



Physiochemical Characteristic of Fermented Coffee with yeast addition (*Hanseniaspora uvarum* and *Candida parapsilosis*)

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ABSTRACT

Robusta coffee (*Coffea canephora*) is one type of coffee that is widely cultivated in Indonesia. Robusta coffee is usually processed using the wet method, which causes the physical and chemical qualities of the coffee beans to change during the process. This research examined the effect of yeast addition during fermentation and the differences in the fermentation method used on the physical and chemical quality of the coffee beans produced. In this research, the fermentation methods used were anaerobic, anaerobic with the addition of *Hanseniaspora uvarum*, anaerobic with the addition of *Candida parapsilosis*, and aerobic. Robusta coffee beans are fermented for 72 hours. After fermentation, the coffee beans are processed into roasted coffee beans. Measurements of the physical and chemical quality of coffee beans in the fermentation process and fermented coffee products included temperature, water content, pH, total soluble solids, and color of coffee beans. The fermentation method has a significant effect on the physical and chemical quality of the fermented coffee. Using yeast as a starter culture can modify coffee beans' physical and chemical characteristics and may affect the final aroma, taste, and quality.

Keywords: Bioreactor, Acidity, Aromatic compound, Caffeine content, Temperature, Quality

1. INTRODUCTION

Coffee (*Coffea* sp.) is one of the leading plantation commodities owned by Indonesia. High coffee production makes Indonesia one of the fourth largest coffee producers in the world after Brazil, Vietnam and Colombia. In 2021, Indonesia produced 774.6 thousand tons of coffee. The production value increased 1.62% from coffee production in 2020 which produced 762.2 thousand tons of coffee [1]. In addition, coffee consumption in Indonesia also increased by 1.7%, from 285 thousand tons to 300 thousand tons in the 2017/2018-2020/2021 period [2] According to the Central Statistics Agency [3], most of these products are exported to foreign countries such as the United States, Egypt, Malaysia, Japan, Italy, Belgium, England, Germany, Russia, Canada, and other countries, while the rest is marketed domestically.

In Indonesia, the most widely cultivated types of coffee plants are robusta coffee (*Coffea canephora*) with

a value of 90% and arabica coffee (*Coffea arabica*) with a value of 10% [4]. Both types of coffee have their respective advantages. Arabica coffee has a more sour taste than Robusta coffee so it has a special market, while Robusta coffee has a bitter taste and has strategic value in empowering the people's economy because it has higher productivity and is more tolerant of pests than Arabica coffee [5] [6] [7]. In addition to coffee varieties, post-harvest handling and coffee processing also play an important role in determining the taste and quality of coffee. Coffee quality is determined through physical and chemical properties [4].

The postharvest process is one of the important stages that can determine the taste and quality of coffee. Post-harvest handling of coffee is divided into three methods, namely the wet method, dry method, and semi-dry method. The difference between the three methods is that there is a fermentation process in wet coffee processing. The fermentation process is carried out to break down the sugar in coffee mucus both anaerobically and aerobically.

This fermentation process is mostly done naturally by relying on microorganisms and enzymes present in coffee cherries [8]. However, the natural fermentation process is not controlled and standardized so that an alternative is needed so that the fermentation process is more controlled, one of the methods is adding starter culture.

The addition of starter culture (yeast) can increase the production of volatile compounds in coffee beans which will also improve the taste and aroma of coffee. Several types of yeast that can be used in the coffee fermentation process, namely *Candida parapsilosis* and *Hanseniaspora uvarum* which can be isolated from coffee fermentation and cocoa fermentation [9] [10] [11]. Both types of yeast were chosen because of their pectinolytic activity and adaptability to stress conditions during fermentation [12] [13].

The use of yeast *C. parapsilosis* and *H. uvarum* in fermentation can increase the aroma of coffee. According to [12], *C. parapsilosis* can produce fruity, nutty, and cocoa aromas. On the other hand, *H. uvarum* will produce several aromatic compounds [13]. However, information related to the physical and chemical characteristics of coffee beans during processing, especially the fermentation process using yeast, is still very limited. Therefore, it is necessary to study the effect of the addition of yeast *C. parapsilosis* and *H. uvarum* on the quality of the coffee beans produced.

