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Improving the Taste of Robusta Coffee by Fermentation with Yeast Inoculum and Its Effect on Caffeine Content

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ABSTRACT

Harvesting coffee process by farmers generally executed entirety, resulting rainbow harvest. Coffee fermentation with yeast starter cultures Saccharomyces cerevisiae Y612, Candida parapsilosis Y207 and Torulospora delbrueckii Y594 was executed separately to determine the role of starter cultures on caffeine and robusta coffee taste at different maturity levels. The study was conducted at the Indonesian Industrial and Beverage Crops Research Institute in Sukabumi,, from June to November 2022. The experiment used a factorial complete randomized design. The first factor was the maturity level of the coffee and starter culture as the second factor. Fermentation was implemented for 48 hours inoculated with 108 cells/mL starter culture. The results showed that the temperature fluctuated, the pH value always decreased to 4.50 and T. delbrueckii was the starter culture with the highest activity during fermentation. Inoculum-fermented robusta coffee caffeine content was higher than non-inoculum. The lowest caffeine content was found in spontaneously fermented red fruit of 1.39%, while the highest caffeine content was produced by red fruit samples inoculated with C. parapsilosis of 2.7%. Robusta coffee with S. cerevisiae inoculation brought the best taste of robusta coffee with 82.10%, there was no significant difference between the red harvest coffee fruit and the fermented rainbow color with a starter culture.

1. INTRODUCTION

Coffee plants have become one of the leading plantation crops produced and traded globally. Indonesia is ranked fourth in the world in terms of coffee production levels. Robusta coffee is known to be Indonesia's favorite commodity because of its high productivity compared to Arabica coffee. The level of Robusta coffee production in Indonesia reached 95.60%, supported by the plantation area which reached 81.96% with an average area of 1.04 million hectares (Martauli, 2018). The high level of Robusta coffee productivity indirectly influences the level of coffee consumption in Indonesia, which is dominated by Robusta coffee, which is around 82.49%.

Robusta coffee has a characteristically more intense taste, with higher caffeine and a lower acidity level than Arabica coffee. Good coffee taste starts from the harvest process. According to Yusianto & Nugroho (2014) there are two ways to harvest coffee fruit, namely selective red harvest and strip harvest or simultaneous harvest (rainbow colors). Although red selective harvesting produces the best quality and taste of brewed coffee if processed using proper processing and promises a better selling price, farmers still use strip harvesting (Puslitkoka, 2021). Apart from red-

picked fruit, what determines the quality of the taste of coffee is wet processing with fermentation to reduce the mucilage layer on the coffee beans (Thalia *et al*., 2020).

Robusta coffee is known for its high caffeine content, around 1.2 – 2.7%, while the caffeine content in Arabica coffee is only around 0.9 – 1.5% (Wang & Lim 2015). Caffeine levels contribute around 10 – 30% to create a bitter taste in coffee, and is one of the reasons why Robusta coffee is better known for having a bitter taste (Sinaga *et al*., 2021). Caffeine in coffee also has different levels depending on the level of maturity of the coffee used in the coffee processing process (Latunra *et al*., 2021). Different levels of caffeine at coffee maturity levels are related to the formation of metabolites to reach coffee maturity levels. Caffeine in coffee berries naturally functions as self-defense against insects or pests (Olechno *et al*., 2021).

Caffeine is a group of alkaloids derived from xanthine which is obtained from secondary metabolite processes. The process of forming caffeine comes from breaking down xanthosine compounds with several enzymes to become caffeine. Coffee is known to have caffeine which is sought by coffee consumers to relieve drowsiness, increase focus or as a trigger for muscle performance, but excessive caffeine consumption also has an impact on health such as heart health, blood pressure problems and insomnia (Latunra *et al*., 2021). The process of reducing caffeine levels or decaffeination is a useful way for consumers to continue consuming coffee without worrying about these impacts (Larassati *et al*., 2021). The decaffeination method basically softens the compound bonds in the coffee beans so that the caffeine can be easily separated from the coffee. One of these methods is soaking, evaporating coffee beans as a residue cleaner, drying coffee beans, extraction with subcritical carbon dioxide, using enzymes or certain starter culture microorganisms in the fermentation process, and decaffeination by extraction using polar solvents (Daisa *et al*., 2017; Kartasasmita & Addyantina, 2012).

The coffee processing process plays a role in determining caffeine levels, because during the coffee processing process, especially coffee fermentation, there is some compound degradation caused by microorganisms present during fermentation. Changes in caffeine levels may occur during the coffee fermentation process, namely the change in caffeine into ester compounds (Larassati *et al*., 2021). Reducing caffeine levels through fermentation is more natural, so it is hoped that it will not reduce the taste of the brew. Coffee fermentation using yeast starter culture affected the caffeine content of the Arabica coffee produced Bressani *et al*. (2018). The aim of this research is to find the type of yeast starter culture that has an effect on improving the taste of robusta coffee in different types of coffee harvest and its effect on the caffeine content of coffee.

2. METHODOLOGY AND METHODS

2.1. Materials and Research Design

The research was carried out from June to November 2022 at the Integrated Laboratory and Bioindustry belonging to the Balai Penelitan Tanaman Industri dan Penyegar (BALITTRI), Sukabumi. The material used was robusta coffee beans (*Coffea canephora*) harvested from the Pakuwon Experimental Garden, BALITTRI, Sukabumi. Inoculum *S. cerevisiae* Y612 (S1), *C. parapsilosis* Y207 (S2), *T. delbrueckii* Y594 (S3) was obtained from the Indonesia Culture Collection (InaCC). Inoculum growth media involved YPG (yeast, peptone, glucose) and YPGA (yeast, peptone, glucose, agar) with a composition of yeast 10 g/L, peptone 20 g/L, glucose 20 g/L, agar 20 g/L. Other chemicals included sodium chloride (NaCl 0.85%), calcium carbonate ($CaCO_3$), chloroform ($CHCl_3$), and caffeine standards.

