

Production of dry culture from *luwak*-isolated *Lacticaseibacillus paracasei* using soy-based flour: its viability and activity

Mukhammad Fauzi^{1*}, Winda Almira Prihandini², Jayus¹, Asmak Afriliana¹,
Soni Sisbudi Harsono³, Abi Bakri⁴, Gregorio Quintao⁵

¹Jember University, Department of agricultural product technology, Jember, East Java, Indonesia

²Department of Head Quality System, PT Torabika Eka Semesta (Mayora Group), Jakarta, Indonesia

³University of Jember, Department of Agricultural Engineering, Jember, East Java, Indonesia

⁴Jember Polytechnic, Department of Agricultural Technology, Jember, East Java, Indonesia

⁵National Directorate of Agricultural Research, Ministry of Agriculture, Livestock, Fisheries, and Forestry, Dili, Timor Leste

*Corresponding author: fauziah@yahoo.com

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ABSTRACT

Luwak coffee is a unique coffee produced by the civet digestive system. Its rarity is a key characteristic, but this can be overcome by producing artificial *luwak* coffee using a dried culture of an isolate of *Lacticaseibacillus paracasei*, whose physiological properties have not been studied. This study aims to assess the physiology of the isolate and to characterize the *luwak* coffee-dry culture (LCDC), which various amounts of soy-based flour, including soybean, tempeh, and soybean sprouts, as a Nitrogen source. The stages include a study of the physiology of the isolate, and the addition of soy-based flour to the starter growth medium. The findings indicate that isolate cells are bacillus-shaped, gram-positive, facultative anaerobic, and homo-fermentative. They can grow at temperatures of 15 °C, 37 °C, and 45 °C, as well as at salt concentrations of 4 % and 6.5 %. LCDC supplemented with 10 g of tempeh flour showed promising results, with the highest viability ($9.45 \log_{10}$ CFU/g) and fermentation power, and a water content of less than 8%. As for fermentation capacity, the glucose fermentation capacity is $109.17 \text{ mg glucose (hg)}^{-1}$ of LCDC, the total titratable acid is $5.11 \text{ mg LA (hg)}^{-1}$, and the pH value is 4.30. Robusta *luwak* coffee, a specialty coffee, is produced by fermenting LCDC for 18 hours, achieving the highest total score of 81.38. The flavor profile is described as chocolate-like, with a hint of brown sugar, a spicy aftertaste, a sweet aftertaste, a hint of rubber, chocolate fruit, and a hint of medicine.

Key words: Specialty coffee; flavor profile; *luwak* coffee-dry culture; nitrogen sources; fermentation capacity.

1 INTRODUCTION

Indonesia is known for producing three main types of coffee: robusta coffee (*Coffea canephora*), arabica coffee (*Coffea arabica* L.), and liberica coffee (*Coffea liberica* var.). In addition to these, specialty coffee has gained significant popularity in the global market, with *luwak* coffee being one of the most renowned varieties. *Luwak* coffee is made from beans that have been consumed by civets (*Paradoxurus hermaphroditus*) and then excreted. This coffee, often referred to as “cat poop coffee,” can fetch extraordinarily high prices, reaching up to \$600 per pound (Febrianto & Zhu, 2023; Lamoureux, 2022). It has been reported that *luwak* coffee has a different caffeine content compared to other processed or unprocessed coffees (Yulia et al., 2016).

The coffee fruit undergoes a natural fermentation process as it passes through the civet’s digestive system. This fermentation alters the chemical composition of the coffee beans, thus enhancing the flavor of the final product (Adadi et al., 2024). *Luwak* coffee exhibits several distinctive characteristics, including lower caffeine content, reduced acidity, low fat, and diminished bitterness (Ifmalinda et al., 2019).

The demand for civet coffee often exceeds its supply due to the limited number of civets capable of producing it (Lewis-Whelan et al., 2022). One proposed solution to this

scarcity is the development of artificial civet coffee produced by fermenting coffee beans with a dried civet-coffee culture containing microflora derived from civet feces, thereby mimicking the metabolic processes occurring in the civet’s digestive tract (Muzaifa et al., 2016). Previous studies have identified bacteria such as *Corynebacterium genitalium*, *Kytococcus sedentarius*, *Streptococcus sanguinis*, and *Streptococcus faecium* in civet feces (Putrama et al., 2022). Fauzi et al. (2023) further identified five lactic acid bacteria (LAB) isolates (A–E) from fresh civet-coffee beans in fecal form, all classified as *Lactobacillus paracasei*. However, the physiological characterization of these isolates has not yet been conducted. These five isolates hold potential as fermentation agents for producing artificial civet coffee.

In recent years, *Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*) has been reported as an isolate from artisanal fermented beverages with health-promoting properties (Bengoa et al., 2021). Others reports of Hill et al. (2018), Bengoa et al., 2019, and Patterson et al. (2020) also state that *Lacticaseibacillus paracasei* is one of the species isolated from both artisanal fermented beverage starters. Although not the dominant species in kefir or water kefir, this species may contribute to improved health status. This species is closely related to, among others, *Lactobacillus casei*, *L. paracasei*, and *L. rhamnosus*, with a long history of safe use

in food and agricultural applications that have been studied for their health-promoting properties. *Lacticaseibacillus paracasei* is an important species in the production of yogurt, cheese, and other fermented milk products. This species not only improves the taste and texture of these products but also provides various health benefits to consumers (Wu et al., 2025). Previous studies reported that different strains within the species express different functional genes, resulting in unique metabolite profiles and functions in fermented milk (Torres-Miranda et al., 2022; Colautti et al., 2024).

In the production of *luwak* coffee-dry culture, various fillers can be utilized; this study specifically focused on rice flour. Rice flour is advantageous as it meets the nutritional needs of microbes, containing a high carbohydrate content specifically, 100g of white rice holds 85.06-90.16g of carbohydrates (Fitriyah et al., 2020). Fauzi et al. (2023) reported that the sugar fermentation capacity of dried civet coffee culture made from 100% rice flour inoculated with *L. paracasei* was 249.94 mg glucose (gh)⁻¹ (dry basis).

