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Research Progress on the Influence of Microbial Fermentation on Tea Quality

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Abstract. Microbial fermentation technology plays a vital role in tea processing. By decomposing and transforming tea components, it enhances the flavor and taste of tea while producing a variety of beneficial nutrients. These transformations significantly improve the overall quality of tea and enhance its health-promoting value. This paper reviews the working mechanisms of microbial fermentation technology from the perspectives of different types of fermentative microorganisms and varying fermentation conditions. Particular attention is given to the roles and mechanisms of bacteria, yeasts, molds, and other microorganisms during the tea fermentation process. Furthermore, the effects of fermentation time, temperature, and environmental factors on tea quality are discussed. Through this analysis, the study aims to provide new insights and technical support for optimizing tea fermentation processes, improving the quality of fermented tea beverages, and innovatively developing functional tea products.

Keywords: Fermentation, Tea, Physicochemical Properties, Microorganisms

1. Introduction

Microbial fermentation, through vigorous metabolic activity and complex enzymatic reactions, effectively regulates the transformation of active components in tea infusions and participates in the synthesis of key flavor substances [1]. It has multidimensional impacts on tea quality formation, flavor characteristics, and health functions, making it a critical process for flavor development and value enhancement [2]. Rui Yang [3] and colleagues focused on the quality and functional properties of tea beverages fermented with single or specific mixed strains, while Ran Ran [4] systematically reviewed liquid fermented tea beverages across different product types, mainly emphasizing the types of fermentation, with relatively limited discussion on the effects of fermentation conditions. Xiaofeng Mao [5] concentrated on basic classifications and conventional processing techniques of liquid tea beverages without deeply exploring the key factors by which microbial fermentation imparts unique flavor and functional characteristics. This paper discusses both the types of fermenting microorganisms and fermentation conditions, analyzing the roles of bacteria, yeasts, molds, and other microorganisms such as Calocybe indica during tea fermentation. It systematically examines the effects of fermentation time, temperature, inoculum size, tea concentration, and environmental factors on tea quality formation. Such analysis supports comprehensive utilization of tea and provides a theoretical basis for research and development of liquid fermented tea beverages.

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2. Influence of microbial types on fermented tea

Studies have shown that microorganisms are widely involved in key stages of tea processing, fermentation, and aging. Specific functional microorganisms can actively regulate their microenvironment, thereby influencing the formation of tea quality [6].

2.1. Effects of bacteria on fermented tea

Bacteria are common microorganisms widely distributed in nature and living organisms. Through their specific metabolic activities, they deeply participate in and regulate tea quality formation. Important bacterial groups include lactic acid bacteria, acetic acid bacteria, Bacillus subtilis, and Weissella confusa, all of which have significant ecological and applied value. Lactic acid bacteria are core microorganisms in traditional fermented foods, effectively inhibiting harmful microbes while ensuring fermentation safety [7,8]. Lihua Zhang et al. [9] compared Du Zhong leaf teas prepared by different processing methods and found that lactic acid bacteria may produce β-glucosidase, which releases soluble bound phenols in tea, reducing the oxidative consumption of polyphenols and maintaining antioxidant activity. Wenjian Yang et al. [10] used Lactobacillus bulgaricus and Lactobacillus plantarum for fermentation. The polyphenol-degrading capacity of the former synergized with the acid-producing capacity of the latter, promoting glycoside hydrolysis and the degradation of chlorogenic acid and forsythiaside A. The microbial growth generated various metabolites and enzymes that facilitated polyphenol release, increasing total flavonoid content, moderately reducing tea polyphenols, and mitigating the bitterness and cold nature of Forsythia leaves, resulting in a mellow and sweet tea infusion. Acetic acid bacteria [11] also regulate the quality and functional properties of fermented foods. Chen et al. [12] studied red tea vinegar prepared from low-quality loose black tea with different acetic acid bacteria strains, finding that these bacteria oxidized ethanol to acetic acid via aerobic metabolism, produced succinic and citric acids, and promoted the formation of thearubigins. This significantly increased organic acid and volatile compound content, reduced excessive oxidation of polyphenols and theaflavins, maintained antioxidant activity, improved flavor, and enhanced both added value and health functions.

