Study on the Correlation between Microorganism and Quality Formation of Pu'er Tea during Fermentation

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Abstract: Pu'er tea is a solid-state fermented tea by microorganisms, and its fermentation cannot be separated from the role of microorganisms. The dynamic changes of microorganisms in the fermentation process of Pu'er tea have an important effect on the quality of Pu'er tea. In this paper, the correlation between microorganism activity and its metabolites and the flavor quality of Pu'er tea during the fermentation process of its fermentation is studied by analyzing the changes of microorganisms in the process of Pu'er tea fermentation at various stages, which has provided a scientific theoretical guidance for the fermentation process of Pu'er tea.

1. Introduction
Pu'er tea is a traditional tea in Yunnan tea. The Yunnan local standard DB53/103-2003 "Pu'er Tea", which was implemented on March 1, 2003, defines Pu'er tea as follows: "Pu'er Tea is a kind of loose tea and compacted tea made from Yunnan large-leaved sun-dried green-wool tea in a certain area of Yunnan Province after fermentation. Its color is brown-red, endoplasmic soup is red and bright, with a unique aroma, mellow and sweet taste, and brown-red at the bottom of the leaves." Studies have shown that Pu'er tea, as a kind of functional drink, has the effects of anti-oxidation, anti-mutagenesis, anti-bacterial, anti-virus, anti-tumor, cholesterol reduction, weight loss, hypoglycemia and anti-allergy [1]. Pu'er tea is a kind of special fermented tea. In the production process, raw materials need to be fermented by solid-state fermentation method to "pile" fermentation, "pile" is also called post-fermentation. This is the key process to form the quality of Pu'er tea. The operation is to transform the contents of Yunnan large-leaf sun-dried green-wool tea into a series of substances under specific environmental conditions by synthesizing the functions of microorganisms, enzymes, humidity and heat, and oxidation, thus forming the special quality characteristics of Pu'er tea [2]. In the fermentation process of Pu'er tea, the growth, reproduction and activity metabolism of microorganisms play a key role in the quality of Pu'er tea.

2. The Main Microorganism during the Fermentation of Pu'er Tea
According to the current research in China, the microbial flora isolated from the pile fermentation process and Pu'er tea include Aspergillus, Penicillium, Rhizopus, Mucor, Yeasts and Bacterium. The number of bacteria is so small that no pathogenic bacteria are found. Among these microorganisms, the dominant position of Aspergillus niger, Yeasts and Rhizopus alternately changes under different environmental conditions or during processing. Due to the role of the dominant flora at different stages, Pu'er tea, which has a variety of flavors of Pu'er tea manufacturers, has been formed. Through traditional methods of microbial isolation and identification, microbial automatic identification system
and molecular biology, researchers have studied the microorganisms in the fermentation process of Pu'er tea, which includes the following types of microorganisms.

2.1 Mould

2.1.1 Aspergillus
Aspergillus niger belongs to Deuteromycotina, Hyphomycetes: Moniliales; Moniliaceae, Aspergillus. It is a lower eukaryote. It is universally acknowledged that it is safe and edible and plays an important role in industrial production and academic research. Some studies have found that Aspergillus niger in Pu'er tea accounts for about 80% of the total number of microorganisms. Zhao Longfei et al. have studied the number of microorganisms in the fermentation process of Pu'er tea and showed that the number of Aspergillus niger is always in the growth advantage compared with the number of other microorganisms in both the early and late stages of fermentation. In addition, Aspergillus oryzae, Aspergillus foetidus, Aspergillus glaucus and other Aspergillus microorganisms are isolated from Pu'er tea during the process of pile.

2.1.2 Rhizopus
Rhizopus belongs to Zygomycotina Mucoraceae. In addition to bacteria, Rhizopus is another important strain producing L-lactic acid in filamentous bacteria up to now. Zhao Longfei and others have shown that the number of Rhizopus from the beginning of the pile to the third turn, has been increasing, and reached the highest point, but the increase is not as large as Aspergillus, and then decreases.

2.1.3 Penicillium
Most species of Penicillium have only asexual stages in life history, belonging to Deuteromycotina; Moniliaceae, and only a few species have sexual stages. Bi Tingju et al. have found that Penicillium is a common bacterium in the fermentation process, often accompanied by Aspergillus and Penicillium is detected in sun-dried green tea, Chaoshui tea and fermented tea samples. Another study has found that the number of Penicillium has reached the highest point at the second turn, the lowest point at the third turn, and increased slightly at the fourth turn. The amount of Penicillium is slightly higher than that of Rhizopus in the pile fermentation process.

2.2 Yeasts
Eumycota, Asconycetes, Hemiasconycetes, Endonycetales and Sacckaromyces Toideae. More than 500 kinds of Yeasts have been discovered, belonging to 41 genera. A large number of studies have shown that Yeasts is a common microorganism in the fermentation process of Pu'er tea, especially in the late fermentation period, the number of Yeasts increases significantly.

