Journal of Bio & Geo Material and Energy (BiGME)
Volume 6 No. 2, April 2026
P-ISSN: 2808-1560 E-ISSN: 2808-1404

Optimization of Fermentation Time and Ghalkoff Microbial Concentration on Organic Robusta Coffee

Ika Putri Sulistiana[1], Suharyono[2], Subeki[2], Medina Alia Rahmawati[1], Suci Putri Ramadani[1], Tesy Pratami[1*]

[1] Departement of Agriculture Technology, Faculty of Agriculture, University of Jambi, Jambi Province, Indonesia
[2] Departement of Agriculture Technology, Faculty of Agriculture, University of Lampung, Lampung Province, Indonesia

*Email: tesypratami@unja.ac.id

Article info:

Submitted: March 2026
Revised: April 2026
Accepted: April 2026
Published: April 2026

Abstract :
Civet coffee (*kopi luwak*) is a distinctive Indonesian coffee produced by feeding coffee cherries to civets, after which the beans excreted in their feces are collected and processed into ground coffee; however, this method raises ethical concerns due to its potential involvement in animal abuse. This study aimed to optimize fermentation time and Ghalkoff microbial concentration to improve the chemical and microbiological characteristics of organic Robusta coffee as an alternative to civet coffee. The method employed was Response Surface Methodology (RSM) with a two-factor Central Composite Design (CCD), involving fermentation time (24, 48, and 72 hours) and microbial concentration (20%, 25%, and 30% w/v). Parameters analyzed included caffeine content, chlorogenic acid content, total microbial count, and sensory evaluation (cupping test). The results showed that optimal conditions were achieved at 72 hours of fermentation and 30% (w/v) microbial concentration, producing the lowest caffeine content (1.55%), the highest chlorogenic acid content (6.81%), and a cupping score of 84, comparable to commercial civet coffee (85). The caffeine content met SNI 01-3542-2004 standards for grades I and II. Thus, Ghalkoff fermentation demonstrates strong potential as a more ethical and sustainable alternative.

Keywords: Fermentation, Ghalkoff, organic Robusta coffee, response surface methodology

1. Introduction

Coffee is a leading commodity for the Indonesian plantation sector, playing a crucial role in national economic growth (Directorate General of Plantations, 2020). One type of coffee widely produced and consumed in Indonesia is Robusta coffee *(Coffea canephora)*. Robusta coffee has a better market share due to its low risk of crop failure and relatively affordable price (Wijaya et al., 2023).

One type of fermented coffee popular in Indonesia is civet coffee. Civet coffee is the result of a short fermentation process that occurs in the digestive system of the civet *(Paradoxurus hermaphroditus)*. The enzymes present in the civet's digestive system contribute to the distinctive flavor and aroma of coffee (Kusmiati et al., 2020). However, the fermentation process in civet coffee still relies on the natural processes occurring within the animal's body. This dependence raises several concerns, including the potential for animal abuse, low production levels, and threats to wild civet populations. Furthermore, because fermentation involves the animal's digestive tract, the halal status of civet coffee remains a matter of debate (Kiyat et al., 2019).

These negative issues have prompted research to develop coffee with similar characteristics through alternative methods. In vitro fermentation (outside the civet's body) may be a solution for producing coffee with similar characteristics. Fermenting coffee beans can enhance flavor due to the metabolites produced by microbes (Pereira et al., 2015). During fermentation, bacteria break down complex compounds into simpler compounds, including reducing sugars and metabolites such as organic acids (Suprihatin, 2010).

One important factor in the coffee fermentation process is the quality of the raw materials. This study used organic Robusta coffee from Hanakau, West Lampung. The use of organic fertilizers on coffee plants does not produce environmentally harmful residues. The application of organic fertilizers serves to enrich and restore nutrient content in the soil and plants. The organic coffee used in this study was fermented using cellulolytic, proteolytic, and xylanolytic microbes developed by PT. Ghaly Roelies Indonesia, known as Ghalkoff microbes. Ghalkoff microbes are active microorganisms that produce enzymes similar to those found in the digestive system of civets, which function to ferment coffee and alter its chemical composition. These microbes were isolated and selected from civet feces (Wijaya et al., 2023).

