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The Impact of Fermentation on the Physical and Chemical Properties of Green and Roasted Robusta Coffee Beans

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Abstract: Conventional coffee decaffeination often relies on chemical solvents that can alter quality and raise processing costs. Although biological fermentation has been studied, research typically evaluates fermentation and roasting separately. Additionally, the use of civet-derived microorganisms as fermentation inoculants is less explored compared to spontaneous, yeast, or lactic acid bacteria-based fermentations. Therefore, this study investigated the interaction between civet-derived microbial inoculum and fermentation duration on the physicochemical characteristics of Robusta coffee before and after roasting. Fully ripe Robusta coffee cherries were fermented in sealed fermenters using civet-derived inoculum at 0%, 50%, and 100% concentrations for 24, 48, and 72 h under controlled conditions; each treatment was replicated 3 times. Density, moisture content, and caffeine concentration were determined in green and medium-roasted beans using standard methods and HPLC analysis. Fermentation significantly affected caffeine concentration and several physical properties of coffee beans. In roasted beans, caffeine content ranged from 0.20% to 0.34%, with the lowest value observed at 100% inoculum for 72 h and the highest at 50% inoculum for 24 h. Extended fermentation reduced caffeine by up to 35.3% (0.34% to 0.22%). These findings demonstrate that civet-derived microbial fermentation can serve as a sustainable biological decaffeination strategy while influencing coffee responses during roasting.

Keywords: Caffeine; Civet inoculum; Decaffeinated; Microbial fermentation; Robusta coffee

Introduction

Robusta coffee (*Coffea canephora*) is one of Indonesia's leading agricultural export commodities and plays a significant role in the national economy. Indonesia is among the world's largest coffee-producing countries, with Robusta accounting for approximately 78.78% of national coffee production (Badan Pusat Statistik, 2024). Jember Regency, East Java, is recognized as one of Indonesia's major Robusta-producing regions, contributing substantially to national coffee production through thousands of hectares of plantations. Given the increasing global demand for specialty and functional coffee products, improving the quality and commercial value of Indonesian Robusta coffee has become a priority.

Robusta coffee is characterized by its strong flavor, full body, and relatively high concentrations of bioactive compounds, particularly caffeine, proteins, and lipids (Lumbanraja et al., 2023). Compared with Arabica coffee (Rodriguez et al., 2020), Robusta contains considerably higher levels of caffeine (Rahmawati & Asrori, 2024). Although caffeine provides several physiological benefits, including improved alertness and cognitive performance, excessive intake may induce adverse effects such as anxiety, insomnia, increased heart rate, and sleep disturbances in caffeine-sensitive individuals (Chauhan, 2025). Consequently, consumer demand for decaffeinated coffee has increased worldwide, spurring the development of processing technologies that reduce caffeine while preserving desirable sensory attributes (Shofinita et al., 2024).

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Commercial decaffeination is commonly achieved using organic solvents like ethanol-methanol (Kartasasmita & Addyantina, 2012) and palm oil (Shofinita et al., 2023), supercritical carbon dioxide, water-ethanol extraction (Lolongan et al., 2021), using pineapple extract (Wicaksono & Kurniawati, 2023), or with boiling and steaming treatment (Wardahni et al., 2026). Despite their effectiveness, these methods often require expensive equipment, high energy consumption, and may reduce desirable volatile compounds that contribute to coffee aroma and flavor (Mussatto et al., 2011). These limitations have stimulated interest in biological decaffeination as a more environmentally sustainable alternative. Biological approaches employ microorganisms or microbial enzymes capable of degrading caffeine naturally while maintaining the integrity of coffee quality (Hamad et al., 2023; Mazzafera, 2004; Ran et al., 2025; Utama et al., 2022).

Among biological methods, microorganisms isolated from the digestive tract of the Asian palm civet (*Paradoxurus hermaphroditus*) have attracted considerable attention because they naturally produce hydrolytic enzymes, including proteases, amylases, and lipases, which modify the biochemical composition of coffee beans during fermentation (Marcone, 2004). Previous studies have demonstrated that bacteria isolated from civet feces, particularly lactic acid bacteria, can reduce caffeine concentration and alter the physicochemical properties of coffee beans (Fitri et al., 2019; Usman et al., 2015). In addition, post-harvest processing methods and roasting conditions substantially affect coffee quality by modifying carbohydrates, proteins, chlorogenic acids, lipids, and alkaloids through enzymatic and thermal reactions, thereby determining the final sensory characteristics of brewed coffee (Alwi et al., 2024; Harijono et al., 2025). Fermentation and roasting are therefore two critical processing stages that jointly influence coffee chemistry.

Several studies have reported that roasting significantly affects the chemical composition of coffee, including caffeine, chlorogenic acids, pH, and moisture content (Ramanda et al., 2024; Rahmawati & Gustiani, 2023). Roasting temperature significantly affects caffeine and chlorogenic acid contents, with higher roasting temperatures resulting in lower concentrations of both compounds (Virhananda et al., 2022). Likewise, biological and physical decaffeination methods have been shown to reduce caffeine levels and modify physicochemical characteristics of coffee beans (Nugroho et al., 2025; Wardahni et al., 2026). Previous studies have generally investigated microbial fermentation and roasting as independent processing factors. Fermentation studies have primarily focused on microbial succession, acid-forming bacteria identification (Salma et al., 2024), enzymatic activity, and their effects on coffee chemistry and sensory quality (Lee et al., 2015; De Bruyn et al., 2017), whereas roasting studies have mainly examined the influence of roasting conditions on caffeine, chlorogenic acids, volatile compounds, and flavor development (Salsabila et al., 2024; Farah, 2012). Although microorganisms isolated from civet feces have been reported to reduce caffeine content during fermentation (Usman et al., 2015), the influence of fermentation duration on the subsequent roasting response has received little attention. Nugroho et al. (2025) stated that the length of fermentation affects caffeine levels.

However, these studies generally evaluated decaffeination and roasting as separate processing stages. Information regarding how decaffeination treatments influence the chemical response of coffee beans to subsequent roasting remains limited, particularly for Indonesian Robusta coffee. Therefore, investigating the interaction between fermentation-based decaffeination and roasting level is necessary to develop an optimized processing strategy that achieves caffeine reduction while maintaining desirable chemical quality. To the best of our knowledge, no previous study has systematically evaluated the interaction between fermentation duration using civet-derived bacteria and roasting level on the chemical properties of Indonesian Robusta coffee. This knowledge gap limits the development of optimized biological decaffeination strategies capable of reducing caffeine while preserving coffee quality.