The fermentation process requires adequate carbon and nitrogen sources, which can be provided by incorporating different types of flour into the fermentation medium. *L. paracasei* is a group of lactic acid bacteria that requires a nitrogen source for its growth, such as proteose peptone which is found in MRS broth media at an amount of 10 g/L (Aryal, 2022). The original material of proteose peptone is soybeans. Remembering that it is made from the same material, namely soybeans, because this material has undergone a fermentation process (tempeh) or a biosynthesis process (soybean sprouts), it will have an impact on the availability of nitrogen elements needed by *L. paracasei*. This research explored the use of three types of flour: soybean flour, tempeh flour, and soybean sprout flour. According to Taufik et al. (2017), 100g of soybean flour contains 347 kcal of energy, 35.9g of protein, 20.6g of fat, and 8mg of iron. In contrast, 100g of tempeh flour provides 448.57 kcal of energy, 46.19g of protein, 22.64g of fat, and 5.77g of fiber (Astawan et al., 2016). Meanwhile, 100g of soybean sprout flour contains 33.22g of protein (Sholihah et al., 2017), 20.79g of fat, and 40.84g of carbohydrate (Hartoyo & Sunandar, 2006).

Given these findings, further research is needed to identify the physiological properties of the five *L. paracasei* isolates including gram stain, catalase test, growth at different temperatures, CO₂ test, and growth at various salt concentrations. Additionally, it is important to determine the characteristics of *luwak* coffee-dry culture inoculated with starter cultures enriched with soybean flour, tempeh flour, and soybean sprout flour as nitrogen sources. These characteristics should include water content, total microflora content, and fermentation power. Furthermore, the potential of these *luwak* coffee-dry culture will be studied as fermentation agents for Robusta coffee beans in the production of artificial civet coffee.

2 MATERIAL AND METHODS

The materials were used in the production of *luwak* coffee-dry culture included *L. paracasei* (isolate A, B, C, D and E). Meanwhile, the chemicals for pro analysis specifications (>99%) from merck-product included: glucose, phenolphthalein indicator, NaOH, buffer solution (pH 4 and 7), oxalic acid, MRSA media (deMan Rogosa Sharp Agar), and MRSB (deMan Rogosa Sharp Broth). The equipment used included containers, blenders, autoclaves, 60 mesh sieves, ovens (Memert), analytical scales (Toledo), measuring cups, beakers, screw test tubes, test tubes, incubators, hot plates, pH meters (Yana), weighing bottles, desiccators, Gluco-dr, measuring flasks, and burettes. In this study, there are two aspects studied, namely, first, the identification of the morphology and physiology of 5 lactic acid bacteria isolates, and second, studying the production of *luwak* coffee-dry culture based on the lactic acid bacteria isolate (*L. paracasei*). The study of *luwak* coffee-dry culture, an experiment was carried out, namely with nine combinations of treatment comparisons between variations in types of flour (A: soybean flour, tempeh flour, and sprouts flour) and flour concentration (B: 7 g, 10 g, and 13 g) in 200 mL of liquid media as a starter culture. Each treatment was repeated three times. Meanwhile, for the trial production of artificial civet coffee, Robusta coffee beans were used as a control treatment for natural civet coffee of Robusta and dry-processed Robusta coffee.

2.1 Characterization of morphological and physiological identification of lactic acid bacteria isolate (*Lacticaseibacillus paracasei*)

The isolates obtained from research results of Fauzi et al. (2023). There were 5 lactic acid bacteria (LAB) isolates identified with codes A, B, C, D, and E. They were streaked again on MRSA agar slants according to requirements for morphological and physiological analysis.

- Gram stain

One loop of needle of lactic acid bacteria isolates is scratched on the glass object, then fixed using one drop of distilled water, then air-dried by passing it over low heat until a stain forms, which is pressed with crystal violet dye, Lugol's solution, ethanol 95%, and safranin. The next step was to dry it and cover it with glass, then observe it under a microscope using magnification 1000x. If the color appeared red, then the lactic acid bacteria were gram-negative bacteria, whereas if the color formed was purple, then the lactic acid bacteria were gram-positive bacteria.

- Catalase test

One needle loop of lactic acid bacteria culture is applied to a glass slide, then above it is dripped with H₂O₂ 3% and let

stand for ± 1 minute. If on a glass object, there were bubbles, then it was categorized as catalase positive. On the contrary, if there were no bubbles, then categorized as catalase negative.

- Growth at different temperatures

One loop of lactic acid bacteria culture was inoculated into a test tube containing 1- 1.5 mL of sterile MRS broth. The reaction tube was incubated in an incubator for 48 hours at varying temperatures of 15 °C (refrigerator), 37 °C, and 45 °C. The growth of lactic acid bacteria was observed.

- CO₂ test

A total of 1 mL of lactic acid bacteria culture was inoculated into sterile MRS broth with a Durham tube in a test tube. The test tube was incubated at 37°C for 48 hours. The growth of lactic acid bacteria culture was indicated by the formation of air bubbles in the Durham tube.

- Growth at different salt concentrations

A total of one loop of lactic acid bacteria was inoculated into various sterile MRS broth media, with different concentrations of NaCl added, including 4% NaCl, 6.5% NaCl, and a control without any salt. The tubes were incubated at 37 °C for 48 hours. The growth of lactic acid bacteria was observed.

