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Development of freeze-dried lactic acid bacteria starter for coffee fermentation: Optimizing incubation time and coating formulation

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Abstract

This study aimed to develop a freeze-dried lactic acid bacteria (LAB) starter for coffee fermentation by optimizing incubation time and coating formulation. The research was conducted in two stages: determining the optimal incubation time for the liquid coffee starter and formulating the dry starter coating material. The liquid starter was prepared using coffee skin powder, glucose, peptone, and minerals, and inoculated with *Lactobacillus plantarum*, *Weissella* sp., and *Leuconostoc mesenteroides*. Incubation was performed at room temperature for 24, 28, 32, and 36 h. The optimal incubation time was found to be 32 h, resulting in a LAB viability of $4.2x10^{13}$ CFU/mL and a total acid content of 1.76%. The dry starter was prepared by encapsulating the liquid starter with various coating materials (gelatin and gum arabic) at different concentrations (5, 6, and 7%) using the freeze-drying method. The formulation with 5% gum arabic best maintained cell viability at 46.77% over a 4-week storage period. Coffee fermentation using the dry starter showed improved organoleptic qualities, with the best results obtained after 48 h of fermentation. This study presents a sustainable approach to producing high-quality fermented coffee, such as Luwak coffee, without the use of animals.

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Lactic Acid Bacteria, Starter, Freeze Dryer, Viability.

1. Introduction

Luwak coffee, whose unique natural fermentation process occurs inside the digestive system of the civet, is a high-quality coffee commodity with a unique flavor and aroma that differentiates it from other coffees (1–3). The distinctiveness of this process through the involvement of microorganisms in the digestive system creates a rich and complex maturation process for coffee cherries sought after by global coffee aficionados. However, with the growing global market demand, the employment of civets in producing Luwak coffee has been accompanied by several ethical and sustainability issues. The exploitation of civets for coffee production has the risk of interfering with natural ecology and violating

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animal rights, and alternatives that are more sustainable and environmentally friendly have been encouraged (2,4).

Studies of coffee fermentation that do not involve civets are expanding to address these challenges. One prospective method is the use of lactic acid bacteria (LAB) starter cultures to mimic naturally occurring fermentation processes (5–8). Bacteria have been extensively recognized as useful for the fermentation of many food and beverage products and can synthesize volatile compounds responsible for aroma and sensory profiles and enhance the quality and safety of products (9,10). Although studies on the utilization of LAB starters in coffee fermentation have been extensively conducted, starter development in dry forms through the freeze-drying process remains relatively selective. Dry forms present distinct advantages in terms of stability, longer shelf life, easier transportation feasibility, and more realistic application than liquid starter forms (11,12).

This study aimed to improve an efficient and stable production process and specifically investigate the method of encapsulation through freeze-drying and coating material variations. In-depth investigations into the optimal incubation time, shelf life of dry starters, and optimal formulation of coating materials will pave the way for the development of innovative and ethical coffee fermentation technologies. The results of this study are expected to provide a strong scientific foundation for producing coffee with characteristics similar to Luwak coffee but with more sustainable practices that support an ecologically and ethically responsible coffee industry.

2. Materials and Methods

2.1. Experimental material

The materials used in this study are distilled water, aluminum foil, cling wrap, peptone, rice flour, gelatin, glucose, gum Arabic, maltodextrin, gauze, cotton, KH_2PO_4, coffee skin, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Weissella* Sp. (13), latex, $MgSO_4$, MRS Agar, MRS Broth, $FeSO_4$, NaCl, $CaCO_3$.

