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Identification of lactic acid bacteria isolated from developed dried coffee starter culture used as a fermentation agent to produce Robusta civet coffee

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Abstract. *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: 3715-3722.* Native civet coffee is a popular coffee bean, produced by the processes which undergo spontaneous fermentation in the civet digestive system. Since the native civet coffee has a higher selling value and its production depends on the uncontrolled civet life activities, the effort to produce a similar quality of this coffee product has been made by designing a dried starter culture isolated from civet caecum, and utilizing coffee cherry peels as the potential inducer and rice flour as the matrix for coating and immobilizing the isolates. The proportion of rice and coffee cherry peel flour mixtures was formulated to fabricate the dried starter culture by determining the viability and fermentability of lactic acid bacteria (LAB) survive in the starter culture. Furthermore, the starter culture was analyzed for the number of microflora and identification of LAB, water content, glucose fermentability, total titrated acid production rate, and pH value. The most viable starter cultures were obtained from the formula of 80% rice flour and 20% coffee cherry peel flour exhibiting the number of microflora of 5.6 log MPN/g and fermentation capacity of 245.73 mg glucose/gh. The total titrated acid production rate was 91.07 mg lactic acid/gh, and a pH value of 5.33. The LABs survived in the starter culture were identified as *Lacticaseibacillus paracasei*. The quality of hot brewed roasted Robusta coffee prepared by fermentation using this dried starter culture was higher than that of unfermented one, as observed from their organoleptic quality. These findings indicate that the LAB isolated from civet caecum is potential to be developed as a dried starter culture in coffee cherry fermentation processes.

Keywords: Civet coffee, coffee peel flour, dried starter culture, fermentation capacity, *Lacticaseibacillus paracasei*, rice flour

INTRODUCTION

As a typical processed coffee in Indonesia, the well-recognized *kopi luwak* (civet coffee) known as *cat poop coffee* has a highest selling value (Febrianto and Zhu 2023), where it can cost for $600 dollars per pound quoted by Lamoureux (2022). It has been reported that the civet coffee has a different level of caffein content compare to that of other processed fermented or unfermented coffee (Yulia et al. 2016). Civet coffee is produced by the coffee beans that have been eaten and spontaneous-processed fermentation in the stomach of the mongoose (*Paradoxurus hermaphroditus*) and excreted within the caecum. Inside the civet's stomach, there is a microflora that can ferment coffee beans. The microbial community within the coffee fermentation has been reported to affects its quality (Braga et al. 2023; Huch and Franz 2015). The research conducted by Fauzi (2008) discovered 5 dominant species of lactic acid bacteria (LAB) isolated from civet caecum, namely *Lactobacillus plantarum*, *L. brevis*, *Leuconostoc paramesenteroides*, *L. mesenteroides*, and *Streptococcus faecium*. Moreover, a selected lactic acid bacterium, *Lactobacillus plantarum* LPBR01 has been implemented to promote an accelerated coffee-pulp acidification process reducing the time required for coffee mucilage removal (Pereira et al. 2016).

Based on those finding, many efforts to produce artificial civet coffee were carried out by introducing fermentative processes using starter cultures of LAB (Evangelista et al. 2014a; Evangelista et al. 2014b; Pereira et al. 2015a; Pereira et al. 2016), which have shown a significant increase in the quality of coffee beans. Furthermore, the stirred tank reactor has also been introduced for coffee fermentation added with starter culture enabled the production of coffee beans with richer aroma composition and beverages with increased quality compared to the conventional process (De Carvalho Neto et al. 2018). Apparently, the LAB growth during coffee fermentation stimulates the production of lactic acid and volatile organic compounds (i.e., 1-hexanol, nonanal, 2-phenethyl acetate, 2-methyl-butanoic acid), which ultimately affecting the final quality of the coffee drinks (Elhalis et al. 2023). The occurrence of these compounds can be directly linked to LAB metabolism (Montanari et al.

2018; Pereira et al. 2019). Although, not much experimental evidence has been given yet, it is often speculated that the diffusion of such metabolites may occur and they modulate the chemical and sensorial profile of the coffee beans (Pereira et al. 2017).

The LABs group can be used to manufacture a dry coffee starter culture that will later produce artificial civet coffee *in vitro*. The investigation by Fauzi et al. (2012) presented that civet coffee starter culture can be created using various fillers such as rice flour, tapioca, and corn starch. Attempts to enhance its implementation have been investigated by Putra (2013) through development a starter culture from fresh civet fecal microflora employing liquid media (MRS Broth). However, this media is quite expensive. Therefore, implementing such liquid media starter culture application is not economical and cannot be afforded by coffee farmers.