2.2 Production of luwak coffee-dry culture

- Preparation of soy-based-flour

a. Making soybean flour

Sorting 500 g of soybean flour to get good and clean soybeans. Sorted soybeans are washed until clean and then soaked in water (soybean:water ratio of 1:3) for 8 hours. Soaked soybeans was peeled to remove the epidermis. The soybeans were washed again to remove remaining epidermis. The cleaned soybeans were steamed for 60 minutes and then dried using an oven at 60 °C for 48 h. The dried soybeans were ground using a blender and sifted using a 60mesh sieve until they become soybean flour (Rahmawati et al., 2020).

b. Making soybean sprout flour

Soybean germination was done by sorting 500 g of soybeans to obtain clean soybeans. The sorted soybeans were soaked in water for 8 hours and drained. The soybeans were put into a container with holes and covered with tissue or a dry cloth to protect them from sunlight. The soybean germination process was carried out at room temperature for 28 h and watered with water every 3 h. The Sprout was dried using an oven at 60 °C for 48 h. The dried sprout was ground using a blender and sifted using a 60 mesh sieve until they become soybean sprout flour (Astawan et al., 2016).

c. Making tempeh flour

The traditional way of making tempeh is that 500 g of soybeans was sorted and washed then soaked for 12 h using

± 750 mL of boiling water. The soybeans were washed again using water until the soybean skin peels off. The soybeans were steamed for 30 mins until soft. The soybeans were poured into a clean, dry and airy container, then 5 g of tempeh inoculant was added and stirred until evenly distributed. The mixed soybeans were packaged in plastic packaging with holes to facilitate the aeration process then stored on a flat surface and incubated for 24 h (Alvina et al., 2019). The tempeh was cut into thin slices of ± 0.5 -1.0 mm and then dried using an oven at 80 °C for 24 h. The dried tempeh was ground using a blender and then sifted using 60 mesh sieve until it became tempeh flour (Mustakim et al., 2016).

- Preparation of filler

Making as much rice flour as possible 2000 g were washed until clean of dirt, then the rice is put into a container and soaked for 6 h. The soaked rice is drained and ground using a hammer mill machine to make flour. Rice flour is dried in the sun until dry and then stored in a dry container.

- Production stages of luwak coffee-dry culture

a. Preparation of inoculum from lactic acid bacteria isolate

The isolates are multiplied on MRSA slant agar media (de Man Rogosa Sharpe Agar) and incubated at 37 °C for 24 to 48 h. A selected LAB isolate is then transferred into 10 mL of sterile MRS broth and incubated at 37 °C for another 24 to 48 h as the initial culture. Second, sterile liquid media are prepared by heating 200 mL of distilled water on a hot plate until it reaches a boil. Various types and concentrations of flour, along with 3% sucrose, are added to the boiling water. The mixture is homogenized using a magnetic stirrer until no lumps remain. The media is then divided into two portions: 20 mL and 180 mL, which are sterilized using an autoclave at a temperature of 121.1 °C for 30 minutes. Finally, after the sterile media has cooled, the initial culture is inoculated into the 20 mL portion of sterile media and incubated at 37 °C for 24 to 48 hours. Subsequently, this culture is further inoculated into the 180 mL portion of sterile media and incubated again at 37 °C for 24 to 48 h. The resulting culture, known as a starter, serves as the inoculant in the production of *luwak* coffee-dry culture (Fauzi et al., 2017).

b. Production of luwak coffee-dry culture

To create *luwak* coffee-dry culture, start by placing 30 g of rice flour into a plastic bag. Then, transfer the bag into a glass beaker and cover it with aluminum foil to sterilize it at 121°C for 15 minutes. Next, add 13 mL of a starter made from a mixture of various types of flour and concentrations into a plastic container with sterile rice flour. Homogenize the flour and starter by thoroughly mixing and then incubate the mixture at 37 °C for 24 to 48 h. Finally, dry the mixture using a vacuum oven at 45 °C for approximately 48 h let the starter culture become dry slowly (Fauzi et al., 2023).

- Characterization of produced *luwak* coffee-dry culture

a. Microbial viability test of *luwak* coffee-dry culture

Viability testing was conducted by suspending 1 g of *luwak* coffee-dry culture in a concentration of 10^{-8} . A total of 1 mL of this suspension was inoculated into a sterile dish containing liquid MRSA media (Safrida, 2021). The suspension in the petri dish was mixed thoroughly and then incubated at 37 °C for 24 to 48 h. The colonies that developed on the petri dish were counted, following the criteria of 25 to 250 colonies per dish. The total number of colonies was calculated using Equation 1 (BSNI, 2009).

$$N = \sum \text{colony in dish} \times \frac{1}{D} \quad (1)$$

Description:

N = number of colony (cfu/g)

D = dilution factor

b. Water content

The determination of air content is conducted using the gravimetric method. Initially, a sample weighing between 1 and 2 grams is placed in a previously weighed, dry weighing bottle. The sample is then dried in an oven at a temperature of 100 to 105 °C for 3 to 5 h. After drying, the sample is allowed to cool in a desiccator before being weighed again. Next, the dry sample is placed back in the oven for an additional 30 to 60 minutes and then returned to the desiccator for cooling. This weighing process is repeated until a constant weight of the dry sample is achieved. Finally, the water content is calculated using the appropriate Equation 2 (Subagio et al., 2004).

$$\text{Water content (\%db)} = \frac{\text{initial weight} - \text{final weight}}{\text{final weight}} \times 100\% \quad (2)$$

c. Fermentation power of *luwak* coffee-dry culture test

In the previous fermentation power test, a 3% glucose solution was fermented with *luwak* coffee-dry culture. 10 mL of 3% (w/v) glucose solution was fermented with 0.1 g of *luwak* coffee-dry culture for 12 h, and then the pH value, total of titrated acid rate, and glucose fermentation power were measured.

i. pH of fermented media

To measure the pH of a 3% glucose fermentation solution, start by calibrating the pH meter using pH 7 and pH 4 buffer solutions. After the fermentation process has continued for 12 h, immerse the calibrated pH meter probe into the glucose solution. For accuracy, pH measurements were repeated three times (Association of Official Analytical Chemists -AOAC, 2005).

ii. Total titrated acids rate (TTAR) of fermented media

To measure the total titratable acidity of the sample that has been fermented for 12 h, it is started by taking 5

mL and diluting it in a 50 mL measuring flask. From this diluted solution, 5 mL is taken and transferred into an Erlenmeyer flask. Next, 2-3 drops of 1% phenolphthalein are added, and the solution is titrated with 0.01 N NaOH until a pink color appears. The total titratable acidity rate (TTAR) is calculated using Equation 3 (Sudarmadji et al., 2010).

