ASIAN J TROP BIOTECHNOL
Volume 22, Number 2, December 2025
Pages: 49-55

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c220201

Artificial *luwak* coffee fermentation using submerged bioreactor and backslopping methods

CHANDRA UTAMI WIRAWATI[1,♥], WINARTO[2]

[1]Department of Food Technology, Politeknik Negeri Lampung. Jl. Soekarno Hatta No 10 Rajabasa, Bandar Lampung 35144, Lampung, Indonesia. Tel./fax.: +62-251-701789, ♥email: cutami@polinela.ac.id

[2]Department of Agriculture Mechanization, Politeknik Negeri Lampung. Jl. Soekarno Hatta No 10 Rajabasa, Bandar Lampung 35144, Lampung, Indonesia

Manuscript received: 6 April 2025. Revision accepted: 27 August 2025.

Abstract. *Wirawati CU, Winarto. 2025. Artificial* luwak *coffee fermentation using submerged bioreactor and backslopping methods. Asian J Trop Biotechnol 22: 49-55. Luwak* coffee is considered a special variant of coffee with a premium price tag. It is currently produced in extremely limited amounts through in vivo fermentation in the digestive tract of Asian palm civets, with the beans collected from droppings. To avoid the industrialization of civet in *luwak* coffee production, a bioreactor combine with backslopping methods is needed. Therefore, this study aims to develop a submerged bioreactor and to isolate and identify the lactic acid bacteria (LAB) involved in artificial *luwak* coffee fermentation. During the study procedures, cupping test quality was assessed, followed by the isolation and identification of the LAB. The fermentation process was guided by a central composite design (CCD), which included two independent variables: fermentation time (12-24 hours) and backslopping culture concentration (3-5%). The results demonstrate the higher cupping test score i.e., 80 was obtained from 12-hour fermentation with 5% culture concentration, which produced an astringent aftertaste, spicy-chili taste, and brown sugar flavor, leading to the classification of the product as specialty coffee. The research have successfully developed a bioreactor prototype that can be used at the farm level. This supports animal welfare and minimizes the industrialization of civets. The bioreactor is also potentially scalable for the in vitro processing of *luwak* coffee.

Keywords: Backslopping, bioreactor, central composite design, LAB, specialty coffee

Abbreviations: CCD: Central Composite Design, LAB: Lactic Acid bacteria

INTRODUCTION

The appreciation of various types of commercial coffee, including specialty coffees with special flavor profiles, is common amongst enthusiasts around the world. A major specialty coffee is the highly demanded *luwak* variety, which commands a high price of 100 USD per 450 g, or approximately 1,500,000.00 IDR/450 g of roasted beans (Fitri et al. 2021). However, its supply in the market is very limited, despite the continuously growing demand. The limitation of *luwak* coffee is mainly due to the difficult production process involved. The commodity is produced in vivo from the drying process of coffee beans that have previously passed through the Asian palm civet's (*Paradoxurus hermaphroditus*) digestive tract. Fermentation occurs through various biochemical and enzymatic reactions, with the beans digested in the civet's stomach, leading to the production of coffee with a unique and distinctive flavor, which is very different from the taste of traditionally processed coffee (wet or dry methods). In addition, it has a light, pillowy texture that almost "floats in the mouth" for a few seconds before being swallowed. The coffee also has lower acidity than traditional variants, together with pleasant sweetness and hints of caramel and chocolate. Despite its potential, the flavor of *luwak* coffee can vary depending on the region of production. In some Southeast Asian countries, such as China and Japan, drinking *luwak* coffee at a shop has become a luxury activity, considering the price of a cup can reach 35-80 USD, or 525,000-1,200,000 IDR.

Due to the high demand, several coffee farmers have "industrialized" wild civets to produce *luwak* coffee. The beans are given as food to the animals kept in cages to obtain larger quantities. However, the practice is considered as animal abuse and a violation of animal welfare, as civets are wild animals that are nocturnal and live by eating fruit, insects, mollusks, and other small animals (Tatin 2024). This situation creates the opportunity to produce large quantities of *luwak* coffee through an in vitro fermentation process using a bioreactor, with civets' digestive tract conditions duplicated in a controlled, simple bioreactor. A previous study showed that the use of a bioreactor in coffee fermentation could yield products with more uniform quality through the control of various variables, such as pH, temperature, brix degree, and culture starter (Reyes et al. 2024).