2. MATERIALS AND METHODS

The main ingredient used in this study was ripe robusta coffee (*Coffea canephora*), which was obtained from the Ngipiksari Coffee Garden located at Jalan Kaliurang Km 23, Ngipiksari, Hargobinangun, Pakem, Sleman, DI Yogyakarta. In this study, 30 kg of cherry coffee was used for one fermentation process. Before being fermented, the cherry coffee peeled off the skin and flesh through a pulping process. From this process, approximately 19 kg of coffee beans were used in the fermentation process with three fermentation methods.

Yeast isolate is used as the main ingredient in addition to robusta cherry coffee. The use of yeast isolates is one of the treatments of the fermentation method that will be carried out. There are two types of yeast isolates used, namely *Hanseniaspora uvarum* and *Candida parapsilosis* due to their pectinolytic activity and adaptability to stress conditions during fermentation [12] [13]. The isolates were obtained from the Indonesian Culture Collection (InaCC).

The physical characteristics of coffee beans measured in this study were temperature, moisture content, total soluble solid, and color, while the chemical characteristics measured were pH during the fermentation process. In this study, aerobic and anaerobic fermentation were repeated 6 times, while for

fermentation with yeast *H. uvarum* and *C. parapsilosis* were repeated 3 times.

Red-picked robusta cherry coffee cherries were processed by the wet method. Cherry coffee cherries are peeled and fermented for 72 hours. After the fermentation process is completed, the coffee beans are washed to remove the remaining mucus and pulp that is still attached. The clean coffee beans are then dried for approximately 24 hours using a cabinet dryer. The coffee beans are still wrapped in parchment skin which needs to be removed by hulling. From the hulling process, rice coffee beans are obtained as raw materials for the roasting process. The roasting process will produce roasted coffee beans.

The fermentation process was carried out using three methods, namely anaerobic fermentation, anaerobic fermentation with the addition of yeast, and aerobic fermentation for 72 hours. The coffee beans used in the fermentation process were 2.1 kg for each reactor. For the anaerobic method, an additional 1750 mL of distilled water was used in each reactor. In anaerobic fermentation with the addition of yeast, the amount of yeast used is 2 g (0.1% gram yeast per gram of coffee). The addition of aquadest and yeast is based on research conducted by [14] with some modifications. The experimental design was shown in Figure 1.

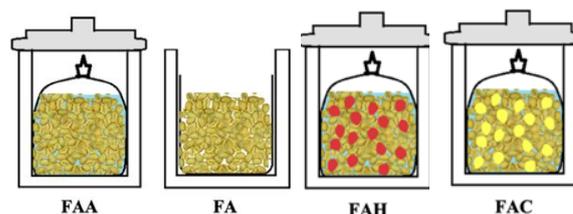


Figure 1 Experimental design of the fermentation process: FAA (anaerobic fermentation); FA (aerobic fermentation); FAH (Anaerobic fermentation with *H. uvarum*, a concentration of 0.1% for 2 kg of coffee); and FAC (Anaerobic fermentation with *C. parapsilosis*, a concentration of 0.1% for 2 kg of coffee).

The reactor used in the fermentation process is connected to a type K thermocouple cable which is connected to a data logger as a temperature sensor to detect and record temperature changes that occur during the fermentation process. During the fermentation process, several measurements were carried out to determine the characteristics of the coffee beans. These measurements include temperature, moisture content, color, pH, and Total soluble solids.

Data analysis was performed using SPSS software. The statistical test begins with the data normality test (Shapiro Wilk). Furthermore, a homogeneity test (Levene's test) was conducted. If the data is homogeneous, then a one-way ANOVA test is carried out

to determine the effect of treatment on the final fermented coffee.