$$\text{TTAR} \left(\frac{\text{mg LA}}{\text{g.h}} \right) = \frac{V \text{ NaOH} \cdot N \text{ NaOH} \cdot M \cdot D}{\text{sample weight} \cdot 12\text{h}} \quad (3)$$

Description:

V NaOH : volume (mL) of NaOH

N NaOH : concentration (Normality) of NaOH

D : dilution factor

M : molecular weight of lactic acid (LA) (90 g/mole)

iii. Glucose fermentation power

Measurement of glucose fermentation power was carried out using the electrochemical method by dissolving 0.1 g of *luwak* coffee-dry culture in 10mL of 3% glucose solution and homogenizing it, then incubating at 37 °C for 12 h. The sample was measured by dropping it on the GlucoDr strip attached to the glucometer and waiting until the sample glucose level value in mg/dL appears. Previous glucometer device calibrated using three different concentrations of anhydrous glucose, namely 75 mg/100 mL, 150 mg/100 mL, and 225 mg/100 mL. The results of these calibration measurements were made into a standard curve, the relationship between anhydrous glucose concentration versus the value displayed by GlucoDr and the regression equation is used as a guideline for calculating glucose levels and glucose fermentation power was calculated using the following Equation 4 (Fauzi et al., 2017).

$$\text{Glucose fermentation power} \left(\frac{\text{mg glucose}}{\text{h.g}} \right) = \frac{(3000 - c) \cdot 10.8}{\text{sample weight} \cdot 12\text{h} \cdot 100} \quad (4)$$

Description:

3000 : 3% glucose conversion where 3% = 3 g/100 mL = 3000 mg/100 mL

c : residual glucose concentration based on the curve of glucose standard

10.8 : final volume of glucose solution + 0.1g *luwak* coffee-dry culture (mL)

100 : conversion dL to mL

2.3 Trial of artificial *luwak* coffee fermentation utilizing *luwak* coffee-dry culture

The best characteristics of *luwak* coffee-dry culture is used for the fermentation process of artificial civet coffee for 18 h at a dose of 1 % by weight of coffee beans. Fresh robusta coffee cherries were removed from the fruit skin

with a pulper. 10 kg of wet coffee beans were added with 1 % of *luwak* coffee-dry culture and placed in a plastic bag and incubated at 37-39 °C for 18 h. The fermented coffee was washed thoroughly with running water and dried in the sun until the water content reaches approximately 12 %. Dry processed robusta coffee and natural robusta civet coffee, both as control samples for treatment and artificial civet coffee were measured for organoleptic characteristics using the analysis service of the Cocoa and Coffee Research Center, Indonesia. The data obtained were then analyzed (Fauzi et al., 2023).

2.4 Sensory test of grounded coffee

The coffee powder cupping test was conducted by three professional panelists from the Indonesian Coffee and Cocoa Research Center in Jember using the Lingle and Menon method (2017). 10 g of ground coffee in a bowl, added with 200 mL of boiling water. Sensory parameters will be assessed by several professional panelists covering the following attributes: Aroma as the character of the coffee taste smelled after 5 minutes of brewing; Flavor as a combination of taste on the tongue and the aroma of steam that permeates from the mouth to the nose; Aftertaste as the impression received after the coffee brew leaves the mouth; Acidity as a delicious and fresh fruity taste when the brew is inhaled; Sweetness as the sweet taste of coffee brewed carbohydrates; body/mouthfeel, the thick taste of coffee; uniform cup as the uniformity of aroma from each bowl; balance as the balance of taste, aftertaste, acidity, and body; clean cup, the clarity of off-flavors from the beginning to the aftertaste; overall as the overall sensory attributes displayed by the coffee brew (Specialty coffee association – SCA, 2022). The average value obtained from the assessment of several panelists was then categorized into the following levels: Average (5.00-5.75), Good (6.00-6.75), Very good (7.00-7.75), Excellent (8.00-8.75), Outstanding (9.00-9.75), and Specialty coffee (total score: ≥ 80). The results of the sensory evaluation of the samples was in the form of a LOA (letter of analysis)

2.5 Data analysis

The physiological and morphological characteristic data of LAB isolate obtained were processed and presented in graphs or tables, then analyzed descriptively. Meanwhile, data on the characteristics of *luwak* coffee-dry culture were analyzed for variance at a 95% confidence level, and the average was tested using the Duncan Multiple Range Test (DMRT) at a 95% confidence level, while the sensory evaluation data of ground robusta coffee from the trial using luwak coffee-dry culture were displayed in a spider graph using Microsoft Excel 2019 and then analyzed descriptively.

3 RESULTS

3.1 Morphology and physiology of isolated lactic acid bacteria (LAB)

The results gram staining, catalase and CO₂ test of the five isolates, namely A, B, C, D, and E isolate are presented in Table 1.

Table 1: Results of gram staining, catalase, and CO₂ production test from 5 LAB isolates.

Isolates	Cell shape	Gram type	Catalase test	CO ₂ test
A	Bacillus	+	Negative	Negative
B	Bacillus	+	Negative	Negative
C	Bacillus	+	Negative	Negative
D	Bacillus	+	Negative	Negative
E	Bacillus	+	Negative	Negative

Based on Table 1, it can be seen that the five isolates had a bacillus (rod) shape and gram-positive bacteria type, and which were shown in purple when observed under a microscope, as seen in Figure 1. Based on the catalase test results presented in Table 1 and Table 2, the five isolates were found to be negative for catalase, as indicated by the absence of gas bubbles in the preparations. Additionally, the results of the CO₂ test shown in the same table indicate that the five isolates would not produce CO₂ gas.

