

Arabica-Robusta Coffee-Blend Fermentation Enhanced by Microbial Starters

Nadiah Nur^{1*}, Reta², Abu Bakar Tawali¹, Amran Laga¹

¹Department of Agricultural Technology, Faculty of Agricultural Technology, Hasanuddin University, Makassar, South Sulawesi, Indonesia

²Department of Agriculture Technology, Pangkep State Polytechnic of Agricultural, Makassar, South Sulawesi, Indonesia

*Corresponding author: nnadia2501@gmail.com

Editor: Samuel Pereira de Carvalho

Received in May 9, 2025 and approved in December 14, 2025

ABSTRACT

This study investigates the use of specific microbial starter cultures in enhancing the quality and flavor profile of Arabica-Robusta coffee blends through controlled fermentation. The research focuses on the application of lactic acid bacteria—particularly *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, and *Weissella* sp., which are known to dominate the bacterial communities during coffee fermentation. These microorganisms play a pivotal role in the demucilagenation and acidification of coffee cherries, processes that are crucial in developing desirable sensory attributes such as flavor and aroma. Furthermore, the study explores how the strategic blending of Arabica and Robusta varieties can contribute to the formation of fruity and floral aromas, as well as caramel-like compounds, thereby enhancing the overall complexity of the coffee's flavor profile. The moisture content of starter culture-inoculated coffee powder was lower (6.01%) than that of the control batch (7.39%), indicating more efficient demucilaging and drying processes facilitated by the microbial starter culture. The metabolic activities of these starter cultures significantly influence the flavor profile of coffee by producing lactic and acetic acids, which decrease the pH of the fermentation environment and the formation of flavor-active compounds such as esters, aldehydes, and ketones. By strategically using microbial starter cultures and optimizing post-fermentation treatments such as washing, drying, and roasting, coffee producers can effectively enhance the overall quality and sensory attributes of Arabica-Robusta coffee blends. These amendments address the evolving preferences of discerning coffee consumers. This research offers valuable insights for stakeholders seeking to utilize microbial starters to enhance the quality and consistency of coffee blends, thereby fostering innovation within the coffee industry.

Key words: Arabica-robusta; *in vitro*; starter cultures.

1 INTRODUCTION

Coffee is a globally significant beverage, deeply rooted in diverse cultures, with *Coffea arabica* and *Coffea canephora* constituting the principal commercial species. Blending these two varieties is a common practice in the coffee industry, allowing for the combination of their respective desirable flavor and aroma attributes. The fermentation stage during coffee processing is particularly critical, as it involves the activity of a range of microorganisms responsible for degrading the mucilage of the coffee cherry, suppressing the growth of mycotoxin-producing fungi, and contributing to the development of the beverage's final flavor and aromatic characteristics (Nur et al., 2023; Calderon et al., 2023).

Traditionally, coffee fermentation has been a spontaneous process, dependent on the coffee cherries' indigenous microbial communities. However, the use of starter cultures has emerged as a promising alternative, offering enhanced control and optimization of the fermentation process. The deliberate inoculation of specific microorganisms, including yeasts and lactic acid bacteria, has been shown to promote the production of fermented coffee with distinct and improved sensory qualities (Calderon et al., 2023; Rodrigues et al., 2020)

Lactobacillus plantarum is a lactic acid bacterium known for its adaptability and ability to ferment a wide range

of carbohydrates. It can be used as a starter culture in food fermentation, contributing to the flavor and aroma of the final product (Da Silva Sabo et al., 2014). The metabolic activity of *L. plantarum* during coffee fermentation can produce lactic acid and other compounds that influence the taste and flavor precursors of coffee beverages (De Melo Pereira et al., 2020). A consortium of *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Bacillus sphaericus* has been shown to significantly improve coffee bean demucilization (Siridevi et al., 2019). Furthermore, *Lactobacillus rhamnosus* has been shown to modify flavor-related constituents in green coffee beans, modulating coffee volatiles during roasting.

Isolates from *Weissella* species, which are a group of lactic acid bacteria (LAB), a diverse group of Gram -positive, non-spore forming, catalase negative, acid intolerant, facultative anaerobic, or microaerophilic bacteria; wherein lactic acid is the predominant end product of carbohydrate fermentation – are also frequently isolated in other coffee fermentation methods. For instance, *Weissella thailandensis* was detected in coffee fermentation in Taiwan. These bacteria may contribute to the microbial diversity in the fermentation process and could potentially affect the flavor profile of the final coffee product (De Melo Pereira et al., 2020)

Recent studies have investigated the application of starter cultures in the fermentation of Arabica-Robusta coffee bean blends. The targeted inoculation of these blends with

defined microbial consortia has demonstrated promising outcomes in improving both the quality and consistency of the resulting coffee products (Calderon et al., 2023; De Melo Pereira et al., 2020; Iqraini D et al., 2021).