Bacillus subtilis [13], a Gram-positive rod-shaped bacterium, is widely present in naturally fermented products. It can be used alone or in combination with other strains to enhance product quality, ensure safety, and optimize production efficiency. Min Su et al. [14] compared B. subtilis fermentation with unfermented and mixed-strain fermentation of kidney tea, finding that B. subtilis exhibited strong metabolic capacity, producing enzymes and metabolites that promoted phenolic acid synthesis or transformation. This significantly increased total phenolic acid content and elevated levels of rosmarinic acid and catechol derivatives, reducing ineffective consumption of active phenolic components and maintaining antioxidant and anti-inflammatory properties. Yanqin Xiao et al. [15] studied Weissella confusa fermentation of Pu’er ripe tea, finding that it synergized with Aspergillus and Staphylococcus species to produce carbohydrate-active enzymes. These enzymes participated in polyphenol, amino acid, and sugar metabolism, promoted catechin conversion to theaflavins, reduced bitter compounds such as caffeine, and increased tea sweetness, jujube aroma, and antioxidant-related components, enhancing flavor and potential health benefits of Pu’er ripe tea.

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2.2. Effects of yeasts on fermented tea

Yeasts are simple unicellular fungi that are rich in various nutrients and bioactive compounds. They also possess antibacterial properties, cholesterol-lowering effects, and immune-enhancing abilities [16]. Tu et al. [17] successfully identified a strain of Starmerella davenportii Do18 from kombucha. During fermentation, this yeast can lower pH and produce characteristic flavor compounds, such as 2-phenylethanol, while exhibiting strong antioxidant and antibacterial activities. Wang et al. [18] used green tea as the research material and fermented it with Saccharomyces cerevisiae 71B. They observed a significant increase in key aroma compounds, including linalool and 2-phenylethanol, which enhanced the floral and fruity fragrance of green tea. Meanwhile, caffeine and catechins decreased, whereas active acids such as gallic acid increased and theanine was retained, ensuring the stability of green tea's antioxidant capacity and effectively modulating its quality. Chen Zhouqin et al. [19] isolated Debaryomyces hansenii (strain T12) from fresh Osmanthus fragrans leaves and sun-dried green Osmanthus tea. Co-fermentation with green tea resulted in increased levels of polyphenols, total flavonoids, and total catechins, significantly enhancing antioxidant activity and providing a new approach for inoculated fermentation of green tea. Ma Hui et al. [20] isolated eight yeast strains and four acetic acid bacteria strains from black tea cultures in Hefei (Anhui), Zhanjiang (Guangdong), and Jining (Shandong). Using Zygosaccharomyces bisporus Y1-1 and Y3-2 together with Gluconobacter oxydans A3, they constructed a synthetic microbial community (SMC) for kombucha fermentation based on raw Pu'er tea. The yeast hydrolyzed sucrose into fructose and glucose, which were then metabolized via glycolysis to produce ethanol, while acetic acid bacteria further converted ethanol into acetic acid and other metabolites, effectively reducing ethanol and acetic acid content in the tea. This SMC not only regulated the composition of volatile flavor compounds in the fermentation liquid, enhancing fruity, floral, and sweet aroma notes for a smoother and more harmonious taste, but also increased total polyphenol and total flavonoid contents, exhibiting excellent in vitro antioxidant activity and significantly improving the functional quality of kombucha. Xu et al. [21] demonstrated that joint fermentation of vine tea with a novel cellulase and probiotic yeast significantly enhanced the antioxidant capacity and free radical scavenging ability of the fermented tea liquid, showing potential anti-aging and cellular protective effects. Additionally, this fermentation liquid effectively inhibited α-melanocyte-stimulating hormone (α-MSH)-induced melanogenesis and reduced inflammatory cell recruitment in a zebrafish model.

2.3. Effects of molds on fermented tea

Fungi are filamentous microorganisms with complex morphological structures. They can produce abundant enzymes and a variety of secondary metabolites, and are widely used in food fermentation, antibiotic production, and organic pollutant degradation. Zhuoyuan Ma et al. [22] isolated and purified "Golden Flower" fungi from commercially available Jingyang Fuzhuan tea and identified the strain as Aspergillus chevalieri. Artificial inoculation of this fungus in tea fermentation produced tea with excellent sensory and physicochemical qualities: the golden flowers were abundant and evenly distributed, the tea liquor was bright reddish-brown, the fungal aroma was rich, and the taste was mellow with a lasting sweet aftertaste. This approach addressed the instability of tea processing and the uneven quality of the products. Jiangang He et al. [23] studied tea samples from the automated piling fermentation process of Changshengchuan Qingzhuan tea. Eight morphologically stable dominant fungal strains were isolated and purified, and seed solutions were prepared for single-strain simulated fermentation. It was found that fermentation with Trichoderma microsporum

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L-1 imparted a unique tea aroma with a strong sweet aftertaste, while Aspergillus tubingensis N-1 accelerated the fermentation process, improving production efficiency. Both strains produced water-soluble extracts and total sugars at high levels, highlighting the rich nutrient content in Qingzhuan tea. Li et al. [24] investigated Pu'er tea and found that during storage, dominant fungi such as Aspergillus spp. promoted the hydrolysis of gallotannins into gallic acid and digested polysaccharides, lipids, and proteins, thereby altering the flavor profile of the tea.