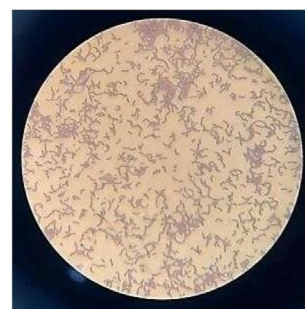


Figure 1: Example of gram staining microscopic identification results of 5 LAB isolate.

Table 2: Results of growth tests at different temperatures and salt concentrations on 5 LAB isolates.

Isolates	Temperature (°C)			Salt concentrations (%)		
	15	37	45	0	4	6.5
A	++	+++	+++	++	+++	+++
B	++	+++	+++	++	+++	+++
C	++	+++	+++	++	+++	+++
D	++	+++	+++	++	++	++
E	++	+++	+++	++	+++	+++

Notation +: Isolate grows (+: low grows; ++: medium; +++: high grows).

Based on Table 2, the five isolates will thrive at temperatures between 15°C and 45°C. Meanwhile, the growth of the five isolates (A, B, C, D, and E) tested at various salt concentrations, namely 4 %, 6.5 %, and 0 % (control, without salt) also showed the same growth. The results indicated that isolates A, B, C, and E produced more sediment than isolate D at both 4 % and 6.5 % salt concentrations. In contrast, all five isolates exhibited similar growth conditions at the 0 % salt concentration (without added salt).

3.2 Characteristic of *luwak* coffee-dry culture (agent of fermentation)

The viability test results are shown in Figure 2a. The use of soy-based flour and its amount did not significantly affect the viability of *luwak* coffee-dry culture microbes at a 95% confidence level. However, It is evident that producing *luwak* coffee-dry culture with varying amounts of flour added to the fermentation media yields fluctuating results. The highest result was obtained from the combination A2B2, which involved adding 10 g of tempeh flour, while the lowest result came from A1B1, with the addition of 7 g of sprout flour.

Figure 2b depicts significant variations in the percentage of water content at the 95% confidence level.

The highest percentage, 4.43 % (db), was observed in A1B1, which pertains to the *luwak* coffee-dry culture with the addition of 7 g of sprouted flour. In contrast, the lowest percentage, 2.60 % (db), was recorded in A3B3, where 13 g of soy flour were added.

The fermentation power of *luwak* coffee-dry culture includes aspects of pH, sugar fermentation rate and titratable acid formation rate. The pH value of the fermentation of glucose solution with *luwak* coffee-dry culture for 12 hours was significantly at the 95% confidence level (Figure 3). In Figure 3, it is evident that the highest pH value, 4.65, was recorded in sample A1B1, which corresponds to the *luwak* coffee-dry culture inoculated with starter media made from 7 g of sprout flour. Conversely, the lowest pH value, 4.30, was observed in sample A2B2, which used starter media with the addition of 10 g of tempeh flour.

The glucose fermentation capabilities of the *luwak* coffee-dry culture are illustrated in Figure 4. The type of flour has a significant effect on the glucose fermentation power of *luwak* coffee-dry culture. The *luwak* coffee-dry culture, A2B2, demonstrates the strongest glucose fermentation due to a higher concentration of lactic acid bacteria (LAB), as shown in Figure 5.

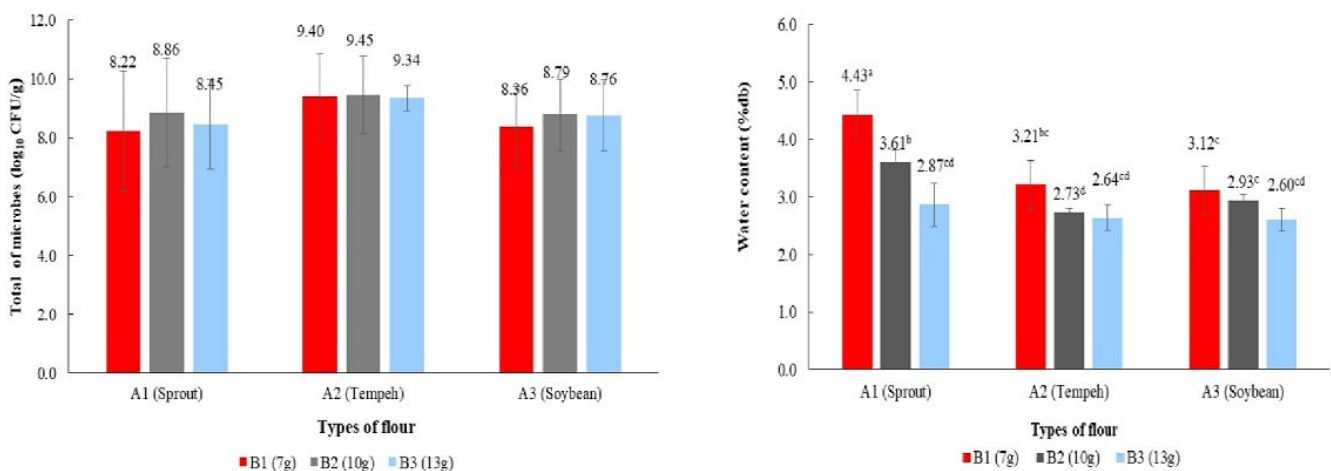


Figure 2: Bacterial viability value (a) and water content (b) of *luwak* coffee-dry culture.

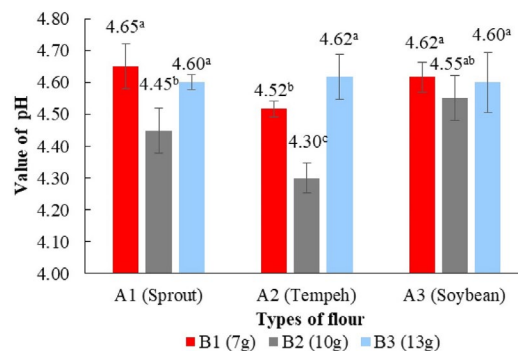


Figure 3: pH value of glucose fermentation for 12 hours in *luwak* coffee-dry culture.