The application of LAB and yeast as a culture starter in coffee fermentation has been extensively used to enhance cup quality (Cassimiro et al. 2023; Dorta et al. 2024; Rivera et al. 2024). Previous studies have been conducted on the fermentation of coffee beans in vitro using LAB isolated from civet feces (Wang et al. 2020; Fauzi et al. 2023). In addition, Hadipernata and Nugraha (2018) integrated LAB isolates from civet stomachs with a

bioreactor engineered to mimic the digestive tract of the animals. The quantity and content of LAB in the digestive tract significantly influence the fermentation process of coffee beans. *Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, and *Streptococcus faecium* have been effectively isolated from civets and used in the in vitro fermentation of coffee (Fitri et al. 2019), as well as *Enterobacter cloacae* and *L. brevis* (Suhandoyo et al. 2016)

On the other hand the backslopping method offers a promising approach to producing *luwak* coffee in a bioreactor. The method is conducted by adding products from the previous fermentation batch to initiate a new cycle. It is closely related to the "carry-over" phenomenon of microorganisms, in which a small portion of the previously fermented product containing diversity and relative abundance functions as a culture for the next fermentation batch (Wirawati and Widodo 2021). This leads to the production of a similar product and aims to speed up fermentation; simplify the application process; and increase the chances of successful production (Wirawati et al. 2019). Research on the use of bioreactors to mimic civet digestive tracts has been conducted by Hadipernata and Nugraha (2018), although the combination of bioreactors and the backslopping fermentation method has never been attempted. This study therefore aims to develop a submerged bioreactor model and to isolate and identify the LAB involved in artificial *luwak* coffee fermentation.

MATERIALS AND METHODS

Tools and materials

The instruments used in the study comprised a styrofoam box, drill, saw, plastic tray, plastic jar, thermometer, pH meter (HANNA), tray dryer, roaster, peeler, grinder, oven, analytical scale, desiccator, and a water content measuring device. The components used included Arabica red cherry beans obtained from the Kota Agung district, backslopping culture (fresh civet feces), and authentic *luwak* coffee beans (control) obtained from Yahman Luwak Coffee, Kota Agung Sub-district, Tanggamus District, Indonesia. In addition, 0.1 N HCl solution, 0.1 N NaOH solution, 0.85% NaCl solution, de Mann Rogosa Sharpe Agar (Merck) media, Kit API® 50 CHL (bioMérieux, France) and a 1-way check valve were also employed.

Bioreactor design

The bioreactor was configured as an anaerobic system with multiple components, namely a reactor tube, an incandescent bulb as a heat source, a heating mantle using a styrofoam enclosure, and manual thermoregulation. In this study, the bioreactor contained three boxes designated for the fermentation of coffee beans. The bioreactor box was a 8 liters volume plastic (Figure 1). The material was selected due to its non-corrosive properties and its lack of interference with biological processes. The apparatus featured an entrance for the installation of a 1-way valve to expel surplus air during fermentation, and a thermometer was incorporated to monitor the temperature during the fermentation process.

An incandescent light bulb situated within a styrofoam container served as the heat source for the bioreactor throughout the coffee fermentation process. This material was selected due to its superior thermal insulation properties and its potential as a substitute for a civet's body. The temperature of the container was regulated to align with the animal's body temperature. Figure 2 shows the arrangement for positioning the bioreactor within the styrofoam box.

Preliminary bioreactor test

Before its application in the coffee bean fermentation process, the bioreactor's efficacy was evaluated in relation to leaks in the rubber seal of the cover and the temperature and pH sensors.

Fermentation procedure

The fermentation process was guided by the central composite design using Minitab 12 software, which included two independent variables, namely fermentation time (12-24 hours) and backslopping culture concentration (3-5%). The CCD experimental design is available in Supplementary Table 4. Whole red cherry coffee beans, processed with a pulper machine, were weighed at 3 kg and subsequently acidified in a 0.1 N HCl solution for 2 hours. The step following acidification was neutralization with a 0.1 N NaOH solution until the pH attained a range of 6.5 to 7.5. The final phase involved incorporating the backslopping culture by using 3 to 5% of civet feces as a culture initiator. In this study, 2 components (coffee bean and backslopping culture) and 2.5 liters of a 0.85% sodium chloride solution were introduced into bioreactor. The fermentation process was adapted to replicate the conditions of the civet's digestive system at 25 to 35°C. Each sample was quantified for total lactic acid bacteria (LAB) and the pH of the medium.

