Advances in Food Science and Human Nutrition (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/afshn.2025.070110
ISSN 2616-258X Vol. 7 Num. 1

Anaerobic Fermentation with Commercial Microbes for Enhanced Sensory Quality of Yunnan Arabica Coffee: A Practical Approach for Small-Scale Processors

Bowen Zhu

HD Beijing School, Beijing, China

Keywords: Yunnan Arabica Coffee; Anaerobic Fermentation; Commercial Microbes; Sensory Quality; Small-Scale Processors; Microbial Community; Metabolomics

Abstract: Yunnan, China's primary Arabica coffee-producing region, faces a critical sensory challenge: its dominant Catimor variety, accounting for over 90% of plantings, typically exhibits nutty and caramel notes but lacks sweetness and complex flavor layers [1]. Chemical analyses have identified that Yunnan coffee beans are characterized by insufficient levels of fruity and sweet aroma compounds such as ethyl propionate, while containing higher levels of furans and pyrazines associated with woody or earthy undertones[2]. Sensory studies using descriptive analysis of 25 representative samples further confirm these limitations, noting that Yunnan coffee frequently lacks intricate flavor profiles, with "sweet" attributes ranking low among 57 identified sensory descriptors[3]. Compounding this issue, small-scale processors rely on traditional processing methods due to limited access to advanced fermentation equipment, restricting technical interventions for flavor enhancement[4]. To address these flavor-related quality issues, this study explored a low-cost solution: improving sensory attributes through anaerobic fermentation with commercial microbes. Three anaerobic groups (3 biological replicates each) were designed: spontaneous fermentation (Group A), commercial lactic acid bacteria (LAB, Group B), commercial yeast (Group C); traditional sun-drying (Group D) was the control. High-throughput sequencing, LC-MS metabolomics, and double-blind SCAA sensory evaluations (by 5 CQI Q Graders) were used for analysis. Results showed commercial microbes reshaped microbial communities: on day 15, Group A had *Levilactobacillus* / *Wickerhamomyces*, Group B had *Lactiplantibacillus* / *Lentilactobacillus*, and Group C had increasing *Saccharomyces*. LC-MS revealed distinct metabolite profiles (e.g., organic acids, phenolics) in anaerobic groups. Sensory scores: samples fermented without anaerobic conditions scored significantly lower than all anaerobic treatments in flavor, aftertaste and overall aspect respectively, compaired with samples from LAC treatment group . Among the treatment groups, Group B exhibited the best performance in flavor balance, cleanliness, and aftertaste, followed by Group A, while Group C scored slightly lower but remained significantly superior to the sample fermented without anaerobic conditions. Although no significance statistically, decreased total score can be observed in non-anaerobic group when compared with samples from A, B and C group, from 83.05~82.5 in fermented group A, B and C, to 81.65 in average in non-anaerobic group. This method—commercial microbes + simple sealed containers, no sterilization (preserving terroir via native microbiota)—is easy and low-cost, ideal for small-scale processors. It provides a feasible way to improve Yunnan coffee's quality and economic value.

1. Introduction

1.1. Research Background and Problem Statement

Coffee (*Coffea arabica* L.) stands as one of the world's most valuable agricultural commodities and a globally cherished beverage. Its complex flavor profile is heavily influenced by post-harvest processing methods, with fermentation playing a pivotal role in degrading mucilage, developing precursors for aroma and flavor compounds, and ultimately determining final cup quality and sensory characteristics[5,6]. While fermentation practices (e.g., wet, dry, honey) are well-documented in traditional coffee-producing regions like south America and Africa, research on region-specific fermentation dynamics, particularly for unique terroirs, remains crucial[7,8].

Yunnan is China's largest coffee-growing region. Its unique combination of high altitude, subtropical climate, distinct soil types, and local microbial ecosystems creates a terroir with substantial potential for producing specialty coffees[9,10]. However, Yunnan Arabica coffee (*Coffea arabica*) is generally characterized as "nutty flavour mainly, balanced body with mild acidity" [3,11]; its flavor profile still shows noticeable limitations, lacking the sweetness, complexity, and aromatic richness found in high-quality Arabica beans from regions such as South America and East Africa. As a result, Yunnan coffee has often been purchased in bulk by international traders at prices $0.10–0.20 per pound below the New York futures market, limiting the realization of its full value.

The development of coffee flavor is a complex biochemical cascade that initiates in the coffee cherry and persists through post-harvest processing. A pivotal phase in this process is fermentation, where indigenous or inoculated microbial communities interact dynamically with coffee's intrinsic compounds to drive flavor precursor formation[10]. Coffee green beans contain a diverse array of biochemical substrates, among which carbohydrates constitute a major fraction of dry matter—these substrates serve as the primary energy source for fermentative microbes, laying the foundation for flavor metabolism[10]. Specifically, microorganisms such as lactic acid bacteria (LAB, e.g., *Levilactobacillus* and *Lactiplantibacillus*) and yeasts (e.g., *Wickerhamomyces* and *Saccharomyces*) metabolize these carbohydrates via pathways including glycolysis and the pentose phosphate pathway. This metabolic activity generates key flavor-active compounds: for instance, esterification reactions produce ethyl acetate (contributing fruity notes), while microbial pyruvate metabolism yields 2,3-butanedione (imparting creamy aromas)—these compounds collectively shape the initial flavor profile of coffee[10,12].