The uniqueness of the current study is found in its comprehensive assessment of fermentation time using bacteria derived from civet feces, paired with various roasting levels as interacting processing factors. In contrast to earlier research that looked at fermentation (Usman et al., 2015; De Bruyn et al., 2017) or roasting (Farah, 2012) in isolation, this study explores their combined impacts on moisture content and caffeine levels, offering a deeper insight into how biological fermentation and thermal processing interact. Furthermore, this work proposes a sustainable biological decaffeination approach utilizing indigenous microorganisms from the luwak fermentation process, thereby reducing dependence on solvent-based decaffeination methods and supporting environmentally friendly coffee processing technologies (Mazzafera, 2004; Mussatto et al., 2011).

This research is important for several reasons. First, it provides scientific evidence for optimizing biological decaffeination of Robusta coffee using naturally occurring microorganisms, offering a sustainable alternative to conventional chemical decaffeination (Mazzafera, 2004; Mussatto et al., 2011). Second, understanding the interaction between fermentation

duration and roasting level enables processors to better control caffeine reduction while maintaining chemical quality before sensory evaluation (Farah, 2012; Lee et al., 2015). Third, the findings may contribute to the development of value-added, naturally decaffeinated Robusta coffee products that meet the increasing consumer demand for healthier coffee beverages. Finally, this study supports the improvement of post-harvest processing technologies for Indonesian Robusta coffee, thereby enhancing its competitiveness in the international specialty coffee market (ICO, 2023).

Therefore, this study evaluated the combined effects of fermentation duration using bacteria isolated from civet feces and different roasting levels on the moisture content and caffeine concentration of Robusta coffee beans. The results are expected to provide a scientific basis for developing a controlled, sustainable, and environmentally friendly fermentation process capable of improving the chemical quality of Robusta coffee before sensory quality development.

Method

This research was conducted from June to November 2025 at the Coffee Product Processing Teaching Factory (TEFA) Politeknik Negeri Jember, Agricultural Product Processing Laboratory Politeknik Negeri Jember, Indonesian Coffee and Cocoa Research Institute, and the Food Chemistry and Biochemistry Laboratory of the Faculty of Agricultural Technology, Universitas Gadjah Mada.

Tools and Materials

The tools applied in this research included a fermentor, pulper, solar dome dryer, huller, grinder, roaster, digital scales, measuring cups, digital moisture tester (Digimost), High Performance Liquid Chromatography (HPLC), drying oven, desiccator, water bath, vortex mixer, centrifuge, micropipette, beaker, and measuring flask.

The materials utilized in this study consist of Robusta coffee cherries (*Coffea canephora*) at an optimal stage of ripeness, a microbial inoculum, clean water, standing pouches, methanol, and aquabidest as a solvent. Additionally, chemical compound standards were employed for the calibration of the HPLC instrument used for caffeine analysis. The research stages are explained in Figure 1.

Pre Treatment

The Robusta coffee cherries utilized in this study were carefully selected at optimal ripeness through a multi-step sorting process. The initial sorting involved assessing the density of the cherries using a water flotation method, where high-density cherries sank and were deemed suitable for processing, while those that floated were discarded due to their presumed low physical quality. The second sorting stage was conducted visually, focusing on cherries that displayed a uniform red color, which serves as an indicator of physiological ripeness. Any cherries that appeared unripe or overripe, characterized by green, yellow, or blackish hues, were excluded from this study. Subsequently, the cherries were processed using a pulper to extract the slimy coffee beans. These beans were then washed and served as the primary raw material for the fermentation process, which was carried out in a controlled fermenter according to the research design (Nuraisyah et al., 2026).

Treatment

The fermentation of coffee fruit was conducted in a sealed fermenter, utilizing civet coffee bacteria inoculum as the primary treatment. The concentration of the civet coffee bacteria was designated as the first experimental factor (L), which included three levels: a control group without added civet coffee bacteria (L0), a group with a 50% concentration of civet coffee bacteria (L50), and a group with a 100% concentration of civet coffee bacteria (L100). Alongside the inoculum concentration factor, the second factor involved the duration of fermentation (H), which had three levels: 24 hours (H1), 48 hours (H2), and 72 hours (H3). All combinations of treatments were fermented under identical conditions to reduce the impact of external variables on the fermentation process.

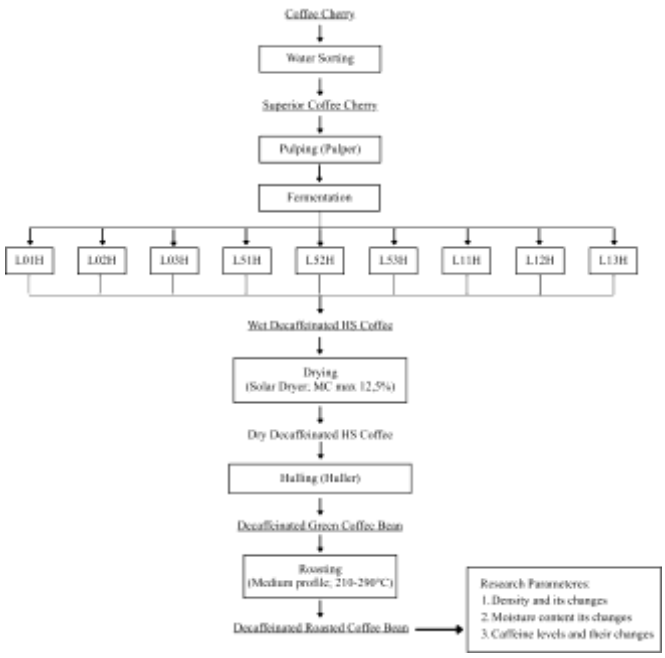

Figure 1. Research flowchart

Once the fermentation process is finished, the coffee cherries are rinsed with running water to eliminate any

remaining mucus on the surface of the beans. Following this, the coffee beans are dried in a solar dome dryer for three days until their moisture content reaches 11% for further processing. The dried coffee cherries are subsequently hulled to separate them from their husks, resulting in green beans. The green beans are then sorted by size using a grinder to ensure consistency, and they are packed into standing pouches. After that, the green beans are roasted with a roaster machine to a medium roast level, and then repackaged in standing pouches before going through physical and chemical quality assessments in line with the research objectives (Nuraisyah et al., 2026).

