Effect of civet-derived bacteria and fermentation duration on basic physical characteristics of robusta green coffee beans

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Abstract. This study investigates the effect of civet-derived bacterial fermentation on the yield, moisture content, and density of Robusta green coffee beans. The research focused on basic physical characteristics as preliminary indicators of bean quality before further chemical and sensory evaluation. Robusta coffee cherries were fermented using civet-derived bacteria at three inoculum concentrations (0%, 50%, and 100%) and for different fermentation durations (1, 2, and 3 days). The resulting beans were analyzed for yield, bulk density, and moisture content. The experiment was arranged in a Completely Randomized Design (CRD) with two factors: civet-derived bacterial concentration and fermentation duration, each replicated three times. Data were analyzed using ANOVA followed by DMRT at a 5% significance level. The results showed that the civet-derived bacterial concentration significantly affected the yield and density, but did not influence the moisture content of the green coffee beans. In contrast, fermentation duration and its interaction with bacterial concentration had no significant effects on any of the observed parameters. The highest yield (38.06%) and stable physical characteristics were obtained at the 50% inoculum concentration, indicating that moderate bacterial activity provided the most efficient mucilage degradation without causing excessive bean-mass loss. These findings suggest that civet-derived bacterial fermentation at a moderate concentration can enhance the physical quality and structural integrity of Robusta green coffee beans, serving as a potential pre-treatment step for improving their chemical and sensory properties.

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1 Introduction

Robusta coffee (*Coffea canephora*) is one of Indonesia's main export commodities, and its productivity has increased steadily in recent years. East Java is the sixth-largest producer of Robusta coffee, contributing 47,995 tons from 91,254 hectares of plantation area, with Jember Regency producing 3,268 tons in 2022 and showing a 1.49% growth in 2023 [1]. Indonesian Robusta coffee is globally recognized for its strong flavor and body, which are influenced by its relatively high fat, protein, and caffeine contents [2].

The quality of coffee is determined by its physical, chemical, and sensory attributes, which collectively influence the formation of flavor compounds during roasting and brewing. Green coffee beans are more stable than roasted beans; however, prolonged storage can lead to the degradation of flavor precursors and a decline in overall quality [3]. Therefore, maintaining the physical quality of green beans is essential. Coffee quality is commonly evaluated based on bean size, shape, color, density, and defect score, which are regulated in the Indonesian National Standard (SNI 01-2907-2008) [4]. Among post-harvest handling techniques, fermentation plays a critical role in improving these quality attributes [5].

Civet coffee, commonly known as kopi luwak, is one of Indonesia's most distinctive and valuable coffee products. It is characterized by a smoother taste, lower caffeine, and reduced bitterness, resulting from microbial and enzymatic processes within the civet's digestive system [6]. Enzymes such as proteases, cellulases, and pectinases produced by civet-associated microbes degrade macromolecules (proteins, polysaccharides, pectin) during digestion, leading to modifications in the structure and composition of coffee beans [7]. These enzymatic transformations influence both chemical composition and basic physical characteristics such as density and porosity [8].

However, natural civet digestion has a limited fermentation capacity and is not scalable for industrial production. Therefore, fermentation using civet-derived bacterial inoculum has been explored as a biotechnological alternative to reproduce the biochemical environment of civet digestion [9]. By introducing civet-derived bacteria during controlled fermentation, it is possible to achieve selective enzymatic degradation of the mucilage layer and minor structural modifications of the endosperm, potentially influencing yield, density, and moisture content, which are fundamental indicators of the physical quality of green coffee beans [10].

The present study investigates the effect of civet-derived bacterial fermentation on the fundamental physical properties of Robusta green coffee beans, with a primary focus on yield, bulk density, and moisture content as key indicators of physical quality. Understanding how microbial activity influences these parameters is essential for developing sustainable and controlled fermentation processes that enhance coffee bean quality before chemical and sensory evaluation.

2 Materials and Methods

2.1 Place and time

This study was conducted at the Agricultural Product Processing Laboratory, Politeknik Negeri Jember, East Java, Indonesia. Coffee cherry samples were obtained from Rembangan, Jember (8°03'14.1"S, 113°41'05.5"E).

2.2 Harvesting of coffee fruit

Ripe Robusta coffee cherries (*Coffea canephora* var. Argopuro) were manually harvested and sorted to remove defective fruits [11]. The outer red pericarp (exocarp) was removed using a mechanical pulper. Each treatment consisted of 5 kg of depulped coffee cherries placed in sterile polypropylene bags to prevent cross-contamination during fermentation.

