymes are not clear. Analysis of the correlation between changes, further analysis of the specific role and mechanism of fungi and its secreted extracellular enzymes in the solid-state fermentation process of cooked tea.

Fund Project

Research on the relationship between storage and aging technology and quality of Pu'er tea (Project No.: 2018JS513, Department of education, Yunnan Province); agricultural experimental practice training base and processing technology innovation service center (No. 2015-56, Yunnan University of Higher Education); scientific research project of Pu'er college, development of Pu'er tea paste (Project No.: 2015xjkt25, Pu'er University); high-level talents of Pu'er College Scientific research start-up project: occurrence conditions of Yunnan tea leaf blight and its influence on tea related components (Project No.: k2015032, Pu'er University)

References

- [1]. 2019. Basic understanding of the mechanism of microbial influence on Pu'er tea fermentation. Seed Industry Guide, no. 12, pp. 35.
- [2] Wei, Lin., Lu, F.M., Shao W.F., Yuan W. (2019). Changes in sensory quality and main components during the fermentation of sour tea. Food Research and Development, vol. 40, no. 14, pp. 69-74.
- [3] Pang, Y.K., Zhu, Q.Q., Zhang X.J., Huang Y.W. (2019). Analysis on the quality formation mechanism of Pu'er ripe tea. Modern Food, no. 07, pp. 43-44 + 54.
- [4] Song, C.X. (2019). Analysis on the application of puer tea microbial fermentation technology. Fujian Tea, vol. 41, no. 03, pp. 21-22.
- [5] Zheng, Xiaoyan., Zhang, Qianru. (2017). Current status of microbial research in the fermentation process of Pu'er tea. Food Safety Review, no. 23, pp. 26-27.
- [6] Ji, A.B., Gong, W.Y., Peng, W.S., Liu, C., Zeng, Y., Yan, L. (2016). Research progress of microorganisms in Pu'er tea. Modern Agricultural Science and Technology, 2016 (21): 253-255.
- [7] Li Y.P. (2016). Analysis on the influence of different kinds of microorganisms on the fermentation process of Pu'er tea. Food Safety Review, no. 21, pp. 110-111.
- [8] Wang, Q.M., Yang R.J., Yan L., Liu L., Lin S. (2016). Effect of Instant Pu'er Tea on Fermentation of Kombucha Beverage. Journal of Food Science and Biotechnology, vol. 35, no. 02, pp. 197-204.
- [9] Zhou, C.B., Chen, W.P., Zhao, Z.J., Mu, R.L., Wu, Z.L., Zhang, M.X. (2015). Study on the function and safety of the dominant strain of Pu'er tea, *Aspergillus oryzae*. Food Research and Development, vol. 36, no. 24, pp. 160-163.
- [10] Zhou, C.B., Chen W.P., Wu Z.L., Mu R.L., Zhang M.X. (2015). Study on the function and safety of the dominant strain of Pu'er tea *Penicillium oxalicum* .Food Science and Technology, vol. 40, no. 08, pp. 63-66.