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Identification of Proteolytic and Pectinolytic Yeasts from Civet (*Paradoxorus hermaphroditus*) and Evaluation of their Potential to Modify Coffee Bean Quality

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***Abstract*— Civet coffee is one of the world's most popular and expensive coffee types. The problem recently is the difficulty of finding natural civet coffee. The study of artificial civet coffee production using microorganisms to ferment the coffee in recent years has attracted the attention of many researchers. This study aims to characterize and identify yeast from civets and evaluate its potential as a starter in coffee fermentation. Three types of yeast isolated from civet feces have been isolated and characterized. They have varied morphological and biochemical characteristics. Only two yeast isolates Kh1 and Kh2 have the potential to be used as starters in fermentation due to the ability to degrade pectin and protein. The 18S rRNA gene fragment analysis results indicated that the isolates Kh1 and Kh2 were identified as *Trichosporon asahii* isolate E22922_ITS and *Wickerhamomyces anomalus* CNRMA10.1139. Applying both isolates as starters in coffee fermentation can improve the quality of coffee flavor and produce a higher score. Fermented coffee using *W. anomalus* starter provides more flavor, aftertaste, body, and overall advantages, while coffee fermentation using *T. asahii* isolate gives more value to the aroma. The highest cupping score was obtained for processed coffee using *W. anomalus*, reaching 84.42. Furthermore, it is necessary to carry out a more complex metabolite analysis to ensure the performance of the two isolates in modifying the chemical composition and taste of coffee.**

***Keywords*— Civet; coffee bean; fermentation; molecular identification; yeast.**

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I. INTRODUCTION

Civet coffee is known as one of the most exotic and expensive types of coffee in the world, as evidenced by the naming of civet coffee in the Guinness Book of World Records as the 1st Excellent and Most Expensive Coffee in the world. This is certainly inseparable from enjoying the taste and the uniqueness of the civet coffee production process. Civet coffee is obtained from coffee cherries that have been eaten by civets (*Paradoxurus hermaphrodites*), but because the civet's digestive tract is unable to break down the coffee beans, these beans are secreted when the civet excretes its feces. This coffee is then collected by farmers, cleaned, and processed as regular coffee [1].

The problem recently is the difficulty of finding natural civet coffee. The civet coffee production process is extremely limited as it relies solely on the civet's biological system. This condition causes civet coffee to be awfully expensive and is often the target of counterfeiting. In addition to the problem of counterfeiting or mixing, there are other civet coffee problems that are quite complex. The halalness of civet coffee as an unclean product is still controversial, the quality of civet coffee products is inconsistent and highly dependent on what the civet eats [2].

The study of the artificial production of civet coffee using microorganisms to ferment coffee has attracted the attention of a number of researchers in recent years. This study is considered to be a possible alternative in creating coffee that can match the quality of natural civet coffee. The microorganisms involved in civet fermentation are certainly an important factor to study. This discovery implies the exploration of various microorganisms of civet either from feces [3] or directly from the civet digestive tract [2].

Lactic acid bacteria (LAB) are one of the most studied microorganisms in civet microorganism research. LAB is one of the indigenous bacteria of the gastrointestinal tract, commonly involved in fermented food products and generally recognized as safe (GRAS) [4]. Yeast is another important microorganism suspected to play a role in civet coffee fermentation. Studies on the presence of yeast from civet origin have been very limited. The ability to degrade substrates efficiently is a superior characteristic of yeast. The ability of yeasts with specific enzyme activities, such as proteolytic and pectinolytic, must be discovered because these microorganisms are thought to play an important role in forming the unique civet coffee flavor. It is known that the decomposition of proteins will produce a number of peptides, while the decomposition of pectin will generate simple sugars. These various components are precursors for forming aroma and flavor compounds in coffee. Some yeast species detected in coffee processing are *Saccharomyces, Schizosaccharomyces, Candida, Hanseniaspora, Pichia, Debaryomyces, Cryptococcus* and *Rhodotula,* and *Torulaspora* [5].