2 MATERIAL AND METHODS

2.1 Time and Place of Research

This study was conducted from July to November 2024 at the Microbiology Laboratory, the Food Processing and Chemical Analysis Laboratory, and the Food Quality Control Laboratory of the Food Science and Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Makassar. In addition, cupping test analyses were performed by 2 trained cuppers with Q-grader certificate at the Coffee and Cocoa Research Center in Jember, East Java.

2.2 Tools and Materials

For analytical purposes, the tools used were stirring rods, coffee cups, separating funnels, high-performance liquid chromatography (HPLC) model DGU-20A5; Shimadzu Sim-pack HPLC packed column/Shimpack GIST C18; Made in Shimadzu Corporation in Kyoto, Japan, UV-Vis spectrophotometer single beam UV-1280 Serial Number A120661 Made in Shimadzu Corporation in Kyoto, Japan, beaker glasses, 100 mL volumetric flasks, Erlenmeyer flasks, an oven, a pH meter, a thermometer, a stopwatch, and a water pot.

The primary materials employed in this study were green beans of Arabica and Robusta coffee. Analytical procedures utilized various reagents and supplies, including calcium carbonate (CaCO_3), *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, Weissella sp., MRS Broth, MRS Agar, latex, physiological saline solution, and buffer solutions with pH values of 4 and 7.

2.3 Research Procedures

2.3.1 Research Stages

1. Dry Starter/Inoculum Storage

The best results of dry starter treatment varied from those of previous studies and were obtained in the first stage, namely, the storage of a dry starter (inoculum). The dry starter was then stored with vacuum packaging for 90 days and sampled every 15 days for LAB viability testing via the total plate count (TPC) method. Furthermore, the moisture content was observed with a moisture analyzer (Fitri et al., 2021).

2. Fermentation of Arabica and Robusta Coffee Beans.

After harvesting, the coffee cherries were sorted and washed to remove impurities, followed by blending the Arabica and Robusta varieties. Mechanical pulping was then conducted to eliminate the outer skin of the cherries. Subsequently, the selected microbial starter cultures were introduced to initiate a controlled fermentation process, which was maintained at a constant temperature of 38°C for 48 h. During the fermentation period, samples were collected at 12-h intervals for analytical evaluation.

After fermentation, the coffee beans were subjected to designated drying and roasting procedures and were subsequently ground to a medium-fine consistency without the use of mesh filters. The coffee particles that did not meet the specified size criteria were reprocessed to achieve uniformity. The resulting product was subjected to a comprehensive series of quality assessments to ensure high standards and consistency. These evaluations included sensory analysis, determination of hydrogen ion concentration (pH), measurement of moisture content, quantification of caffeine levels, and assessment of total acidity, all of which contributed to rigorous quality control (Fitri et al., 2021).

3 RESULTS

3.1 LAB Viability Testing (Shelf-Life Testing on Dried Starter)

Viability refers to the ability of cells to maintain life and function normally under optimal conditions. The objective of the LAB viability test was to assess microbial colony formation in a dry starter culture using the total plate count (TPC) method. The primary independent variable was the incubation period following microencapsulation, monitored over 90 days with sampling conducted at 15-day intervals. The dependent variable was the number of viable microbial colonies. This method was employed to evaluate the impact of microencapsulation on the growth and long-term survival of LAB (Fitri et al., 2021).

An analysis of the temporal dynamics of LAB viability, as presented in Table 1, revealed a marked decline in microbial populations throughout the experimental period. Quantitative enumeration indicated a decrease in LAB colony counts by about one logarithmic unit, from log 7,60 cfu/mL on day 15 to log 6,98 cfu/mL on day 30. This represents an approximate 76.3% reduction in viable cell concentration over the two-week interval. The downward trend in LAB viability continued through the later stages of the study, with further significant reductions recorded between days 45 and 52. This progressive decline suggests the presence of environmental stressors adversely affecting

bacterial survival, potentially including nutrient depletion, accumulation of metabolic byproducts, or alterations in the physicochemical conditions of the growth medium. The observed pattern of diminishing LAB viability carries important implications for microbial stability, functional efficacy, and the shelf life of the fermented product. These findings underscore the need for effective preservation strategies to sustain probiotic viability throughout the intended storage period (Fitri et al., 2021).

Table 1: Calculation of the colony count of the storage-dried starter.