Research Parameters

Density

Density measurements were conducted on both green and roasted coffee beans to assess variations in their physical properties. These measurements were executed utilizing a volumetric method implemented with a 100 mL measuring cylinder. Green bean samples were added to the measuring cylinder until a stable volume of 100 mL was attained, ensuring no compaction occurred, and were subsequently weighed to determine the mass of the green beans (Mg). The density of the green beans was calculated as the ratio of the mass of the sample to the volume of the measuring cylinder (Alwi et al., 2024).

The same procedure was applied to measure roasted bean density. Roasted bean samples were placed in 100 mL measuring cylinders until they reached the same volume, then weighed to obtain the roasted bean mass (Mr). Measurements were performed uniformly to minimize variations due to bean filling and orientation:

$$Green\ bean\ density\ \left(\frac{g}{mL}\right) = \frac{Mg\ (g)}{100\ mL} \quad (1)$$

$$Roasted\ bean\ density\ \left(\frac{g}{mL}\right) = \frac{Mr\ (g)}{100\ mL} \quad (2)$$

Moisture Content

Moisture content was measured on green and roasted beans to evaluate the effect of fermentation on the physical characteristics of coffee beans. Moisture content was measured directly using a digital moisture tester (Digimost). Green bean samples from each treatment were placed into the Digimost, and the moisture content was automatically displayed on the display.

The measurement of the moisture content in roasted beans was conducted indirectly, utilizing the gravimetric method. A sample of the roasted beans was weighed in a pre-measured cup. The cup containing the sample was subsequently placed in an oven at a temperature of 105 °C until a constant weight was achieved. Following the drying process, the cup was cooled in a desiccator to avoid the absorption of atmospheric moisture and was then weighed again. The moisture content of the roasted beans was determined by calculating the percentage difference between the mass of the sample before drying and its mass after drying, relative to the initial mass of the sample (Kusumaningtyas et al., 2026). The calculation of moisture content was performed using the following equation:

$$Moisture\ content(\%) = \frac{m1-m2}{m1-m0} x\ 100\ \% \quad (3)$$

Annotation:
m0 = mass of empty cup (g)
m1 = mass of cup + sample before oven (g)
m2 = mass of cup + sample after oven to constant weight (g)
(m1 − m0) = initial sample mass (g)
(m1 − m2) = mass of water lost (g)

Caffeine Content

Caffeine levels were measured using the High-Performance Liquid Chromatography (HPLC) method. Green bean or roasted bean samples were ground into a fine powder and weighed. The samples were extracted using hot methanol or distilled water with the aid of a water bath or controlled stirring to dissolve the caffeine compounds. The extract was then filtered using filter paper or a 0.45 µm pore membrane filter before analysis. HPLC analysis was performed using a reversed-phase C18 column with a mobile phase consisting of a mixture of methanol and water or acetonitrile and water, which was flowed isocratically at a constant flow rate. Caffeine detection was performed using a UV–Vis detector at a wavelength of approximately 272 nm. Caffeine identification was based on the match in retention time between the sample and the caffeine standard, while quantification was performed using a calibration curve obtained from the caffeine standard solution. Caffeine levels were expressed in milligrams per gram of dry sample. All analyses were repeated to ensure data accuracy and reproducibility, and the results were used as a basis for evaluating the effect of fermentation treatment on changes in the main chemical components of Robusta coffee (Awwad et al., 2021).

Experimental Design

This study was conducted utilizing a factorial Completely Randomized Design (CRD) that incorporated two treatment factors. The first factor examined was the concentration of civet coffee bacteria (L), which included three levels: a control group with no bacteria added (L0), a 50% concentration (L5), and a

100% concentration (L1). The second factor investigated was the fermentation time (H), which also encompassed three levels: 24 hours (1H), 48 hours (2H), and 72 hours (3H). The interplay of these two factors produced a total of nine treatment combinations (3×3). Each treatment combination was replicated three (3) times, resulting in a total of 27 experimental units. All experimental units were randomly assigned to minimize the influence of uncontrolled environmental factors.

The observational data were examined through analysis of variance (ANOVA) to assess the impact of civet coffee bacteria concentration, fermentation duration, and the interaction of these two variables on the measured parameters. If the results of the analysis showed a significant or highly significant effect, additional testing was performed using Duncan's Multiple Range Test (DMRT) at a 5% confidence level to pinpoint specific differences among the treatments.

Result and Discussion

Density and Its Changes

Density serves as a key physical characteristic that reveals the nature of the internal composition of coffee beans, specifically relating to the level of tissue compactness, porosity, and how the beans react to post-harvest processes. Figure 2 illustrates the density of both green and roasted beans, whereas Figure 3 displays the variations in density of green and roasted beans across different fermentation treatment combinations. Figure 2 illustrates that the fermentation process did not influence the density of the green beans. The density of the green beans across all treatments varied from 0.63 to 0.70 g/mL. The L51H treatment recorded the lowest density at 0.63 g/mL, whereas the L11H treatment exhibited the highest density at 0.70 g/mL.