The role of yeasts in coffee fermentation has started to be explored. The potential of yeasts isolated from spontaneous coffee fermentation to produce aromatic compounds and their pectinolytic activity tests showed that *Saccharomyces cerevisiae* had the best pectinase activity while *Pichia fermentans* generated the highest concentration of active flavor ester compounds. Their application to wet fermentation showed a significant increase in volatile aroma compounds with higher sensory scores for fruit, butter, and fermentation aromas. Previous studies have shown that yeast starters can improve coffee quality and provide the possibility to control and standardize the fermentation process toward a final product with a new and desired flavor profile [6], [7].

Muzaifa [2] has identified bacteria in the civet digestive tract and applied them in coffee fermentation. The yeast from the civet gastrointestinal tract and its ability to modify coffee's chemical components and flavor have not been studied so far. This study aims to identify and characterize yeast from the civet digestive tract and evaluate its potential in coffee fermentation.

II. Materials and Method

This research is divided into several stages as shown in Figure 1.

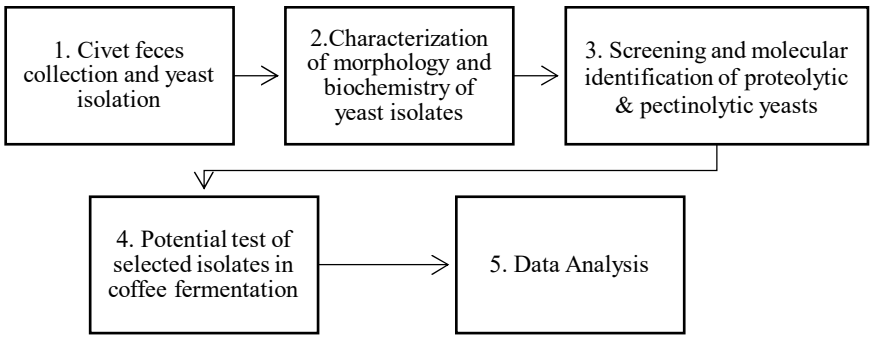

Fig. 1 Research stages

A. Civet feces Collection and Yeast Isolation

Yeasts were isolated from fresh wild civet feces obtained from Arabica coffee plantations in Central Aceh, Indonesia. Sabouraud Dextrose Agar (SDA) (Merck, Germany) was the media used to isolate the yeast. Aseptically, 1 g of fresh civet feces was taken and suspended in peptone solution, homogenized, and vortexed for 2 minutes. 1 ml of this suspension was inoculated into a petridish, and sterilized liquid SDA was added. Next, 1% chloramphenicol was added to prevent bacterial growth and incubated at 30°C for 48 hours. Observed the growing colonies; different colonies were streaked and purified repeatedly [8]. The purified culture obtained was then inoculated into a tilted SDA medium and stored at 4°C.

B. Characterization of Morphology and Biochemistry of Yeast Isolates

Pure yeast isolates were characterized by colony morphology test (color, shape, size, edge, and elevation) and yeast cell shape. Furthermore, the ability of yeast isolates to ferment sugar, namely glucose, lactose, mannitol, maltose, and sucrose and grow at temperatures of 10°C, 37°C, and 45°C was tested [9].

C. Screening and Molecular Identification of Proteolytic and Pectinolytic Yeasts

The ability of yeasts to degrade proteins (proteolytic activity) was screened using 2% skim milk-enriched media, while screening the ability of yeasts to degrade pectin was using 1% pectin-enriched media [2]. Isolates that showed clear zones were selected for further molecular identification by rDNA gene identification [10]. Isolation of yeast DNA and Polymerase Chain Reaction (PCR) amplification were performed simultaneously using a direct PCR kit (KOD FX Neo, Toyobo) following the kit protocol. The PCR machine used was an Eppendorf brand personal master cycler using universal Primer F: ITS-1 (F)/Sequence: TCC GTA GGT GAA CCT GCG and Primer R: ITS-4/Sequence: TCC TCC GCT TAT TGA TAT GC. PCR products were sent to PT Genetika Science Jakarta for sequencing. The raw sequencing data were then edited using the MEGA 7 program and analyzed using Basic Local Alignment Search Tool (BLASTN) software with references from the Gen Bank National Center for Biotechnology Information (NCBI). This procedure is used to define which yeast species have the greatest homology and are molecularly closest.