Despite the rapid growth of Yunnan's coffee industry and increasing international recognition, scientific research dedicated to optimizing and understanding the fermentation processes specific to its small-grain coffee is relatively nascent[13,14]. Current fermentation practices in Yunnan often rely on empirical knowledge or adaptations of methods from other regions. This approach potentially overlooks the critical interplay between the intrinsic properties of Yunnan beans, the indigenous microbiota present on the cherries and in the local environment, and the fermentation parameters (time, temperature, pH, oxygen availability)[15,16]. This gap is significant, as spontaneous fermentations driven by native yeasts, bacteria (especially lactic acid bacteria - LAB), and fungi exhibit high variability. This variability directly impacts the metabolic pathways involved in flavor development and can lead to inconsistencies or defects in the final cup profile[17,18]. Understanding and harnessing this microbial diversity is key to achieving controlled fermentation for enhanced and reproducible cup quality[18,19].

Controlled fermentation techniques, particularly anaerobic fermentation, have emerged as a promising strategy to precisely regulate coffee flavor development. The oxygen-deficient environment of anaerobic fermentation suppresses the proliferation of aerobic microorganisms (e.g., *Acetobacter*, which may produce excessive acetic acid and off-flavors) while enhancing the metabolic

activity of facultative anaerobes like LAB and non-*Saccharomyces* yeasts[12]. This selective microbial activation facilitates the accumulation of more complex and refined flavor compounds, avoiding the harsh or unbalanced notes associated with uncontrolled aerobic fermentation[10]. Among anaerobic approaches, self-induced anaerobic fermentation (SIAF)—a technique where CO_2 produced by microbial metabolism gradually creates anoxic conditions—has been shown to significantly promote the growth of beneficial microbes, including *Torulaspora* yeasts and heterofermentative LAB. Concurrently, SIAF inhibits the growth of toxigenic fungi (e.g., *Aspergillus niger*, which produces ochratoxin) and spoilage-related *Enterobacteriaceae*, reducing health risks while improving flavor stability[12]. For Yunnan Arabica coffee specifically, studies have observed a strong positive correlation between the abundance of such beneficial microbes and sensory quality: higher counts of LAB and functional yeasts correspond to improved cup scores, particularly in terms of flavor balance and clean aftertaste—this provides a direct scientific basis for applying microbial inoculants to optimize the quality of Yunnan-grown coffee[10].

Nevertheless, many advanced fermentation technologies—such as precision anaerobic tanks and automated oxygen control systems—though effective, require substantial capital investment (e.g., an automated processing line can cost hundreds of thousands of RMB) and operational expertise. These factors make them difficult for small and medium-sized coffee farmers and processors in Yunnan to adopt and replicate. Therefore, there is a pressing need to develop a standardized, easy-to-implement fermentation method that utilizes readily available commercial yeast and LAB strains, simple sealed containers (e.g., barrels with one-way valves), and procedures that can be easily adopted by typical coffee processing units. Such an approach would have significant practical implications for broadly enhancing the flavor quality of Yunnan coffee.

The decision to refrain from sterilizing the indigenous microbiota was based on two considerations: firstly, coffee is an agricultural product strongly influenced by terroir, and the native microbial community contributes significantly to its regional identity and value; secondly, while sterilization may improve process controllability, it entails higher costs, advanced equipment, and stricter environmental controls, making it less feasible for smallholders in Yunnan. Thus, all experimental groups (A, B, and C) retained their natural microbiota without intervention.

1.2. Research Objectives and Significance

1.2.1. Research Objectives

This study aims to comprehensively investigate the impact of distinct indigenous microbial consortia on the fermentation quality and sensory profile of Yunnan small-grain coffee (*Coffea arabica*). It seeks to develop a low-cost, easy-to-operate, and highly reproducible anaerobic fermentation process using combined microbial cultures to enhance the flavor profile and economic value of Yunnan Arabica coffee.

It is worth noting that Yunnan Agricultural University recently established China's first dedicated microbial strain resource bank for coffee fermentation (CFMRD), which preserves 3,030 strains with potential applications[20]. This lays the foundation for systematically developing specialized fermentation starters tailored to different regions of Yunnan. This study employs commercially available strains combined with simple anaerobic processing, aiming to explore a more accessible technical pathway for small and medium-scale processors and provide practical basis for regional fermentation strategies.

Specifically, the research will utilize indigenous microbial communities, commercially available ordinary yeast powder, and LAB to ferment coffee cherries under anaerobic conditions (using simple, readily available sealing equipment). Through comparison with samples processed by ordinary sun-drying methods, the impact on the final quality of coffee beans will be evaluated.

The specific research contents include:

• Characterize the dynamic succession of microbial communities (bacteria, yeasts, fungi) throughout spontaneous fermentation using high-throughput metagenomic sequencing;

• Monitor the evolution of key flavor-related metabolites (volatile organic compounds, organic acids, sugars) during fermentation using GC-MS and LC-MS;

• Five Q Grader coffee tasters certified by the Coffee Quality Institute (CQI) conducted blind evaluations in accordance with SCAA standards, focusing on sensory indicators such as dry/wet aroma, sweetness, acidity, body, flavor complexity, and aftertaste. The assessments aimed to determine the impact of anaerobic fermentation on the sensory characteristics of coffee. This approach was designed to ensure the objectivity, authority, and international recognition of the evaluation results.

1.2.2. Research Innovations and Significance

Although Yunnan small-grain coffee has developed distinct sensory characteristics, the fermentation mechanisms and microbial interactions specific to this region remain underexplored, limiting further quality improvement[3,11]. Current practices often rely on empirical knowledge or adopted techniques from other regions, overlooking the critical role of native microbiota and local terroir in flavor development[15,16]. While anaerobic fermentation has been shown to effectively enhance cup quality[17,18], existing advanced technologies—such as precision anaerobic tanks—are often impractical for smallholders due to high costs and operational complexity.