Inoculation of civet feces isolate into a dry starter culture filler as the media is expected to grow LABs. The nutrients needed by LABs are carbohydrates and simple sugars. LABs will catabolize sugar into lactic acid. The main component in constructing the initial starter culture is mainly using the extract of Robusta coffee skin. This component is mostly containing simple sugars. Since the intended starter culture will be implemented in Robusta coffee fermentation, this study was focused on the potency of Robusta coffee cherry skin flour as filler to construct artificial civet coffee dried starter culture in combination with rice flour to support the survival of isolated LABs from civet feces. Thus, this study aims to determine the right proportion of the mixture between rice flour and Robusta coffee skin flour in the production of dry civet coffee culture, followed by the implementation of it in artificial civet coffee production.

MATERIALS AND METHODS

Production of coffee skin extract

Fresh Robusta coffee cherry skin was blanched using boiling water for 5-10 minutes, followed by the size reduction using blender until its puree was formed. This Robusta coffee skin puree was then extracted in stages by adding 4 times distilled water of coffee cherry skin puree. The extractions were carried out twice followed by filtration to obtained coffee cherry skin filtrate. The filtrate was then sterilized at 121ºC for 15 minutes (Fauzi et al. 2012).

Preparation of liquid inoculum

The inoculum was prepared according to the method of Fauzi et al. (2012), with some modification. A single unwashed civet coffee bean, obtained from caecum of civet, was immersed into 10 mL MRS Broth medium and incubated at 37-39ºC for 24-48 hours to attain the initial culture. The initial culture was then inoculated on selected sterile media consisting of liquid coffee cherry peel extract and sugar (3%), which had been divided into two parts of 10% and 90%. The first 10% sterile media, which has been inoculated by the initial culture, was incubated at 37-39ºC for 48 hours. This culture was then inoculated on the remaining 90% sterile media, and incubated further at 37-39ºC for another 48 hours. This liquid inoculum will then be used to prepare dry civet coffee starter culture, as described in the next section.

Coffee cherry skin flour preparation

Dried Robusta coffee skin was grounded using a blender. The crushed dry coffee cherry skin was then sieved using a 60-mesh strainer to obtain a fine coffee cherry skin powder. This coffee cherry skin flour will then be used to prepare dry civet coffee starter culture, as will be described in the next section.

Civet coffee starter culture production

In this study, artificial civet coffee starter cultures were developed using 5 different formulas, designated as A, which consist of 200 g rice flour and 0 g coffee skin flour; B with the formula of 160 g rice flour and 40 g coffee skin flour; C representing 120 g rice flour and 80 g coffee skin flour; and D which composed of 80 g rice flour and 120 g coffee skin flour. The mixture containing of rice flour and coffee fruit skin (30 g of each formula), was then packed in an aluminum foil to be sterilized at 121ºC for 15 minutes. The sterile mixtures as the filling material were then separately transferred into a sterile plastic bag, followed by the aseptic inoculation of 13 mL of the prepared liquid inoculum (as described above), and then homogenized by mashing up the mixture several time. The inoculated mixture was then incubated for 48 hours in an incubator at 27-29ºC. The incubated mixture was then dried in a vacuum oven at 45ºC for 48 hours to let the starter culture become dry slowly. The quality of this dry starter cultures was then observed, which include its viability and glucose fermenting power, as well as the identification of its microflora.

Measurement of cell viability of dried starter culture

A gram of dry starter culture was distributed in a higher dilution series using the pouring method with sterile distilled water. The 3 series of dilution (10^{-5}, 10^{-6}, 10^{-7}) were selected to be grown in tubes containing nutrient broth media (7 test tubes) filled with 10 mL of the last dilution suspension in the first 5 test tubes, 1 mL for 1 second test tube, and 0.1 mL for 1 third tube. After that, incubating for 48-72 hours, followed by the observing the positive tube and looking at the probable number in the MPN (Most Probable Number) table (BSN 2006).