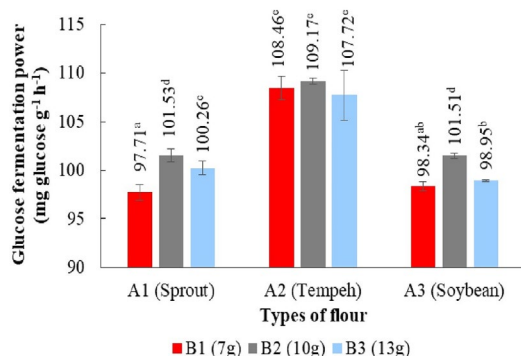


Figure 4: Glucose fermentation power (fp) of *luwak* coffee-dry culture.

Based on the test results, we conclude that the most effective fermentation agent is the *luwak* coffee-dry culture inoculated with a starter containing 10 g of tempeh flour as a nitrogen source. This specific *luwak* coffee-dry culture demonstrates a glucose fermentation capacity of 109.17 mg glucose (hg)⁻¹ of *luwak* coffee-dry culture.

The total titrated acid rate represents the amount of lactic acid formed during the fermentation process. The type of flour has a significant effect on the rate of titratable acid production of *luwak* coffee-dry culture. The total titrated acid rate can be seen in Figure 5. The highest total titrated acid rate resulting from glucose fermentation by *luwak* coffee-dry culture occurred in sample A2B2, which included the addition of 10 g of tempeh flour as an inoculant.

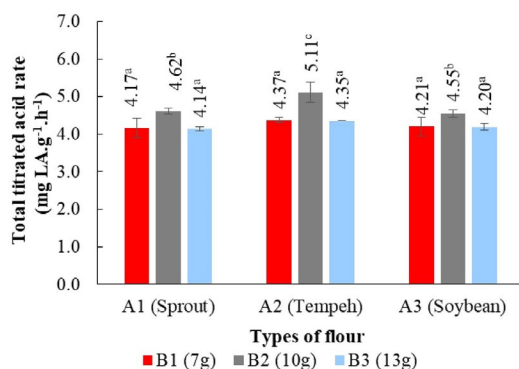


Figure 5: Total acid production rate of glucose fermentation in *luwak* coffee-dry culture.

Among the various samples tested, the *luwak* coffee-dry culture that included 10 g of tempeh flour exhibited the highest total acid titration value at 5.11 mg.g⁻¹.h⁻¹. This increase is attributed to its higher microflora count, measured at 9.45 log₁₀ cfu/g, compared to other *luwak* coffee-dry cultures (see Figure 2a)

3.3 Grounded coffee cupping test

The cupping test for grounded coffee was conducted by an expert panel from the Indonesian Coffee and Cocoa

Research Institute (ICCRI) Jember. The results indicated that robusta coffee beans fermented with a *luwak* coffee-dry culture received a higher overall organoleptic score compared to the control sample, which consisted of civet coffee and dry-processed robusta coffee (see Figure 6). This finding suggests that using a *luwak* coffee-dry culture can significantly influence the taste of fermented coffee.

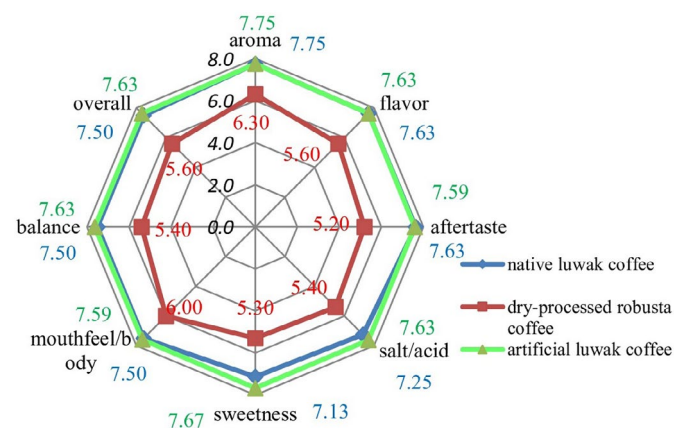


Figure 6: Spider web diagram of cup test values for 3 types of roasted robusta coffee beans.

The cupping test value and overall value of imitation civet coffee were the cupping test results revealed that the artificial *luwak* coffee had the highest overall value when compared to both original civet coffee and robusta coffee. However, original *luwak* coffee excelled in the aftertaste attribute, receiving a score of 7.63, while artificial *luwak* coffee scored 7.59, and native robusta coffee only scored 5.20. In addition to these attributes, there are 2 other attributes, namely the uniformity of the cup (10) and the cleanliness of the cup (10), where the artificial *luwak* coffee has a total of 20, so this coffee has a final score of 81.38 (20+61.38).

4 DISCUSSION

4.1 Morphological and physiological characteristics of isolated lactic acid bacteria (LAB)

Based on the bacillus shape, Gram-positive staining test, and purple color, the five isolates are identified as lactic acid bacteria, as stated by Kurnia et al. (2020), which exhibit the characteristic features of Gram-positive and rod-shaped bacteria. The purple color was formed because gram-positive bacteria retained their crystal violet color after being rinsed with alcohol. The existence of maintaining color ability was caused by 50% of the cell walls of gram-positive bacteria consist of peptidoglycan and have low lipid content (Kesuma

et al., 2021). The low lipid content caused the cell pores to become smaller, so their permeability was reduced, and the violet dye could not exit from the cells.