Duration (days)	Number of colonies (log cfu/mL)
15	log 7,60
30	log 6,98
45	log 7,25
52	log 6

3.2 Dry Starter Moisture Content Test

To determine the moisture content of the dry starter, a 1-g sample was weighed using a moisture analyzer cup. The analyzer was set to 105 °C, sealed, and operated until the moisture content was displayed on the monitor. The resulting water content data were subsequently recorded. Moisture content analysis of the Arabica-Robusta coffee grounds indicated no statistically significant differences among the samples. Triplicate measurements yielded values of 10.3%, 8.8%, and 8.3%, with a mean moisture content of 9.0% (n = 3). These results suggest a consistent level of hydration across the analyzed coffee ground samples (Rukmawati, Hartini, & Cahyanti, 2017).

3.3 Fermentative Process

Based on the data presented in Table 2, which reports the average water content of the optimal treatment, the use of starter cultures combined with temperature-controlled fermentation proves essential for producing high-quality, well-fermented coffee. This controlled approach contributes significantly to enhancing the sensory attributes and overall quality of the final product (Silamba et al., 2024; Adam, Agustina, & Fadhil, 2022).

Table 2: The average water content value of the best treatment.

1st test	10.3%
2nd test	8.8%
3rd test	8.3%
Average	9%
Standard Deviation	± 4.45

The role of starter cultures is to utilize selected microorganism starter cultures, and ohmic heating technology during coffee fermentation significantly enhances both its sensory quality and consumer appeal. The complete fermentation process for Arabica and Robusta coffee, as (Fitri et al., 2021), a standardized of steps designed to maintain optimal conditions microbial activity and biochemical beans are sorted and washed to remove materials and homogeneity are blended and pulped to the outer layers. A microbial starter culture is, and fermentation at a temperature of 38°C for 48.

The consistency and quality control guarantee microbial stability throughout the coffee fermentation process, aiding in maintaining uniformity in quality and sensory profiles. The controlled process results in the development of specialty-grade coffee, enhancing desirable characteristics, such as acidity, sweetness, and complexity. Consequently, it yields a more refined and consistent flavor profile in the final product (Silamba et al., 2024).

3.4 Cupping Test

Figure 1, the cupping test results, shows the panelists' responses to the 10 attributes assessed for coffee with 5 different fermentation times, namely, B1W0 (control, 0 hours fermentation), B1W1 (12 hours fermentation), B1W2 (24 hours fermentation), B1W3 (36 hours fermentation), and B1W4 (48 hours fermentation). Overall, the characteristics of the coffees were not significantly different. Based on Figure 2, the analysis of variance indicated no statistically significant impact.



Figure 1: Flavor profile of a brewed blend of Arabica and Robusta coffees.

Figure 1 shows the average scores of the panelists for the five different fermentation times. The panelists preferred the aroma/flavor of Arabica and Robusta coffee from B1W4, which was fermented for 48 h. This finding shows that the

optimum fermentation time with a starter to obtain the best flavor of Arabica-Robusta blend coffee is 48 h with the starter. The factors that influence coffee fermentation are the duration of fermentation, the amount of bacterial inoculum, the substrate (medium), oxygen, temperature, water, and acidity (pH) (Calderon et al., 2023; Da Silva Vale et al., 2019).

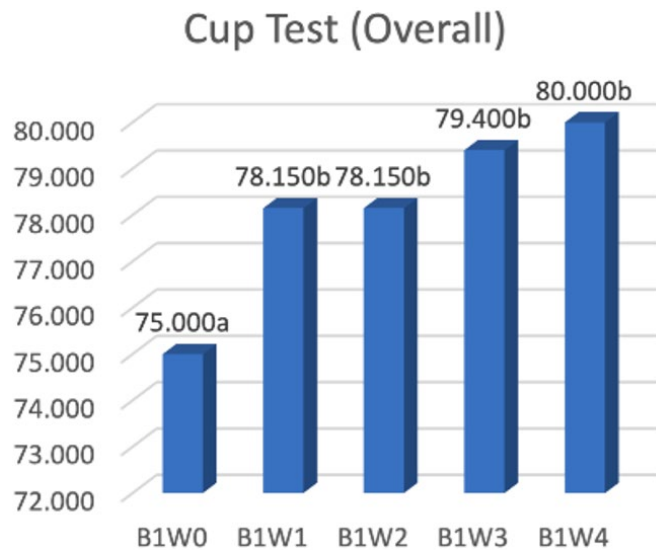


Figure 2: Cupping test chart.