3. RESULT AND DISCUSSION

3.1. Temperature During Fermentation Process

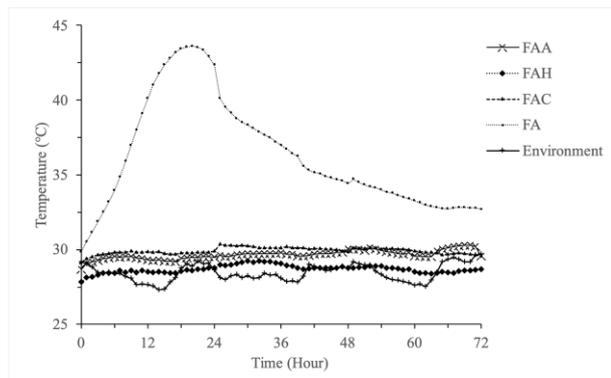


Figure 2 Changes in the temperature of coffee beans during fermentation

During the fermentation process, observations were made on temperature changes in the reactor and the environment. Changes in temperature that occur during fermentation can be seen in Figure 2. The temperature during the anaerobic (FAA) fermentation process, the anaerobic with *H. uvarum* (FAH), and the anaerobic with *C. parapsilosis* (FAC) tended to be stable, while the temperature in the aerobic fermentation increased at the beginning of the fermentation.

Based on Figure 2, the temperature of both anaerobic and aerobic fermentation is not affected by ambient temperature. This is because the anaerobic fermentation is carried out in a heat-resistant reactor, while the aerobic fermentation is carried out in a reactor covered with styrofoam and glass wool. In aerobic fermentation, there is an increase in temperature in the first 24 hours of the fermentation process. This is because the release of mucus and pulp that is still attached to the coffee beans under aerobic conditions is faster than under anaerobic conditions where the mucus and pulp of coffee beans are still present in the reactor. Aerobic conditions at the beginning of fermentation cause an increase in temperature in the seeds and accelerate the formation of acetic acid which is more volatile when dried so that the acid content in the seeds will be reduced [15].

In aerobic fermentation, aeration is also carried out to supply oxygen. After stirring (aeration) the temperature decreases because the hot air produced during fermentation will be replaced with new air. The decrease in temperature can also be caused by microorganisms that have reached the peak of growth, resulting in a decrease in activity because the sugar content in the mucilage of coffee beans has been widely used at the beginning of fermentation [16].

The average temperature of aerobic fermentation is higher than the average temperature of anaerobic fermentation. In aerobic fermentation, the average fermentation temperature was 36.26 ± 0.70 °C with the maximum temperature reaching 43.75 ± 1.61 °C. In anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis*, respectively, the maximum temperatures achieved were 30.68 ± 1.12 °C, 29.27 ± 0.06 °C, and 30.37 ± 0.29 .

The temperature of aerobic fermentation is higher than that of anaerobic fermentation. This can be caused by the anaerobic fermentation process carried out in the condition of the material submerged in water. Anaerobic fermentation produces heat of 185 kJ/mol glucose, while in aerobic fermentation it produces heat of 2.872 kJ/mol glucose [17]. Although the anaerobic fermentation process produces heat, the heat may not be high enough to raise the temperature of the water used for soaking the coffee because the specific heat of water is quite high, namely 75.6 kJ/mol of water. As a result, the heat generated in the anaerobic fermentation process only increases the water temperature (very small) because the heat is not enough to increase the temperature of the material as well as the temperature of the water. This study is in line with that conducted by [18] which states that aerobic fermentation can produce temperatures reaching 40–45 °C. This study is also in line with [19], which states that the temperature in anaerobic fermentation ranges from 28–31 °C. According to [20], the optimum temperature for yeast growth ranged from 20–30 °C so that the temperatures during anaerobic fermentation, anaerobic with *H. uvarum* and anaerobic with *C. parapsilosis* obtained were suitable as conditions for yeast growth. From the analysis of variance, the value of sig. <0.05 was obtained, so it can be concluded that there is a significant difference between the fermentation treatment and the average temperature during the fermentation process.