This study addresses the practical needs of Yunnan coffee production by systematically comparing microbial succession, metabolite profiles, and sensory outcomes under anaerobic conditions using spontaneous fermentation versus commercial lactic acid bacteria (LAB) and yeast inoculation. The key innovations of this study are:

• Developing a low-cost anaerobic fermentation process using commercially available microbial strains and simple sealing equipment, suitable for small and medium-scale applications;

• Clarifying the regulatory effects of different inoculation strategies on flavor quality while preserving the native microbial background;

• Integrating high-throughput sequencing and metabolomics to systematically elucidate fermentation mechanisms from the perspective of microbiota-metabolite-sensory relationships.

The significance of this research is twofold: Theoretically, it enriches the understanding of microbial fermentation in Yunnan coffee, providing a scientific basis for region-specific fermentation strategies[13]. Practically, the proposed approach requires neither complex equipment nor sterilization processes, making it highly accessible and adoptable for local farmers, thereby contributing substantially to the quality and economic value of Yunnan coffee[7,19].

2. Materials and Methods

2.1. Coffee Anaerobic Fermentation and Sampling Process

Coffee cherries (*Coffea arabica*) were harvested and stored under ventilated and shaded conditions at ambient temperature for 7 days prior to processing. The fruits were subsequently washed with a 0.9% saline solution (prepared with boiled cooled water and non-iodized salt) and air-dried at room temperature.

The fermentation experiment was designed with three treatment groups, each with three biological replicates:

• Indigenous microbial communities (Group A): 720 g of whole coffee cherries were placed in sealed containers, subjected to oxygen exclusion, and allowed to undergo spontaneous fermentation.

• Lactic acid bacteria inoculation (Group B): 720 g of whole coffee cherries were mixed uniformly with 60 g of a lactic acid bacterial inoculum before being sealed under anaerobic conditions. Commercial brand of Kefir Yogurt was purchased online and used as LABs to conduct fermentation, containing *Lactobacillaceae spps*.

• Yeast inoculation (Group C): 720 g of whole coffee cherries were combined with 58 g of water and 2.4 g of commercial yeast powder, mixed thoroughly, and then transferred to sealed containers under anaerobic conditions. Commercial brand of Angelyeast was purchased online and used as yeast to conduct fermentation, containing *Saccharomycetaceae spps*.

All containers were kept in a dark, cool place with an average ambient temperature of 20 °C and relative humidity of 60–70% throughout the fermentation period. Sampling was performed at three time points: before fermentation (Day 0), after 7 days of fermentation (D7), and after 15 days of fermentation (D15). For each replicate, 20 g of cherries were aseptically collected and rinsed with 20 mL of sterile physiological saline. The wash solution was retained as a sample and stored at –20 °C. The remaining cherries were returned to their containers to continue fermentation, except at the final time point (Day 15), where the cherries were sun-dried on mesh trays after sampling.

All equipment used during experimentation and sampling was sterilized or rinsed with physiological saline to minimize external contamination. All samples were stored in a household freezer at –20 °C until further analysis.

Regarding the control group: A control group using traditional sun-drying (Group D) was established: 720 g of whole coffee cherries were placed on mesh trays under natural sunlight and ventilation conditions without any sealing or anaerobic treatment. All other conditions—including fruit origin, pre-processing, sampling time points, and storage—remained consistent with the experimental groups. All cherries were sourced from the same farm, same harvest batch, and same maturity grade.

2.2. Microbial Diversity Analysis

Microbial community genomic DNA was extracted from each sample using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop2000 spectrophotometer (Thermo Scientific, United States).

• For the bacterial community, the bacterial 16S rRNA genes were amplified using the universal bacterial primers 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-RGYTACCTTGTTACGACTT-3')[21].

• For the fungal community, the ITS sequences were amplified using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') [22].

Primers were tailed with PacBio barcode sequences to distinguish each sample. Amplification reactions (20-μL volume) consisted of 5 × FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and DNase-free water. The PCR amplification was performed as follows: initial denaturation at 95 ℃ for 3 min, followed by 27 cycles of denaturing at 95 ℃ for 30 s, annealing at 60 ℃ for 30 s, and extension at 72 ℃ for 45 s, and a single extension at 72 ℃ for 10 min, ending at 4 ℃ (T100 Thermal Cycler PCR thermocycler, BIO-RAD, USA).

After electrophoresis, the PCR products were purified using AMPure® PB beads (Pacific Biosciences, CA, USA) and quantified with Qubit 4.0 (Thermo Fisher Scientific, USA). Purified products were pooled in equimolar amounts, and a DNA library was constructed using the SMRTbell prep kit 3.0 (Pacific Biosciences, CA, USA) according to PacBio's instructions. Purified SMRTbell libraries were sequenced on the Pacbio Sequel IIe System (Pacific Biosciences, CA, USA) by

Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

High-fidelity (HiFi) reads were obtained from the subreads, generated using circular consensus sequencing via SMRT Link v11.0. HiFi reads were barcode-identified and length-filtered:

- For bacterial 16S rRNA gene, sequences with a length <1,000 or >1,800 bp were removed.
- For fungal ITS, sequences with a length <300 bp or >900 bp were removed.

Bioinformatic analysis of the coffee bean microbiota was carried out using the Majorbio Cloud platform (https://cloud.majorbio.com)[23]. Based on the ASVs information, rarefaction curves and alpha diversity indices (observed ASVs, Chao1 richness, Shannon index, and Good's coverage) were calculated with Mothur v1.30.1[24]. The similarity among microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity using the Vegan v2.5-3 package. The PERMANOVA test was used to assess the percentage of variation explained by the treatment and its statistical significance using the Vegan v2.5-3 package.