Fermentation time and microbial concentration are two key factors that influence the quality of the resulting coffee (Larassati et al., 2021). According to Tika et al. (2017), fermentation is one step in producing low-caffeine coffee with a strong flavor. Factors affecting the coffee fermentation process include the amount of bacterial inoculum, fermentation time, substrate (medium), temperature, oxygen, water, and acidity level (Usman et al., 2015).

Tawali et al. (2018) reported that the optimal coffee fermentation time using yogurt-derived lactic acid bacteria was 48 hours, yielding a caffeine content of 1.09% and a pH of 4.73. Meanwhile, a study by Usman et al. (2015) found that coffee fermentation using lactic acid bacteria isolates from civet feces for 24 hours resulted in a caffeine content of 0.72%. Fermentation of robusta coffee beans using Saccharomyces cerevisiae starter with varying concentrations (20-30%) and fermentation times (24-72 hours) was shown to reduce caffeine, reducing sugar, and protein levels, as well as lower pH, which improved flavor and aroma (Afriliana, 2024). A challenge in organic robusta coffee fermentation is the lack of appropriate microbial concentration and fermentation time using Ghalkoff microbes to achieve better chemical and microbiological properties of civet coffee.

Based on the above description, this study was conducted to determine the optimal fermentation time and microbial concentration in the coffee processing process so as to increase the economic value of organic Robusta coffee beans.

2. Methodology

Materials and equipment

The main materials for this study were organic Robusta coffee beans obtained from Hanakau, West Lampung, and Ghalkoff microbes obtained from PT. Ghaly Rolies Indonesia. Other materials used included chlorogenic acid standards, methanol (E. Merck), acetonitrile for LC (Merck Millipore), 0.45 µm PTFE filter paper, phosphate buffer, caffeine standards (Sigma-Aldrich), and distilled water. Unfermented organic coffee (negative control) and commercial civet coffee (positive control) were used as controls.

The equipment used in this study included a high-performance liquid chromatography (HPLC) (Shimadzu type LC 200), a hot plate, a water bath, an autoclave (Hirayama), an analytical balance, plastic jars, a coffee roaster, a coffee grinder (Masema MS-CG600), a micropipette, a Bunsen burner, a Leica ICC 50 HD microscope, and a hemocytometer.

Fermentation of organic Robusta coffee beans

Organic Robusta coffee beans were fermented in plastic containers, with each treatment using 1 kg of coffee beans. The fermentation process was carried out for 24, 48, and 72 hours, using Ghalkoff microbes at concentrations of 20%, 25%, and 30% (w/v). After fermentation, the coffee beans were soaked in 5 liters of water, then washed and sorted. The next step was drying in the sun until the moisture content reached 12%. Once dry, the coffee beans were roasted for 11 minutes at 180°C, then ground using a coffee grinder and sieved through a 100-mesh sieve. The resulting organic Robusta coffee grounds were then analyzed for caffeine content, chlorogenic acid content, and total microbial count.

Fermentation optimization using response surface methodology (RSM)

In this study, two independent variables were defined: fermentation time (X_1) and microbial concentration (X_2), each at three levels. The range of each factor was determined based on preliminary experiments. Optimization was performed using Response Surface Methodology (RSM) with a two-factor Central Composite Design (CCD) implemented in Design-Expert 12.0.0 software. The measured responses included caffeine content, chlorogenic acid content, and total microbial count.

Table 1. Central composite design

Central Composite Design	Total		Total
Factor	2	Replicates	1
Base runs	13	Total runs	13
Base blocks	1	Total blocks	1
Two-level full factorial			
Cube points	5		
Center points in cube	4		
Axial points	4		
Center points in axial	0		
α: 1,414			

Table 2. Independent variable and its levels

Independent Variable	Level -1	Level 0	Level +1
Fermentation time (hour)	24	48	72
Microbial concentration (%, w/v)	20	25	30

Table 3. Factorial experimental design with 2 independent variables

Running	Microbial Concentration (%)	Fermentation Time (h)
1	25	14
2	30	72
3	25	48
4	25	48
5	25	48
6	30	24
7	20	72
8	18	48
9	25	48
10	25	48
11	32	48
12	25	82
13	20	24