2.2. Determination of Optimum Incubation Time of Liquid Starter

The first stage focused on identifying the optimal incubation time for LAB liquid starter. The coffee skin was prepared by drying and grinding it into a powder. A total of 1 kg of dried coffee skin that had been ground was used to prepare 1 liter of the liquid starter. The liquid starter was comprised of 2% coffee skin powder, 1% glucose, 1% peptone, and 5% minerals (0.5% $MgSO_4$, 0.5% KH_2PO_4, and 0.01% $FeSO_4$). This mixture was homogenized and sterilized before the addition of 1% of each type of LAB (*Lactobacillus plantarum, Weissella sp*., and *Leuconostoc mesenteroides*). Incubation was performed at room temperature at 120 rpm for 24, 28, 32, and 36 h. LAB viability was measured using the Total Plate Count (TPC) method by taking 1 mL of liquid starter sample, performing a multilevel dilution, pouring the last three-level dilution results into a petri dish containing MRSB, and incubating for 48 h before counting the number of colonies. In addition, total acid was measured by titrating 4 mL of liquid starter sample that had been added with 100 mL of aquadest and 1% PP indicator using 0.1 N NaOH until it turned pink. The optimal incubation time obtained at this stage was used in the next stage of the study. Workflow for determination of optimum incubation time for a coffee skin–based LAB liquid starter can be seen in figure 1.

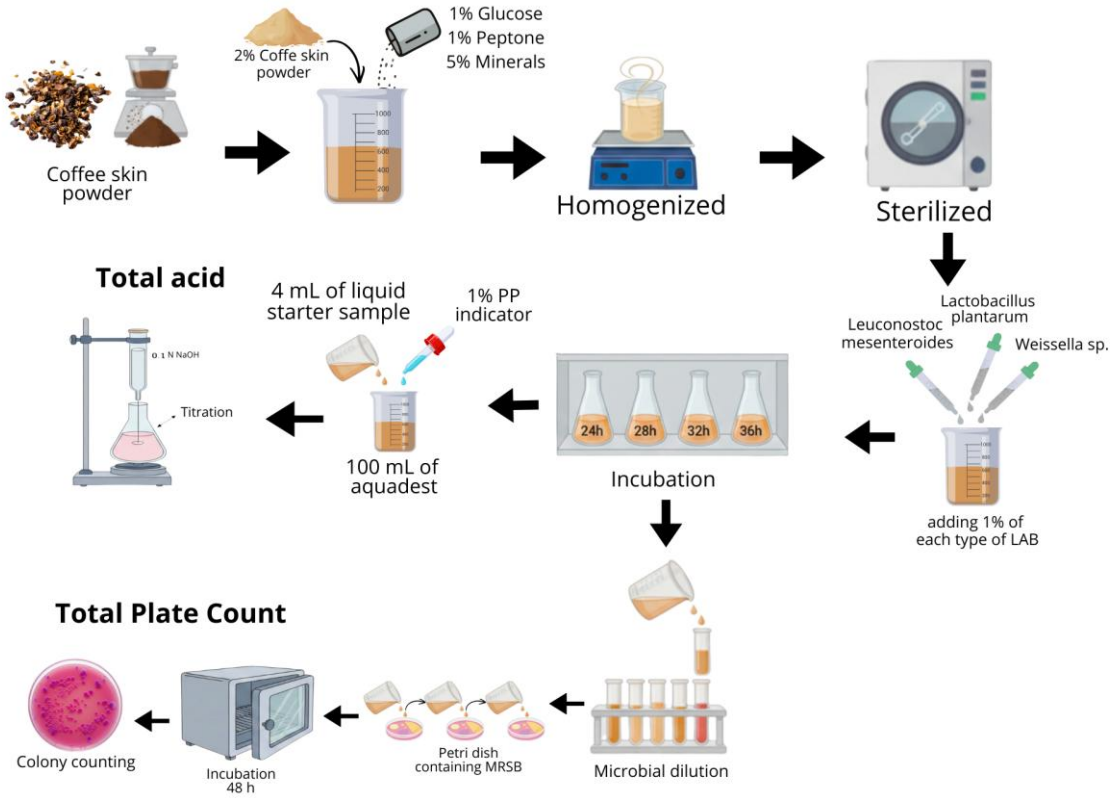

Figure 1. workflow for determination of optimum incubation time for a coffee skin–based LAB liquid starter