Identification of LAB from dried artificial civet coffee starter culture

To identify the lactic acid bacteria found in dry starter culture, a series of dilution series was carried out between 10^{-6} to 10^{-8}. A total of 1 mL of suspension from the 10^{-6} to 10^{-8} dilution series was poured into a sterile petri dish, then MRSA-Ca media was poured and evenly distributed. The petri dishes were incubated at 37-38ºC for 24-48 hours. Colonies showing clear zones were sampled, isolated, streaked onto NA slanted agar, and incubated at 38-39oC for 24-48 hours. Isolates grown on slanted agar were streaked onto MRSA in a petri dish using zigzag and then

incubated at 38-39ºC for 24-48 hours. Colonies separated from the ends were sampled and streaked onto the oblique agar again as isolated material ready to be identified based on its specific DNA sequences, followed by analyzing the homology of its DNA sequence using Basic Local Alignment Search Tool (BLAST) program (Stackebrandt and Goebel 1994). The genomic DNA extraction of the isolate was carried out using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). Its PCR amplification was conducted using (2x) My Taq HS Red Mix (Bioline, BIO-25048) and Bi-directional Sequencing.

Moisture content of dried artificial civet coffee starter culture

The dry starter culture water content was measured using the gravimetric method (Sudarmadji et al. 2010). Dry coffee starter culture powder weighed 1-2 g in a weighing bottle, then dried in an oven at 100-105ºC for 3-5 hours. This dried starter cultures, were then let it cool in a desiccator, before their weight was measured again. This step is repeated until a constant weight is reached (the difference in successive weighing is less than 0.2 mg). Weight reduction is the amount of water in the material and then it is presented to the weight of the material.

Glucose fermentability (GF) of starter culture

The glucose fermentability of the starter culture was measured using the method of Fauzi et al. (2012). Dry starter culture (0.1 g) was dissolved into 10 mL of 3% sterile glucose solution in a test tube, then incubated for 12 hours. A glucometer which has been calibrated with glucose standard solution, was used to measure the glucose level of the fermented liquid. Their glucose fermentation power was then calculated using the following equation:

$$GF\left(\frac{\text{mg}}{\text{g.h}}\right) = \frac{(\text{Initial g.c.} - (\text{final g.c}/100)) \text{ x } 10.08}{12\text{x}0.1}$$

Where:
g.c : glucose concentration

Production rate of total titrated acid (PRTTA)

Total acid was measured by taking 5 mL of the fermented sample, put it in a 50 mL measuring flask and dilute it to the mark. Furthermore, 5 mL is taken and put into a 250 mL Erlenmeyer and added 2-3 drops of 1% phenolphthalein and titrated using a standard solution of 0.01N NaOH until the end point of the titration is reached, with the formation of a pink color. The number of mL of NaOH used is equivalent to the total amount of lactic acid produced by coffee dried culture acid bacteria.

$$\text{PRTTA}\left(\frac{\text{mg}}{\text{g.h}}\right) = \frac{\text{V NaOH x N NaOH x MW x DF}}{\text{sample weight}}$$

Where:
V NaOH : volume NaOH
N NaOH : concentration of NaOH
DF : dilution factor
MW : lactic acid molecular weight (90)

Degree of acidity (pH)

Measurement of the pH was carried out using a pH meter that has been calibrated with buffer solutions pH 7.0 and 4.0. The pH meter electrode was rinsed with distilled water and then dried out with tissue paper. The pH meter electrode is dipped into a fermented sugar solution until it shows a constant pH value (Sudarmadji et al. 2010).

Trial production of artificial civet coffee with dry coffee dried culture

Coffee dried culture made from a mixture of rice flour and coffee skin flour with the best characteristics is used for the fermentation process of civet coffee for 24 hours at a dose of 1% by weight of coffee beans. Fresh coffee cherries were removed from the fruit skin with a pulper. Wet coffee beans were added with 1% dried culture and put in a plastic bag and incubated at 37-39ºC for 24 hours. 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 civet coffee along with artificial civet coffee were measured for their organoleptic characteristics using the analysis services of the Cocoa and coffee Research Center, Indonesia. Dried starter culture with the best characteristics was tested on artificial civet coffee fermentation process. Furthermore, the data obtained were analyzed descriptively according to method of SCA (2022).