Caffeine content

Caffeine content was determined using the Bailey-Andrew method (AOAC, 2015). A 5 g sample was placed in an Erlenmeyer flask. 5 g of MgO and 200 mL of distilled water were then added. The solution was then heated at 100°C for 20 minutes with stirring. The solution was then cooled to room temperature, filtered through a 0.2 µm filter cartridge, and injected into the HPLC. Caffeine content was calculated using the Eq.1.

$$\text{Caffeine content} = \frac{\text{Geometric series x standard injection volume x } \frac{\text{final volume}}{\text{injection volume}}}{\text{sample weight}} \text{ x DF} \quad (1)$$

Chlorogenic acid content

Chlorogenic acid content was determined using method described previously (Mangiwa et al., 2015). The test solution was prepared from coffee bean extract by dissolving 1 g of ground coffee in 37.5 mL of methanol and water. The test solution was homogenized for 1 hour and filtered using a 0.45 µm PTFE filter paper before use. Chlorogenic acid was determined by HPLC using a C-18 column, 150 mm long and 4.6 mm in diameter, with a mobile phase of methanol:10 mM phosphate buffer, pH 2.6 (30:70), a flow rate of 1 mL/min, and an injection volume of 20 µL. The separation results and chlorogenic acid content obtained are shown by the resulting chromatogram. The peaks formed in the chromatogram indicate the presence of chlorogenic acid, while the peak area in the chromatogram represents the chlorogenic acid content in the coffee bean extract.

Total microbial count

Total microbial counts were performed using a hemocytometer (Fardiaz, 2012). Dilution was performed nine times by taking 1 mL of water after fermentation and placing it in a test tube containing 9 mL of distilled water. The microbial cell count of the mixture was calculated under a microscope (Prihantini, 2007). The total number of microbes was calculated on a scale of 30-300 colonies and expressed in CFU/g. Total microbial counts was calculated using Eq. 2.

$$\text{Total mikrobial count} = \frac{\text{Number of colonies counted}}{5} \text{ x } \frac{25}{0.1\text{mm}^3} \quad (2)$$

3. Result and Discussion

Caffeine content

Based on Table 4, the prediction model for caffeine content is a quadratic model. The lack-of-fit value for caffeine content was 0.4060 ($p > 0.05$), indicating that the quadratic model is suitable for predicting the response variable. The quadratic model describing caffeine content is presented in Eq.3, while the response surface contour illustrating the effects of microbial concentration and fermentation time on caffeine content in organic Robusta coffee is shown in Fig. 1.

Table 4. A comparison of the caffeine content of organic Robusta coffee with other variants

Coffee variant	Microbes	Caffeine (%)	Reference
Robust coffee	Lactic acid bacteria from yakult	0.76-1.75	Izzati, 2022
Arabica coffee	-	0.49-1.40	-
Organic robust coffee	Ghalkoff	1.58-2.17	Wijaya et al., 2023
Robust coffee	Yeast from tape	1.1-1.4	Nafisyah, 2024
Robust wine coffee	*Saccharomyces cerevisiae*	0.76-1.17	Fatima, 2024
Organic robust coffee	Ghalkoff	1.55-2.26	Research analysis

$$\bar{y} = 1.70 + 0.1189x_1 + 0.2189x_2 \quad (3)$$

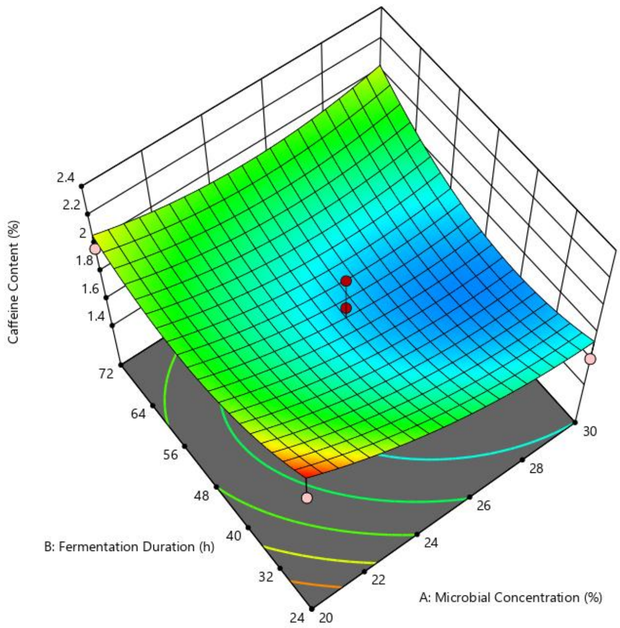

Figure 1. Surface plots showing the effects of microbial concentration and fermentation time on caffeine content in organic robusta coffee