2.3. Preparation and Characterization of Encapsulated Dry Starter

The second stage involved encapsulating the liquid LAB starter with various coating materials. The experimental design for this stage was a Completely Randomized Design with two factors: type of encapsulant (A) and concentration of the encapsulant (B). Factor A consisted of two levels: A1 (gelatin) and A2 (gum Arabic). Factor B consisted of three levels: B1 (5%), B2 (6%), and B3 (7%). The coating materials used were gelatin, gum Arabic, and maltodextrin. The dry starter formulation consisted of 1% liquid starter, 10% maltodextrin, various concentrations of gum Arabic and gelatin (5%, 6%, and 7%), 1% peptone, 1.5% glucose, and 5% minerals. All materials were homogenized and heated, and bacterial cultures were added at a ratio of 1:3. The encapsulation process was carried out using the freeze-drying method at -55 °C for 24 h. Water content testing was conducted using a moisture analyzer, and TPC testing was performed on the starter to assess cell viability during storage.

2.4. Coffee Fermentation

Red Arabica and Robusta coffee cherries were sorted, pulped, mixed, and fermented using a dry starter from the previous step, using a fully washed method. Fermentation lasted 48 h, with sampling every 12 h. The beans were then washed, dried, and evaluated for organoleptic quality using cupping tests. Coffee fermentation process using a dry starter can be seen in figure 2.

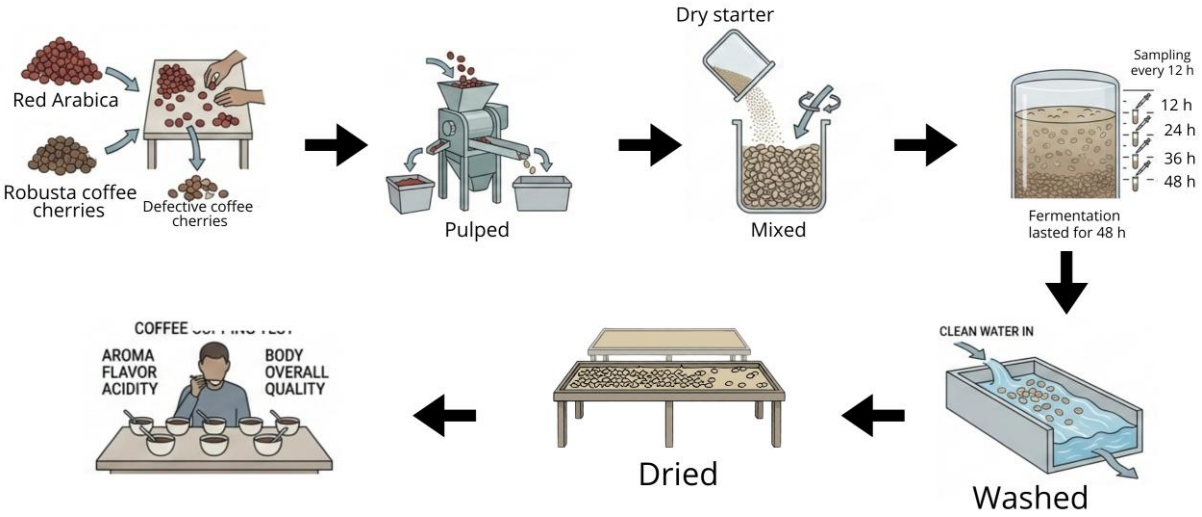

Figure 2. Coffee fermentation process using a dry starter

3. Results and Discussion

3.1. Liquid Starter LAB Viability Measurement

Viability analysis was performed to test the ability of lactic acid bacteria to survive in a sample. This test can be related to the shelf life of products containing LAB, and the number of bacterial cells present in the sample during storage is known (14). The purpose of the LAB test was used to determine the best results from the variation of samples tested, which were indicated by the growth of bacterial colonies that met the standards. Viability testing was performed by the TPC (Total Plate Count) method. The results of viability tests of the liquid coffee starter with varying incubation times are shown in Table 1.