The linear discriminant analysis (LDA) effect size (LEfSe)[25] (http://huttenhower.sph.harvard.edu/LEfSe) was performed to identify significantly abundant taxa (from phylum to genera) among different groups (LDA score > 2, $P < 0.05$). Due to multicollinearity among soil physicochemical properties, the variance inflation factor (VIF) for each variable was estimated using the vif function in the car package (https://cran.r-project. org/web/packages/car/car.pdf). Distance-based redundancy analysis (db-RDA) was performed using the Vegan v2.5-3 package to investigate the effect of soil physicochemical properties on soil bacterial community structure. Forward selection was based on Monte Carlo permutation tests (permutations = 9999). The x- and y-axis values and arrow lengths represented the importance of each physicochemical property in explaining taxon distribution across communities. Linear regression analysis was applied to determine the association between major physicochemical properties identified by db-RDA and microbial alpha diversity indices. Co-occurrence networks were constructed to explore internal community relationships across samples[26]. A correlation between two nodes was considered statistically robust if the Spearman's correlation coefficient was >0.6 or <–0.6, with $P < 0.01$.

2.3. LC-MS Analysis for Sensory Properties

100 mg of solid matter from each sample was placed in a 2 mL centrifuge tube with a 6 mm diameter grinding bead. 800 μL of extraction solution (methanol: water = 4:1 (v:v) containing four internal standards (0.02 mg/mL L-2-chlorophenylalanine, etc.)) was added for metabolite extraction. Samples were ground using a Wonbio-96c (Shanghai Wanbo Biotechnology Co., LTD) frozen tissue grinder for 6 min (-10 ℃, 50 Hz), followed by low-temperature ultrasonic extraction for 30 min (5 ℃, 40 kHz). The samples were left at -20 ℃ for 30 min, centrifuged for 15 min (4 ℃, 13000 g), and the supernatant was transferred to an injection vial for LC-MS/MS analysis.

As part of system conditioning and quality control, a pooled quality control (QC) sample was prepared by mixing equal volumes of all samples. QC samples were processed and tested identically to analytic samples to represent the entire sample set and were injected at regular intervals (every 5–15 samples) to monitor analysis stability.

LC-MS/MS analysis was conducted on a UHPLC-Orbitrap Exploris 240 system equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The mobile phases consisted of 0.1% formic acid in water: acetonitrile (2:98, v/v) (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The flow rate was 0.40 mL/min, the column temperature was 40°C, and the injection volume was 5 μL.

The UPLC system was coupled to a UHPLC-Orbitrap Exploris 240 Mass Spectrometer with an electrospray ionization (ESI) source operating in positive and negative modes. Optimal conditions were: source temperature 400°C; sheath gas flow rate 40 arb; aux gas flow rate 10 arb; ion-spray

voltage floating (ISVF) -2800V (negative mode) and 3500V (positive mode); normalized collision energy 20–40–60V rolling for MS/MS. Data acquisition was performed in Data Dependent Acquisition (DDA) mode, with detection over a mass range of 70–1050 m/z.

Bioinformatic analysis of coffee bean sensory properties was also carried out using the Majorbio Cloud platform (https://cloud.majorbio.com)[23].

2.4. Sensory Properties Characterization

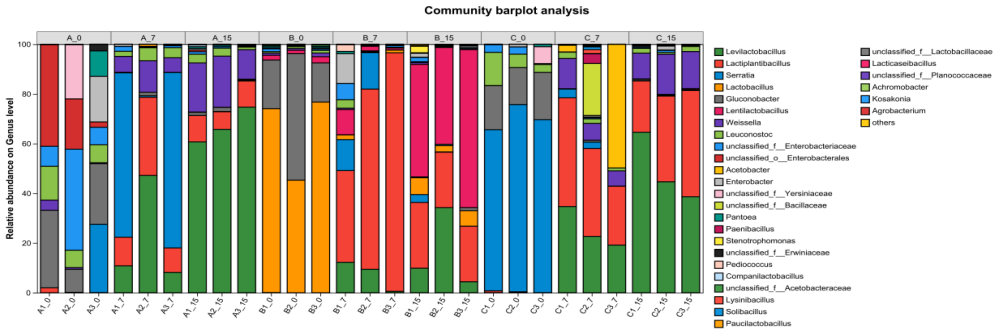
Five Q Grader coffee tasters certified by the Coffee Quality Institute (CQI) conducted sensory evaluation of coffee samples. The cup test was performed under strict double-blind conditions; both panelists and the server were unaware of sample identities to prevent bias.

Evaluation followed the official protocol of the Specialty Coffee Association of America (SCAA). Sensory attributes were assessed and scored using the standardized SCAA Coffee Cupping Form (2003 version), which quantifies key qualities such as fragrance/aroma, flavor, aftertaste, acidity, body, balance, and overall impression, in addition to detecting potential defects [5,27].

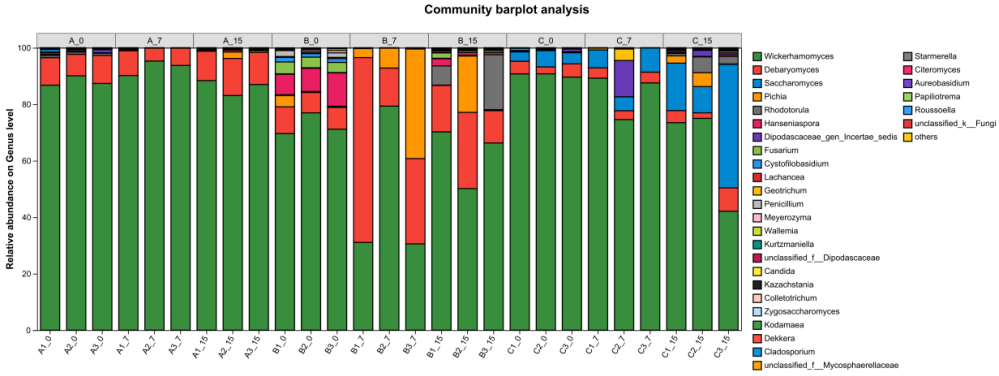
All samples were coded with random three-digit numbers and presented in randomized order to each panelist. The tasting environment was controlled for quietness, cleanliness, and minimal odor interference.