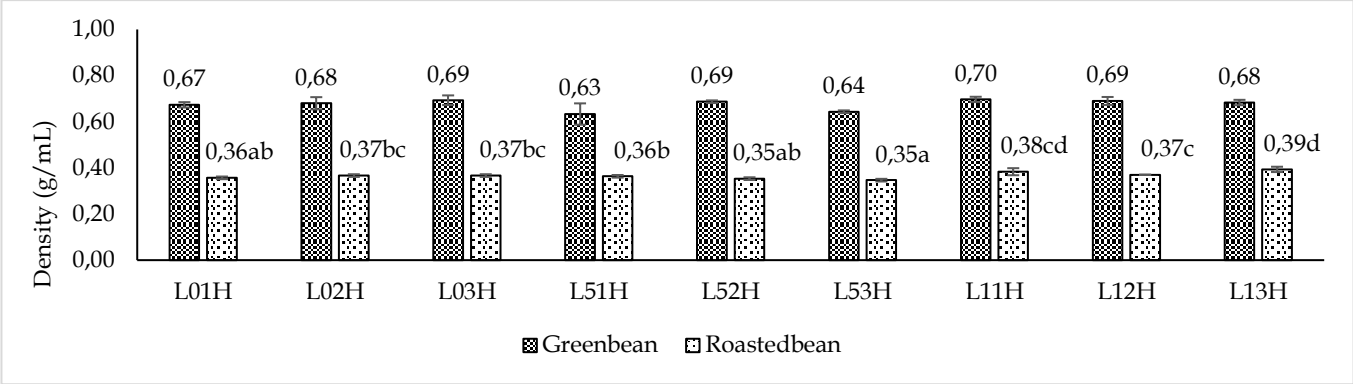

Figure 2. Density of green beans and roasted beans in each research treatment

Variations in the density of green beans according to the concentration of inoculum suggest that fermentation processes influence the physical characteristics of coffee beans before roasting. The treatment involving 50% inoculum (L5) yielded the lowest green bean density at 0.65 g/mL (a), while both the treatment without inoculum (L0) and the one with 100% inoculum (L1) resulted in higher densities of 0.68 g/mL (b) and 0.69 g/mL (b), respectively. This demonstrates that the impact of inoculum on green bean density is non-linear and is contingent upon the level of microbial activity during fermentation.

Green bean density serves as an indicator of the degree of compaction within the endosperm tissue and the distribution of microvoids within the seed. At an inoculum concentration of 50%, microbial activity is considered sufficiently vigorous to soften the tissue and alter the composition of cell wall components, leading to a looser and more porous structural configuration. This modified structure contributes to a reduction in seed mass per unit volume, which is evidenced by decreased green bean density values. In contrast, treatments that do not utilize inoculum exhibit a slower and more restricted fermentation process, resulting in a relatively intact and compact seed tissue structure following the drying phase. Consequently, this leads to higher density measurements.

The introduction of a 100% inoculum led to an increase in green bean density, which nearly matched that of the control treatment. This suggests that extremely high microbial activity does not necessarily enhance the loosening of the bean's tissue structure. An excessively intense fermentation process can lead to more uniform structural changes or may cause shrinkage of the tissue during drying, thereby resulting in a rise in green bean density. Consequently, a moderate level of inoculum concentration yields the most effective structural changes in lowering green bean density.

The lack of any impact from fermentation duration on the density of green beans suggests that alterations in the structure of the bean tissue due to fermentation occur early on and remain relatively constant as fermentation time increases within the examined period. This implies

that once the tissue structure is altered by microbial activity, extending the fermentation time does not lead to significant changes in density. The density of green beans is affected by the concentration of the fermentation inoculum, while the duration of fermentation does not exert a significant influence. A 50% inoculum concentration results in the lightest and most porous structure of green beans, whereas the absence of inoculum and a 100% inoculum concentration yield denser green beans. Green bean density plays a crucial role in determining how the beans respond during the roasting process and in the density variations observed in the roasted beans at later stages.

Figure 2 illustrates that the density of the roasted beans varied between 0.35 g/mL and 0.39 g/mL, which is less than the density of the green beans. This reduction in density indicates volume expansion and the development of a porous structure throughout the roasting process. The L53H treatment exhibited the lowest density among the roasted beans, while the highest density was observed in the L13H treatment.

According to the treatment with varying inoculum concentrations, there was a notable difference in the density of roasted beans. The control treatment that did not include any inoculum (L0) resulted in a roasted bean density of 0.36 g/mL (b). The treatment that had 50% inoculum added (L5) yielded the lowest roasted bean density at 0.35 g/mL (a), whereas the treatment with 100% inoculum (L1) resulted in the highest roasted bean density of 0.38 g/mL (c). This indicates that the concentration of inoculum significantly influences the density of roasted beans. Conversely, fermentation time did not affect roasted bean density. At each inoculum concentration level, roasted bean density remained relatively stable and showed no tendency to increase or decrease with increasing fermentation time. This indicates that changes in bean structure that affect roasted bean density are determined more by fermentation intensity related to inoculum concentration, rather than by fermentation time.

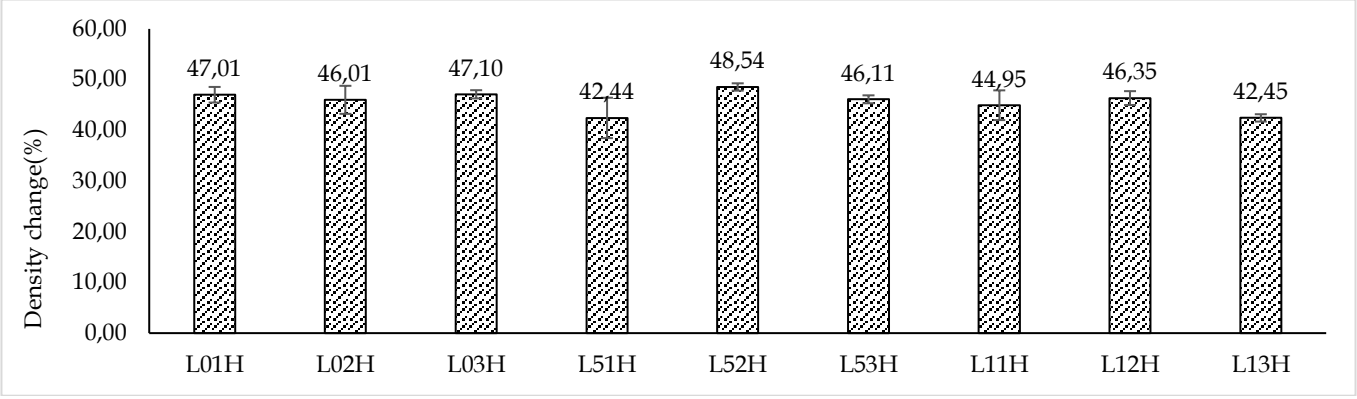

Figure 3. Changes in the density of green beans and roasted beans in each research treatment

Even though fermentation time did not have an impact, roasted bean density was affected by the interaction between inoculum concentration and fermentation duration. This interaction was evident in the differences in roasted bean density among the treatment combinations, with L53H exhibiting the lowest density and L13H showing the highest. This indicates that, under specific conditions of inoculum concentration, fermentation time can alter the bean's response to roasting, though its effect alone was not substantial enough to be considered a significant factor.