The surface plots (Fig. 1) shows a color gradient from orange to blue, indicating that increasing microbial concentration and longer fermentation time are associated with lower caffeine content. This suggests that the fermentation process in organic coffee contributes to a reduction in caffeine levels. Caffeine is a compound that contributes to the aroma and flavor of coffee. Variations in fermentation time and microbial concentration affect the caffeine content of organic Robusta coffee grounds. The caffeine content of organic Robusta coffee grounds ranges from 1.55-2.26%.

These results align with research by Wijaya et al. (2023), who reported that fermentation of organic Robusta coffee with Ghalkoff microbes produces caffeine levels of around 1.55-2.17%. The caffeine content of organic Robusta coffee grounds decreases with the duration of the fermentation process and increasing microbial concentration. This is in line with the findings of Fatima et al. (2024) stated that the decrease in caffeine content in coffee is directly proportional to the fermentation period and the increase in microbial concentration.

The decrease in caffeine content can be caused by the esterification process. Caffeine is an alkaloid compound that is esterified with alcohol during fermentation. The esterification process causes caffeine to break down into esters such as chlorogenic acid (Larassati et al., 2021). The caffeine compound becomes free with a smaller molecular size and weight, making it more easily diffuse through cell walls and dissolves in water (Adrianto et al., 2020).

Furthermore, the decrease in caffeine content is also caused by the activity of Ghalkoff microbes during the fermentation process. Nafisyah et al. (2024) reported that the decrease in caffeine content in Robusta coffee is related to increased microbial activity during fermentation. According to Usman et al. (2015), during fermentation, microbes produce enzymes that break down complex compounds in coffee beans into simpler ones, facilitating the diffusion of caffeine from the cell membrane and its dissolution in water (Utama et al., 2022).

Coffee with high caffeine content and excessive consumption can have negative health effects, such as causing heart palpitations, anxiety, stomach aches, and disrupted sleep quality (Pratiwi et al., 2023). The caffeine content of the organic Robusta coffee powder produced is lower than that of civet coffee (positive control). Based on SNI 01-3542-2004 concerning the quality requirements for ground coffee, the resulting caffeine content meets the requirements for quality I and II, which is in the range of 0.45-2%.

Chlorogenic acid

The prediction model for chlorogenic acid levels was a quadratic model. The lack-of-fit value for chlorogenic acid levels was 0.0813 (P<0.05), indicating that the quadratic model was suitable for predicting the response variable. The quadratic model describing chlorogenic acid content is presented in Eq.4, while the response surface plots illustrating the effects of microbial concentration and fermentation time on chlorogenic acid content in organic Robusta coffee is shown in Fig. 2.

$$\bar{y} = 2.19 + 0.6534x_1 + 0.6584x_2 \quad (4)$$

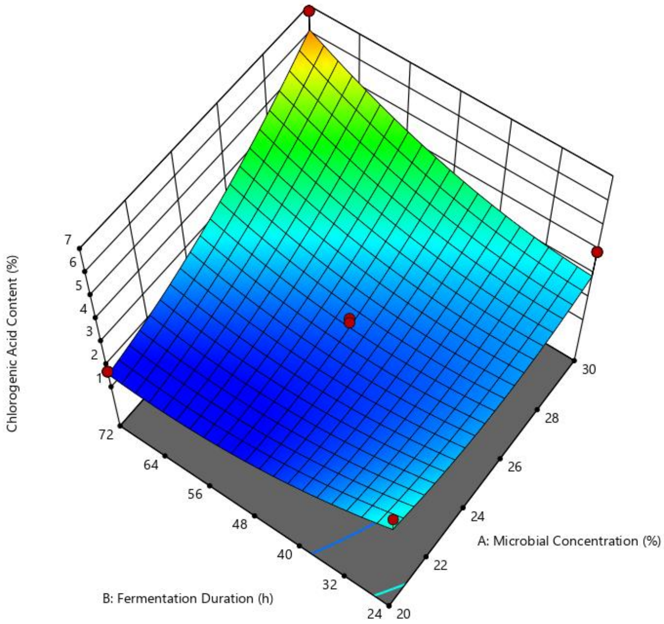

Figure 2. Surface plots showing the effects of microbial concentration and fermentation time on chlorogenic acid content in organic Robusta coffee