Table 1. Viability testing results of LAB starter liquid coffee TPC method.

Time (hours)	Colony Count (CFU/mL)
0	4.96×10^{7}
24	3.8×10^{9}
28	5.2×10^{10}
32	4.2×10^{13}
36	3.06×10^{12}

Based on the results of the calculation of LAB viability of lactic acid bacteria using the TPC method, there was an increase in viability of the starter after–incubation for 24-32 hours. After an incubation time of 36 h, the viability of the starter decreased. At the peak of the incubation period, namely at 32 hours with 4.2×10^{13} CFU/mL of colonies, it is assumed to be the stationary period of the viability test. Therefore, the best time to use was chosen as, 32 h before the death period, which was 36 h.

This decline marks the beginning of the death phase, which is characterized by a greater number of dead cells than living cells (15,16). Some factors that lead to a decrease in viability, such as environmental conditions, temperature, pH, and the amount of nutrients present in the starter, also affect LAB growth. Sugar molecules serve as microbial nutrients

and are converted into organic acid products. The longer it is, the greater the nutrients it contains will be decreased, so the viability of LAB will decrease as long as the fermentation takes a long time (14). In addition to nutrient availability, fermentation duration also affects LAB growth (17).

3.2. Total Acid of Liquid Starter

Total acid measurement is a crucial parameter in studies involving Lactic Acid Bacteria (LAB) because it directly reflects the metabolic activitiy of these microorganisms. LAB are widely known for their ability to ferment carbohydrates (sugars) into lactic acid and other organic acids, such as acetic acid (18). Therefore, an increase in total acid levels in a sample strongly indicates optimal LAB growth and activity. The total acid testing in this study was aimed at determining the effect of variations in the incubation time of the coffee liquid starter on the total acid produced. The results of the total acid measurements are shown in Figure 3.

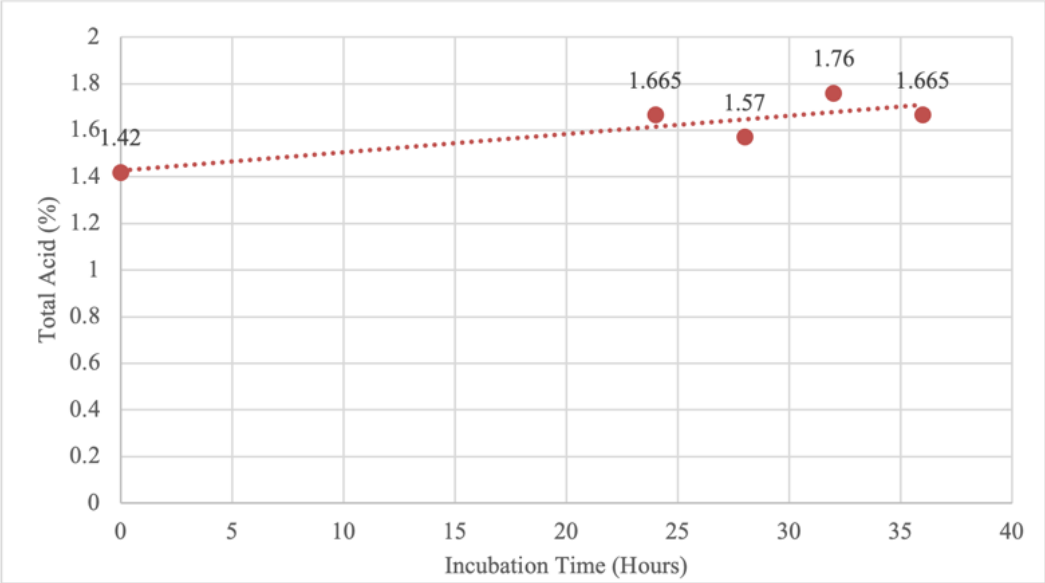

Figure 3. Total acid testing results of coffee liquid starter.