Organoleptic Test for grounded artificial civet coffee

Cupping test for the grounded coffee was carried out by three trained panelists from the Indonesian Coffee and Cocoa Research Institute in Jember using the method of Lingle (2001). For the purposes of the taste test, 10 g of grounded coffee was prepared in a bowl hot brewed with 200 mL of boiling water. The quality measured via this organoleptic test included the following attributes: Aroma, representing the character of the coffee taste captured by the sense of smell (smell) after 5 minutes of brewing; Flavor, reflecting the combination sensed on the tongue and the aroma of vapor flowing from mouth to nose; Aftertaste, the impression that arises after brewed coffee leaves the mouth; Acidity, describing the delicious and fresh fruity taste when the coffee is inhaled; Sweetness, a pleasant sweet taste because carbohydrates content of coffee; Body/mouthfeel, the thick taste of coffee in mouth; Uniform cup, which is the uniformity of the aroma from each bowl; Balance, namely all aspects of flavor, aftertaste, acidity, and body are balanced; Clean cup, off-flavor clarity from start from the beginning until aftertaste; Overall, reflecting the overall organoleptic attributes captured by the panelist (SCA 2022). The average scores obtained from the test were then categorized into the following grade: Average (5.00-5.75), Good (6.00-6.75), Very good (7.00-7.75), Excellent (8.00-8.75), and Outstanding (9.00-9.75).

RESULTS AND DISCUSSION

Number of microflora test and identification of coffee dried culture acid bacteria

The total amount of microflora grown from the dried starter culture was appear to be declining when the starter culture filler was mixed by coffee bean skin flour. The number of microorganisms dropped from 7.393 log MPN/g (rice flour only as a filler) to 5.640 log MPN/g when coffee bean skin flour was added to the rice flour filler. The higher the amount of coffee bean skin flour added, the lower the number of microorganisms observed, as seen in Figure 1.

The higher number of microflora obtained from dried starter culture prepared by rice flour only as the filler, this may because the rice flour contains more reducing sugars which can nourish lactic acid bacteria (LAB). LABs are a group of bacterial species that have the ability to form lactic acid from carbohydrate metabolism and grow at low environmental pH (Braga et al. 2023). Among the formulations used, the more coffee peel added the lower the number of colony detected. It is suspected that the alkaloid content such as tannin in coffee cherry skin (Campos et al. 2021) powder inhibits the growth of its microflora. The secondary metabolite compound from the coffee skin may works cellularly as an antibacterial compound, as has been demonstrated by Li et al. (2021) that the coffee skin extracts inhibit the activity of enzymes, which may be causing disturbances in the metabolism of any bacteria grown in that coffee skin containing media. Even though the number of colonies in starter culture prepared by coffee peel is lower compare to the one without, the colony is expected to be more adaptable the coffee cherry fermentation since the coffee cherry skin is not only containing reducing sugars but also crude fiber and pectic substances (Campos et al. 2021) which may induce a certain LAB growth and aroma component during the coffee cherry fermentation.

Genotypic characteristics of acidic bacteria of civet coffee dried culture

The coffee dried starter culture prepared with 160 g rice flour plus 40 g coffee husk flour (B) was selected for the isolation of LABs. The five isolates obtained designated as A, B, C, D, and E, were rejuvenated on NA slanted to identify the species name using PCR method to obtain genetic material (DNA) ready for sequencing. The PCR product appear in the gel is illustrated in Figure 2.

The size of PCR fragment of lactic acid bacteria is in between 1000-2000 bp (Figure 2). The results of DNA sequences of 5 isolates showed that was around 1400-1500 bp in size. The isolates analyzed with the BLAST tool showed similarities or 97.5% closely related to *Lacticaseibacillus paracasei*. The phylogenetic tree of all isolates shown in Figure 3 is divided into two large clusters (x; y). Cluster x contains two sub cluster (x1 and x2). Sub cluster x1 contains isolate A, B, C, and E. Cluster x2 contains isolate D and *Lcb. paracasei* VHProbiF11. Cluster y consist of one species control, namely *L. delbrueckii* subsp. *bulgaricus* strain thank come BC01.