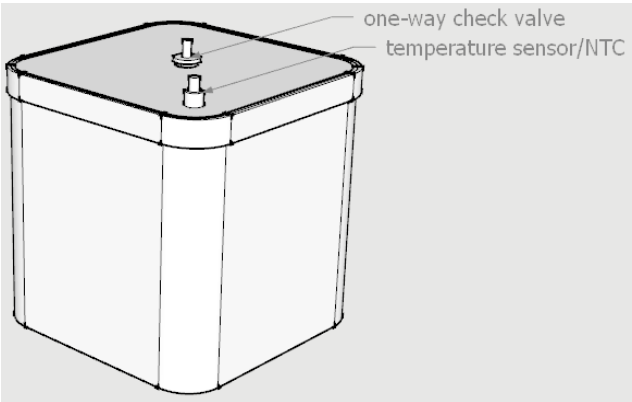

Figure 1. Bioreactor one way check valve and NTC

Cupping test

A cupping test was conducted according to the Specialty Coffee Association (SCA) guidelines (Specialty Coffee Association 2021). Fermented coffee beans were washed and dried to achieve a moisture level of 11 to 12%. The beans' endocarp was subsequently split and removed with a peeler. The peeled coffee beans were subsequently roasted in a roasting machine and pulverized using a grinder. Subsequently, the ground coffee was evaluated for sensory attributes through a final score cupping test at the Coffee and Cacao Study Centre (Puslitkoka) in Jember, East Java. Variables such as fragrance, flavor, aftertaste, acidity, balance, body, sweetness, flaws, uniformity, cleanliness, and total score constituted the comprehensive cupping characteristics. The cumulative ratings for each attribute were aggregated, and deficiencies were deducted to determine the final cupping score. The final score determined the coffee's classification as specialty or non-specialty. Authentic *luwak* coffee is also subjected to cupping tests as a control measure.

Isolation and identification of LAB species

Isolation and identification of LAB in fermented coffee samples were conducted based on the guidelines of Wirawati and Widodo (2021). Colonies from the coffee sample with the highest final score were separated and examined for morphological characteristics. Bacteria isolates exhibiting spherical or rod-like, gram-positive, and catalase-negative characteristics were purified on fresh plates until colonies of consistent shape, size and color were achieved. Phenotypic identification of LAB isolates was conducted by examining carbohydrate fermentation patterns with the API® 50 CHL Kit (bioMérieux, France). The fermentation profile of the isolates was assessed using APIWEBTM software version 1.3.0 from bioMérieux.

Data analysis

The levels of LAB and the pH medium during the fermentation process were analyzed descriptively, and CCD was applied on the cupping test response. ANOVA at 95% significance levels ($P<0.05$) was used to calculate the differences between the two independent variables using the Minitab 12 software. Authentic *luwak* coffee (from civet secretion at Yahman Luwak Coffee) was used as a control.

RESULTS AND DISCUSSION

Bioreactor design

Figure 4 shows the design of the bioreactor used. It was constructed from styrofoam, with overall dimensions of 33 × 41 × 74 cm and a wall thickness of 3 cm, ensuring that external environmental temperatures did not influence its internal temperature. Within the bioreactor, three plastic containers were installed, each fitted with a 1-way valve and a temperature control system that included a thermistor sensor (NTC) and a heater comprising three light bulbs (25-watt).

Preliminary bioreactor test

The initial experiments was conducted to test the bioreactor's operation. In the first step, the test was configured and the temperature threshold was entered into the bioreactor (data were collected 10 times). If the temperature fell below the range (with the ideal fermentation temperature for coffee is being approximately 28-30°C), the system switched on the heater until the temperature reached 28-30°C. On the other hand, when it neared the maximum temperature, the digital thermostat turned off the power to the heater so that it did not overheat. This happened automatically and continually. Table 1 presents the temperature profile of the bioreactor during the preliminary test.

Table 1. Bioreactor temperature profile during the preliminary test

No	Bioreactor temperature (°C)	Heating state
1	29.9	ON
2	30.0	ON
3	31.5	ON
4	33.0	ON
5	34.6	ON
6	35.0	ON
7	35.1	OFF
8	36.0	OFF
9	25.0	ON
10	24.9	OFF

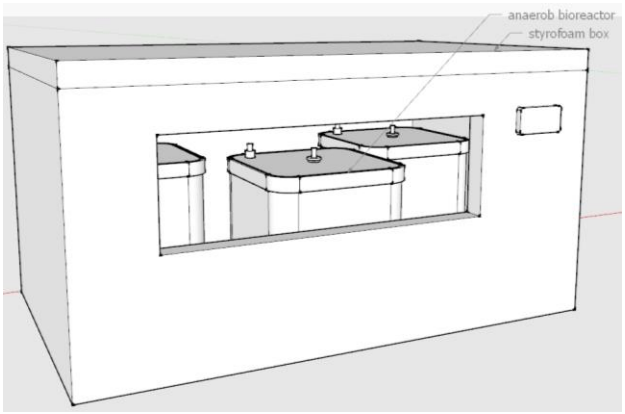

Figure 2. Submerged bioreactor

Figure 3. Simple bioreactor

Fermentation process

The incorporation of backslopping cultures initiated the fermentation process within the bioreactor. LAB were considered to play a significant role in the digestive tract; they are microorganisms frequently present in the digestive and genitourinary tracts of humans and animals. The total LAB count is shown in Figure 5.