3. Results

3.1. Microbial Diversity Evaluation during Coffee Anaerobic Fermentation



Composition of bacteria



Composition of fungi

Figure 1: Composition of bacteria and fungi of samples collected in the natural fermentation group (A), Lactic acid bacteria inoculation (B), and Yeast inoculation (C). Bacteria and fungi were shown according to prevalence, respectively. The number after each letter represents the fermentation time (e.g., B0= Lactic acid bacteria inoculation at 0 h)

Bacterial community composition (Figure 1) showed distinct succession patterns across groups:

• In indigenous microbial communities,(Group A), the D0 microbiota was dominated by heterogeneous contaminants with limited *Gluconobacter*. By D7, lactic acid bacteria (LAB) became enriched, primarily *Levilactobacillus* and *Lactiplantibacillus*, accompanied by *Weissella* and *Serratia*. At D15, *Levilactobacillus* was highly dominant, with substantial *Weissella*; *Leuconostoc* was consistently detected.

• In LAB-inoculated samples (Group B), D0 microbiota included *Gluconobacter* and *Lactobacillus*. By D7, *Lactiplantibacillus* dominated, succeeded by *Lentilactobacillus* at D15. *Levilactobacillus* enrichment was significantly lower than in Groups A and C.

• In yeast-inoculated samples (Group C), D0 microbiota was dominated by *Serratia* (similar to Group A), with *Gluconobacter* and *Leuconostoc*. Over time, *Weissella*, *Lactiplantibacillus*, and *Levilactobacillus* gradually became dominant LAB.

Fungal community composition (Figure 1) also varied:

• Group A maintained *Wickerhamomyces* as a major component throughout fermentation, with *Debaryomyces* constituting ~10% from D0 to D15.

• Group B (LAB-inoculated) introduced *Hanseniaspora* and minor *Fusarium* at D0. By D7, additional genera (*Pichia*, *Debaryomyces*) were observed; at D15, *Rhodotorula* was detected, and *Wickerhamomyces* abundance was lower than in Group A.

• Group C (yeast-inoculated) showed consistent *Saccharomyces* detection, with increasing abundance over fermentation.

3.2. LC-MS Analysis of Different Fermentation Methods

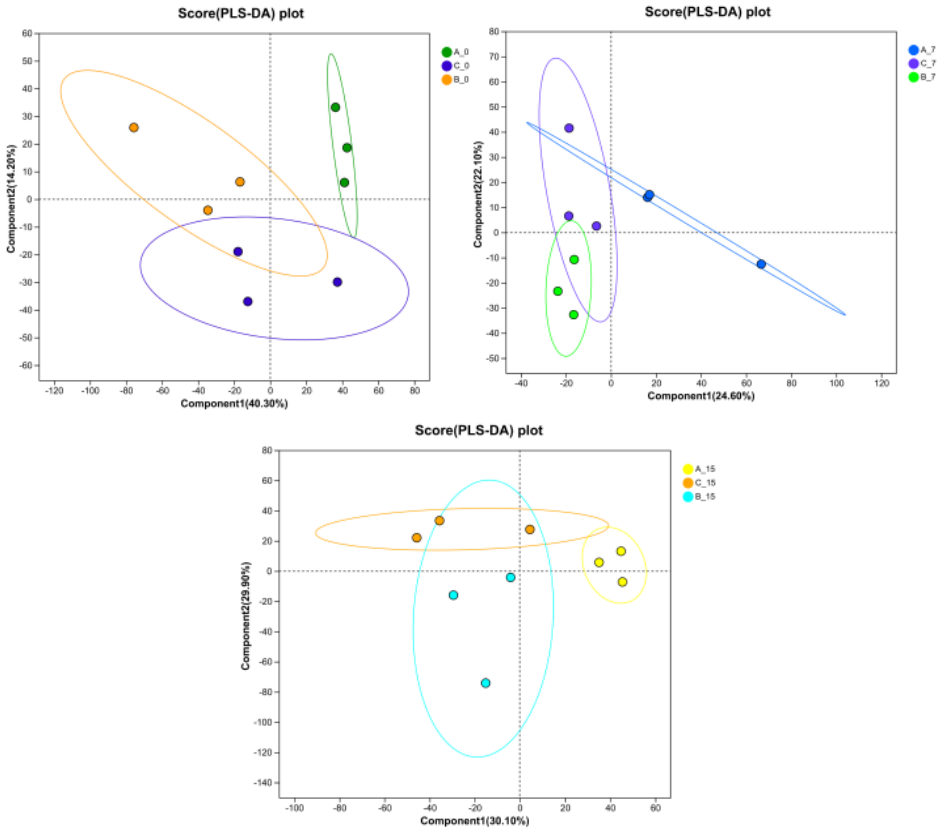


Figure 2: Principal Component Analysis (PCA) score plot derived from the GC-MS volatile compounds of coffee samples undergoing different fermentation processes.

PCA analysis of LC-MS data (Figure 2) revealed distinct metabolite profiles among groups post-fermentation, despite variability in parallel replicates.

The heatmap of volatile compounds (Figure 3) further highlighted differential accumulation of metabolites, including organic acids, fatty acids, and phenolic derivatives, across treatments and time points, consistent with previous metabolomic studies on coffee fermentation[7,27].