D. Potential Test of Selected Isolates in Coffee Fermentation

Selected yeasts were tested for their ability to ferment coffee beans. Coffee fermentation was carried out experimentally on a laboratory scale using the selected starter. The yeast starters were prepared by following the modified procedure of Muzaifa [2]. Starters prepared from selected yeast were grown on NB media and incubated for 24 hours. Furthermore, a sterile medium of coffee fruit peel extract enriched with 2% (w/v) sugar was prepared. The incubated yeast culture from NB was pipetted as much as 10 ml put into 90 ml of sterile media of coffee fruit peel extract and incubated for 24 hours. The culture that was incubated was taken as much as 50 ml, put into 450 ml of sterile coffee extract media, and incubated for 24 hours. This culture will be used as a starter in the coffee fermentation treatment.

Coffee fermentation was conducted on a laboratory scale and designed with three different treatments, namely coffee fermentation without the addition of starter (control), coffee fermentation with the addition of starter *Trichosporon asahii* isolate E22922_ITS (*T. asahii*), and *Wickerhamomyces anomalus* (*W. anomalus*). The study began with weighing fresh coffee beans that had been separated from the skin by as much as 500 g each and put into a 1000 ml beaker glass. Next, 10% (v/w) yeast starter (according to treatment) was inoculated and stirred evenly. The beaker glass was covered with aluminum foil and fermented at room temperature for up to 48 hours. The fermented coffee was then washed thoroughly until smooth, dried to reach a moisture content of about 12%, and removed from the husk. The resulting green bean was then stored in a clean and tightly closed container until ready for analysis. Each treatment was conducted three times. Chemical analysis (pH and total titratable acidity) of coffee liquid at the beginning and end of fermentation was recorded, and sensory analysis (cupping test) of fermented coffee was conducted by coffee brewing referred to Specialty Coffee Association of America (SCAA) [11].

E. Data Analysis

The research data was compiled using Microsoft Excel 2013. Data were displayed in the form of tables and figures and analyzed descriptively. Raw data from sequencing results were analyzed using BLASTN software with references from the Gen Bank National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST). Analysis of variance (ANOVA) was performed using SPSS to confirm variations among chemical data coffee fermentation.

III. Results and Discussion

A. Morphology and Biochemical Characterization

Three different isolates were obtained based on the growth of yeast colonies on SDA media. The three isolates were further purified and characterized morphologically and biochemically, as presented in Table 1. Table 1 shows that isolate Kh1 has a circular shape, white colony color with flat edges, convex elevation, and small size. Isolate Kh2 has a circular colony shape, cream color with jagged edges, convex elevation, and medium size. The Kh3 isolate has a circular shape, beige colony color with flat edges, flat elevation, and medium size. The cell shape of the three isolates is uniform, which is oval, but the three isolates have varied characteristics during biochemical testing.

TABLE I
MORPHOLOGY AND BIOCHEMICAL CHARACTERIZATION

Characteristics		Yeast Isolates		
		Kh1	**Kh2**	**Kh3**
Morphology	Colony:			
	Shape	Circular	Irregular	Circular
	Color	White	Cream	Cream
	Margin	Entire	Undulate	Entire
	Elevation	Raised	Raised	Flat
	Size	Small	Moderate	Moderate
	Cell:			
	Shape	oval	oval	oval
Biochemical	Fermentation:			
	Glucose	+	-	-
	Lactose	+	+	+
	Mannitol	+	+	+
	Maltose	-	+	-
	Sucrose	-	-	-
	Growth:			
	15^{o} C	+	+	+
	35^{o} C	++	++	++
	45^{o} C	-	-	-

Fermentation Test: (-) cannot ferment, (+) can ferment
Temperature Growth Test: (-) did not grow; (+) grew slowly and slightly; (++) grew rapidly and abundantly

Table 1 shows a difference in the carbohydrate (sugar) fermentation test of the three isolates in fermenting sugar. The sugar fermentation test is conducted to evaluate the ability of isolates to degrade or ferment certain sugars such as lactose, glucose, maltose, sucrose, and mannitol. This result is known from the change in color of the test media, which was initially clear purple to yellow, indicating that the isolate can ferment certain sugars. Fermentation test results showed that isolate Kh1 was able to ferment glucose, lactose, and mannitol, isolate Kh2 was able to ferment lactose, mannitol, and maltose, while isolate Kh3 was only able to ferment lactose and mannitol. Maicas [12] mentioned that the sugar fermentation ability of yeast can specify or classify a yeast. During fermentation, sugar has a role as the final electron acceptor, with the final metabolites being acid and gas. Thus, yeast isolates that have the ability to ferment different sugars are thought to be different species of yeast.