Figure 4 shows that the fermentation time and backslopping culture proportion produced different total LAB counts. Sample C3 (24 hours fermentation; 5% backslopping culture) yielded total LAB very similar to C12 (12 hours fermentation; 5% backslopping culture) sample, namely 8.53 and 8.64 log cfu/g, respectively. In other samples, the LAB count ranged from 7.44 to 7.65 log cfu/g. The quantity of LAB presented in each sample also influenced the final pH product in our study (additional final pH product is available in supplementary Figure 5). It is shows the pH levels of the fermented coffee beans corresponding to each independent variable. Samples C3 and C12 had the lowest pH values of 4.1 and 4.08, respectively.

Cupping test

The ANOVA results showed no significant differences between the samples ($p>0.05$), so it was concluded that all combinations of fermentation duration and backslopping culture concentration did not influence the final cupping test scores (ANOVA is available in supplementary Table 4). However, the actual data, as shown in Figure 5, shows that C12 (a combination of 12-hour fermentation and 5% backslopping culture) attained the highest final score (80) in the cupping test, while the authentic *luwak* coffee (control) scored just 73.25.

Isolation and identification of LAB species

The C12 sample contained four gram-positive bacteria with rod or cocci morphology and the catalase test was negative (Table 2).

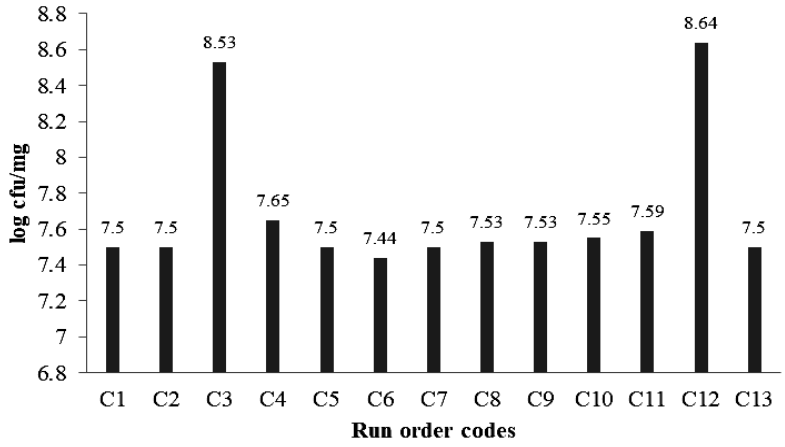

Figure 4. Total LAB count in the fermented coffee beans

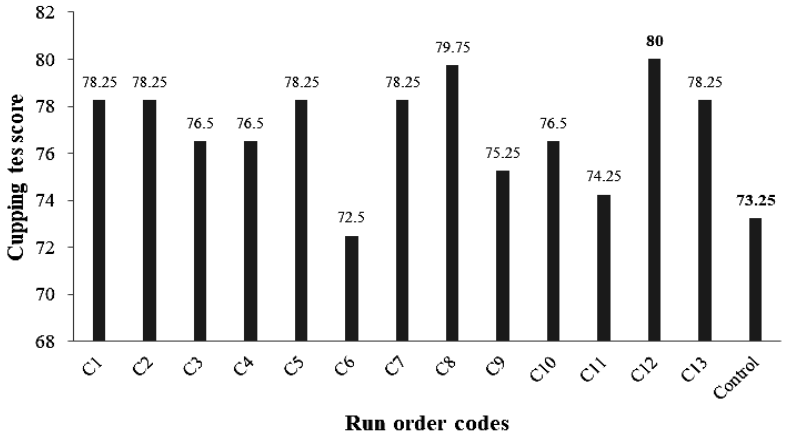

Figure 5. Cupping test final scores

All colonies exhibited a positive reaction in the Gram staining procedure. The identification of LAB isolates was conducted through phenotypic analysis, focusing on their capacity to ferment different sugars, using the API® 50 CHL kit. The results of the identification are presented in Table 3. The phenotypic identification results, represented by fermentation profiles of LAB isolates across different carbohydrates, validated the earlier genotypic identification results, achieving an accuracy level exceeding 96%.

Discussion

As shown in Table 1, the bioreactor is functioning properly and producing the desired results. The installed heating system and calibrated digital temperature sensors ensured continuous temperature stability during the fermentation process with a tolerance for temperature fluctuations not exceeding ±1°C. The important component of the temperature control system was the negative temperature coefficient (NTC) sensor, a thermistor in which resistance falls with rising temperatures. In the fermentation chamber, the NTC sensor operates as a live-time temperature measurement tool in the bioreactor. The system first reads the real temperature inside the bioreactor with the NTC sensor. This resistance value is then converted by the digital thermostat to temperature data. This bioreactor is designed to regulate the temperature between 25-35°C, which replicated civets' body temperature. According to Silva et al. (2024), the ideal temperature for coffee fermentation is typically between 25 and 30°C where maximum metabolism of the microorganisms involved in aerobic and anaerobic fermentation, such as bacteria and yeast occurs.