Figure 3 shows the variation in incubation time (h) of the liquid coffee starter. For time 0, the results were 1.42%, 24 hours obtained results of 1.665%, 28 hours obtained results of 1.57%, 32 hours obtained results of 1.76%, and 36 hours obtained results of 1.665%. The total acid yield increased with an increase in incubation time. A decrease in the value was observed at an incubation time of 28 h. This could be caused by suboptimal environmental conditions and decreasing nutrients in the sample, so the total acid sample at 28 h also decreased. Total acid production in the 32-hour sample increased because the longer the fermentation time, the more LAB produced lactic acid from its metabolic process. At 36 h, the LAB in the liquid coffee starter experienced a period of death, which was marked by a decrease in the total acid value.

3.3. Moisture Content of Dried Starter

Moisture content was defined as the amount of water in the food. Moisture content determines the quality of food, especially during the shelf life, and is related to the activity of water in the material. The higher the water content of a material, the lower its shelf life

the material will have. In dry starter containing microbes such as LAB, the moisture content determines the stability level of the sample during storage. The moisture content of each sample is shown in Figure 4.

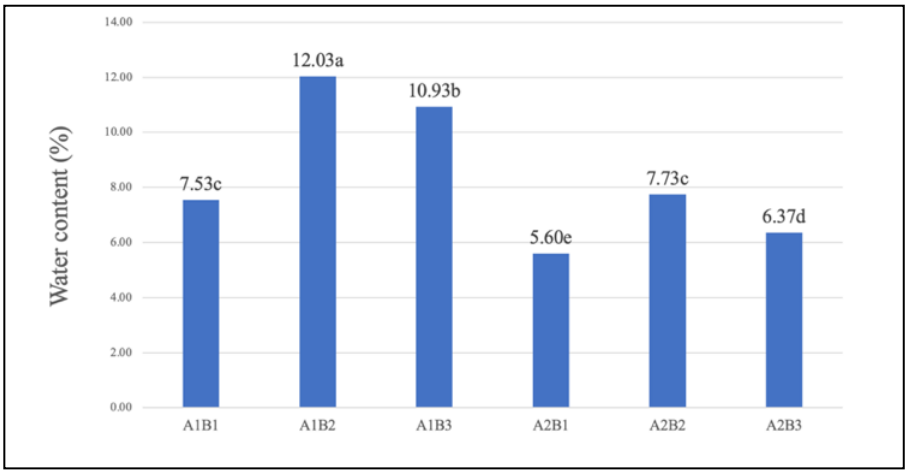

Figure 4. Water content of dry starter with various coating materials.

Based on the test results (Figure 4), the variations in the dry starter water content of the gelatin coating material with the use of 5% gelatin (A1B1), 6% gelatin (A1B2), and 7% gelatine (A1B3) were 7.53 %, 12.03 %, and 10.93%, respectively. While the results of the dry starter water content using variations of gum Arabic coating materials obtained the results, namely, the use of 5% gum Arabic (A2B1) was 5.60%; the use of 6% (A2B2) was 7.73%; the use of 7% (A2B3) was 6.37%. Based on the results of the analysis using variance (ANOVA), a significant value of (0.000), which means there was a significant difference (<0.05), and further testing (Duncan) was carried out, and the results showed that all results were significantly different between treatments. However, A1B1 and A2B2 showed results that were not significantly different between treatments.

Moisture content testing dry lactic acid bacteria (LAB) starter cultures revealed significant variation, depending on the type and concentration of the coating material used, as shown in Figure 4. The use of gelatin as a coating material resulted in a higher water content than gum Arabic at the same concentration, except for A1B1 (7.53%) and A2B2 (7.73%), which showed no significant difference. Gelatin has a reasonably high water absorption capacity therefore, the use of gelatine as a coating affects the water content of the dry starter (19). The water content of the starter affects bacterial activity during the fermentation process. The water content of a good dry starter range from 5-10% (20). The water content of dry cultures can affect product stability during storage. Low water activity causes bacterial cells to dehydrate, thereby inhibiting their growth (21,22).