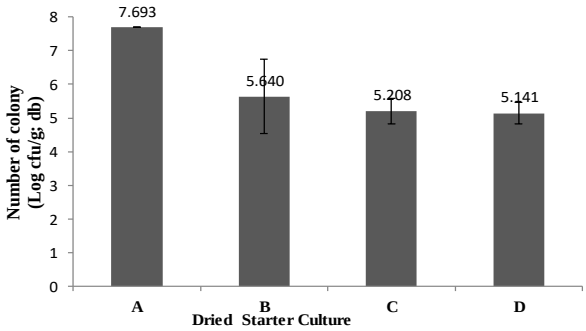

Figure 1. MPN of dried coffee starter culture. A. Prepared with rice 200 g+coffee skin 0 g; B. Prepared with rice 160 g+ coffee skin 40 g; C. Prepared with rice 120 g + coffee skin 80 g; D. Prepared with+ rice 80 g+ coffee skin 120 g

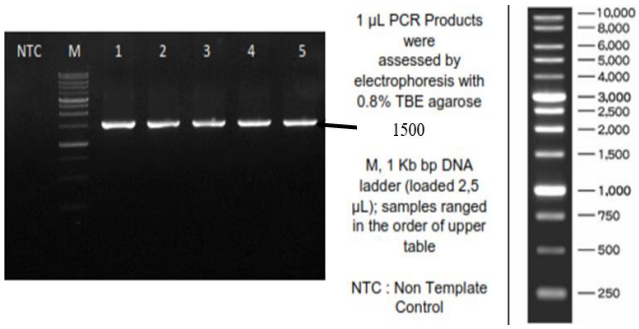

Figure 2. Electrophoresis results from PCR product of five isolate bacterial genomic DNA (1, 2, 3, 4 and 5) and 1 kb bp DNA ladder (M)

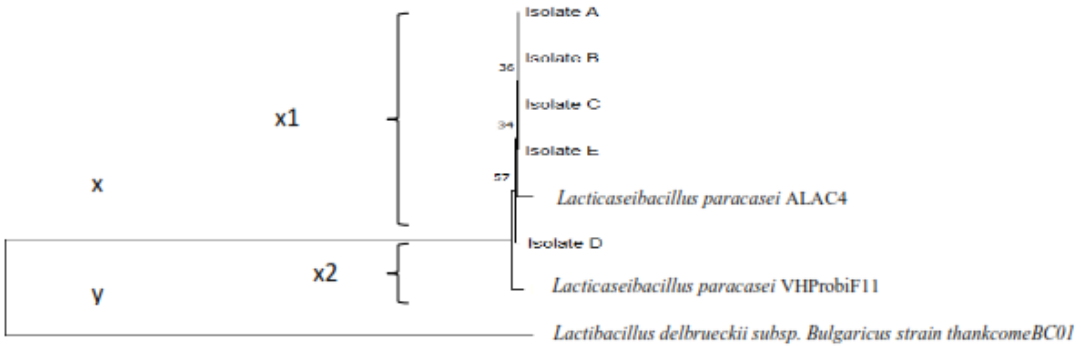

Figure 3. The phylogenetic tree of 5 isolates in comparison to other specified species

The phylogenetic tree analysis based on the nucleotide sequence dataset showed that the closest relatives of isolates A, B, C and E are *Lcb. paracasei* ALAC4, meanwhile the isolate D was closely related to *Lcb. paracasei* VHProbiF11. This two major strain of LABs detected are different to other countries coffee fermentation where it were reported that the predominant LABs found in Brazil coffee fermentation belonged to the group of Leuconostocaceae family (De Carvalho Neto et al. 2018), as also observed in Australia which include *L. mesenteroides* and *Lactococcus lactis* (Elhalis et al. 2020). The dominancy of LABs strain during coffee fermentation may also depended on the preparation of stater culture used. As observed by Fauzi et al. (2008), five dominant species of LABs isolated from civet caecum, namely *Lactobacillus plantarum*, *L. brevis*, *L. paramesenteroides*, *L. mesenteroides*, and *Streptococcus faecium* were not dominantly appear when the starter culture used was prepared by the addition of coffee cherry peel powder which may possibly able to repress the growth of those strain, as the number of microflora is obviously dropped (Figure 1) when the starter culture was prepared with coffee cherry peel powder.

The role of LABs during fermentation of coffee have been contributed to the removal of coffee mucilage. A selected lactic acid bacterium, *Lactobacillus plantarum* LPBR01 was able accelerate coffee-pulp acidification process (Pereira et al. 2016), since this bacterium is able to produce several lactic acids as their metabolite (De Carvalho Neto et al. 2018). This bacterium strain may also able to produce some glycolytic (Lovabyta et al. 2020) and proteolytic enzymes (Anggraeni et al. 2022) during fermentation, which may be able to degrade complex carbohydrate and protein presence in the coffee mucilage layer (Febrianto and Zhu 2023).