During fermentation process, the LAB count in the fermented samples showed that the quantity of back-slopping culture introduced influenced final LAB concentration. These results were similar to those of previous studies, which have shown, for example, that the level of LAB in the wild Asian palm civet's stomach, small intestine, and colon was 7.1, 8.4, and 8.6 log cfu/mL, respectively (Muzaifa et al. 2019b).

Similar activity has been noted in several fermented product matrices, including yogurt (Santoso 2019). The addition of backslopping culture to ready-to-ferment coffee beans generated biochemical changes in the beans. LAB fermented the mucilaginous carbohydrates in coffee beans, resulting in the production of lactic acid and several volatile aromatic chemicals. The mucilage encasing coffee beans is abundant in sugars, chiefly sucrose, glucose and fructose. During fermentation, microorganisms, including bacteria and yeasts, can metabolize sugar, transforming it into diverse products such as acids, alcohols and other chemicals, in a metabolic process crucial for the creation of coffee's flavor profile (Silva et al. 2024). Previous studies have shown that the ideal microbial metabolites created during fermentation were thought to permeate coffee seeds, influencing the flavor, fragrance and sensory quality as well as the acidity and body of the final product (Pereira et al. 2016; Elhalis 2023).

The pH value was directly correlated with the quantity of LAB in each sample. It is shows the pH levels of the fermented coffee beans corresponding to each independent variable. Samples C3 and C12 had the lowest pH values of 4.1 and 4.08, respectively. Furthermore, the concentration of LAB in both samples exceeded that of the others, leading to a greater accumulation of organic acids during fermentation, and thus resulting in a lower pH. The pH value was consistent with previous results, when LAB culture was used in artisanal bioreactors for coffee fermentation to produce specialty coffee (Reyes et al. 2024). Acetic and lactic acid have been shown to be the primary and most prevalent organic acids produced during coffee fermentation, responsible for the reduction in pH (Cassimiro et al. 2023).

Cupping is a standardized tasting method used to assess the quality and characteristics of coffee beans. This process allows coffee experts to compare coffees side by side and determine their scores. Figure 5 show the cupping test score from each samples. The final artificial *luwak* coffee score was derived from the aggregate of scent, flavor, aftertaste, salt/acid, bitter/sweet, body, uniformity of cups, balance, cleanliness of cups, and overall scores (Specialty Coffee Association 2021). Sample C12, which have 12 hours of fermentation and the addition of 5% backslopping culture, had the highest cupping test score. It is classified as specialty coffee, surpassing the final score of the control. The product possessed characteristics of brown sugar, a spicy chili-like flavor, and astringent aftertaste.

Table 2. Morphological characteristics of LAB isolates

Isolates code	Cell shape	Gram staining	Catalase test
C121	Bacilli	+	-
C122	Coccus	+	-
C123	Coccus	+	-
C124	Coccus	+	-
C125	Bacilli	+	-

Note: +: Presence, -: Absence

Table 3. Analysis profile index (API) of LAB in C12 coffee samples

Isolates code	Database accuracy level (%)	Identified species
C121	99.9 excellent identification	*Lactobacillus plantarum*
C122	99.8 very good identification	*Lactococcus lactis* subsp. *lactis*
C123	96.7 good identification	*Pediococcus* spp.
C124	99.9 excellent identification	*Lactococcus lactis* subsp. *lactis*
C125	99.9 excellent identification	*Lactobacillus plantarum*

The higher cupping scores of artificial *luwak* coffee compared to authentic *luwak* coffee indicate that backslopping acts as a bridge between fully spontaneous fermentation and pure culture fermentation by facilitating successful microbial consortium replication, while maintaining the benefits of natural microbial diversity. Backslopping also helps stabilize the microbial population, maintaining beneficial species and metabolic capabilities across batches, together with kombucha fermentation (Liao et al. 2024). Previous studies showed that the final *luwak* coffee, score both in vitro and in vivo, varied between 84.47 and 84.75 (Hadipernata and Nugraha 2018; Patria et al. 2018; Muzaifa et al. 2019b). Despite not using the final cupping test score variable, the results of Kang et al. (2023) showed that Luwak coffee fermented in vitro with an enzyme mixture of yeast and LAB had nearly identical sensory qualities to the original coffee. The myriad chemical compounds contributed significantly to the flavor and aroma of coffee.