According to the panelists, coffee fermented for 48 h exhibited flavor characteristics of nutty, floral (coffee blossom), fruity-lemon, and fruity-lemon-grape notes. In addition to these primary attributes, other flavor notes identified included an astringent aftertaste, black tea aftertaste, as well as spicy and sweet corn nuances. Research has shown that the longer the fermentation time, the higher the acidity, body, and balance scores. However, the aroma score for the body attribute decreased after an increase. The fermentation process increases acidity due to the formation of compounds such as reducing sugars, amino acids, organic acids, trigonelline, chlorogenic acid, lipids, and peptides (Adam, Agustina, & Fadhil, 2022) (Kalschne et al., 2018)

Lactic acid and acetic acid are produced gradually over the course of fermentation. The increase in body parameters is attributed to the formation of reducing sugars, amino acids, lipids, peptides, and other organic acids during this (Adam; Agustina; Fadhil, 2022) (Licandro et al., 2020). The enhancement in balance value is primarily due to the degradation of macromolecules such as carbohydrates and proteins into simpler compounds—namely simple sugars and organic acids—facilitated by the metabolic activity of yeast and bacteria throughout fermentation. Flavor attributes are significantly influenced by temperature during the roasting process, where increased complexity arises from the Maillard reaction and the development of sweetness through sugar

caramelization (Calderon et al., 2023) (Da Mota et al., 2020). However, excessive roasting can lead to a burnt taste, resulting from intensified phenolic reactions in the coffee beans. Additionally, extended roasting tends to reduce the acidity of the brewed coffee, often producing a ‘flat’ taste or diminishing flavor complexity (Da Silva Vale et al., 2019; Kinasih et al., 2021; Lee, B. et al., 2023; Lee et al., 2015; Lee, Y. et al., 2023; Lessy et al., 2023; Ly et al., 2019).

3.5 pH Changes and Acidity Levels

Our experimental analysis of coffee acidity revealed notable variations in pH when precisely measured quantities of ground coffee (10 g) were extracted with 100 mL of distilled water under controlled laboratory conditions. Based on Table 3, triplicate measurements yielded pH values of 5.01, 4.21, and 4.35, resulting in a mean pH of 4.52. While the literature generally identifies an optimal pH range of 4.8–5.0 for high-quality coffee extraction, our findings indicate a broader acidity spectrum. The first trial, with a pH of 5.01, approached slight alkalinity, whereas the second trial exhibited significantly greater acidity with a pH of 4.21. These variations underscore the sensitivity of coffee acidity to extraction parameters and highlight their potential impact on flavor development (De Melo Pereira et al., 2020).

Table 3: The average pH changes and acidity levels of the best treatments.

1st test	5.01
2nd test	4.21
3rd test	4.35
Average	4.52
Standard Deviation	± 0,48

4 DISCUSSION

Microencapsulated lactic acid bacteria (LAB) exhibited a substantial decline in viability over a 90-day storage period, with a 76.3% reduction observed by day 30. These results suggest that while microencapsulation offers initial protection, factors such as nutrient depletion and the accumulation of metabolic byproducts significantly influence long-term bacterial survival. This underscores the need for improved preservation strategies to maintain probiotic viability in dry cultures. Analysis of Arabica-Robusta coffee grounds revealed an average moisture content of 9.0%, indicating consistent hydration, an essential parameter for microbial regulation during fermentation. Maintaining optimal moisture levels, carefully monitoring starter cultures, and controlling fermentation temperature are critical for ensuring favorable fermentation outcomes. Fermentation conducted at 38°C for 48 h effectively stimulates microbial activity, resulting in

enhanced sensory attributes such as acidity and sweetness, thereby emphasizing the importance of standardized processing protocols for high-quality specialty coffee production. Sensory evaluations revealed that coffee fermented for 48 h (B1W4) was particularly well-received by panelists, who noted its distinctive nutty, floral, and fruity notes (Nur et al., 2023). Although extended fermentation may further enhance acidity and body, the aroma was observed to peak and subsequently decrease, indicating the existence of an optimal fermentation window. Measured pH values ranging from 4.21 to 5.01 highlight the role of acidity in flavor development. Roasting also plays a significant role, with moderate roasting enhancing flavor complexity, while over-roasting tends to produce burnt or flat sensory notes.

5 CONCLUSIONS

The application of microbial starter cultures improved the fermentation quality of Arabica–Robusta coffee, with the B1W4 treatment (48 h) achieving specialty grade at 85.75 points and earning perfect scores for sweetness, uniformity, and clean cup. The sample showed distinctive fruity, floral, nutty, and light spicy notes, confirming the effectiveness of directed microbial fermentation in enhancing coffee quality. Further research is needed to assess commercial feasibility and clarify microbial succession for more consistent, high-quality production.