The density of roasted coffee beans is influenced by the extent of volume increase and the formation of microvoids during roasting. Green beans that initially have a lower density or a more open structure tend to expand more during roasting, resulting in lower-density roasts. In contrast, green beans that have a denser structure will see limited expansion, resulting in roasted beans with higher density. This variation in initial structure is closely tied to the inoculum concentration used during fermentation (Galarza & Figueroa, 2022).

Referring to Figure 3, the change in density, determined by the difference between the density of green beans and that of roasted beans, varied between 42.44% and 48.54%. This variation suggests that the roasting process led to a significant and relatively consistent reduction in density throughout all treatments. Consequently, the fermentation process had no impact on the change in density.

Changes in density indicate how coffee beans react to the roasting process, particularly through their increase in volume and the development of a porous structure due to heat exposure. At this point, the primary factors influencing the degree of density decrease are the release of water, the creation of gases, and the softening of the tissue matrix. Since the roasting process was consistently applied across all treatments at a medium level, the expansion responses were also quite

similar, resulting in no significant differences in density changes among the treatments.

The density transformation from green beans to roasted beans was not notably impacted by the duration of fermentation or the level of inoculum concentration. Instead, alterations in density were determined by the roasting process itself. This suggests that changes in density can serve as a reliable measure of roasting uniformity and offers a solid foundation for understanding variations in other parameters without being influenced by differences in fermentation processes (Elhalis et al., 2020).

Moisture Content and Its Changes

The moisture content evaluated in this study aimed to assess the influence of fermentation treatment on the physical characteristics of Robusta coffee beans. The moisture content for both green and roasted beans is illustrated in Figure 4, while Figure 5 depicts the variation in moisture content from green beans to roasted beans.

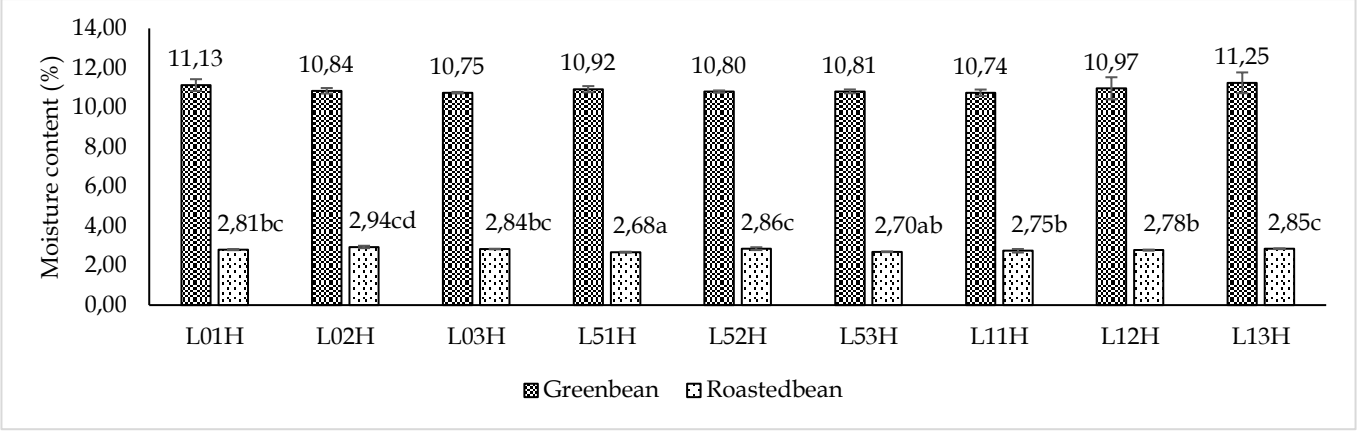

Figure 4. Comparison of moisture content in green beans versus roasted beans across different research treatments

The data presented in Figure 4 indicate that the fermentation treatment does not significantly impact the moisture content of green beans. The moisture content observed in the green beans varies between 10.74% and 11.25%. Specifically, the lowest moisture content recorded was 10.74% under the L11H treatment, while the highest was 11.25% in the L13H treatment.

The drying process utilizing a solar dome dryer is essential in regulating water mass transfer; consequently, the moisture content of green beans is predominantly influenced by the drying conditions rather than the fermentation process. In this drying system, the primary factors that govern the rate of water release include the humidity gradient between the beans and the drying air, temperature, and airflow rate. These elements collectively contribute to the attainment of a near-equilibrium moisture state in the beans.

The fermentation of mucilage beans alters surface components, including the mucilage and outer layer, through enzymatic and metabolic processes. These modifications can influence the soluble compounds present on the seed surface, but they do not change the inherent water retention ability of the endosperm. During the drying process, free water on the outer surface and mobile water within the seed tissue are released until a new balance is established. According to Figure 4, the moisture content of roasted beans varies between 2.68% and 2.94%, which is lower than that of green beans. The L51H treatment recorded the lowest moisture content in roasted beans, while the L02H treatment showed the highest.

The variance in roasted bean moisture content across different inoculum concentration levels demonstrates that fermentation significantly influences the beans' response to water release during the roasting process. The treatment without inoculum addition (L0) yielded the highest roasted bean moisture content of 2.86% (c), whereas the inclusion of 50% inoculum (L5) resulted in the lowest moisture content of 2.74% (a). This finding suggests that moderate levels of inoculum can effectively alter the structural properties of the bean tissue, facilitating a more efficient release of water during roasting. The microbial activity associated with fermentation is believed to soften the surface tissue and enhance the permeability of the bean matrix, thereby promoting easier diffusion of water during heating.

In comparison, with the inclusion of 100% inoculum (L1), the moisture level of the roasted beans rose again to 2.795 (b) compared to L5, even though it remained lower than the control sample. This suggests that an excessively high inoculum concentration does not necessarily enhance the effectiveness of water extraction during roasting. Highly active microbial processes can lead to alterations in tissue structure, such as the development of layers or the presence of specific metabolite residues, which can cause some water to be

retained for a longer period within the bean matrix during the roasting process. In comparison, with the inclusion of 100% inoculum (L1), the moisture level of the roasted beans rose again to 2.795 (b) compared to L5, even though it remained lower than the control sample. This suggests that an excessively high inoculum concentration does not necessarily enhance the effectiveness of water extraction during roasting. Highly active microbial processes can lead to alterations in tissue structure, such as the development of layers or the presence of specific metabolite residues, which can cause some water to be retained for a longer period within the bean matrix during the roasting process.