3.2. Water Content

In this study, the water content is expressed on a wet basis. Changes in water content during 72 hours of fermentation at intervals of 24 hours can be seen in Figure 3. The water content during the anaerobic (FAA) fermentation process, the anaerobic with *H. uvarum* (FAH), and the anaerobic with *C. parapsilosis* (FAC) tended to increase slightly, while the water content in the aerobic fermentation (FA) tended to decrease. The increase in water content in anaerobic fermentation was caused by the addition of aquadest in the reactor so that water was absorbed into the coffee beans, while in aerobic fermentation no aquadest was added. Aerobic fermentation carried out in dry conditions will reduce the water content in coffee beans due to evaporation of the water content in the beans.

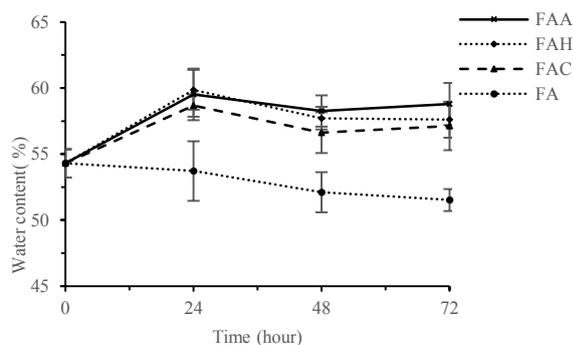


Figure 3 Changes in water content of coffee beans during fermentation

In this study, the water content of coffee beans at the beginning of fermentation was $54.30 \pm 1.07\%$. The water content of coffee beans from anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis* increased at the end of fermentation, while in aerobic fermentation there was a decrease in water content.

Water content at the end of anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis* showed similar results, which was between $57.13 \pm 1.84\%$ to $58.79 \pm 1.59\%$. In aerobic fermentation, the lowest final moisture content value was $51.52 \pm 0.84\%$. From the analysis of variance, the value of $\text{sig.} < 0.05$ was obtained, so it could be concluded that there was a significant difference between the fermentation treatment and the final moisture content of the fermentation process. For the treatment of anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis* did not show any significant difference.

The water content of fermented coffee beans, which is quite high, needs to be reduced through a drying process carried out after the mucus on the coffee beans is removed through the washing process. The drying process is carried out to reduce the water content in coffee beans so as to reduce the potential for fungal growth that can reduce the quality of coffee beans [21].

Water content is one of the conditions used to determine the quality of coffee beans. Water content that exceeds the standard indicates that the coffee beans do not meet the eligibility standards. The average moisture content of anaerobic and aerobic fermented coffee beans at the end of drying ranged from $9.36 \pm 0.42\%$ to $10.69 \pm 0.93\%$. This showed that the average water content of all fermented coffee beans has met the standard of SNI 01-2907-2008, namely the maximum water content of coffee beans is 12.5% [22].

The drying rate of the total moisture content of all treatments ranged from 1.72 to 1.92%/hour. From the analysis of variance, the value of $\text{sig.} < 0.05$ was obtained for the initial moisture content of drying and the value of

$\text{sig.} > 0.05$ for the final moisture content of drying and drying rate of the total moisture content of the coffee beans. Thus, it was concluded that the initial moisture content of drying showed a significant difference, while the final moisture content of drying and drying rate of total moisture content did not show any significant difference. This can be because the water content at the beginning of drying is still affected by the fermentation carried out, while at the end of drying the water content of the coffee beans must meet SNI standards so that the water content is relatively uniform.

3.3. Acidity Level (pH)

The degree of acidity (pH) of coffee beans is influenced by the fermentation process. According to [23], anaerobic fermentation produces the main product in the form of lactic acid, while aerobic fermentation produces acetic acid. Overall changes in pH during the fermentation process are presented in Figure 3.