2.3 Processing of coffee beans

The treatments in this study comprised two experimental factors. The first factor was the concentration of civet-derived bacteria, consisting of three levels (K): K1 (0%, without bacterial addition but using water), K2 (50%), and K3 (100%). The second factor was the fermentation duration, which also consisted of three levels (T): T1 (1 day), T2 (2 days), and T3 (3 days). Accordingly, the experiment involved nine treatment combinations, each replicated three times, resulting in a total of 27 experimental units. After fermentation, the coffee beans were thoroughly washed to remove any residual mucilage adhering to the surface. The beans were then dried in a solar dryer for four days. The dried parchment (horn-skin) coffee was subsequently hulled using a hulling machine.

2.4 Analytical parameters

2.4.1 Yield analysis

Sorted Robusta coffee cherries were weighed using a digital analytical scale with an accuracy of 0.01 g to record the initial weight (W_1) of depulped coffee. After fermentation, washing, drying, and hulling, the resulting green beans were reweighed to obtain the final weight (W_2) [7]. Yield was calculated using the equation:

$$Yield = W2/W1 \times 100 \quad (1)$$

2.4.2 Density analysis

Particle density was measured by immersing 50 g of green coffee beans into a 100 ml graduated cylinder partially filled with water and recording the displaced volume. Density (ρ) was calculated using the equation m/v, where *m* is the mass and *v* is the displaced volume [12].

2.4.3 Moisture content analysis

Fermented coffee beans are dried in a solar dryer for 4 days, and then the moisture content is measured. The moisture content of fermented green coffee beans was determined using a DIGI-MOST (ICCRI, Indonesia) moisture analyzer, which operates based on infrared and capacitance principles for rapid and non-destructive measurement. Approximately 50 g of green coffee beans were analyzed in triplicate, and the results were expressed as a percentage [10].

2.5 Research design

The experiment was arranged using a Completely Randomized Design (CRD). The physical characteristics analyzed included yield, bulk density, and moisture content. The research data were analyzed using ANOVA and Duncan's advanced test at the 5% level.

3 Results and Discussion

3.1 Yield of green coffee beans

As illustrated in Figure 1, the interaction between the concentration of civet-derived bacteria and fermentation duration showed no significant effect on the yield of green coffee beans. Similarly, fermentation duration alone did not significantly influence yield, whereas the bacterial concentration exhibited a notable impact (Table 1).

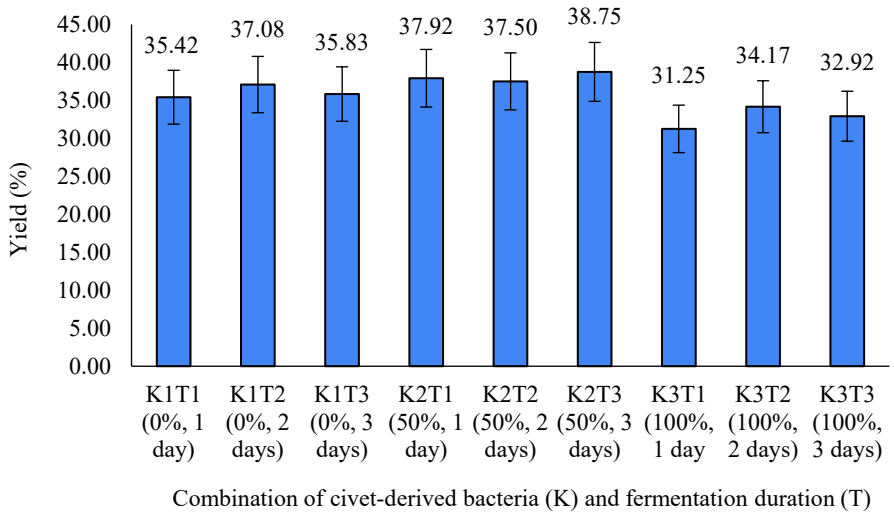

Fig. 1. Effect of the combination of civet-derived bacteria concentration and fermentation duration on the yield of green coffee beans (%)

The stability of yield values across different fermentation times indicates that the enzymatic degradation of mucilage primarily depends on the availability and concentration of active microbial enzymes, rather than the length of fermentation. Once the mucilage is sufficiently degraded during the early stages (within 24 hours), extending fermentation up to 72 hours does not further enhance mucilage removal and may even increase the risk of uncontrolled microbial activity, which can lead to minor bean-mass losses.

The absence of interaction effects suggests that the optimal yield achieved at 50% inoculum (K2) is maintained regardless of fermentation duration. This reflects a robust enzymatic system capable of rapidly decomposing mucilage without over-degradation of the bean's structural layer. At 100% inoculum, however, excessive microbial activity likely accelerates fermentation beyond the optimal point, promoting over-fermentation, surface softening, and fragmentation, resulting in a decline in yield across all time intervals [13].

As presented in Table 1, the concentration of civet-derived bacteria significantly affected the yield of green coffee beans (DMRT, 5% level). The highest yield (38.06) was obtained from the K2 (50%) treatment, followed by K1 (36.11), while the lowest yield (32.78) occurred at K3 (100%). The different superscript letters indicate statistically significant differences among treatments.