All attributes in the highest final score are considered several metabolic reactions of LAB that occur during the fermentation process. Previous studies identified the microbial strains in palm civet biomass that facilitate caffeine catabolism in coffee berries through N-demethylation and xanthine oxidation. Similar results were also obtained in coffee bean wet fermentation with *L. plantarum*, which showed a caffeine decrease during the 12-hour fermentation (Swasti et al. 2024). Amino acid residues from fermentation have also been shown to enhance the sensory characteristics of coffee and improved the scent resulting from the maillard reaction during roasting (Fitri et al. 2021). In their study, valine, glutamine, sspartic acid, alanine, arginine, and glutamic acid were regarded as the predominant amino acids present in *luwak* coffee, with the level of glutamic acid the highest (Muzaifa et al. 2019a; Fitri et al. 2021). This unique aroma profile of *luwak* coffee was believed to be generated by guaiacol, pyrazine, and furan derivatives. Additional metabolites that impact the aroma profile of *luwak* coffee have been demonstrated to include trace amounts of alkaloid compounds such as caffeine, trigonelline, and santin, along with kahweol and difurfuryl ether (Farag et al. 2023). The authentic roasted *luwak* coffee beans contained higher levels of acetic acid, lipid, trigonelline, quinic acid, citric acid, and malic acid than those of regular coffee. The most discriminant metabolites in the caged *luwak* coffee were lipid and acetic acid (Febrina et al. 2023).

Table 2 is the morphological properties of colonies successfully isolated from sample C12. The binding of crystal violet dye to the peptidoglycan layer in gram-positive bacteria is a feature of this phenomenon. Gram-positive bacteria possess thick cell walls, primarily consisting of 50 to 90% peptidoglycan, which retain crystal violet effectively during Gram staining (Tripathi et al. 2024). Furthermore, gram staining enhanced the clarity of cell shape observation. All isolates were identified as either rod-shaped or cocci and exhibited a negative reaction to the catalase test. No bubble formation was observed after the cell reacted with a few drops of hydrogen peroxide (H_2O_2). LAB could not produce catalase, which catalyzes the conversion of hydrogen peroxide (H_2O_2) into water and oxygen (Vos et al. 2009). Consequently, LAB was classified as belonging to the group of anaerobic or facultative anaerobes that did not require oxygen for growth under acidic conditions.

The identification outcomes using the API® 50 HCL kit showed an accuracy of over 99%, except for isolate C123 (Table 3). The fermentation profile exhibited by each isolate corroborated the results of genotypic identification derived from the nucleotide sequence of the 16S rRNA gene. Each species identified based on its base sequence had a fermentation profile that could fit the biochemical features of the species. The LAB in sample C12 that were successfully isolated and identified included *Lactococcus lactis* subsp. *lactis*, *L. plantarum*, and *Pediococcus* spp. A study conducted by Muzaifa et al. (2019) showed that *L. brevis, Lactobacillus fructivorans, Pediococcus pentasaceus,* and *Lactococcus lactis* spp. *lactis* were the predominant LAB in the digestive tract of wild civets. Other studies have isolated *L. plantarum, L. brevis, Leuconostoc paramesenteroides, Leuconostoc mesenteroides*, and *Streptococcus faecium* from the feces of the Jember local civet (Munandar et al. 2022). In Peru, *misha* coffee is a type of coffee comparable to civet coffee. The product is derived from the digestive processes of ring-tailed coati (*Nasua nasua*) animals. The study conducted by Pinillos-Miñano et al. (2022) successfully isolated various LAB species from the feces of ring-tailed coatis, including *Leuconostoc lactis, Leuconostoc citreum, Lactobacillus pentosus, Lactobacillus vaccinostercus, L. plantarum, L. brevis,* and *Pediococcus pentosaceus*. The API 50 CH system has been widely applied due to its convenience and ability to characterize isolates based on carbohydrate fermentation patterns. These methods are biochemical in nature and rely on the differential metabolism of a broad set of carbohydrates to generate a profile intended as a species or subspecies fingerprint. However, such methods are often limited by sample variability, environmental influences, and strain-specific metabolic diversity. Many researchers have combined classical morphological and biochemical analyses (via API 50 CHL) with molecular genetic methods, including RAPD, ARDRA, 16S rDNA sequencing, and species-specific PCR assays. This multi-tiered strategy has facilitated precise delineation of closely related species, thus overcoming limitations faced by either method conducted separately (Urshev et al. 2024).

In conclusion, the research has successfully developed a simple bioreactor prototype that *luwak* coffee artisans can use at the farm level, thereby minimizing the industrialization of *luwak* animals. The bioreactor is designed to be scaled up for larger-scale commercial use. The use of a 5% backslopping culture and 12-hour fermenting process yielded specialty-grade coffee. The identified LAB isolates included *Lactococcus lactis* subsp. *lactis*, *L. plantarum*, and *Pediococcus* spp. This result also have the potential to be scaled up for the in vitro production process of *luwak* coffee. Due to limited funds, more comprehensive analyses are needed to verify research

results, such as amino acid profiles, flavor components, and PCR to identify lactic acid bacteria species.