2.4. Effects of other microorganisms on fermented tea

Beneficial microorganisms in tea fermentation are not limited to bacteria, fungi, and yeasts. For example, Sittisart et al. [25] studied kombucha fermentation using black tea leaves, pandan leaves, and sucrose, supplemented with hemp leaves and Calocybe indica powder as functional ingredients, along with a highly symbiotic microbial consortium (a mixed fermentation approach). The study showed that kombucha fermented with these innovative ingredients had significantly increased total polyphenols and total flavonoids, indicating enhanced levels of plant-derived nutrients and improved functional properties. Yuan Lin et al. [26] used lotus leaves as the substrate and inoculated three fungal mycelia: Ganoderma lucidum, Hericium erinaceus, and Flammulina velutipes. By measuring mycelial growth rate and the contents of quercetin and tannins, Ganoderma lucidum mycelium was selected as the optimal strain for debittering fermentation. After fermentation, quercetin and tannin contents decreased, while the various breakdown products of tannin oxidation enriched the taste and complexity of lotus leaf tea. The resulting fermented lotus leaf tea demonstrated a significantly superior sensory profile compared with commercially available ordinary lotus leaf tea.

3. Effects of microbial fermentation conditions on tea fermentation

3.1. Effects of fermentation temperature on tea fermentation

Fermentation temperature is a key environmental factor regulating the formation of tea quality, and an appropriate temperature positively influences both microbial safety and the physicochemical properties of tea. Xian Li et al. [27] studied Jin Xuan black tea from Shengzhou, Zhejiang, under fermentation temperatures of 20, 25, and 30 °C. Using HPLC, LC-MS non-targeted metabolomics, and in vitro enzyme inhibition assays, they found that a lower fermentation temperature of 20 °C increased the content of tea polyphenols, soluble sugars, theaflavins, and theanine A (TSA), showing stronger bioactivity. These compounds were closely associated with enhanced α-glucosidase inhibitory activity, with the 20 °C fermented tea exhibiting superior α-glucosidase inhibition compared to α-amylase inhibition, indicating better hypoglycemic potential. Sadok et al. [28] investigated kombucha under home-brewing conditions using temperature gradients of 17, 22, and 25 °C. Compared with the low temperature of 17 °C, fermentation at 22 °C and 25 °C significantly reduced the risk of mycotoxin contamination. Among them, fermentation at 25 °C showed the lowest positive detection rates for aflatoxin M1 and penicillic acid, ensuring the highest product safety. Moreover, fermentation at 22 °C and 25 °C maintained pH within a more palatable range and better preserved total polyphenols and flavonoids, thereby enhancing the nutritional value and sensory acceptability of the final product. Jiandong Lai et al. [29] used Cha brick tea from Xianning and Dan Guihua as raw materials, fixing the osmanthus addition at 7% and fermentation time at 18 h in a single-factor experiment, and studied the effects of fermentation temperature from 25 °C to 45 °C. Using solid-phase microextraction coupled with gas chromatography–mass spectrometry

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(SPME-GC-MS) to analyze volatile components, they found that increasing fermentation temperature accelerated the conversion of substances in osmanthus Cha brick tea, with a trend of increasing water-extractable components, which improved the release efficiency of bioactive compounds during brewing. When the fermentation temperature was 30 °C, sensory scores reached the highest level, with a prominent and harmonious osmanthus aroma, mellow taste, and bright reddish-brown liquor. Cohen et al. [30] used black tea soup as the substrate, setting sucrose concentrations at 5%, 7.5%, and 10%, and fermentation temperatures at 20 °C and 30 °C. They found that fermentation temperature had a more significant impact on the sensory quality of black tea than sucrose concentration. Higher fermentation temperatures accelerated fermentation, leading to increased titratable acidity and decreased pH, which intensified astringency and suppressed sweetness. Ultimately, in sensory evaluations, low-temperature fermented black tea was preferred for its superior flavor profile.