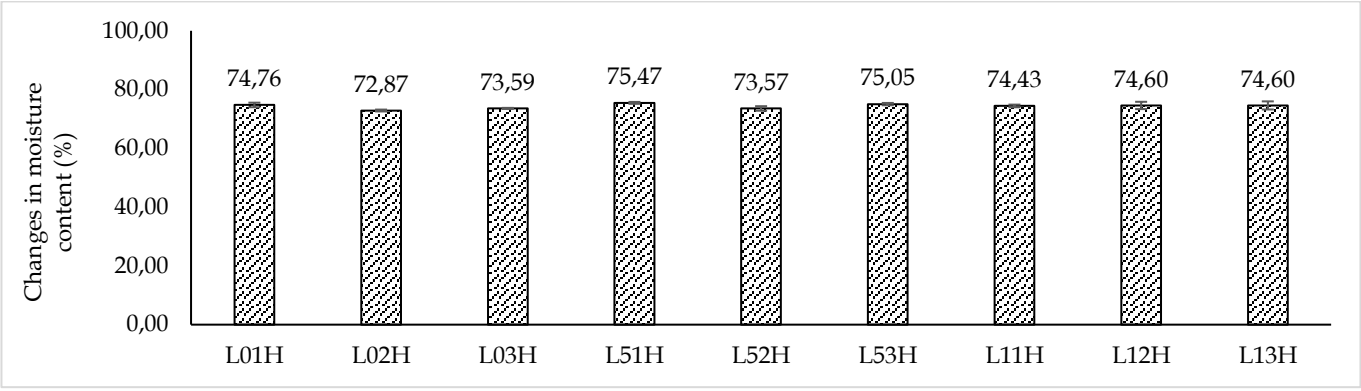

Figure 5. The moisture content changes as green beans are roasted in different research treatments

The duration of fermentation also had a notable impact on the moisture levels of the roasted beans. The lowest level of moisture in the roasted beans was found in the 1-hour fermentation treatment, registering at 2.755% (a), which then rose in the 2-hour fermentation treatment to 2.86% (c), before slightly decreasing again in the 3-hour fermentation treatment to 2.8% (b). This suggests that there is a non-linear relationship between fermentation time and the moisture content of roasted beans. During shorter fermentation periods, the structure of the beans remains relatively open, facilitating a quicker release of water during roasting. With longer fermentation, the alterations in the more intricate network structure can lead to some water being bound more firmly or trapped within the matrix, increasing the moisture content of the roasted beans (Nizori et al., 2021).

The moisture level in roasted beans is more affected by fermentation treatment compared to green beans. The concentration of the inoculum and the duration of fermentation contribute to the development of the bean structure, which affects how efficiently water is released during roasting. This highlights the significance of managing fermentation as an initial phase that impacts the physical traits of roasted beans, which may subsequently affect the density, grind size, and extraction properties of coffee during brewing. The variation in moisture content, determined by the difference between the moisture content of unroasted and roasted beans, was observed to be between 72.87% and 75.47% (Figure 5). This slender range suggests that the release of water during the roasting process was consistent across all combinations of fermentation treatments. The minimum change in moisture content was observed in the L02H treatment (72.87%), whereas the maximum change was noted in the L51H treatment (75.47%).

Changes in moisture content involve the release of both free and bound water from the tissue of the bean as a result of heating. During the roasting process, the vapor pressure within the bean increases rapidly, which forces water out through the pores and microcracks present. While fermentation may influence pore formation and tissue permeability, existing data indicate that the extent of this effect is insufficient to significantly alter the overall quantity of water lost. Fermentation had an impact on the moisture content of roasted beans individually; however, the extent of the change in moisture content from green to roasted beans remained relatively consistent across all treatment groups. This observation supports the conclusion that the roasting process significantly influences the total water release from coffee beans, while fermentation primarily alters the structural characteristics rather than the quantity of water released (Setiaboma et al., 2025).

Caffeine Levels and Their Changes

Caffeine content is one of the main chemical parameters used to evaluate the quality and functional characteristics of coffee. In this study, caffeine content was analyzed in green beans and roasted beans (Figure 6). Changes in caffeine content, which represent the amount of caffeine lost during the roasting process, can be seen in Figure 7.

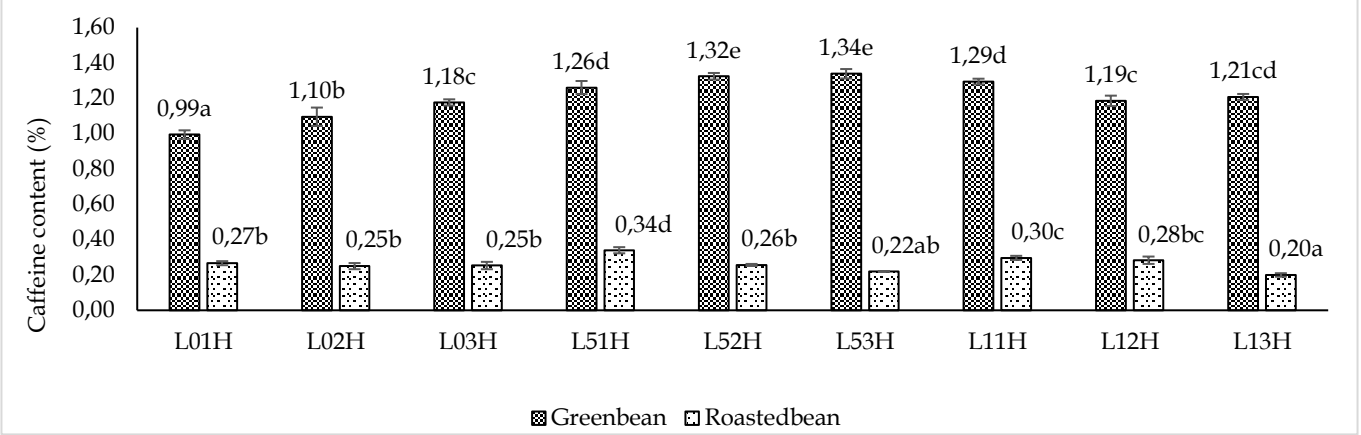

Figure 6. Caffeine content of green beans and roasted beans in each research treatment

According to Figure 6, the fermentation process had a significant impact on the caffeine levels in green beans. The caffeine concentration in the green beans varied from 0.99% to 1.34%. The L01H treatment produced the lowest caffeine concentration at 0.99%, while the L53H treatment yielded the highest caffeine level at 1.34%. Figure 5 indicates that both the duration of fermentation and the concentration of the inoculum influenced the caffeine levels in the green beans.