Furthermore, the observation of the growth of yeast isolates at several different growth temperatures showed varying results. Temperature is a physical parameter that has an important effect on yeast growth and its performance during fermentation. In this study, all yeast isolates can grow at 15°C and 35°C but not at 45°C. At 35°C the isolates grew well and quickly, but at 15°C the growth was relatively slow. This outcome is thought to be related to the character of yeast, which is generally a mesophilic organism. The mesophilic microorganism is an organism that grows at moderate temperatures. Trip et al. [13] have observed that the growth of yeast slows down at low temperatures. Furthermore, Segal-Kischinevzky et al. [14] mentioned that the optimal temperature for yeast growth ranges from 20 to 25°C.

B. Screening of Proteolytic and Pectinolytic Yeasts

The screening result of the ability of yeast isolates to degrade protein and pectin can be seen in Table 2.

TABLE II
ENZYME ACTIVITY OF YEAST ISOLATES

Yeast Isolates	Enzyme Activity	
	Proteolytic	Pectinolytic
Kh 1	+	+
Kh 2	+	+
Kh 3	-	-

(+): has enzyme activity; (-): has no enzyme activity

Table 2 illustrates the differences in the ability of isolates to degrade protein and pectin. Isolates Kh1 and Kh2 could degrade protein and pectin, while Kh2 could not degrade both. This characteristic is important to know because protein and pectin degradation results in simpler molecules that will contribute to the formation of flavor components in coffee. Peptides and amino acids are components of protein degradation, whereas simple sugars are components of pectin degradation that will be precursors in forming aroma and flavor compounds in coffee [2]. Therefore, isolates Kh1 and Kh2 have the potential to be used as a starter to ferment coffee.

The presence of yeasts in feces and the digestive tract is still not fully understood. Knowledge of the presence of yeasts in the gut is fundamental to understanding the reciprocal relationship between yeasts and bacteria and their role in nutrient and energy bioavailability. Though some are pathogenic, gastrointestinal yeasts are generally harmless [15], [16].

C. Molecular Identification of Yeast

Molecular yeast identification was only carried out on potential starter isolates for civet coffee fermentation, specifically isolates Kh1 and Kh2 (both indicated proteolytic and pectinolytic activities). The stages of molecular identification include DNA isolation, PCR amplification, electrophoresis of PCR results, and DNA data analysis by sequencing. The results of PCR amplification of Kh1 and Kh2 yeast isolates can be seen after electrophoresis with agarose gel, as shown in Figure 2. The electrogram shows that the amplification process produces DNA fragments with a base sequence length of about 500 bp. Each isolate indicates a single band and is quite thick, indicating that the results of the amplification process went well.

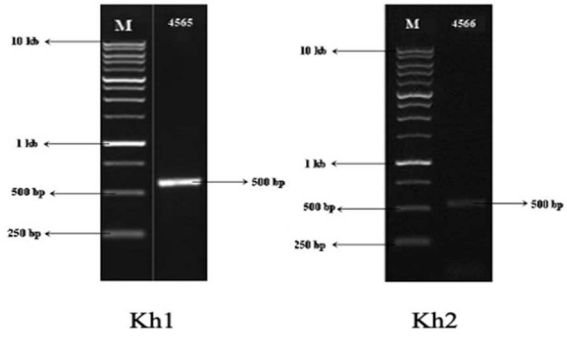

Fig. 2 Electrogram of yeast isolates from civet (M= marker (DNA Ladder), 4565=Kh1, 4566=Kh2)

The amplification results above are followed by an analysis of the DNA base sequence of the 18S rRNA fragment (sequencing analysis). The principle of this process is to read the sequence of bases (sequences) that encode the 18S rRNA gene region. The results of the 18S rRNA gene fragment analysis of Kh1 and Kh2 isolates can be seen in Table 3.