In the three-dimensional response surface graph (Fig.2), a color gradient from light blue to yellow-green indicates an increase in chlorogenic acid content with increasing microbial concentration (A) and fermentation time (B). Chlorogenic acid is the primary phenolic compound in coffee and plays a crucial role in preventing diseases associated with oxidative stress (Farhaty & Muchtaridi, 2016). Variations in fermentation time and Ghalkoff microbial concentration affected the chlorogenic acid levels of organic Robusta coffee grounds. The highest chlorogenic acid levels were found after a fermentation time of 72 hours and a microbial concentration of 30%, at 6.81%, which was higher than that of civet coffee (the positive control).

The longer the fermentation time and the higher the microbial concentration, the higher the chlorogenic acid levels. These findings are consistent with those of Narko et al. (2020), who reported an increase in chlorogenic acid content in Robusta coffee during fermentation. The increase in chlorogenic acid levels is expected due to the esterification process during fermentation. According to Tawali et al. (2018), the esterification process results in the breakdown of the complex compound caffeine into chlorogenic acid.

The longer the dissolution time, the more chlorogenic acid is dissolved in the fermentation medium. These results align with the statement by Febrianti & Setyaningtyas (2021), who stated that chlorogenic acid in coffee is formed through the esterification process between quinic acid and several types of trans-cinnamic acids, such as caffeic acid, ferulic acid, and p-coumaric acid.

Total microbial content

The prediction model for total microbial content was a linear model. The lack of fit value for total microbial content was 0.0813 ($P<0.05$), indicating that the linear model was suitable for predicting the response variable. The linear model describing total microbial count is presented in Eq.5, while the response surface plots illustrating the effects of microbial concentration and fermentation time on total microbial count in organic Robusta coffee is shown in Fig. 3.

$$\bar{y} = 7.69 + 00699x_1 - 0.5835x_2 \quad (5)$$

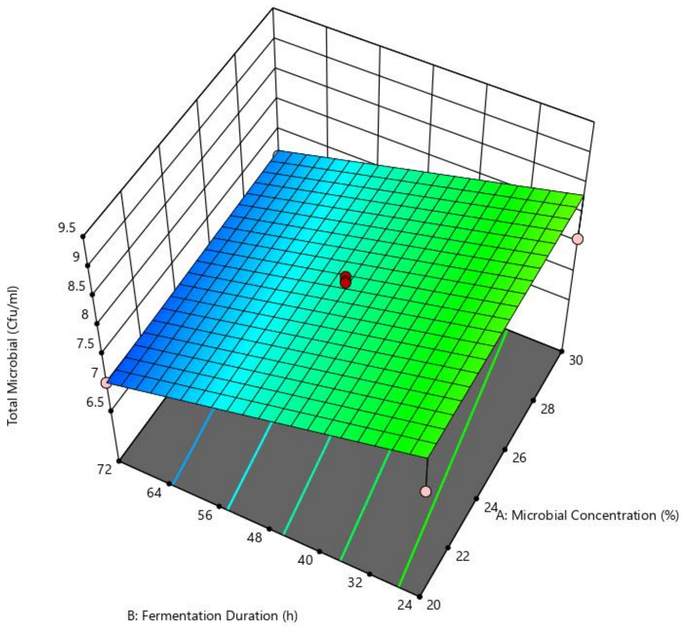

Figure 3. Response surface showing the effects of microbial concentration and fermentation time on total microbial count in organic Robusta coffee

The three-dimensional response surface graph (Fig.3) shows a color gradient from green to blue, indicating that increasing microbial concentration (A) and longer fermentation time (B) are associated with higher total microbial count in organic coffee. This increase reflects enhanced microbial activity during fermentation, which may contribute to the biochemical transformation of substrates such as sugars into organic acids.

The results of the total microbial content analysis indicated that fermentation time and Ghalkoff microbe concentration influenced the total microbial content of organic Robusta coffee grounds. Run 1 coffee beans had the highest microbial count, at 9.36 log CFU/g, followed by Run 11 at 8.05 log CFU/g, and the lowest at 6.83 log CFU/g. These results align with research by Larassati et al. (2021), which found that fermentation time and the addition of *Saccharomyces cerevisiae* cultures affected the total microbial count in Robusta coffee beans.