Civet coffee dried culture water content

The results of the analysis of the water content of civet coffee dried culture ranged from 8.51-9.26%. The water content of dried starter culture composed by 200 g rice without coffee skin flour reached 9.26% as the highest compared to the other three starter culture composed with coffee skin powder. It appeared that the higher the amount of coffee skin powder added, the lower the water content of starter culture obtained. This possibly becomes an advantage for lengthening its self-life. The water content of dried starter culture constructed from rice and robusta coffee cherry flour is illustrated in Figure 4.

As can be seen from Figure 4, the more Robusta coffee cherry flour added, the lower water content of the dried culture obtained. This may because of the higher water holding capacity (WHC) of rice when compared to coffee skin flour. The presence of crude fiber in coffee peel (Campos et al. 2021) may lower the WHC of the starter culture prepared, which release more water during drying.

Glucose Fermentability of coffee dried culture fermentation

The highest fermentation power (249.94 mg/gh) was found in starter culture prepared with 200 g of rice without coffee skin flour. The presence of coffee skin flour in the starter culture formula, lower its glucose fermentation power. Among those starter culture composed by coffee skin flour, the higher the coffee skin flour added, the lower its glucose fermentation power. The lowest fermentation power (242.77 mg/gh) was observed in the starter prepared with 80 g rice and 120 g of coffee skin flour. The glucose fermentation power of all starter cultures can be seen in Figure 5.

As can be seen from Figure 5, the higher the proportion of coffee cherry flour, the lower the fermentation power of coffee dried starter culture to ferment glucose. It is suspected that increasing the proportion of coffee cherry flour reduces the viability of microflora, as shown in Figure 1, which will also have an impact on reducing the glucose fermentative power. This phenomenon is also observed by Nurhayati et al. (2020), where the addition of dry coffee skins powder reduce the pH of kombucha cascara as a result of the high phenol or tannin which inhibit the growth of microflora as the ability of microorganism used to ferment sugar. In contrast to this finding, Campanella et al. (2017) reported that the LABs can grow well on grape marc media containing phenol which indicate that LABs use some phenols, such as gallic acid, for their metabolism.

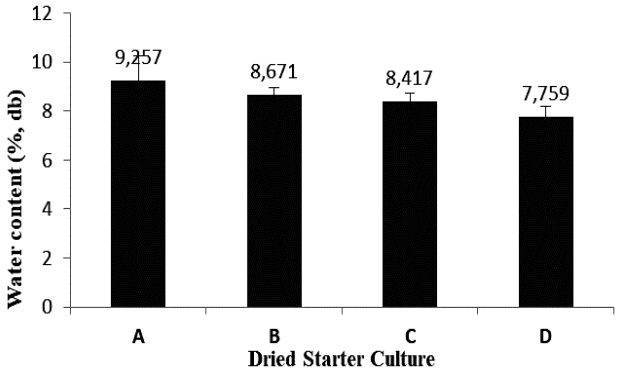

Figure 4. Water content of civet coffee dried culture. A. Prepared with rice 200 g + coffee skin 0 g; B. Prepared with rice 160 g+ coffee skin 40 g; C. Prepared with rice 120 g + coffee skin 80 g; D. Prepared with+ rice 80 g+ coffee skin 120 g

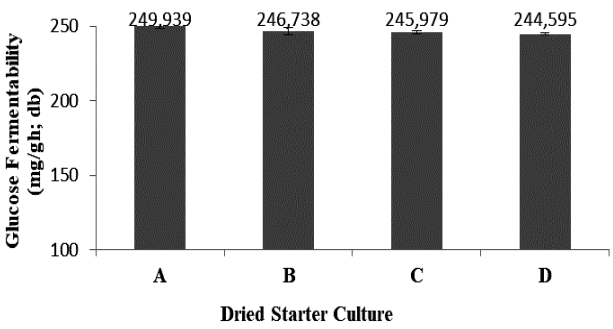

Figure 5. Glucose fermentability of civet coffee dried culture. A. Prepared with rice 200 g + coffee skin 0 g; B. Prepared with rice 160 g + coffee skin 40 g; C. Prepared with rice 120 g + coffee skin 80 g; D. Prepared with + rice 80 g + coffee skin 120 g

Total titrated acid production rate of civet dried starter culture

The ability of civet starter culture apparently influenced by the composition fillers used. Total titrated acid production rate of starter culture prepared with 200 g rice flour only, without any addition of coffee skin flour, it was 149.38 mg/gh, which is the highest among the starter culture produced. The incorporation of the coffee skin flour as the fillers decreases the total titrated acid production rate of the starter culture. The lowest acid production rate (46.40 mg/gh) was found in starter culture formulated with more of coffee skin flour (80 g rice and 120 g coffee skin flour). The results of the total titrated acid production rate in civet coffee dried culture are presented in Figure 6.