ACKNOWLEDGEMENTS

This study was funded by DIPA of Politeknik Negeri Lampung, Indonesia in 2023, with contract number 208.70/PL.15.8/PP/2023.

REFERENCES

Cassimiro DMJ, Batista NN, Fonseca HC, Naves JAO, Coelho JM, Bernardes PC, Dias DR, Schwan RF. 2023. Wet fermentation of Coffea canephora by lactic acid bacteria and yeasts using the self-induced anaerobic fermentation (SIAF) method enhances the coffee quality. Food Microbiol 110: 104161. DOI: 10.1016/j.fm.2022.104161.

Dorta C, Pardo RB, Martins AN, Otoboni AMMB, Oshiiwa M, Shigematsu E, Favoni SP, Machado SMF, Giannoni JA, Marinelli PS, Tanaka AY. 2024. Addition of commercial lactic acid bacteria as starter cultures in anaerobic fermentations of *Coffea arabica* L., in cherry or raisin stages. Contribuciones a Las Ciencias Sociales 17 (10): 1-16. DOI: 10.55905/revconv.17n.10-390.

Elhalis H, Cox J, Zhao J. 2023. Coffee fermentation : Expedition from traditional to controlled process and perspectives for industrialization. Appl Food Res 3 (1): 100253. DOI: 10.1016/j.afres.2022.100253.

Farag MA, Tarik AM, Enas AEl, Amr A. 2023. Metabolite profiling of premium civet luwak bio-transformed coffee compared with conventional coffee types, as analyzed using chemometric tools. Metabolites 13 (2): 173. DOI: 10.3390/metabo13020173.

Fauzi M, Subagio A, Restanto DP, Jayus J. 2023. Identification of lactic acid bacteria isolated from developed dried coffee starter culture used as a fermentation agent to produce Robusta civet coffee. Biodiversitas 24 (7): 3715-2722. DOI: 10.13057/biodiv/d240708.

Febrina L, Happyana N, Syah YM. 2023. Metabolic profiling, antioxidant activity, and alpha-glucosidase inhibitory activity of the roasted beans of luwak (civet) coffee. ACS Food Sci Technol 3 (11): 1864-1876. DOI: 10.1021/acsfoodscitech.3c00249.

Fitri, Tawali AB, Laga A. 2019. Luwak coffee in vitro fermentation : literature review. IOP Conf Ser Earth Environ Sci 230 (1): 1-6. DOI: 10.1088/1755-1315/230/1/012096.

Fitri, Laga A, Dwyana Z, Tawali AB. 2021. Composition of amino acids and fatty acids on luwak coffee processing. Food Res 5 (3): 60-64. DOI: 10.26656/fr.2017.5(3).637.

Hadipernata M, Nugraha S. 2018. Process technology of luwak coffee through bioreactor utilization. IOP Conf Ser: Earth Environ Sci 101 (1): 012092. DOI: 10.1088/1755-1315/102/1/012092.

Kang HM, Shin YO, Hye MN, Joong HK, Yoon J. 2023. Quality characteristics of in vitro luwak coffee produced using enzyme and microbial complexes. Korean J Food Preserv 30 (2): 287-299. DOI: 10.11002/KJFP.2022.30.2.287.

Liao T, Li XR, Fan L, Zhang B, Zheng WM, Hua JJ, Li L, Mahror N, Cheng LH. 2024. Nature of back slopping kombucha fermentation process: Insights from the microbial succession, metabolites composition changes and their correlations. Front Microbiol 15: 1433127. DOI: 10.3389/fmicb.2024.1433127.

Munandar K, Afriayanti D, karimah I. 2023. Isolation and characteristics of lactic acid bacteria in feces of jember local mongoose. Intl Appl Sci 1 (1): 43-47. DOI: 10.32528/ias.v1i1.46.

Muzaifa M, Hasni D, Yunita D, Febriani, Patria A, Abubakar A. 2019a. Amino acid and sensory profile of kopi luwak (civet coffee). IOP Conf Ser: Mater Sci Eng 523 (1): 012028. DOI: 10.1088/1757-899X/523/1/012028.

Muzaifa M, Hasni D, Patria A, Febriani, Abubakar A. 2019b. Phenotypic identification of lactic acid bacteria from civet (*Paradoxorus hermaphroditus*). Intl J Adv Sci Eng Inf Technol 9 (5): 1681-1686. DOI: 10.18517/ijaseit.9.5.10222.

Patria A, Abubakar A, Febriana, Muzaifa M. 2018. Physicochemical and sensory characteristics of luwak coffee from Bener Meriah, Aceh-Indonesia. IOP Conf Ser: Earth Environ Sci 196 (1): 012010. DOI: 10.1088/1755-1315/196/1/012010.