For the LC-MS results, considerable variations were observed among parallel replicates within individual samples, which did not align with the expected outcomes. However, PCA analysis revealed certain differences between groups after fermentation were completed, which may account for the variations in cupping scores.

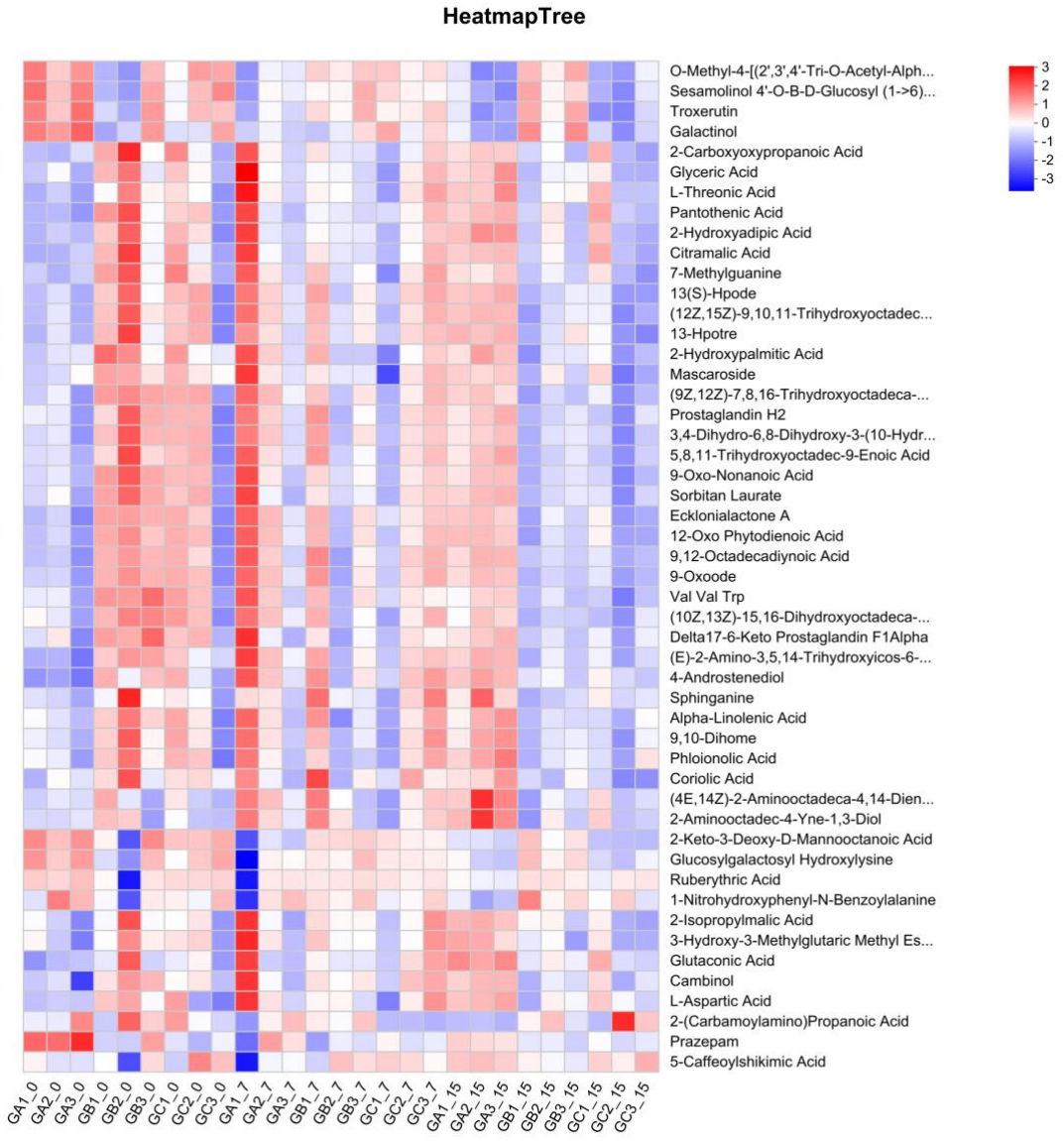
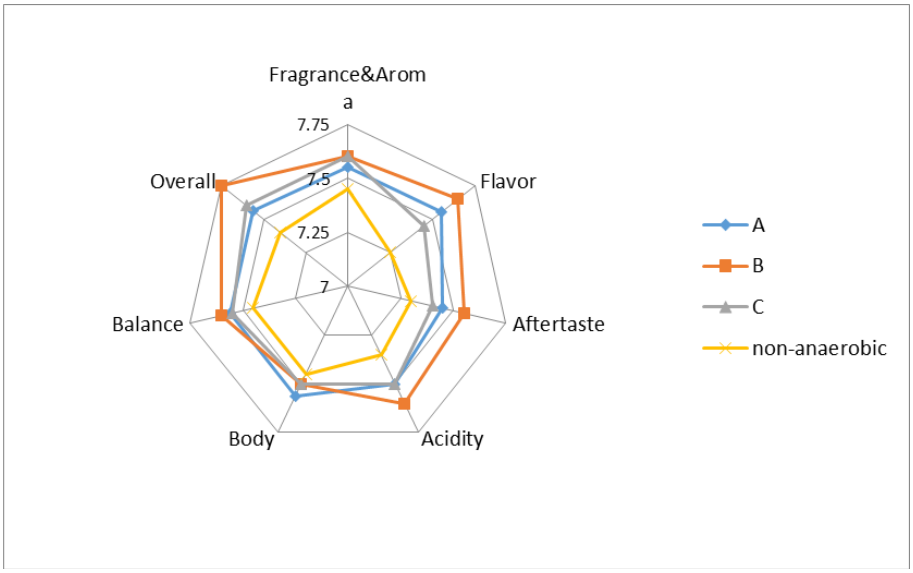


Figure 3: Heatmap of the volatile compounds identified in each groups, The number after each letter represents the fermentation time (e.g., GB0= Lactic acid bacteria inoculation at 0 h) the number after bottom-dash represents the parallel repetition.