This pattern indicates that a moderate inoculum concentration yields the most efficient conversion of wet-processed cherries into dried green beans. At 50% inoculum, the enzymatic activity of civet-derived bacteria—particularly pectinase, cellulase, and protease—is sufficient to degrade the mucilage layer and loosen the parchment–endosperm interface without damaging the bean structure. This selective depolymerization facilitates

mucilage removal and decreases mechanical stress during washing and hulling, thereby reducing solid losses and improving recovery yield [14].

Table 1. Effect of civet-derived bacteria concentration on the yield of green coffee beans (%)

Civet-derived bacteria concentration	Yield of green coffee beans (%)
K1 (0%)	36.11[a]
K2 (50%)	38.06[b]
K3 (100%)	32.78[c]

Note: Numbers followed by the same letter in the same column show no significant difference at the DMRT Test 5%

At a higher concentration (100%), excessive microbial activity may lead to over-fermentation, partial degradation of structural polysaccharides, and micro-fissures on the parchment surface, causing bean fragmentation and reduced mass recovery. Conversely, the control (0%) produced a lower yield than K2 because mucilage removal depended solely on native microbiota and manual washing, leaving sticky residues that required stronger agitation, which in turn increased surface abrasion.

These results indicate that yield differences were primarily driven by microbial efficiency and mucilage degradation, rather than by moisture or time effects, since post-drying moisture content remained constant (11–12) and fermentation duration was not significant. The optimum yield at the 50% inoculum level confirms that moderate bacterial activity provides an ideal balance between effective mucilage removal and preservation of bean integrity, consistent with previous reports on microbial fermentation improving coffee processing efficiency [15].

3.2 Density of green coffee beans

As shown in Figure 2, the interaction between civet-derived bacterial concentration and fermentation duration had no significant effect on the density of green coffee beans. Similarly, fermentation duration alone did not significantly influence density, while the bacterial concentration had a measurable but limited effect (Table 2).

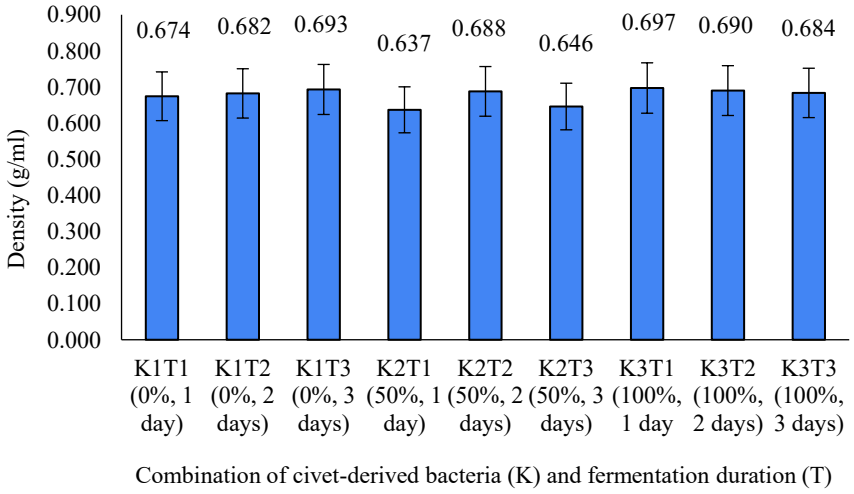

Fig. 2. Effect of the combination of civet-derived bacteria concentration and fermentation duration on the density of green coffee beans (g/ml)

The density of green coffee beans ranged from 0.637 to 0.697 g/cm³, indicating relatively stable structural integrity among treatments. The slight increase in density observed at moderate inoculum levels (K3T1 = 0.697 g/cm³) suggests that microbial fermentation may enhance mucilage removal and compact bean structure by facilitating uniform dehydration. However, at higher bacterial concentrations (100%) or longer fermentation times, the density tended to decrease slightly, possibly due to excessive enzymatic degradation of polysaccharides and proteins within the parchment layer, leading to micro-porosity and a less compact internal structure [16].

The absence of a significant interaction between inoculum and time confirms that the fermentation process reached equilibrium before 72 hours, and that enzyme activity, rather than time, primarily determined bean physical properties. Overall, the stable density profile across treatments demonstrates that civet-derived fermentation does not negatively affect bean compactness, preserving structural integrity and quality consistency of the green beans during processing [13].

As presented in Table 2, the concentration of civet-derived bacteria had a significant effect on the density of green coffee beans (DMRT, 5% level). The highest density (0.690 g/mL) was obtained at K3 (100% inoculum), followed by K1 (control, 0.683 g/mL), while the lowest density (0.657 g/mL) was observed at K2 (50% inoculum). Although the numerical differences were small, they were statistically significant, indicating that microbial concentration influences the compactness and structural properties of the beans after fermentation.