6 AUTHORS' CONTRIBUTIONS:

Conceptual Idea: Nur, N.; Tawali, A. B; Laga, A.; Reta. Methodology design: Nur, N.; Tawali, A. B; Laga, A.; Reta. Data collection: Nur, N. Data analysis and interpretation: Nur, N. ; Tawali, A. B; Laga, A. ; Reta. Writing and editing: Nur, N. ; Tawali, A. B; Laga, A. ; Reta.

7 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

8 REFERENCES

- Adam, F., Agustina, R., Fadhil, R. (2022). Pengujian cita rasa kopi arabika dengan metode cupping test (Arabica Coffee Taste Testing With Cupping Test Method). *Jurnal Ilmiah Mahasiswa Pertanian*, 7(1):517-520.
- Siridevi, G. B. et al. (2019). Coffee starter microbiome and in-silico approach to improve Arabica coffee. *LWT*, 114:108382.
- Calderon, N. et al. (2023). A consumer assessment of fermented green coffee beans with common beer/wine yeast strains for novel flavor properties. *Fermentation*, 9(10):865.
- Da Mota, M. C. B. et al. (2020). Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. *Food Research International*, 136:109482.
- Da Silva Sabo, S. et al. (2014). Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. *Food Research International*, 64:527-536.
- Da Silva Vale, A. (2019). et al. Effect of Co-Inoculation with *Pichia fermentans* and *Pediococcus acidilactici* on metabolite produced during fermentation and volatile composition of coffee beans. *Fermentation*, 5(3):67.
- De Melo Pereira, G. V. et al. (2020). Lactic acid bacteria: what coffee industry should know? *Current Opinion in Food Science*, 31:1-8.
- Fitri, F. et al. (2021). Identification of lactic acid bacteria from luwak (*Paradoxurus hermaphroditus*) gastrointestinal tract. *International Journal of Agriculture and Biology*, 26(6):717-721.
- Iqraini, D. N. et al. (2021). Effect of the fermentation process using a consortium of probiotic bacteria on the taste of Arabica coffee (*Coffea arabica*). *IOP Conference Series: Earth and Environmental Science*, 807:032020.
- Kalschne, D. L. et al. (2018). Steam pressure treatment of defective *Coffea canephora* beans improves the volatile profile and sensory acceptance of roasted coffee blends. *Food Research International*, 105:393-402.
- Kinasih, A. et al. (2021). Karakteristik sensori kopi arabica dan robusta menggunakan teknik brewing berbeda. *Jurnal Teknologi Pangan dan Hasil Pertanian*, 16(2):1-11.
- Lee, B. H. et al. (2023). Microbial diversity of anaerobic-fermented coffee and potential for inhibiting ochratoxin-produced *aspergillus niger*. *Foods*, 12(15):2967.
- Lee, M. E. et al. (2015). Starter cultures for kimchi fermentation. *Journal of Microbiology and Biotechnology*, 25(5):559-568.
- Lee, Y. G. et al. (2023). Value-added products from coffee waste: a review. *Molecules*, 28(8):3562.
- Lessy, S. N. et al. (2023). Pengaruh metode fermentasi dan lama penyangraian terhadap cita rasa kopi tuni asal maluku. *Jurnal Agrosilvopasture-Tech*, 2(2):386-393.
- Licandro, H. et al. (2020). How fermentation by lactic acid bacteria can address safety issues in legumes food products?. *Food Control*, 110:106957.

- Ly, S. et al. (2019). Engineering synthetic microbial communities through a selective biofilm cultivation device for the production of fermented beverages. *Microorganisms*, 7(7):206.
- Nur, N. et al. (2023). Production of drip bag coffee filled with blended arabica and robusta coffee beans by ohmic fermentation technology. *IOP Conference Series: Earth and Environmental Science*, 1228:012029.
- Rodrigues, S. D. et al. (2020). Sensory q-grader evaluation of fermented Arabica coffees by yeast (*Saccharomyces cerevisiae*) and lactic bacteria (*Pediococcus acidilactici*) cultures. *Coffee Science*, 15:e151690.
- Rukmawati, Y. E. A., Hartini, Sri., & Cahyanti, M. N. (2017). Isoterm sorpsi air pada tepung ubi jalar terfermentasi dengan angkak. *Jurnal Kimia VALENSI*, 31(1):71-78.
- Silamba, I. et al. (2024). Enhancing coffee sensory quality: Unleashing synergistic effects of selected microorganism starter cultures and ohmic heating technology. *IOP Conference Series: Earth and Environmental Science*, 1356:012034.