The five isolates were found to be negative for catalase, as indicated by the absence of gas bubbles in the preparations. This result suggests that the five isolates are facultative anaerobic lactic acid bacteria. They did not ~~creat~~ produce the catalase enzyme necessary for breaking down hydrogen peroxide (H_2O_2) into Oxygen and water (H_2O) (Finanda et al., 2021). Additionally, the five isolates could not produce CO_2 gas, leading to their classification as homofermentative lactic acid bacteria. Homofermentative lactic acid bacteria ferment glucose primarily to produce lactic acid, without generating CO_2 as a byproduct (Finanda et al., 2021). This is because these bacteria do not form the enzyme pyruvate oxidase, which is responsible for converting pyruvate into CO_2 and H_2O_2 . (Wang et al., 2021)

The optimum growth temperature for lactic acid bacteria ranges from approximately 30 °C to 45 °C (Sionek et al., 2024) and are classified within the genus *Lactobacillus* (König & Fröhlich, 2017), with the ideal growth temperature for these bacteria being 37 °C (Ozogul et al., 2022). As shown in Table 2, the highest cell precipitate production occurred at this temperature. Meanwhile, the growth of five isolates at varying salt concentrations showed good growth except for isolate D. The results indicated that isolates A, B, C, and E produced more sediment than isolate D at both 4 % and 6.5 % salt concentrations. In contrast, all five isolates exhibited similar growth conditions at the 0% salt concentration (without added salt). Thus, the five isolates are halophilic bacteria. Halophilic bacteria can grow optimally at salt concentrations exceeding 2 % (Budiharjo et al., 2017). Increasing the concentration of NaCl, these bacteria can prevent fluid loss from cells due to high osmotic pressure outside the cell. Dishan and Gönülalan (2024) said that rod-shaped lactic acid bacteria (bacilli) from the genus *Lacticaseibacillus paracasei* AD22 can survive at NaCl concentrations of up to 8 %.

4.2 Microbial viability and water content of *luwak* coffee-dry culture

Based on Figure 2a, it is evident that producing *luwak* coffee-dry culture with varying amounts of flour added to the fermentation media yields fluctuating results. The highest result was obtained from the combination A2B2, and the lowest result came from A1B1. According to Nurdini et al. (2015), fermentation during tempeh production promotes the growth of various microorganisms, including lactic acid bacteria. The high cell content in the addition of tempeh flour of 10g/200 mL compared to others. This phenomenon is in line with the research of related to the use of fish peptone of 9.38 g/L in the producing of probiotic *L. paracasei* with a microbial cell count of 9.6×10^9 CFU/mL from the

addition of 6.25 g/L, and 12.5 g/L. The tempeh provides the availability of readily digestible nitrogen and the optimum amount for the growth of *L. paracasei*. Wikandari et al. (2021) reported that the population of lactic acid bacteria continued to grow during the tempeh fermentation process until it reached 10^9 CFU/g.) The high levels of lactic acid bacteria in the fermentation can be attributed to the interaction among molds, which plays a significant role in the fermentation process, along with yeast. Besides obtaining nutrients from the metabolism of mold or yeast, lactic acid bacteria can also hydrolyze proteins into polypeptides, dipeptides, and amino acids. Proteolysis in lactic acid bacteria is initiated by cell envelope proteinase (CEP), which degrades proteins into oligopeptides (Wang et al., 2021). Liu et al. (2023) said that optimizing fermentation culture conditions provided a suitable growth environment for *L. paracasei* B1, which promoted the upregulation of the phosphotransferase system for sugar source uptake and maintained high levels of glycolysis and pyruvate metabolism. Consequently, this strain was able to grow and reproduce rapidly.

Water content refers to the amount of water present in a material, expressed as a percentage of dry weight (% db). Figure 2b illustrates significant variations in water content percentages. The highest percentage, 4.43% (db), was observed in A1B1, which pertains to the *luwak* coffee-dry culture with the addition of 7g of sprouted flour. In contrast, the lowest percentage, 2.60 % (db), was recorded in A3B3, where 13 g of soy flour were added. According to Figure 2b, the water content of the *luwak* coffee-dry culture complies with the SNI 01-2982-1992, which states that the maximum allowable water content should not exceed 8%. This phenomenon is the same as that reported by Maidannyk et al. (2022) which occurs in powdered implants with high protein and low solubility in water which indicated low water content. Among the three types of *luwak* coffee-dry cultures examined, the highest protein content was found in the culture containing tempeh flour. It appears that *luwak* coffee-dry culture added with tempeh flour has a lower water content than the other two flours as a result of the higher protein content and reduced content of hydrophilic components (carbohydrates and ash). Low hydrophilic components will cause low water content. This phenomenon occurs because the hydrophilic compounds in tempeh flour, such as reducing sugars and amino acids, have been greatly reduced due to being consumed by *L. paracasei* during incubation, resulting in the water content of *luwak* coffee-dry culture being lower than the other two flours.

4.3 Fermentation power test of *luwak* coffee-dry culture

One of the properties of the fermentation power of civet coffee dry culture is the pH value of the remaining fermentation media. The acids accumulation as product

from the metabolism of LABs can lower the pH of medium according to Charalampopoulos et al. (2002). Next, Fauzi et al. (2023) said the acid gives real impacts on the pH value of the glucose solution, when the total acid produced is higher, the pH value of the glucose solution will lower.

The fermentation starter medium consisted of these three types of flour as a treatment: The *luwak* coffee-dry culture of civet coffee mixed with 10 grams of tempeh flour, which has the highest microflora content of any flour ($9.45 \log_{10}$ CFU/g) as a result of the nutritional condition of tempeh flour which is ready to be used by *L. paracasei*. The *luwak* coffee-dry culture, rich in microflora, is capable of breaking down glucose ($109.17 \text{ mg glucose} \cdot (\text{gh})^{-1}$) and producing significant amounts of metabolites, primarily lactic acid ($5.11 \text{ mg LA} \cdot (\text{gh})^{-1}$), and so decreasing pH of 4.30. Based on the Fauzi et al. (2023), there is unique fact that shows when the amount of microflora in dry civet culture is higher, their capability in fermenting glucose, producing metabolites and also lactic acid will be better. In addition, all acid produced give the real impacts on the pH of the glucose solution. If the amount of acid produced is higher, the pH of the glucose solution will be lower. This decrease in pH is attributed to the accumulation of lactic acid formed during the fermentation process, which is the primary product of fermentation by homofermentative lactic acid bacteria (Azizah et al., 2019). This increase in microflora enhances the ability to convert glucose into lactic acid (see Figure 2a).