The total microbial count increased during fermentation, whereas longer fermentation time led to a decrease. This phenomenon is thought to be caused by the microbial growth phase. According to Rini & Rochmah (2020), microbial growth is divided into four phases: the lag phase, the exponential phase, the stationary phase, and the death phase. Fermentation at 14 hours showed the exponential phase, while at 82 hours, the death phase had already occurred. These results are similar to those of Hatiningsih et al. (2018), who stated that the microbial count in coffee beans increased gradually, starting from the 0th hour of fermentation, reached a maximum at 12 hours, and then declined after passing the peak.

According to Yusianto & Widyotomo (2013), microbial growth is indicated by the presence of bubbles during the coffee fermentation process. During fermentation, complex compounds are broken down into simpler compounds by microbes. Several factors influencing microbial growth are pH, temperature, oxygen availability, and nutrients (Rini & Rochmah, 2020).

The Lactobacillus genus, a member of the lactic acid bacteria (LAB), can be used in coffee bean processing to reduce fermentation time and improve coffee quality. *L. plantarum* can reduce fermentation time from 24 hours to 12 hours. L. brevis can metabolize p-coumaric, ferulic, and caffeic acids. L. reuteri can hydrolyze chlorogenic acid (da Silva et al., 2023).

Sensory characteristics

Cupping tests were conducted following the Specialty Coffee Association (SCA) protocol by trained panelists. Attributes assessed included fragrance/aroma, flavor, aftertaste, saltiness/acidity, bitterness/sweetness, mouthfeel/body, balance, and overall taste. The results of the cupping test are shown in Table 5.

The highest score was achieved by the civet coffee sample (Run 15), with a total score of 85. The sample closest to the civet coffee score was Run 2, with a score of 84, which achieved 8s in all categories: fragrance/aroma, flavor, aftertaste, salt/acid, bitter/sweet, mouthfeel/body, balance, and overall. The sample with the lowest score was the negative control coffee (unfermented) with a score of 75.75. Based on the Indonesian Specialty Coffee category, almost all samples fell into the very good category (scores 80-85), except for Run 8 (score 76) and the negative control (score 75.75), which was classified as commercial grade. This indicates that coffee bean fermentation with Ghalkoff microbes had a positive effect on cupping test scores.

Caramel notes were the most common notes found in the coffee bean samples. Based on previous research, de Sousa et al. (2023) demonstrated the effect of coffee bean fermentation with S. cerevisiae on cupping test scores and sensory attribute notes. Coffee bean fermentation also produces coffee with different sensory attributes compared to conventional processing (da Silva et al., 2023). Fermentation changed the composition of organic acids in green beans, from malic and succinic acids to citric, malic, lactic, and acetic acids, which can affect the sensory attributes of the brewed coffee (Rocha et al., 2024).

Flavor quality increases with increasing fermentation time and microbial concentration. Lee et al. (2015) stated that improvements in coffee aroma quality can be caused by modifications in the composition of aroma precursors, such as proteins, carbohydrates, and chlorogenic acid, during the fermentation process. Longer fermentation times degraded the proteins in coffee beans into oligopeptides, dipeptides, and amino acids, resulting in a change in flavor. Reducing caffeine levels can also reduce bitterness in coffee (Maligan et al., 2024).

Fermentation is one of the primary processing steps that significantly determines the quality of a coffee brew (Widyotomo & Yusianto, 2013). During fermentation, chemical reactions occur that are crucial for developing the coffee's flavor characteristics, including the formation of flavor precursor compounds such as organic acids, amino acids, and reducing sugars (Lin, 2010).