Table 2. Effect of civet-derived bacteria concentration on the density of green coffee beans (g/ml)

Civet-derived bacteria concentration	Density of green coffee beans (g/ml)
K1 (0%)	0.683^{ab}
K2 (50%)	0.657^{a}
K3 (100%)	0.690^{b}

The relatively higher density at K3 suggests that a high concentration of civet-derived bacteria may enhance mucilage degradation and dehydration uniformity, allowing the coffee beans to retain a more compact microstructure. Enzymes such as pectinase and cellulase produced by the bacteria facilitate the removal of mucilage residues from the parchment surface, improving packing density and minimizing trapped air spaces within the beans. However, at moderate bacterial concentration (50%), excessive enzymatic activity might have slightly softened the cell walls of the parchment or endosperm, leading to a lower apparent density [15].

The control (K1) exhibited intermediate density values, likely due to limited natural microbial activity that produced slower mucilage removal but without excessive degradation of the structural matrix. Overall, these findings indicate that the density of green coffee beans is influenced not only by moisture equilibrium but also by the balance between enzymatic mucilage removal and structural preservation. The result confirms that civet-derived fermentation at 100% concentration can improve bean compactness and physical stability, contributing positively to post-fermentation bean quality [14].

3.3 Moisture content of green coffee beans

As shown in Figure 3, neither the civet-derived bacterial concentration nor the fermentation duration had a significant effect on the moisture content of green coffee beans. Likewise, the interaction between both factors showed no significant difference among treatments. The moisture content of the beans ranged narrowly between 10.74% and 11.24%, indicating that the post-drying process was well-controlled and consistent across all treatments.

This narrow variation suggests that the fermentation process and bacterial inoculation did not substantially alter the water-holding capacity of the beans. Moisture content in green coffee beans is mainly influenced by drying efficiency and environmental conditions rather than microbial activity during fermentation. Since all samples underwent identical drying conditions in a dome dryer for four days, the observed uniformity in moisture content confirms that the drying procedure effectively standardized the final moisture equilibrium [17].

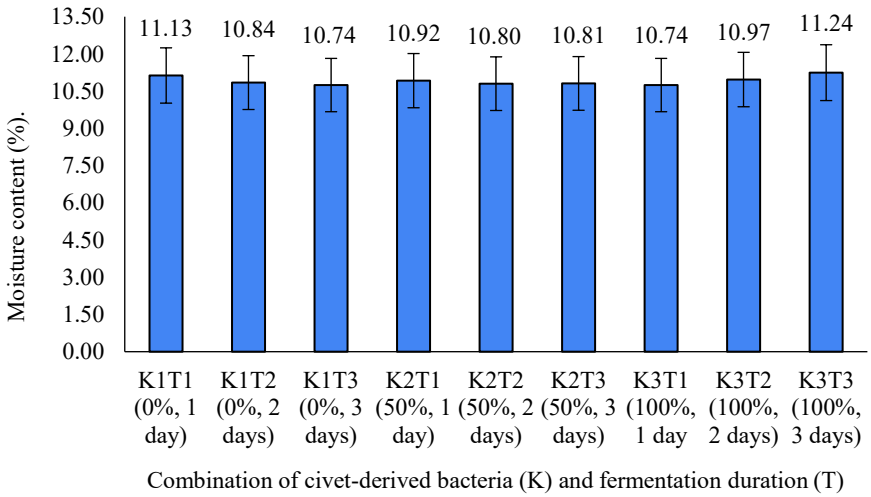

Fig. 3. Effect of the combination of civet-derived bacteria concentration and fermentation duration on the moisture content of green coffee beans (%)

Maintaining moisture content within this range (approximately 10–12%) is crucial for preserving the storage stability, flavour precursors, and resistance to microbial growth of green coffee beans. Excessive moisture could increase the risk of fungal contamination and reduce shelf life, whereas overly low moisture may cause brittleness and quality degradation during roasting [18]. Therefore, the stable moisture values observed across treatments indicate that civet-derived fermentation and controlled drying collectively ensure consistent bean quality, without adversely affecting the final water content [19].

4 Conclusion

The civet-derived bacterial concentration treatment had a significant effect on the yield and density of green coffee beans but did not affect their moisture content. Meanwhile, fermentation duration had no significant effect on yield, density, or moisture content. Similarly, the interaction between civet-derived bacterial concentration and fermentation duration showed no significant effect on all observed parameters. The best result was obtained at the 50% civet-derived bacterial concentration, which produced the highest yield (38.06%) and maintained stable physical characteristics, indicating that moderate bacterial activity provided the most efficient fermentation process and preserved the structural integrity of Robusta green coffee beans.

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