Fermentation is a biocatalytic process that transforms substrates through the action of microbial enzymes produced by bacteria, molds, and yeasts, generating specific products. Several factors influence this process, including the type of microorganisms used, the substrate or medium, pH levels, temperature, oxygen availability, and water activity (Kesuma et al., 2021). One significant factor is pH, or the degree of acidity, which affects both the growth of microorganisms and the formation of products during fermentation. When present in large quantities, yeast, along with various microflora, can effectively break down glucose to produce metabolites like lactic acid, enhancing fermentation efficiency (Fadilah et al., 2018).

The fermentation process generates a variety of acid compounds, including lactic acid, acetic acid, formic acid, butyric acid, and propionic acid. Consequently, foods with higher glucose levels tend to have higher acid content, resulting in a lower pH (Edviani et al., 2014). Based on the test results, we conclude that the most effective fermentation agent is the *luwak* coffee-dry culture inoculated with a starter containing 10g of tempeh flour as a nitrogen source. This specific *luwak* coffee-dry culture demonstrates a glucose fermentation capacity of $109.17 \text{ mg glucose} \cdot (\text{hg})^{-1}$ of *luwak* coffee-dry culture.

The total titrated acid value is significantly influenced by the number of lactic acid bacteria present in the *luwak*

coffee-dry culture, like sample added 10g of tempeh flour. It is believed that the *luwak* coffee-dry culture contains lactic acid bacteria, which play a crucial role in the fermentation process by producing lactic acid (Prastujati et al., 2018). Additionally, Arfianty et al. (2017) noted that a higher total number of lactic acid bacteria correlates with an increased amount of acid produced. Rohman et al. (2019) explain that lactic acid bacteria convert glucose into lactic acid through a process called glycolysis. This process begins with the conversion of glucose into pyruvic acid, which is subsequently transformed into lactic acid. Lactic acid bacteria metabolize glucose via the Embden-Meyerhof pathway (EMP), where monosaccharides are converted into glucose-6-phosphate or fructose-6-phosphate (Burgé et al., 2015).

4.4 Grounded coffee cupping test

The evaluation considered several criteria, including fragrance/aroma, flavor, aftertaste, acidity, sweetness, mouthfeel/body, balance, and overall quality of grounded coffee, as illustrated in Figure 6. The artificial *luwak* coffee beans received a higher overall organoleptic score compared to the two control sample, which consisted of natural civet coffee and dry-processed robusta coffee. However, some specific attributes scored lower than natural civet coffee. It is believed that the presence of lactic acid bacteria (LABs) in the *luwak* coffee-dry culture enhances the breakdown of components in the coffee during fermentation, resulting in improved flavor compared to unfermented robusta coffee, as noted by Pinillos-Miñano et al. (2022).

The cupping test value and overall value of imitation civet coffee were the cupping test results revealed that the artificial *luwak* coffee had the highest overall value when compared to both original civet coffee and dry-processed robusta coffee. However, original *luwak* coffee excelled in the aftertaste attribute, receiving a score of 7.63, while artificial *luwak* coffee scored 7.59, and dry-processed robusta coffee only scored 5.20. This shows that lactic acid bacteria (LAB) from *luwak* coffee-dry culture used in civet coffee production can improve the cupping score of robusta coffee, lifting it from the average-good level (5.20-6.75) to the very good level (7.00-7.75), thus approaching the score of authentic civet coffee. This grade (final score: 81.38) is said to be a specialty coffee and has a taste description of chocolate, brown sugar, spicy, sweet, chocolate fruit, slightly medicinal aroma. The enhanced characteristics of artificial *luwak* coffee can be attributed to the presence of lactic acid bacteria introduced during the fermentation process. These bacteria aid in breaking down the large components found in coffee bean pulp into simpler molecules, organic acids (such as lactic acid), and other flavor precursors that integrate into the coffee bean mass. The impact of LAB on coffee bean quality can vary depending on the dominant strain present in the coffee fermentation region.

This species, as reported by de Melo Pereira et al. (2020) is important in coffee processing and affects coffee quality.

The aroma of coffee is influenced by several factors, particularly the microbial fermentation processes that occur during post-harvest processing (Cao et al., 2023). Fermentation, a crucial post-harvest process, can be strategically manipulated to improve coffee quality. This improvement is achieved through the activity of microorganisms, which produce metabolites that play a key role in shaping the unique organoleptic profile (Silva et al., 2024). The differences in these organoleptic attributes are likely due to the fermentation involved in removing the mucilage of coffee bean during wet processing. Additionally, the breakdown of the mucilage layer of coffee bean during fermentation releases a higher concentration of reducing sugars. A marked increase in sugar will enhance the function of sugar as an important precursor for the caramelization process and Maillard reaction during roasting to form a distinctive flavor in ground coffee. The greater the amount of reducing sugars in green coffee beans due to fermentation, the more pronounced the caramel and sweet flavors will be after roasting (Lee et al., 2015; Elhalis et al., 2020).

5 CONCLUSIONS

The five isolates from the *L. paracasei* group are bacilli, Gram-positive, facultative-anaerobes, homo-fermentative, grow at 15-45°C, and are salt tolerant to 4-6.5%. The *luwak* coffee-dry culture with 10 g tempeh flour is a superior formula, containing 9.45 log₁₀ CFU LABs/g, a fermentation rate of 109.17 mg glucose (hg)⁻¹, titratable acid of 5.11 mg LA (hg)⁻¹, and a final pH of 4.30 and can produce specialty coffee. Stirring studies in specialty coffee fermentation are needed as a civet digestive system model.

6 AUTHORS' CONTRIBUTIONS:

Conceptual Idea: Fauzi, M.; Jayus.; Methodology design: Fauzi, M.; Jayus.; Data Collection: Prihandini, W. A.; Afriliana, A.; Data analysis and interpretation: Afriliana, A.; Jayus; Bakri, A.; Writing and editing: Harsono, S. S.; Quintao, G; Fauzi, M.; Jayus.

7 DATA AVAILABILITY STATEMENT

Data available upon request to authors

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