Table 5. Sensory characteristics of the fermented coffee

Run	Time (Hour)	Concentration (%)	Fragrance/ Aroma	Flavor	Aftertaste	Saltiness/ Acidity	Bitterness/ Sweetness	Mouthfeel/ Body	Uniform Cups	Balance	Clean Cups	Overall	Final Score
1	14	25	7.75	7.75	7.5	7.5	7	8	10	7.5	10	7.25	80.25
2	72	30	8	8	8	8	8	8	10	8	10	8	84
3	48	25	8	8	7.5	7.5	7.5	8	10	7.75	10	7.75	82
4	48	25	7.5	7.5	7.5	7.5	7.5	8	10	7.5	10	7.5	80.5
5	48	25	7.75	7.75	7.5	7.5	7.75	8	10	7.75	10	7.75	81.75
6	24	30	7.75	7.75	7.75	7.75	7.75	7.75	10	7.75	10	7.75	82
7	72	20	8	8	7.75	7.5	8	8	10	7.75	10	8	83
8	48	18	7	7	6.75	7.5	7.5	6.75	10	6.75	10	6.75	76
9	48	25	7.5	7.5	7	7.5	7.5	7.75	10	7.5	10	7.25	80
10	48	25	8	8	8	7.75	8	8	10	8	10	8	83.75
11	48	32	8	8	7.75	7.5	7.5	8	10	7.75	10	7.75	82.25
12	82	25	8	7.75	7.75	7.75	7.5	7.75	10	7.5	10	7.5	81.5
13	24	20	8	8	7.5	7.5	7.5	8	10	7.5	10	7.5	81.5
14 (-)	Negative control	Organic	7.5	7	6.5	6.75	6.75	7.75	10	6.75	10	6.75	75.75
15 (+)	Positive control	Civet	8	8.5	8	8	8	8	10	8	10	8.5	85

4. Conclusion

Optimum organic Robusta coffee was obtained at a fermentation time of 72 hours and a Ghalkoff microbial concentration of 30% (w/v). Under these conditions, the coffee exhibited the lowest caffeine content (1.55%), the highest chlorogenic acid content (6.81%), and a cupping score of 84, which is close to that of civet coffee (85). Fermentation using Ghalkoff microbes has been shown to effectively improve the chemical and sensory characteristics of organic Robusta coffee while producing caffeine levels that meet the SNI 01-3542-2004 standard.

References

Adrianto, R., Wiraputra, D., Agrippina, F. D., & Andaningrum, A. Z. (2020). Penurunan kadar kafein pada biji kopi robusta menggunakan fermentasi dengan bakteri asam laktat *Leuconostoc mesenteroides* (B-155) dan *Lactobacillus plantarum* (B-76). *Jurnal Dinamika Penelitian Industri*, *31*(2), 163–169. https://doi.org/10.28959/jdpi.v31i2.6424

da Silva, M. de C. S., Veloso, T. G. R., Brioschi Junior, D., Bullergahn, V. B., da Luz, J. M. R., Menezes, K. M. S., … Pereira, L. L. (2023). Bacterial community and sensory quality from coffee are affected along fermentation under carbonic maceration. Food Chemistry Advances, 3, 100554. https://doi.org/10.1016/j.focha.2023.100554

de Sousa, L. H. B. P., da Luz, J. M. R., da Silva, M. de C. S., Moreli, A. P., Veloso, T. G. R., … Oliveira, E. C. da S. (2023). Relationship between sensory and microbial profiles of fermented coffee by dry and washed methods. Food Chemistry Advances, 2, 100259. https://doi.org/10.1016/j.focha.2023.100259

Ditjenbun. (2020). Statistik Perkebunan Unggulan Nasional 2020-2022. Direktorat Jenderal Perkebunan, Kementerian Pertanian. Jakarta. https://ditjenbun.pertanian.go.id/template/uploads/2022/08/STATISTIK-UNGGULAN-2020-2022.pdf (Retrieved March 15, 2026).

Fardiaz, S. (2012). Mikrobiologi Pangan I. Gramedia Pustaka Utama. Jakarta.

Farhaty, N., & Muchtaridi. (2016). Tinjauan kimia dan aspek farmakologi senyawa asam klorogenat pada biji kopi. Farmaka, 14(1), 214-227.

Febrianti, K. D., & Setyaningtyas, S. W. (2021). Asam klorogenat pada kopi dan obesitas: A systematic review. *Media Gizi Indonesia*, *16*(3), 256–266. https://doi.org/10.20473/mgi.v16i3.256-266

Kiyat, W. E., Mentari, D., & Santoso, N. (2019). Review: Potensi mikrobial selulase, xilanase, dan protease dalam fermentasi kopi luwak (*Paradoxurus hermaphroditus*) secara in vitro. *Jurnal Kimia Sains dan Aplikasi*, *22*(2), 58–66. https://doi.org/10.14710/jksa.22.2.58-66

Larassati, et al. (2021). Pengaruh waktu fermentasi dan konsentrasi Saccharomyces cerevisiae terhadap mutu kopi robusta. Jurnal Teknologi Pertanian, 10(4), 449-458. http://dx.doi.org/10.23960/jtep-l.v10.i4.449-458.