TABLE III
SEQUENCING RESULTS OF 18S RRNA FRAGMENTS OF YEAST ISOLATES

>STRAIN Kh1
TGAACCTGCGGAAGGATCATTATAGTATTCTATTGCCAGCGCTTAATTGCGCG
GCGATAAACCTTACACACATTGTCTAGTTTTTTTGAACTTTGCTTTGGGTGCAT
CAGCCTAGCTGCGTGCCCAAAGGTCTAAACACATTTTTTTAATGTTAAAACCT
TTAACCATTAGTCATGAAAATTTTTAACAAAAATTAAAATCTTCAAAACTTTC
AACAACGGATCTCTTGGTTCTCGCAACGATGAAGAACGCAGCGAAATGCGAT
ACGTATTGTGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATT
GCACCCTCTGGTATTCCAGAGGGTATGCCTGTTTGAGCGTCATTTCTCTCTCA
AACCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTTAACTTGAAATAT
TGACTTAGCAAGAGTGTACTAATAAGCAGTCTTTCTGAAATAATGTATTAGGT
TCTTCCAACTCGTTATATCAGCTAGGCAGGTTTAGAAGTATTTTAGGCTCGGC
TTAACAACAATAAACTAAAAGTTTGACCTCAAATCAGGTAGGACTACCCGCT
GAACTTAAGCATATCAATAA

>STRAIN Kh2
GTAGGTGAACCTGCGGAAGGATCATTAGTGATTGCCTTTATAGGCTTATAACT
ATATCCACTTACACCTGTGAACTGTTCTACTACTTGACGCAAGTCGAGTATTT
TTACAAACAATGTGTAATGAACGTCGTTTTATTATAACAAAATAAAACTTTCA
ACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAGCTT
GCGCTCTCTGGTATTCCGGAGAGCATGCCTGTTTCAGTGTCATGAAATCTCAA
CCACTAGGGTTTCCTAATGGATTGGATTTGGGCGTCTGCGATTTCTGATCGCT
CGCCTTAAAAGAGTTAGCAAGTTTGACATTAATGTCTGGTGTAATAAGTTTCA
CTGGGTCCATTGTGTTGAAGCGTGCTTCTAATCGTCCGCAAGGACAATTACTT
TGACTCTGGCCTGAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA
TAA

DNA sequence data in Table 3 was then aligned with other yeast DNA sequences in the National Center for Biotechnology Information (NCBI) gene bank with the Basic Alignment Search Tool (BLAST) method. This aims to determine the similarity of Kh1 and Kh 2 with other yeasts in the gene bank. BLAST results of Kh1 and Kh2 isolates can be seen in Tables 4 and 5.

TABLE IV
BLAST RESULT OF KH1 ISOLATE

Strain Name	Isolate code acces	Homology
Trichosporon sp. isolate EE (19)-CHc	MK605936.1	100%

TABLE V
BLAST RESULT OF KH2 ISOLATE

Strain Name	Isolate code acces	Homology
Wickerhamomyces anomalus CNRMA10.1139	KP132877.1	100%

According to Tables 4 and 5, isolate Kh1 has the closest similarity with *Trichosporon asahii isolate* E22922_ITS, while isolate Kh2 with *Wickerhamomyces anomalus* CNRMA10.1139. The presence of these two isolates in the gastrointestinal tract of civet has not been reported so far. However, both types of yeast have been isolated from the gastrointestinal tract of other living things. *Trichosporon sp.* is a ubiquitous fungus commonly found as a commensal on human skin and the gastrointestinal tract. *Trichosporon* species are basidiomycetes yeasts, widely distributed in nature and mostly found in tropical and warm regions. These organisms are usually found in substrates such as soil, aquatic areas, decaying wood, air, food, and animal feces. In humans, this species is found as part of the gastrointestinal and oral microbiota and can colonize the respiratory tract, skin, and vagina [17], [18]. *Wickerhamomyces anomalus*, formerly known as *Pichia anomala, Hansenula anomala, and Candida*

pelliculosa was recently assigned to the genus *Wickerhamomyces* based on phylogenetic analysis of gene sequences [19]. *Wickerhamomyces anomaly* is associated with *Anopheles stephensi* mosquito, a major vector of malaria, and found to be localized in humans' gastrointestinal tract and reproductive system [20].