The duration of fermentation influences the caffeine levels in green beans, but this relationship depends on the concentration of the inoculum utilized. Nugroho et al. (2025) stated that the soaking time will have a significant impact on caffeine reduction. In the treatment that did not involve any inoculum addition (L0), the caffeine content in the green beans rose as fermentation time increased, from 0.99% (L01H) to 1.10% (L02H), and eventually to 1.18% (L03H). A similar trend was observed in the treatment with a 50% inoculum addition (L5), where the caffeine content grew from 1.26% (L51H) to 1.32% (L52H) and peaked at 1.34% (L53H). This indicates that, at low to moderate inoculum levels, there is a correlation between fermentation duration and increased caffeine content in green beans. This aligns with research by Novriani et al. (2025), which found that caffeine levels increased from 1.76% after 6 hours of fermentation to 2.38% after 24 hours in robusta coffee fermented with *Lactobacillus plantarum*.

In contrast, when using a treatment that included a 100% inoculum (L1), the extended fermentation duration did not lead to an increase in the caffeine levels of the green beans. The caffeine levels observed in the L1 treatment displayed a downward trend from 1.29% (L11H) to 1.19% (L12H) and then slightly increased to 1.21% (L13H). This suggests that when the inoculum concentration is too high, the fermentation process fails to enhance caffeine content; it is believed that increased microbial activity results in various alterations in dry matter composition or hampers the diffusion of caffeine from the bean matrix.

The concentration of the inoculum also affects the caffeine levels in green beans. The control group that lacked inoculum (L0) exhibited a lower caffeine content compared to the group with 50% inoculum (L5). Incorporating 50% inoculum led to the highest and fairly consistent caffeine levels in the green beans throughout different fermentation times. However, when 100% inoculum (L1) was added, the caffeine content in the green beans showed a tendency to drop compared to the L5 group, although it remained higher than the control in certain cases.

Fermentation is an important factor that influences changes in caffeine levels (Omega & Wibisono, 2023). Caffeine is a stable alkaloid compound that does not form during the fermentation process. The rise in caffeine levels is believed to occur because there is a decrease in soluble non-caffeine components, such as sugars and organic acids, throughout fermentation and washing. This decrease in these substances raises the ratio of caffeine to the overall mass of dry matter. Additionally, fermentation can alter the microstructure of the coffee bean tissue, making it easier to extract caffeine during analysis, which consequently boosts the caffeine content of green beans (Sarosa et al., 2024).

Fermentation is essential in influencing the caffeine levels in green beans, with a balance of 50 percent inoculum and extended fermentation duration yielding the highest caffeine content in the beans. On the other hand, overly high concentrations of inoculum can diminish the capability of fermentation to raise detectable caffeine levels. These results highlight the importance of fine-tuning inoculum concentration and fermentation duration to manage the chemical characteristics of green beans before roasting.

According to Figure 6, the fermentation process had a notable impact on the caffeine levels in roasted beans. The caffeine levels of roasted beans across all treatments varied from 0.20% to 0.34%. The treatment with the lowest caffeine content was L13H, which had a value of 0.20%, while the treatment with the highest content was

L51H at 0.34%. This suggests that even though roasting typically decreases caffeine levels, the amount of caffeine that remains after roasting is also affected by the specific fermentation treatments used. The fermentation time factor showed a significant effect on the caffeine content of roasted beans at all inoculum concentrations. In the treatment without the addition of inoculum (L0), the caffeine content of roasted beans decreased with increasing fermentation time, namely from 0.27% (L01H) to 0.25% (L02H) and remained low at L03H (0.25%). This indicates that the length of spontaneous fermentation tends to reduce caffeine content after the roasting process.

In the treatment involving the addition of 50% inoculum (L5), a notable decrease in the caffeine content of roasted beans was observed. Specifically, the caffeine content diminished from 0.34% (L51H) to 0.26% (L52H), reaching a recorded minimum of 0.22% (L53H). This trend suggests that the combination of inoculum and fermentation duration significantly alters the structural and compositional aspects of the beans prior to roasting, thereby influencing the caffeine concentration in the roasted product. A similar pattern was evident in the treatment with a 100% inoculum addition (L1). In this case, the caffeine content decreased from 0.30% (L11H) to 0.28% (L12H), ultimately attaining the lowest measurement of 0.20% (L13H). This reduction indicates that at elevated inoculum concentrations, an extended fermentation period further contributes to the reduction of caffeine levels in the roasted beans.

Caffeine is an alkaloid compound that remains relatively stable at the temperatures used in coffee roasting. As a result, variations in caffeine content within roasted beans are believed to arise from alterations in the physical structure and composition of the beans during fermentation, which influence how the beans react to heat. Extended fermentation can alter the endosperm tissue and the outer layer of the beans, leading to changes in their expansion, porosity, and distribution of microcracks while roasting. These structural modifications impact the availability of caffeine during the extraction process, which may lead to a decrease in the caffeine levels of roasted beans.

The caffeine content in roasted beans was greatly influenced by the duration of fermentation and the concentration of the inoculum. While roasting lowered the measurable levels of caffeine across all treatments, fermentation was crucial in influencing the amount of caffeine released post-roasting. The highest caffeine content in roasted beans was achieved with a 50% inoculum and a brief fermentation time, whereas extended fermentation, particularly with higher inoculum levels, significantly diminished the caffeine content of the roasted beans.