3.4. Viability of Dried LAB Starter

Viability is a measure of the ability of the bacteria to survive. Microbial survival was measured using the TPC method. Viability measurements are used to determine the durability of a product containing microbial species so that it can be used to evaluate the quality of a product. The viability of a sample is influenced by several factors, such as the type of microbes used, environmental conditions, oxygen levels, and acidity levels in the sample. In a dry starter, viability measurement determines the shelf life of a dry starter with

a vulnerable time of 1 week for one month. The results of the LAB viability test are shown in Figure 5.

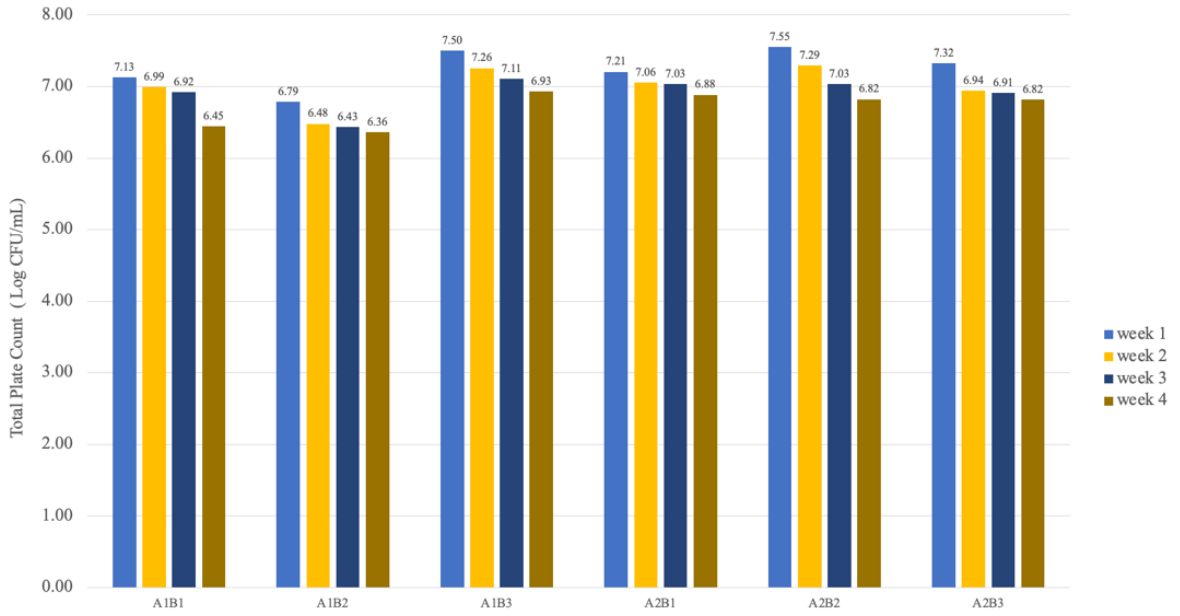

Figure 5. Dry starter viability test results.

Based on the average results of LAB viability measurements using the TPC method, 6% gum Arabic (A2B2) showed the highest number of colonies in the first week, (7.551 log CFU/mL). In comparison, the highest number of colonies in the fourth week was observed in the starter culture using 5% gum Arabic (A2B1), which was 6.884 Log CFU/mL. Based on the results of the analysis using variance (ANOVA), a significance value (0.00) was obtained so that it had a significant effect between the treatments (<0.05), was obtained, which means that there are differences between the types of coating material concentrations during the storage period. Duncans test results showed that all treatments differed significantly between storage times and treatment variations. Based on the results of each variation in the coating concentration, the best result was obtained, that is, the concentration of 5% gum Arabic (A2B1). This is indicated by the viability of each week, which is experiencing a decrease that is not too far, therefore, the 5% gum Arabic coating material can protect LAB during storage. The requirement for a sufficient number of probiotic colonies for consumption or application is a minimum of 10^6 CFU/g (23).