3.3. Sensory Analysis

SCAA cupping results (Figure 4) showed:



Cupping result

	A	B	C	non-anaerobic
Fragrance&Aroma	7.55	7.6	7.6	7.45
Flavor	7.55	7.65	7.45	7.25
Aftertaste	7.45	7.55	7.4	7.3
Acidity	7.5	7.6	7.5	7.35
Body	7.5625	7.5	7.5	7.45
Balance	7.5625	7.6	7.55	7.45
Overall	7.5625	7.75	7.6	7.4
total score	82.9	83.05	82.45	81.65

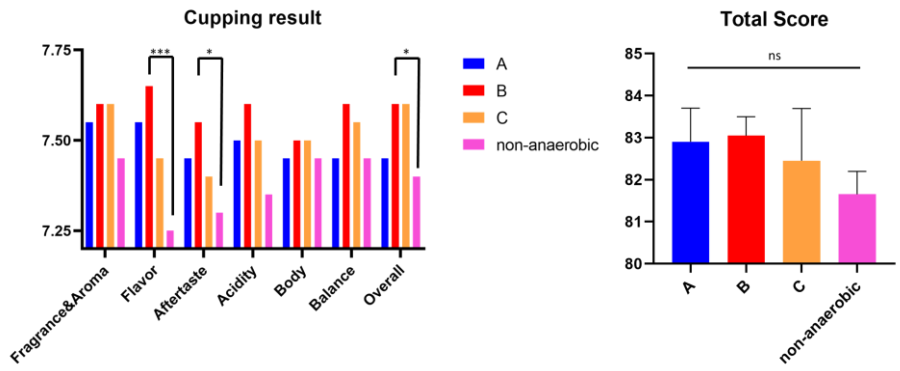


Figure 4: SCAA cupping results

- Groups A and B received identical total scores, slightly higher than Group C.
- Group A was described as "caramel, berry, dark chocolate, sweet potato, bright acidity, well-balanced."
- Group B was characterized by "nutty, caramel, sweet orange, dark chocolate, layered berry notes, clean finish," with superior balance and aftertaste compared to Group A.
- Group C exhibited "nutty, dark chocolate, spicy, slightly woody, somewhat rough aftertaste."

Notably, samples fermented without anaerobic conditions scored significantly lower than all anaerobic treatments in flavor, aftertaste and overall aspect respectively,compaired with samples from LAC treatment group . Among the treatment groups, Group B exhibited the best performance in flavor balance, cleanliness, and aftertaste, followed by Group A, while Group C scored slightly lower but remained significantly superior to the sample fermented without anaerobic conditions. Although no significance statistically, decreased total score can be observed in non-anaerobic group when compared with samples from A, B and C group, from 83.05~82.5 in fermented group A, B and C, to 81.65 in average in non-anaerobic group.The results confirm the positive impact of anaerobic conditions on cup quality, as reported in prior research[17,18].

4. Conclusion

The results indicated that various inoculation strategies significantly influenced the microbial community structure and metabolite composition. Sensory evaluation revealed that all anaerobic fermentation groups (A, B, and C) achieved significantly higher total scores than the control group, which was processed by ordinary sun-drying without anaerobic treatment. Descriptors such as "balanced," "clean finish," and "bright acidity" were associated with anaerobic treatments, underscoring the critical role of controlled anaerobic conditions in enhancing the complexity and overall sensory profile of coffee. These results suggest that anaerobic fermentation serves as a decisive factor in the development of desirable flavors, while microbial inoculants can further modulate community composition and metabolic outcomes.

It should also be noted that microbial communities vary across farms and processing environments. Our study further demonstrates that even using low-cost commercial strains and simple anaerobic equipment can effectively improve coffee flavor quality; however, practical implementation should involve locally adapted trials to identify the most suitable and cost-effective microbial strains and processing protocols. Future efforts could leverage the established microbial resource bank[10,20] to screen strains and optimize processes for specific growing regions, ultimately developing fermented coffee products with stronger regional characteristics and identity.

References

[1] Hu, R., Xu, F., Chen, X., Kuang, Q., Xiao, X., & Dong, W. (2024). The growing altitude influences the flavor precursors, sensory characteristics and cupping quality of the Pu'er coffee bean. Foods, 13(23), 3842. https://doi. org/10. 3390/foods13233842

[2] Bi, X., Yu, H., Hu, F., Fu, X., Li, Y., Li, Y., Yang, Y., Liu, D., Li, G., Shi, R., & Dong, W. (2023). A systematic analysis of the correlation between flavor active differential metabolites and multiple bean ripening stages of Coffea arabica L. Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/acs.jafc.3c00001

[3] Hu, G., Yin, Y., Han, X., Zhang, Y., & Liu, J. (2022). Characteristic flavor compounds and sensory profiles of washed Yunnan Arabica coffee. Molecules, 27(21), 6989. https://doi.org/10.3390/molecules27216989

[4] Global Times. (2025, March 24). Yunnan coffee in global spotlight, thanks to its natural advantages, elevated quality. https://www.globaltimes.cn/page/202503/1330772.shtml

[5] International Coffee Organization (ICO). (2023). Coffee development report 2023: Growing for prosperity. London, UK: ICO.