From Figure 6, it is obvious that the coffee peel flour can repress the activity of the LABs in starter culture. The contribution of LABs to the coffee fermentation processes is not well defined, whether from their quantity or the changes in the consortium of microorganisms, is still questionable. A high account in numbers of one strain of LAB is not always corelate to the quality. In some cases, bacterial diversity had a positive correlation to sensory characteristics (Brioschi-Junior 2020). But in other case, several LAB species that generally present with high counts in the wet and semi-dry processes due to the anaerobic environment. Most of these LABs metabolite detected in coffee bean fermentations including lactic, acetic, butanoic, formic, and glutamic acids (de Bruyn et al. 2017), was speculated responsible for the quality of coffee liquor (Elhalis et al. 2023).

pH value of glucose solution after fermented by dried starter culture

Measurement of the pH value of glucose solution is one of the parameters to determine the ability of the starter culture to ferment the glucose in the solution. The glucose solution after being fermented by starter culture has a pH value ranged from 4.83 to 5.76. The glucose solution fermented by dried starter culture made from 200 g rice flour and 0 g coffee skin flour has a pH value of 4.83. The higher the proportion of coffee skin flour use for the filler of starter culture, the higher the pH value obtained, as can be seen in Figure 7.

The pH value of the fermented glucose solution is related to the fermentation power and the total acid produced. Civet coffee dried culture, which has a higher number of microflora, is able to ferment glucose and produce more metabolites, which include lactic acid. The total acid produced affects the pH value of the glucose solution, the higher the total acid produced, the lower the pH value of the glucose solution. According to Charalampopoulos et al. (2002), the accumulation of acids produced through the metabolism of LABs can lower the pH of the medium. Although the accumulation of acid produced by LABs can lower the pH, the decrease in pH does not always correspond to the amount of acid accumulation. During coffee fermentation, the bacteria grew by utilizing sugars in the mucilage and produced glycerol, mannitol, as well as lactic acid, leading to a significant drop in pH (Elhalis et al. 2020).

Grounded coffee cupping test

Cupping test of grounded artificial civet coffee carried out by the expert panelist from the ICCRI, which include the criteria of fragrance/aroma, flavor, aftertaste, acidity, sweetness, mouthfeel/body, balance, and overall, as illustrated in Figure 8, showed that Robusta coffee beans fermented by civet coffee dried starter culture, has a higher overall organoleptic score than that of the control (civet and Robusta coffee), except for certain attribute which have a lower score. This indicates that the inoculation of civet coffee dried starter culture can affect the taste of fermented coffee obtained. It is suspected that the use of civet coffee dried culture which contain LABs during fermentation causes a better degradation of the components in the coffee than the unfermented Robusta coffee, as has been also observed by Pinillos-Miñano et al. (2022).

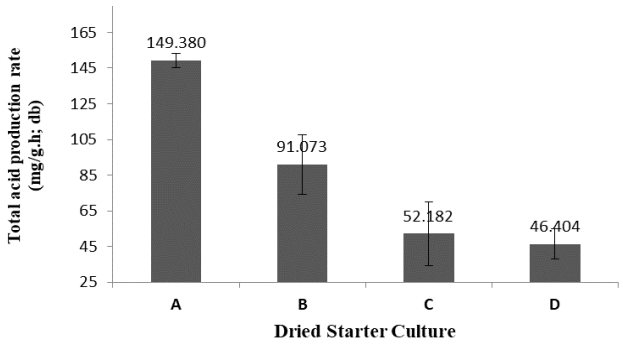

Figure 6. Production rate of total titrated acid from dried starter culture A. Prepared with rice 200 g+coffee skin 0 g; B. Prepared with rice 160 g+ coffee skin 40 g; C. Prepared with rice 120 g + coffee skin 80 g; D. Prepared with+ rice 80 g+ coffee skin 120 g