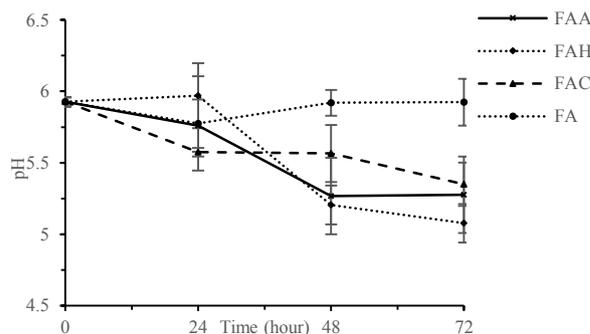


Figure 4 Changes in the pH of coffee beans during fermentation

Based on Figure 4 it is known that the pH in aerobic fermentation (FA) tends to increase, while in anaerobic fermentation (FAA), anaerobic with *H. uvarum* (FAH), and anaerobic with *C. parapsilosis* (FAC) tends to decrease. In aerobic fermentation, the pH tends to rise because the acetic acid formed during the fermentation process easily evaporates when exposed to high temperatures. In anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis*, a decrease in pH can occur due to the accumulation of lactic acid during the fermentation process.

The results of the analysis of variance showed that the coffee fermentation method had a significant effect on the pH of the coffee beans at the end of the fermentation. The difference is influenced by the treatment between aerobic and anaerobic. For the treatment of anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis* did not show any significant difference.

The pH of fermented coffee beans is lower than the pH of unfermented coffee beans (UF) both before and after roasting. The results of this study are in line with the

research of [24], which states that the fermentation process using yeast can reduce the pH value of coffee beans, reaching 5.25-5.37 at the end of fermentation. This study is also in line with research conducted by [25], which states that the use of starter culture can lower the pH more than the natural starter in the material, from 6.90 to 4.09. Based on the analysis of variance, the value of $\text{sig.} < 0.05$ was obtained for the pH of coffee beans before and after roasting. Thus, it can be concluded that there is a significant difference between coffee beans treated with fermentation and without fermentation on the pH value of coffee beans before and after roasting. This can be due to differences in compounds in the coffee beans.

3.4. Total Soluble Solids

The total soluble solids ($^{\circ}$ Brix) test was only carried out in anaerobic fermentation. This is because in aerobic fermentation there is no liquid medium (fermentation is carried out in dry conditions). Total soluble solids tended to increase at 24 hours of fermentation. After 24 hours, the total soluble solids tended to decrease Figure 5.

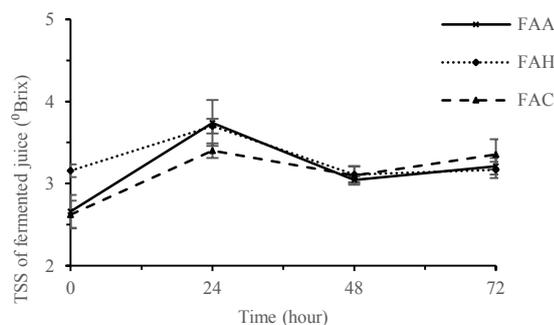


Figure 5 Total soluble solid of fermented juice changes during the fermentation process

The decrease in total soluble solids in anaerobic fermentation (FAA), anaerobes with *H. uvarum* (FAH), and anaerobes with *C. parapsilosis* (FAC) could be caused by a decrease in sugar content in fermented water. The sugar content in the fermented water is influenced by the mucus in the coffee beans. Coffee bean mucus contains sugars that are used by microorganisms to break down complex molecules into simpler ones. The longer the fermentation process, the total dissolved solids will also decrease due to the reduced sugar content in the fermented water [26].

The mean of TSS at the end of the fermentation showed a value that was not much different. The analysis of variance carried out showed that the value of $\text{sig.} > 0.05$ so that it can be concluded that there was no significant difference between the fermentation treatment with TSS at the end of the fermentation. The results obtained are in line with research conducted by [19], which stated that the use of yeast had no significant effect on the total dissolved solids in the fermented water with an TSS value of approximately 2.5 at the end of fermentation.

3.5. Coffee Beans Color

Coffee beans color include lightness, chroma, and hue angle. The values of lightness, chroma, and hue angle at the beginning of fermentation were 38.06 ± 5.80 , respectively; 21.56 ± 1.32 ; and 90.73 ± 9.44 . The lightness value tends to decrease slightly at the end of the fermentation as shown in Figure 6. The chroma value tends to increase at the end of the fermentation as shown in Figure 7. The value of hue angle in aerobic fermentation (FA) tends to decrease, while in anaerobic fermentation (FAA), anaerobic with *H. uvarum* (FAH), and anaerobic with *C. parapsilosis* (FAC) tends to increase at the end of fermentation as shown in Figure 8.