3.2. Effects of fermentation time on tea fermentation

Fermentation time is another critical parameter determining tea quality transformation. Its duration directly affects substrate degradation and the synthesis of flavor compounds, exerting cumulative effects on the final flavor balance and physicochemical properties of the product. Saadah et al. [31] prepared kombucha using Sandoricum koetjape stems as raw material and applied fermentation durations of 6, 7, 8, 9, and 10 days. They found that kombucha fermented for 6 days exhibited the highest vitamin C and total polyphenol contents, maximally preserving trace nutrients such as vitamin C and phenolic compounds, resulting in the highest nutritional quality score and enhanced nutritional value of the product. Yingzhou Zhang et al. [32] studied anaerobic fermentation of De'ang sour tea wet leaves under 10 different process conditions. They found that samples treated with a rolling speed of 30 r/min for 25 min reached peak total lactic acid bacteria counts after 4 months of fermentation, confirming that 4 months is the optimal growth period for lactic acid bacteria. This allows lactic acid bacteria to fully perform their metabolic functions, decomposing sugars and polyphenols in tea to generate lactic acid, acetic acid, and volatile aroma compounds, ultimately forming the unique quality profile of De'ang sour tea wet leaves. Fermentation periods that were too long (≥6 months) or too short (≤2 months) reduced microbial activity and adversely affected tea quality. Li et al. [33] investigated Forsythia leaf fermentation tea prepared from wild Forsythia leaves collected from Lingchuan (LC), Anze (AZ), Pingshun (PS), and Pingding (PD) counties in Shanxi. By varying fermentation time from 1 h to 5 h, they observed that the content of forsythoside A in the fermented tea first increased and then decreased, reaching a peak at 3 h. The increased forsythoside A enhanced the tea's free radical scavenging ability and inhibition of oxidative damage, strengthened antimicrobial activity against common pathogens, and consequently improved the health-promoting properties of the tea. Optimal conditions were determined to be 2 h fermentation at 80% humidity and 35 °C, under which the average forsythoside A content reached 25.87 mg/g, ensuring good process stability and significantly improving overall quality. Xingyu Qian et al. [34] studied honey kombucha fermented with pure cultures of Zymomonas mobilis. Monitoring a 14-day fermentation period, they found that the pH of the kombucha decreased significantly, inhibiting the growth of spoilage and pathogenic bacteria. As fermentation progressed, the content of carboxylic acids (such as acetic acid and isovaleric acid) increased, contributing a mildly acidic flavor; alcohols (such as α-terpineol and phenylethanol) increased, adding clove and rose aromas. In the later fermentation stage (7–14 days), newly formed compounds such as isoamyl acetate and menthol shifted the flavor from initial "citrus floral" notes to a "spicy complex" profile with richer layers. Zhuoyuan Ma et al. [22] conducted solid-state fermentation of Ya'an black Mao

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tea using Aspergillus chevalieri as the inoculum. They found that extending fermentation from 5 to 11 days increased the spore count to a peak of (3.93 ± 0.41) × 10^7 CFU/g on day 11. At this point, tea polyphenol content decreased, substantially reducing bitterness. Vigorous microbial metabolism promoted the accumulation of tea pigments (such as thearubigins) and aromatic compounds, with aroma developing from a “light tea fragrance” to a “rich fungal flower aroma.” The fungal flower aroma, a characteristic fragrance generated by Aspergillus chevalieri, masked undesirable original tea odors and enhanced aroma complexity. Sensory scores also improved, while spore counts declined and the tea liquor darkened with weakened fungal aroma when fermentation exceeded 11 days due to nutrient competition.