2.3 Bacterium
Researchers have isolated and nurtured microorganisms from Pu'er tea in Yunnan and Zhejiang provinces. The results have showed that there are more bacteria in the early stage, then decreases gradually, and very few in the later stage, at the same time, no pathogenic bacteria are found in the fermentation process. Dong Kun et al. have found that bacteria exist in every process of Pu'er tea production (fresh leaf tea, killing green tea, kneading tea, sun-dried tea, pile tea), but the number is very small.

2.4 Other Microorganisms
In addition, a large number of other microorganisms, such as basidiomycetes and actinomycetes, still exist in the fermentation process of Pu'er tea and Pu'er tea. These microorganisms interact and restrict each other and work together to create the quality of Pu'er tea.
3. The Effect of Microorganisms on the Quality of Pu’er Tea

The effect of microorganisms on the quality of Pu’er tea is mainly reflected in three aspects: the color, aroma and taste of Pu’er tea. Studies on the effect of microorganisms on the quality of Pu’er tea have been reported mainly on several dominant strains, such as Aspergillus niger, Penicillium, Rhizopus, Aspergillus foetidus, Yeasts, Streptomyces pink and Streptomyces griseus.

3.1 The Effect of Microorganisms on the Color of Pu’er Tea
Tea pigment is the main component of the composition of Pu’er tea. Tea pigment is a polyphenolic compound mainly composed of catechins in tea. It is a mixture of pigments that are soluble in water and oxidized and polymerized, which mainly includes the orange-yellow theaflavin, the brown-red thearubigin and the brown theabrownine. During the pile process, the levels of thealavin and thearubigins decrease significantly, and the theabrownine accumulated in large amounts. When the content of theabrownine is 7.85%, the best color quality is bright reddish-brown; The color of the soup with 5.12% theabrownine is bright red and orange, which is considered to be insufficient pile fermentation[6]. It can be seen that the large amount of theabrownine formed during the pile fermentation has a crucial role in the formation of the red-brown color of Pu’er tea. The content of theabrownine in the samples of Pu’er tea produced by different microorganisms increased with the increase of the number of turns during the fermentation process. However, the variation rules of theabrownine formed by fermentation of different strains are different, which may be caused by the differences in the growth conditions of different microorganisms and the different extracellular enzymes produced during the fermentation of Pu’er tea[7]. The tannins acid and tannase in Aspergillus niger have the function of degrading and hydrolyzing tannins, producing gallic acid, and makes Pu’er tea dark red[8, 9]. Under the enzymatic action of extracellular enzymes secreted by yeast, a series of reactions, such as tea polyphenols oxidation, condensation, protein degradation, carbohydrate decomposition and mutual polymerization of products, give Pu’er tea soup its unique red-brown color and special fragrance[10]. Streptomyces strains of grey micrococcus and pink micrococcus isolated from Pu’er tea can shorten the fermentation time, deepen the soup color, improve the content of total polyphenols and strengthen the ability of scavenging free radicals[11]. Some rod-shaped bacteria existed in the late stage of Pu’er tea fermentation can produce abundant polyphenol oxidase, peroxidase and so on. These enzymes can oxidize catechin tea polyphenols into benzoquinones, and benzoquinones can be further condensed into dark red polymers, which is conducive to the improvement of quality of Pu’er tea and the shortening of fermentation time[12].

According to the research of Fu Xiujuan[13] et al., during the fermentation of Pu’er tea, molds have the largest influence on the content of theabrownine, followed by yeasts and bacteria.

3.2 The Effect of Microorganisms on the Aroma of Pu’er Tea
The formation of aroma components in Pu’er tea is closely related to microbial metabolism. It is mainly composed of terpenes, aromatic cyclic alcohols, phenols, acid esters, aldehydes and ketones, heterocyclic compounds and hydrocarbons. Aromatic substances only account for 0.03%~0.05% in fresh tea leaves. However, due to the effect of microorganisms in the pile process, the types of aromatic substances in tea have changed greatly, and the content of the same substance has also changed greatly. In the process of pile, the effects of enzymes, redox of phenolic substances such as catechins, hydrothermal and acidic factors can cause and promote the production of aroma components in tea. Studies have believed that during the whole fermentation process of Pu’er tea, except for the gradual decrease in the relative content of alcohol compounds, the relative content of various aroma components, aldehydes, ketones, acids, esters and other substances in the tea samples are generally increasing. The types and quantities of methylated substances have increased with the fermentation process[14]. The effects of different microorganisms on the aroma components of Pu’er tea during fermentation are also different. Zhang Chunhua[15] et al. of Yunnan Agricultural University have studied the effects of solid-state fermentation of different beneficial bacteria on aroma...
components of Pu’er tea. The results show that among the aroma components of Pu’er tea fermented by Trichoderma viride, the content of terpene alcohol compounds and their derivatives is the highest, the taste is mellow and sweet, and the aroma is old and fragrant; Among the aroma components of Pu’er tea fermented by Aspergillus niger and Rhizopus arrhizus, the content of methoxybenzene and its derivatives is the highest; Among them, the Pu’er tea fermented by Aspergillus Niger is mellow in taste and full of fruity flavor, while the Pu’er tea fermented by Rhizopus oryzae is mellow and smooth in taste and full of flavor; Among the aroma components of Pu’er tea fermented by Saccharomyces cerevisiae, the content of the tea terpene alcohol compound and its derivatives and methoxybenzene and its derivatives are relatively high, the taste is sweet and smooth, and the aroma is more obvious. Microorganisms act on tea components through some of their own characteristics, such as unique odors and secreted enzymes, thus forming a unique aroma of Pu’er tea.