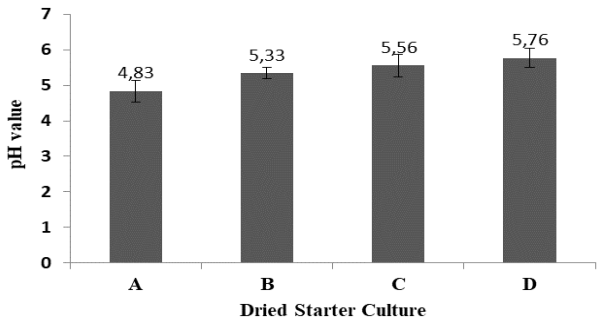

Figure 7. pH value of sugar solution that has been fermented by civet coffee dried starter culture. A. Prepared with rice 200 g+coffee skin 0 g; B. Prepared with rice 160 g+ coffee skin 40 g; C. Prepared with rice 120 g + coffee skin 80 g; D. Prepared with+ rice 80 g+ coffee skin 120 g

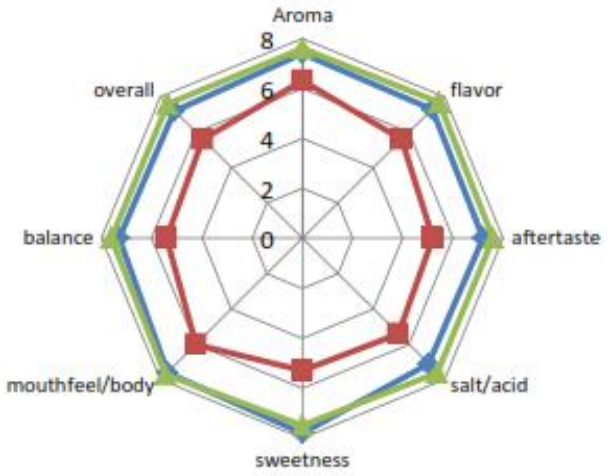

Figure 8. Spider web diagram of cup test values for 3 types of roasted robusta coffee beans. : native civet coffee, : robusta coffee, : artificial civet coffee

The values of aroma, flavor, after-taste, salt/acid, mouth feel/body, balance and overall value of artificial civet coffee is the highest in comparison to the both native civet and robusta coffee. However, natural civet coffee has the highest score on the sweetness attribute, which is 7.8, while the artificial civet coffee has a score of 7.5 and Robusta coffee has a score of 5.3. This proves that LAB from civet coffee dried starter culture increase the cupping test score of Robusta coffee from a good level (6.00-6.75) to a very good level (7.00-7.75) which is comparable to the score of natural civet coffee. The increase in the characteristic score of artificial civet coffee was due to the presence of lactic acid bacteria in from the dry starter culture, which have been inoculated during the fermentation process. These bacteria help the process of degradation of large components in coffee bean pulp into simple molecules, organic acids (lactic acid) and other flavor precursors. The contribution of LABs to the coffee bean quality may differ depending on the strain predominant on the area of coffee fermentation.

The coffee aroma is affected by numerous factors which include fermentation processes during post-harvest coffee processing (Sunarharum et al. 2014). Fermentation processes have been found to modulate more aromatic coffee with fruity and acidic attributes and lower bitterness, woody and burnt notes (Lee et al. 2015). These differences in the organoleptic attributes could most likely be contributed by the fermentation process during the coffee mucilage removal in wet processing. Moreover, the degradation of the mucilage layer would release a higher concentration of reducing sugars during the fermentation, presumably this would had a positive impact on coffee aroma since sugars are important aroma precursors for caramelization and Maillard reactions during roasting. The more reducing sugars presence in green coffee beans as the result of fermentation, the greater caramelic and sweet attributes found reveal after roasting (Lee et al. 2015; Elhalis et al. 2020).

In summary, a good dried coffee cherry bean starter culture can be fabricated by formula of rice and coffee fruit skin flour, and LABs isolates from civet feces which shown to have predominant microflora of *Lcb. paracasei*. Since the viability of LAB consist of predominant *L. paracasei* appear in the starter culture, and its glucose fermentability also high, followed by a higher rate of total titrated acid production, the starter culture then very reliable to elevate the quality of coffee bean obtained from the fermentation processes. It has been proof by the ability of the starter culture to increase the organoleptic value of roasted coffee from a good (score of 6.00-6.75) to a very good score (7.00-7.75). This finding supported the potency of LAB microflora as the promising fermentation agent in coffee bean production, especially for artificial civet coffee development.

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