Lee, L. W., Cheong, M. W., Curran, P., Yu, B., & Liu, S. Q. (2015). Coffee fermentation and flavor – an intricate and delicate relationship. *Food Chemistry*, *185*, 182–191. https://doi.org/10.1016/j.foodchem.2015.03.124

Maligan, J. M., Hernanto, D. P., & Wulan, S. N. (2024). Characteristics of fermented Arjuno Robusta coffee by commercial baker's yeast. Jurnal Pangan dan Agroindustri, 12(3), 143-153. https://doi.org/10.21776/ub.jpa.2024.012.03.3

Nafisyah, S. Z., Saloko, S., & Nofrida, R. (2024). Pengaruh konsentrasi ragi dan lama fermentasi terhadap mutu bubuk kopi robusta (*Coffea canophora*) Karang Sidemen. *EduFood*, *2*(3), 61–70.

Narko, T., Wibowo, M. S., Damayanti, S., & Wibowo, I. (2020). Effect of kombucha culture on caffeine and chlorogenic acid content in fermentation of robusta green coffee beans (*Coffea canephora* l.). *Rasayan Journal of Chemistry*, *13*(2), 1181–1186. https://doi.org/10.31788/RJC.2020.1325675

Pratiwi, et al. (2023). Pengaruh lama fermentasi alami terhadap mutu kopi robusta Bantaeng. Jurnal Pendidikan Teknologi Pertanian, 9(2), 263-272.

Rini, C. S., & Rochmah, J. (2020). Dasar Bakteriologi. Umsida Press. Sidoarjo.

Rocha, R. A. R., Cruz, M. A. D. da, Silva, L. C. F., Costa, G. X. R., Amaral, L. R., Bertarini, P. L. L., … Santos, L. D. (2024). Evaluation of Arabica coffee fermentation using machine learning. Foods, 13(3), 454. https://doi.org/10.3390/foods13030454

SNI 01-3542-2004. Kopi Bubuk. Badan Standardisasi Nasional. Jakarta.

Suprihatin. (2010). Teknologi Fermentasi. UNESA University Press. Surabaya.

Tawali, A. B., Abdullah, N., & Wiranata, B. S. (2018). Pengaruh fermentasi menggunakan bakteri asam laktat yoghurt terhadap citarasa kopi robusta (*Coffea robusta*). *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, *1*(1), 90–97. https://doi.org/10.20956/canrea.v1i1.26

Usman, D., Suprihadi, A., & Kusdiyantini, E. (2015). Fermentasi kopi robusta (*Coffea canephora*) menggunakan isolat bakteri asam laktat dari feces luwak dengan perlakuan lama waktu inkubasi. *Jurnal Akademika Biologi*, *4*(3), 31–40. https://ejournal3.undip.ac.id/index.php/biologi/article/view/19417

Utama, et al. (2022). Pengaruh fermentasi terhadap difusi kafein dalam biji kopi. Jurnal Teknologi Pangan, 8(1): 77-87.

Wijaya, R. Y., Tamrin, T., & Sugianti, C. (2023). Pengaruh lama fermentasi menggunakan mikroba Ghalkoff terhadap perubahan konsentrasi kandungan kafein kopi robusta organik Kabupaten Lampung Barat. Jurnal Agricultural Biosystem Engineering, 2(3), 354-360. https://doi.org/10.23960/jabe.v2i3.7892

Widyotomo, S., & Yusianto. (2013). Optimasi proses fermentasi biji kopi arabika dalam fermentor terkendali. *Pelita Perkebunan*, *29*(1), 53–68.

Yusianto & Widyotomo, S. (2013). Mutu dan citarasa kopi arabika hasil beberapa perlakuan fermentasi: Suhu, jenis wadah, dan penambahan agens fermentasi. *Pelita Perkebunan*, *29*(3), 220–239.