3.3 The Effect of Microorganisms on the Taste of Pu’er Tea
The main material basis of Pu’er tea taste is tea polyphenols, caffeine and amino acids. However, the bio-macromolecule in tea, the metabolites produced by the interaction between tea and microorganisms in the fermentation process, and other trace substances will have an effect on the taste of Pu’er tea.

3.3.1 Tea Polyphenols
Tea polyphenols are a general term for more than 30 polyphenolic compounds in tea. The most important of them are catechins, which are complex compounds. The content of catechins in green tea is the highest. During the processing of fermented tea, catechins are oxidized and polymerized into a series of colored compounds, such as theaflavin, thearubigin and theabrownine. They have important effects on the quality and color of fermented tea. During the fermentation of Pu’er tea, the polyphenol oxidase produced by microorganisms can effectively cause the change of polyphenols. The polyphenols are reduced by about 60% compared with those before fermentation, among which catechins are reduced by about 70%, while water-insoluble tea polyphenols are increased by about 70%. This promotes the astringency and bitter taste of Pu’er tea tea soup significantly lower than before fermentation, thus forming the unique mellow taste of Pu’er tea.

3.3.2 Caffeine
Caffeine is the main purine in tea and is an important flavoring substance in tea soup. Caffeine has a bitter taste, but when combined with polyphenols and oxidation products, it can reduce the bitterness of these substances and form a substance with a fresh taste. During the process of tea fermentation, mold can increase the content of caffeine, while yeast can decrease the content; Among the molds, Aspergillus niger has the greatest effect and can significantly increase the content of caffeine [16].

3.3.3 Amino Acid
Amino acids account for 1% to 5% of the dry weight of tea. There are 28 kinds of amino acids in tea, among which the content of theanine is more than 50% of the total amount of amino acids, which is an amino acid unique to tea. The amino acid has a high water solubility, which makes the tea soup have a distinctly fresh and sweet taste. Aspergillus niger can produce more than 20 hydrolyases in the fermentation process, and the acid protease (peptidase) can hydrolyze proteins into free amino acids, thus affecting the flavor and quality of Pu’er tea.

3.3.4 Other Metabolites
Some of the substances that make up the taste of Pu’er tea are metabolites produced by microorganisms that degrade macromolecules (polysaccharides, proteins, nucleic acids and fats) in tea. Cellulose in tea can be decomposed into simple sugars under the action of cellulase-secreting microorganisms. Microorganisms can use degradation products of proteins and nucleic acids as nitrogen and energy sources for their growth. The protein can be hydrolyzed into a short peptide by the
action of an extracellular enzyme (protease) secreted by the microorganism, and further decomposed into amino acids by the action of the peptidases. Fungi have the ability to break down natural proteins. Certain microorganisms can hydrolyze phosphodiester bonds by secreting nucleases to form lower polynucleotides or single nucleotides. Microorganisms generally do not use fat as a carbon source or energy substance, but when other energy sources and carbon source substances are not present, many microorganisms begin to decompose and utilize fat. Fungi such as Candida, Fusarium, and Penicillium have strong lipase-producing ability to hydrolyze fat into glycerol and fatty acids and enter the TCA cycle[17]. Penicillium microbial mycelial waste is rich in protein, minerals and B vitamins. Moreover, the penicillin produced by its metabolism may have a good elimination or inhibition effect on the bacteria and spoilage bacteria, thereby supporting the formation of Pu’er tea taste[18]. The formation of lactic acid during the growth of Rhizopus fungi is beneficial to the formation of stick-slip and mellow quality of Pu’er tea[19-20].

4. Conclusion
With the development of the times, people pay more attention to scientific diet and health. Pu’er tea is very popular with consumers due to its high health care effect and Pu’er tea industry has been developing very fast. We should study the population, quantity and growth laws of microorganisms in the fermentation process of Pu’er tea, analyze the correlation between microbial community structure, dynamic changes and the quality changes of Pu’er tea, and establish a microbial library of Pu’er tea in Yunnan Province; In addition, we should also clarify the correlation between the differences in regions, processes and storage years and the flavor quality of Pu’er tea, and reveal the effect and mechanism of microorganisms in the production of Pu’er tea; These have important guiding significance for improving the quality of Pu’er, providing theoretical basis for the standardized production of Pu’er tea, and have great significance for the sustainable development of Pu’er tea industry.

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