Concerning the fermentation of food products, *Trichosporon asahii* was reported to have been isolated from ogi, a cereal-porridge from Nigeria [21]. Meanwhile, *Wickerhamomyces anomalus was* reported as an acetate ester producer in wine manufacturing [22], bioaugmentation of Baiju fermentation, a traditional alcoholic beverage from China [23], and starter to improve rice wine flavor [24], volatile terpene compounds in Korean Muscat Bailey a wine [25] and different styles of wines with higher quality [26]. As for its relation to coffee processing, it was discovered that *Wickerhamomyces anomalus* has been studied by several researchers in fermenting coffee [23], [27], [28].

D. Coffee Fermentation using Civet Yeast Isolate as Starter

The potential of the two identified civet yeast isolates, *T. asahii* and *W. anomalus*, was then tested for their ability as starters to ferment coffee beans on a laboratory scale. Fermentation was carried out in a closed container. Changes in pH value and total soluble solids of coffee liquid at the beginning and end of fermentation can be seen in Tables 6 and 7.

TABLE VI
CHANGES IN THE PH VALUE OF FERMENTED COFFEE LIQUID*

Coffee Fermentation	pH	
	Fermentation 0 h	Fermentation 48 h
Control/without starter	4.88[a]	4.00[a]
with starter *T. asahii*	4.89[a]	3.86[b]
with starter *W. anomalus*	4.88[a]	3.84[b]

* Same letter in the same column indicates no significantly different

TABLE VII
CHANGES IN TOTAL TITRATABLE ACIDITY(TTA) OF FERMENTED COFFEE LIQUID*

Coffee Fermentation	TTA (%)	
	Fermentation 0 h	Fermentation 48 h
Control/without starter	0.97[a]	0.43[a]
with starter *T. asahii*	0.93[a]	0.55[b]
with starter *W. anomalus*	0.97[a]	0.55[b]

* Same letter in the same column indicates no significantly different

The results in Table 6 indicate that all treatments experienced a decrease in pH after fermentation for 48 hours, both with the addition of a starter and control. These findings indicate the occurrence of biochemical processes that convert substrates into organic acids through fermentation. The fermentation process in coffee beans occurs with the help of enzymes naturally found in coffee beans and microorganisms around them [29]. The starter also influences this result added, especially in the treatment with the addition of a starter. This outcome can be proven by the lower pH of the final treatment of coffee fermented with the addition of T. *asahii* and *W. anomalus*. It is suspected that with the addition of a starter, the activity of microorganisms to break down the substrate becomes greater so that the acid produced is more and has implications for the decrease in pH value. This conjecture is reinforced by the analysis of total fermentation liquid acid at the beginning of fermentation, which is lower than at the end of fermentation in all treatments, as shown in Table 7.

Table 7 shows that the acid content of the initial coffee fermentation liquid was low (0.93-0.97%). This acid content originated from the raw material of coffee beans that are still covered with mucus, which is known to contain many dissolved organic acids. Osorio-Perez et al. [30] detected the presence of organic acids in coffee beans, including citric, quinic, malic, succinic, and acetic acid. The presence of these organic acids contributes to the acidity of coffee beans.

At the end of fermentation, the total acid increased significantly. In the control treatment, the total acid obtained was not as large as the other two treatments that added a starter. This result was influenced by the addition of other organic acids produced from the activity of microorganisms during coffee fermentation. The activity of microorganisms causes a decrease in pH along with an increase in organic acids produced. Some organic acids produced in coffee fermentation include lactic, butyric, acetic, citric, malic, and succinic acid [31]–[33].

Furthermore, the fermented coffee flavors were tested by 'cupping' following the SCAA protocol. Coffee cupping is a standard protocol for assessing and identifying coffee flavors. The results of the taste quality test can be observed in Figure 2, which shows that fermented coffee with the addition of a starter has a better sensory profile value than the control, especially in aroma, flavor, aftertaste, acidity, body, balance, and overall. Aroma is the smell found in coffee powder and brew. The flavor is a combination of taste and aroma present in coffee. Aftertaste indicates the length of time the "good" flavor remains in the mouth. Acidity indicates acidity, especially the "good" sourness of the coffee. The body indicates the viscosity of the coffee. Balance is the balance of flavor, aftertaste, acidity, and body of coffee, and overall is an assessment that reflects the overall aspects of the cupping attributes above [11].