Pereira GV de M, Neto DP de C, Medeiros ABP, Soccol VT, Neto E, Woichiechowski AL, Soccol CR. 2016. Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing. Intl J Food Sci Techol 51 (7): 1689-1695. DOI: 10.1111/ijfs.13142.

Pinillos-Miñano RM, Rodriguez-Portilla LM, Hatta-Sakoda BA. 2022. Isolation of lactic acid bacteria from the feces of ring-tailed coati (*Nasua nasua*), biochemical and fermentative aspects related to coffee fermentation. Appl Biochem Microbiol 58: S102-S112. DOI: 10.1134/S0003683822100180.

Reyes EIM, Gonzales MAB, Balcazar JOC, Velasco JS. 2024. Controlled fermentation in artisanal bioreactors to produce specialty coffees. Ciência e Agrotecnologia 48: e007524. DOI: 10.1590/1413-7054202448007524.

Rivera AMP, Silva JL, Torres-Valenzuela LS, Dorado JLP. 2024. development of starter inoculum for controlled arabica coffee fermentation using coffee by-products (pulp and mucilage broth), yeast, and lactic acid bacteria. Fermentation 10: 516. DOI: 10.3390/fermentation10100516.

Santoso N. 2019. Pengaruh jumlah inokulum terhadap waktu fermentasi pada pembuatan yoghurt dari susu sapi. Prosiding SNTK Eco-SMART 2018: 129-135. [Indonesian]

Silva LCF, Pereira PVR, da Cruz MAD, Costa GXR, Rocha RAR, Bertarini PLL, do Amaral LR, Gomes MS, Santos LD. 2024. Enhancing sensory quality of coffee: The impact of fermentation techniques on *Coffea arabica* cv. Catiguá MG2. Foods 13 (5): 653. DOI: 10.3390/foods13050653.

Specialty Coffee Association. 2021. Specialty Coffee Association Arabica Cupping Form. https://www.scith.coffee/wp-content/uploads/2021/03/SCA-Cupping-Form.pdf.

Swasti YR, Leong LAIP, Purwijantiningsih E, Pranata FS. 2024. The effects of *Lactobacillus plantarum* addition to robusta coffee (*Coffea canephora*) during wet fermentation. Biodiversitas 25 (9): 3132-3140. DOI: 10.13057/biodiv/d250935.

Suhandoyo S, Setiadi H, Kristianti T, Kusuma AB, Wedaringtyas AW, Djajadi DT, Artantha INP. 2016. Diversity of culturable bacterial in various parts of luwak's (*Paradoxurus hermaprodithus javanica*) gastrointestinal tract. Microbiol Indones 10 (2): 65-70. DOI: 10.5454/mi.10.2.4.

Tatin IAG. 2024. Eco-CDA and counter-discourse: From exotic luxury to nonhuman animal exploitation in civet coffee. Intl J Hum Stud 7 (2): 327-340. DOI: 10.24071/ijhs.v7i2.6592.

Tripathi N, Zubair M, Sapra A. 2024. Gram staining. StatPearls. Treasure Island (FL): StatPearls Publishing: 4-7. Available at: https://www.ncbi.nlm.nih.gov/books/NBK562156/.

Urshev Z, Doynova D, Prasev I, Denkova-Kostova R, Koleva A, Denkova Z, Goranov B, Kostov G. 2024. Identification of lactic acid bacteria strains isolated from sourdoughs prepared with different flour types. Appl Sci 14 (5): 2093. DOI: 10.3390/app14052093.

Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB. DH. 2009. Bergey's manual of systematic bacteriology - Vol 3: The Firmicutes, Springer-Verlag, New York. DOI: 10.1007/978-0-387-68489-5.

Wang CH, Sun JC, Lassabliere B, Yub B, Liu SQ. 2020. Coffee flavour modification through controlled fermentations of green coffee beans by Saccharomyces cerevisiae and Pichia kluyveri: Part I. Effects from individual yeasts. Food Res Intl 136: 109588. DOI:10.1016/j.foodres.2020.109588.

Wirawati CU, Widodo YR. 2021. Assessment of antimicrobial and proteolytic activity of lactic acid bacteria isolated from dadih: Naturally fermented buffalo milk from West Sumatra, Indonesia. Jurnal Ilmiah Peternakan Terpadu 9 (3): 346-361. DOI: 10.23960/jipt. v9i3.p346-361.

Wirawati CU, Sudarwanto MB, Lukman DW, Wientarsih I, Srihanto EA. 2019. Diversity of lactic acid bacteria in dadih produced by either back-slopping or spontaneous fermentation from two different regions of West Sumatra, Indonesia. Vet World 12: 823-829. DOI: 10.14202/vetworld.2019.823-829.