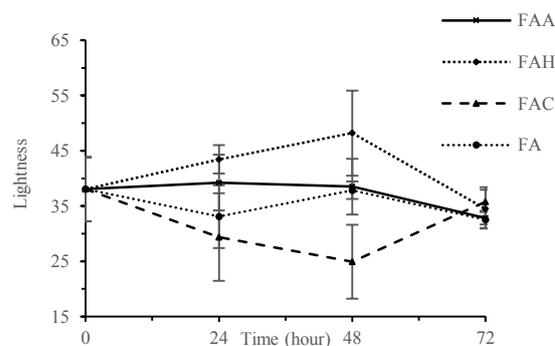


Figure 6 Lightness change during fermentation

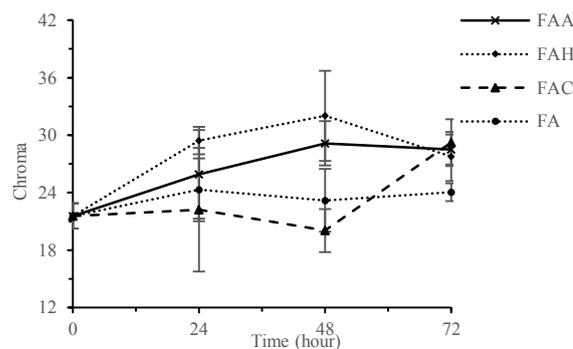


Figure 7 Chroma change during fermentation

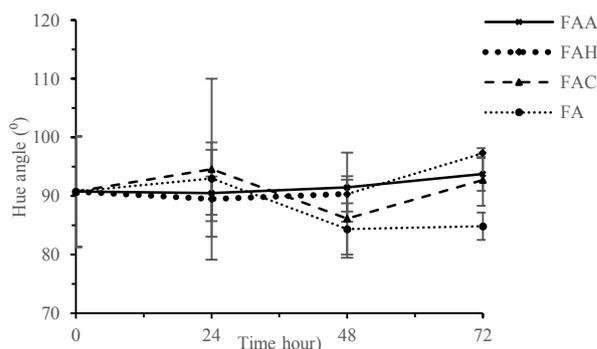


Figure 8 Hue angle change during fermentation

The lightness of the coffee beans at the end of the fermentation did not show any significant difference, while the chroma value increased and the hue angle value

decreased at the end of the fermentation. Based on the analysis of variance carried out on the lightness value, the sig.> 0.05 value was obtained which indicated that there was no significant difference between the fermentation treatment and the lightness of the coffee beans at the end of the fermentation process. However, in the analysis of variance for the chroma and hue angle values obtained sig.<0.05 which indicates that there is a significant difference in the treatment of the results of chroma and hue angle at the end of fermentation. The difference is in the form of treatment differences between aerobic and anaerobic. For the treatment of anaerobic fermentation, anaerobic with *H. uvarum*, and anaerobic with *C. parapsilosis* did not show any significant difference.

4. CONCLUSION

The addition of pure culture yeast in the fermentation process affected coffee beans' properties, such as temperature, moisture content, acidity level, and total soluble solid (TSS) of the fermented juices. The lightness of coffee beans did not have significant differences during the fermentation, while the chroma and hue angle have differences. This indicates that there were color differences between coffee beans with different fermentation methods during the fermentation process. The characteristics of coffee beans during fermentation may affect the final taste and aroma of the coffee bean. However, further research is needed to understand the aromatic compound formed during fermentation.

AUTHORS' CONTRIBUTIONS

Siti Mariyam is responsible for experimental design and analysis. Joko Nugroho is responsible for direction for the experiment and analysis. Rani Juni W and Anisa Kistanti are responsible for data collection and analysis. Hanim Zuhrotul Amanah contributed to the writing style advisory.

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