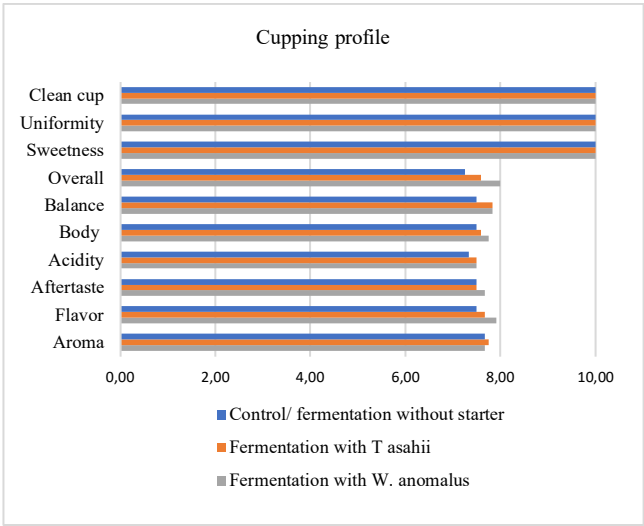

Fig. 3 Cupping profile of coffee fermentation using yeast isolates from civet

Figure 3 illustrates that the aroma of coffee fermented with *T. asahii* starter is higher than the other two treatments, namely with the addition of *W. anomalus* starter and control.

However, for other attributes, coffee fermentation by adding *W. anomalus* starter produced a higher sensory profile than fermentation with *T. asahii* and control. In the results of Haile and Kang [23], coffee fermentation using *W. anomalus*, in addition to being able to improve the functional properties of coffee, can also improve its flavor, especially aroma, bitterness, and astringency.

The overall total cupping score of all attributes for the three fermentation treatments in the study can be seen in Figure 4.

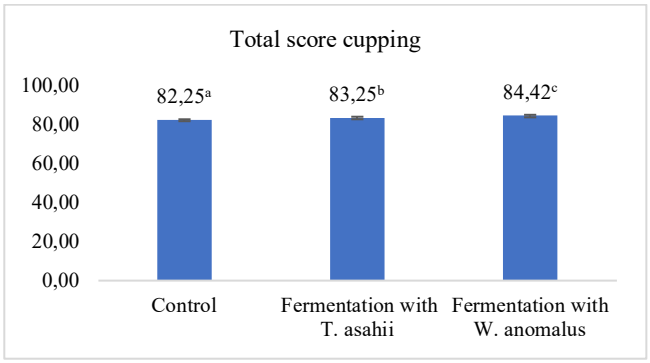

Fig. 4 Score total of coffee fermentation using yeast isolates from civet

Figure 4 demonstrates a significant increase in cupping score with the addition of a starter. The highest cupping score was obtained in the treatment with the addition of *W. anomalus* and the lowest in the control treatment. These results differ greatly from research by Muzaifa [2], who fermented coffee by adding proteolytic bacterial starters from the civet digestive tract. In this study, the resulting cupping score increased and was higher than the control, reaching 84.33. More specifically on the use of yeast, several other studies show a similar profile, namely, the use of yeast as a starter can improve the quality of fermented coffee beverages both chemically and sensory [31], [33], and [23]. These outcomes indicate that yeast has the potential to modify coffee flavors for better flavor. However, especially in the results of this study, research is needed to confirm further by analyzing the metabolites of fermentation results and their correlation with the resulting flavors.

IV. Conclusion

Three types of civet feces-derived yeast isolates have been successfully isolated and characterized. Two yeast isolates Kh1 and Kh2 have the potential to be used as starters in fermentation due to their ability to degrade pectin and protein. The 18S rRNA gene fragment analysis results showed that isolates Kh1 and Kh2 were identified as *Trichosporon asahii isolate* E22922_ITS and *Wickerhamomyces anomalus* CNRMA10.1139. Using both as a starter in coffee fermentation can improve the quality of coffee flavor and produce higher scores. Coffee fermentation using *W.anomalus* starter gives more advantages to flavor, aftertaste, body, and overall, while coffee fermentation using *T. asahii* starter gives more value to the aroma. The highest cupping score was obtained in fermentation using *W.anomalus* starter. Further research is needed to analyze more complex fermentation metabolites so that the performance of the two isolates in modifying the chemical composition and flavor of coffee can be ascertained.

Acknowledgment

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