[6] Velmourougane, K. (2013). Impact of natural fermentation on physicochemical, microbiological and cup quality characteristics of Arabica and Robusta coffee. Proceedings of the National Academy of Sciences India Section B: Biological Sciences, 83(2), 233–239. https://doi.org/10.1007/s40011-013-0473-9

[7] De Melo Pereira, G. V., de Carvalho Neto, D. P., Magalhães Júnior, A. I., Vásquez, Z. S., Medeiros, A. B. P., Vandenberghe, L. P. S., & Soccol, C. R. (2019). Exploring the impacts of postharvest processing on the aroma formation of coffee beans – A review. Food Chemistry, 272, 441–452. https://doi.org/10.1016/j.foodchem.2018.09.070

[8] Puertas, G., Guerrero, K., Cortés, M. P., & Suárez, H. (2022). Cup quality and biochemical attributes of coffee (Coffea arabica L.) varieties grown in different agroecological regions of Colombia. Agronoma Colombiana, 40(2), 189–199. https://doi.org/10.15446/agron.colomb.v40n2.103180

[9] Cheng, B., Furtado, A., Smyth, H. E., & Henry, R. J. (2016). Influence of genotype and environment on coffee quality. Trends in Food Science & Technology, 57, 20–30. https://doi.org/10.1016/j.tifs.2016.08.004
[10] Zhai, H., Dong, W., Fu, X., Li, G., & Hu, F. (2024). Integration of widely targeted metabolomics and the e-tongue reveals the chemical variation and taste quality of Yunnan Arabica coffee prepared using different primary processing methods. Food Chemistry: X, 22, 101286. https://doi.org/10.1016/j.fochx.2024.101286
[11] Zhang, S. J., Yang, M., Liu, C., Chen, X. D., & Wang, J. (2021). Quality characteristics of Yunnan coffee and influencing factors. Agronomy, 11(10), 2153. https://doi.org/10.3390/agronomy11102153
[12] Kartika, A. A., Sunarharum, W. B., & Mahatmanto, T. (2024). The impact of self-induced anaerobic fermentation (SIAF) on coffee antioxidants: A review. Journal of Experimental Life Sciences, 14(1), 40–49. https://doi.org/10. 18052/www. scipress.com/JELS.14.40
[13] Hu, G., Peng, C., Xie, Y., Liu, J., Han, X., & Wang, Y. (2023). Microbial community succession and metabolite changes during Yunnan Arabica coffee bean fermentation. Food Research International, 172, 113163. https://doi. org/10. 1016/j. foodres.2023.113163
[14] Liu, J., Huang, W., Zhou, R., Wang, Y., Zhang, L., & Liu, S. (2020). Microbial diversity and chemical analysis of Shuangjiang wine starter for rice wine fermentation. Journal of the Science of Food and Agriculture, 100(6), 1898–1906. https://doi.org/10.1002/jsfa.10293
[15] Elhalis, H., Cox, J., & Zhao, J. (2021). Ecological succession of the microbial communities of an alkaline-fermented food indigenous to Yunnan Province, China. Frontiers in Microbiology, 12, 701713. https://doi.org/10.3389/fmicb.2021. 701713
[16] Evangelista, S. R., Miguel, M. G. D. C. P., de Souza Cordeiro, C., Silva, C. F., Pinheiro, A. C. M., & Schwan, R. F. (2014). Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. Food Research International, 61, 183–195. https://doi.org/10.1016/j.foodres.2014.03.024
[17] De Melo Pereira, G. V., Neto, E., Soccol, V. T., Medeiros, A. B. P., Woiciechowski, A. L., & Soccol, C. R. (2020). Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing. International Journal of Food Science & Technology, 55(8), 3279–3291. https://doi.org/10.1111/ijfs.14566
[18] Pereira, G. V. d. M., Neto, D. P. d. C., Soccol, V. T., Medeiros, A. B. P., Woiciechowski, A. L., & Soccol, C. R. (2015). Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects. Food Research International, 75, 348–356. https://doi.org/10.1016/j.foodres. 2015. 05. 032
[19] Silva, C. F., Schwan, R. F., Dias, Ë. S., & Wheals, A. E. (2008). Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. International Journal of Food Microbiology, 120(2), 160–169. https://doi.org/10.1016/j.ijfoodmicro.2007.09.017
[20] Yunnan Provincial Department of Agriculture and Rural Affairs. (2024). Yunnan Agricultural University established China's first dedicated microbial strain resource bank for coffee fermentation. Retrieved from https://www.ynagri.gov.cn/
[21] Liu, C., Zhao, D., Ma, W., Zhang, X., & Wang, A. (2016). Denitrifying sulfide removal process on high-salinity wastewaters in the presence of Halomonas sp. Applied Microbiology and Biotechnology, 100(3), 1421–1426. https://doi.org/10.1007/s00253-015-7039-6
[22] Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560
[23] Han, C., Shi, C., Liu, L., Han, J., Zhang, Y., & Wang, J. (2024). Majorbio Cloud 2024: Update single-cell and multiomics workflows. Imeta, 3(4), e217. https://doi.org/10.1002/imt2.217
[24] Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., & Sahl, J. W. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied & Environmental Microbiology, 75(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09
[25] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome Biology, 12(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60
[26] Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome functions. Nature Biotechnology, 38(6), 685–688. https://doi. org/10.1038/s41587-020-0548-6
[27] Zhang, S. J., De Bruyn, F., Pothakos, V., Torres, J., Falconi, C., Moccand, C., Weckx, S., & De Vuyst, L. (2019). Following coffee production from cherries to cup: Microbiological and metabolomic analysis of wet processing of Coffea arabica. Applied and Environmental Microbiology, 85(6), e02635-18. https://doi.org/10.1128/AEM.02635-18