3.3. Effects of other fermentation conditions on tea fermentation

Tea quality is also influenced by other fermentation conditions, including but not limited to inoculum size, tea concentration, relative humidity, and acidity. These factors can directly or indirectly affect microbial metabolic pathways and enzyme reaction efficiency, providing multidimensional opportunities for quality optimization. Variation in inoculum size can influence the functional components and antioxidant activity of tea infusions. Alves et al. [35] prepared kombucha using green tea as raw material and tested inoculum levels of 10%, 20%, and 30%. They found that at 30% inoculum, the total polyphenol content and DPPH and ABTS radical-scavenging activities reached their highest levels. Principal Component Analysis (PCA) indicated a strong positive correlation between inoculum size and total polyphenol content and antioxidant activity, highlighting inoculum size as a key factor influencing polyphenol accumulation and antioxidant function in green tea kombucha. At higher inoculum levels, microorganisms in the Symbiotic Culture of Bacteria and Yeast (SCOBY), such as acetic acid bacteria and yeasts, can release bound polyphenols in tea via enzymatic hydrolysis (e.g., polyphenol oxidase, esterases) and transform them into derivatives with higher antioxidant activity. Meanwhile, sucrose provides a carbon source that supports microbial synthesis of additional polyphenolic metabolites, collectively enhancing antioxidant capacity. Tea concentration provides differentiated substrate levels, directly regulating the enrichment of bioactive compounds and physicochemical characteristics in the infusion. Wang et al. [36] studied black tea kombucha fermented with New Zealand starter cultures and found that tea concentration is a core factor affecting bioactivity, functional activity, and certain sensory and physicochemical properties. Higher tea concentrations significantly increased total polyphenols, total flavonoids, and characteristic phenolics such as caffeine, gallic acid, and theobromine. This increase in phenolic content also enhanced antioxidant and antimicrobial activity, showing clear inhibitory effects against pathogens like Escherichia coli and Staphylococcus aureus, whereas lower tea concentrations had no inhibitory effect. Additionally, higher tea concentrations increased soluble solids without affecting safety indicators such as acidity and ethanol content and enhanced tea redness through the accumulation of thearubigins, thereby improving sensory quality and optimizing the core quality of black tea kombucha. Relative humidity is another key ecological factor that can regulate microbial community structure and microbial growth status, thereby ensuring flavor formation and accumulation of functional components. Shuyan Li et al. [37] studied multi-spike K sweet tea from Liangping District, Chongqing, using single-factor experiments combined with response surface methodology to optimize Monascus fermentation. They found that increasing relative humidity from 5% to 10% maximized total flavonoid content. Adequate humidity promoted biotransformation of flavonoids while avoiding problems associated with excessively low humidity (substrate drying, inhibited microbial adhesion and metabolism) or excessively high humidity (growth of contaminants, intensified nutrient competition), thereby enhancing potential antioxidant

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and metabolic regulation functions and maintaining tea quality. Acidic environments also favor the regulation of bioactive compounds in tea infusions, optimizing flavor and quality. Tuo Zhang et al. [38] prepared solid-state fermented tea using black Mao tea, treating Aspergillus coronatus with a low-acid solution (pH 3) for 48 h. They found that the low-acid group had significantly higher water-extractable components and richer soluble substances in the infusion. Polyphenol, total flavonoid, and caffeine contents were significantly lower, while electronic tongue analysis showed reduced bitterness and astringency and increased umami and sweetness. These changes effectively reduced bitterness, creating a smoother, mellow taste with prominent sweetness and lasting aftertaste. The tea infusion also exhibited bright reddish color, rich aroma, full-bodied flavor with distinctive fungal fragrance, and tender leaf texture, demonstrating comprehensive quality improvement in the solid-state fermented tea.

4. Conclusion

Microbial fermentation plays a crucial role in determining the quality of tea beverages, and different types of fermenting microorganisms exhibit distinct metabolic characteristics. Bacteria primarily promote the transformation and release of polyphenols, flavonoids, amino acids, and other compounds through acid production, enzyme secretion, and organic acid metabolism, thereby improving tea liquor taste and aroma while enhancing antioxidant and other functional properties. Yeasts generate abundant volatile compounds such as alcohols, esters, and aldehydes/ketones during fermentation, imparting unique fruity, floral, or wine-like aromas to tea, while also facilitating the transformation of amino acids and sugars, resulting in a richer, fuller-bodied infusion. Molds, such as species of Aspergillus, secrete multiple active enzymes that accelerate component conversion and promote the formation of pigments like thearubigins and theaflavins, improving tea liquor color and mouthfeel. Other fungi, such as Calocybe indica, Ganoderma lucidum, and Hericium erinaceus, also contribute through synergistic metabolic activity, further enriching aroma layers, stabilizing quality, and enhancing functional properties. In addition, appropriate fermentation temperature balances microbial activity, promoting the conversion of bioactive compounds and the formation of flavor substances, thereby improving both the nutritional and sensory quality of tea. Controlling fermentation time ensures optimal microbial activity, fully harnessing metabolic functions to enhance the health-promoting value of tea and overall quality. Other factors, including inoculum size, tea concentration, relative humidity, and pH, can regulate bioactive compound accumulation and physicochemical properties, maintain efficient microbial metabolism, and influence the formation of sensory attributes.

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