3.5. Cupping Test

Cupping tests of coffee fermented with a dry starter revealed differences in organoleptic quality based on the fermentation time (Table 2). At 0 h of fermentation, the coffee had a total score of 84.81. After 12 h of fermentation, the total score had decreased to 83.52. However, as fermentation time increased, the total score increased. At 24 h, the total score was 84.82, slightly higher than that at 0 h. The increase continued at 36 h with a total score of 85.38 and peaked at 48 h with a total score of 85.75. Specifically, the fragrance/aroma, flavor, and overall attributes showed an increase in value from 0 h to 48 h, with maximum values of 8 at 36 and 48 h for fragrance/aroma and flavor and 8 at 48 h for

overall. Sweetness, uniformity, and clean cup attributes consistently received a perfect score of 10 across all samples (0, 12, 24, 36, and 48 h).

Table 2. Cupping test results for coffee fermented using a dry starter.

Parameters	Time (hours)				
	0	12	24	36	48
Fragrance /aroma	8	7.94	7.94	8	8
Flavor	8	7.87	7.94	8	8
Aftertaste	7.75	7.63	7.69	7.69	7.94
Acidity	7.62	7.44	7.69	7.88	7.88
Sweetness	10	10	10	10	10
Body	7.81	7.57	7.94	8	7.94
Uniformity	10	10	10	10	10
Balance	7.81	7.57	7.82	7.88	8
Clean cup	10	10	10	10	10
Overall	7.82	7.5	7.82	7.94	8
Total score	84.81	83.52	84.82	85.38	85.75

The results of the cupping tests indicated that fermentation time had a significant effect on the organoleptic quality of coffee. The lowering of the overall score for the 12-hour-fermented sample may be caused by suboptimal microbial activity or an initial asymmetry in the compound profile. At the initial stage of pulp incubation with coffee cherries, the degradation of certain compounds is suspected, with the synthesis of undesirable compounds responsible for the suboptimal aroma and flavor of the final coffee. However, as the incubation time increased, particularly during and after 24 h of incubation, the overall organoleptic quality of the coffee improved and reached the best level after 48 h of incubation of the pulp with cherries. This manifests as higher scores for fragrance/aroma, flavor, and overall attributes. This coincides with the activity of lactic acid bacteria (LAB) responsible for fermentation the ability to synthesize volatile constituents responsible for the aroma and flavors of the final product, and improving overall quality (24,25).

4. Conclusions

This study successfully determined the optimal incubation time for a lactic acid bacteria (LAB) liquid starter and developed an LAB dry starter using a freeze-drying method with various coating materials. The best incubation time for the LAB liquid starter was 32 h, resulting in a total acid content of 1.76% and LAB viability of 4.2×10^{13} CFU/mL. The encapsulated LAB dry starter showed a shelf life of up to four months at freezing temperatures owing to its low water content. The best formulation for making a coffee LAB dry starter using the freeze-drying method was 5% gum arabic as a coating material. This formulation exhibited good bacterial viability during storage. The results of this study significantly contribute to providing a sustainable solution for fermented coffee production, especially Luwak coffee, by addressing the ethical and sustainability challenges.

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Author Contributions

Conceptualization, A.B.T and V.A; methodology, Z.D.Z. and Z.Z; software, N.R.; validation, M.M. and I.; formal analysis, F., A.B.T, and M.M; investigation, F.; resources, V.A and A.R.R; data curation, A.B.T and All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not Applicable

Data Availability Statement

Available data are presented in the manuscript

Conflicts of Interest

Author declares no conflict of interest

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