The variation in caffeine levels in this research was determined as the difference between the caffeine concentration in green beans and that in roasted beans, indicating the degree of caffeine decrease as a result of the roasting process. According to Figure 7, the alteration in caffeine content was between roughly 70% and 85%. This indicates that roasting is the most crucial factor in altering caffeine levels, irrespective of the differences in the fermentation treatment used beforehand.

While caffeine degradation was observed in all treatments, the extent of the change in caffeine levels varied. The treatments L53H and L13H exhibited more significant changes in caffeine content, whereas L51H demonstrated fewer changes. This variation suggests that fermentation affects the amount of caffeine that is lost or becomes undetectable after roasting. In other words, fermentation does not directly lead to caffeine degradation, but it does affect how the beans react to the roasting process, thereby influencing the final caffeine levels in the roasted beans.

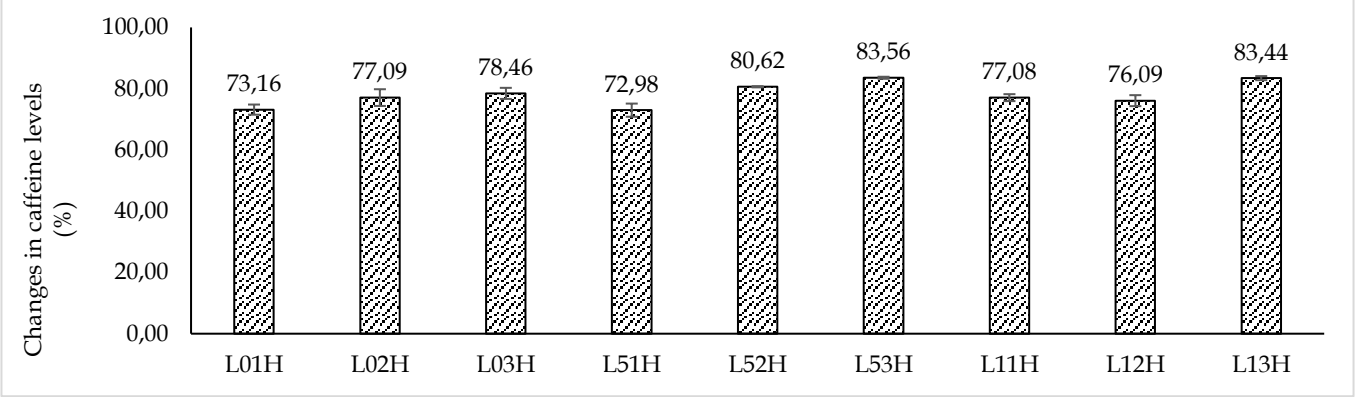

Figure 7. Change in caffeine content from green beans to roasted beans

The variations in caffeine levels are closely linked to alterations in the physical characteristics of the beans throughout the roasting process. Fermentation can influence the integrity of the bean structure and the

extent of tissue softening, subsequently affecting the rate of expansion and pore development during heating. Beans that have a structure that expands more readily typically undergo more significant changes in density, which can affect the distribution of caffeine and volatile compounds during roasting. Treatments that result in greater changes in density may experience a more notable redistribution or measurable loss of caffeine, leading to larger fluctuations in caffeine content (Darwin et al., 2021).

The relationship between changes in caffeine content and roasted bean density is consistent with the physical data obtained. Treatments that produce roasted beans with higher density and a more compact structure tend to show greater changes in caffeine content, because diffusion limitations and matrix changes during roasting can reduce the caffeine measured in the analysis stage. Conversely, treatments that produce roasted beans with a more open and porous structure tend to retain higher levels of measured caffeine, resulting in relatively smaller changes in caffeine content.

The low and relatively uniform moisture content of roasted beans across all treatments indicates that changes in caffeine content are not primarily influenced by residual moisture, but rather by structural changes resulting from the interaction between fermentation and roasting. This reinforces the interpretation that changes in caffeine content are the result of a complex interaction between biological processes during fermentation and thermal processes during roasting, rather than a single factor.

Changes in caffeine content can be used as an integrative indicator reflecting the response of coffee beans to fermentation and roasting. The extent of caffeine reduction is determined by the roasting process, but fermentation plays a role in directing the magnitude of these changes by modifying the structure and physical properties of the beans. Controlling fermentation not only affects sensory characteristics but also the stability and chemical profile of coffee after roasting (Klikarová et al., 2022).

Conclusion

The primary objective of this study is to establish a scientific foundation for the development of indigenous microbial-based coffee fermentation technology, inspired by the civet coffee process. This approach aims to provide a more environmentally sustainable alternative to conventional chemical decaffeination methods. This research investigates the effects of a civet coffee microbial inoculum combined with varying fermentation times on the physicochemical characteristics of Robusta coffee beans, both prior to and following roasting. This study establishes that fermentation significantly influences the physical and chemical properties of Robusta coffee, specifically in both green and roasted beans. The concentration of fermentation inoculum is shown to have a profound effect on the density and caffeine content of green beans, as well as on the moisture content and density of roasted beans. Additionally, the duration of fermentation is critical, as it affects the caffeine content in green beans, along with the moisture and caffeine levels in roasted beans. Notably, there is a significant interaction between inoculum concentration and fermentation time, which influences the caffeine content of green beans, as well as the moisture content and density of roasted beans. While this study successfully characterizes the physical and chemical properties of Robusta coffee under controlled fermentation conditions, further optimization is essential. Future work will involve analyzing population dynamics and microbial activity during fermentation to gain insights into the biological mechanisms that influence coffee quality. Additionally, assessing volatile compounds through gas chromatography-mass spectrometry (GC-MS) and conducting sensory evaluations will be critical in determining how fermentation impacts aroma, flavor, and consumer acceptance. Exploring this microbial-based fermentation method with other coffee varieties and scaling it up for larger production will also be a valuable direction for future research.

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Author Contributions

The authors have contributed together for each stage of research and have read and approved the published version of the manuscript. Conceptualization, writing-original draft, A.N. and A.L.A; methodology, R.N.K; software, A.N.; validation, A.L.A., A.N., and R.N.K; format analysis, A.L.A.; investigation, R.N.K..; resources, A.L.A.; data curation, A.N.; writing-original draft preparation, A.L.A., A.N., and R.N.K.; writing–review and editing, A.L.A.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this research.

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