The experimental design of this research used a factorial Completely Randomized Design (CRD) with 2 factors. The first factor was the level of maturity (K) of the coffee berries included red coffee fruit (K1) and rainbow coffee fruit (K2). The second factor was the type of starter culture (S), namely *S. cerevisiae* Y612 (S1), *C. parapsilosis* Y207 (S2), and *T. delbrueckii* Y594 (S3). The data obtained were analyzed using two-way Analysis of Variance (ANOVA) with a significance level expressed as P value < 0.05. Tukey's further test with a 95% confidence level was carried out if the results have a significant effect.

2.2. Preparation of Culture Media

The yeast inoculum of *S. cerevisiae* Y612, *C. parapsilosis* Y207 and *T. delbrueckii* Y594 which will be inoculated on coffee was first activated using YPG media. Each cell was inoculated with 10 mL of YPG medium for 24 hours at room temperature at a speed of 120 rpm. The media was then transferred to 90 mL of YPG media to be incubated again under the same conditions. The starter culture was grown until a number of around 108 cells/mL which was

inoculated into coffee fermentation. Cells were harvested by centrifuging the media at 3000 rpm for 20 min. The supernatant-free cells were suspended with NaCL (0.85%) for further use as a medium for coffee fermentation treatment.

2.3. Coffee Fermentation Using Yeast Culture

The harvested coffee was sorted to separate red coffee berries and rainbow coffee berries as research treatments. Fermentation was carried out using the wet method for 48 h by inoculating each starter culture into the coffee fermentation process. As much as 5 kg of wet coffee beans were fermented in a plastic container, starter culture with a number of around 108 cells/mL was inoculated into the coffee fermentation, non-inoculum fermentation was used as a control for red coffee and rainbow coffee. Observations on the characteristics of fermentation process including temperature, pH, and enumeration of microbial populations were carried out every 12 h. The fermented coffee was then washed and dried in the sun until it reaches a water content of around 11-12%. The dried coffee beans were then proceeded to the hulling stage to remove the horny skin. The coffee beans were roasted at a medium level at a temperature of 200 °C to obtain roasted coffee beans which were used to measure caffeine levels and analyze the quality of coffee taste.

2.4. Calculation of the Number of Starter Culture Colonies

Calculation of colonies was carried out at fermentation time intervals every 12 h. A wet coffee sample of 1 g was taken at this time interval, then mixed with 9 mL of 0.85% NaCl. Calculations were carried out by serial dilution, up to 10 times, using YPGA media with the spread plate method. Cultures were incubated at 30 °C for 48 h. The number of countable colonies was selected from the petri containing the first 30-300 colonies of each dilution series.

2.5. Measurement of Caffeine Content

The first process to measure caffeine levels was to extract the caffeine from the coffee beans. Roasted coffee powder of 1 g was brewed with 150 mL of water for 5 min, then the solution was filtered to get dreg-free coffee solution. Calcium carbonate ($CaCO_3$) 1.5 g was added to the coffee solution, stirred periodically until the two materials were completely mixed, then put into a separating funnel. Chloroform (25 mL) was added to the coffee solution in a separating funnel to extract caffeine. The bottom layer of the solution was separated as the solution containing caffeine. The addition of chloroform and the process of taking caffeine extract was carried out 3 times. The solution containing caffeine was evaporated using an evaporator until the solution evaporates and leaves the caffeine extract. Caffeine extract was taken by dissolving it with 50 mL of distilled water in a volumetric flask.

The next step was preparing a standard caffeine solution which was carried out before testing the caffeine on the sample. Standard caffeine of 100 mg was dissolved in 100 mL of distilled water in a 100 mL measuring flask, to obtain a stock solution with a concentration of 1000 ppm. As much as 10 mL of the mother solution (1000 ppm) was put into a 100 mL measuring flask and then diluted with distilled water to the limit mark, to obtain a solution with a concentration of 100 ppm.

Standard solutions were made by taking 0; 0.5; 1; 2; 3; 4; 5 mL of 100 ppm caffeine solution into a 50 mL volumetric flask. Modified from Latunra *et al*. (2021), each solution was diluted to the limit mark, so that the standard solutions had concentration of at 0, 1, 2, 4, 6, 8, 10 ppm. Distilled water was used as a blank in determining caffeine standard and in testing caffeine samples. Determination of the wavelength was carried out by detecting standard absorbance at a wavelength of 272 nm using a Thermo Scientific Genesys 10S UV-Vis spectrophotometer with a single beam type. A standard curve was created by relating the absorbance to the concentration of each standard. Measuring the caffeine content of coffee samples was carried out using the same steps as measuring standard caffeine. The caffeine content of each sample was measured at a wavelength of 272 nm.

2.6. Coffee Taste Assessment

The taste of Robusta coffee was assessed using several attributes including fragrance, flavor, aftertaste, salt/acid, bitter/sweet, balance, mouthfeel, uniformity, clean cup, and overall. The fragrance attribute of coffee is how the aroma of coffee is perceived by the sense of smell which was assessed for dry coffee (before brewing) and wet coffee (after brewing). Flavor is a coffee attribute that was assessed by the sense of taste, regarding the taste characteristics of the coffee after sipping it. The aftertaste attribute is an assessment of coffee attributes by describing the part of the coffee that remains after the coffee drink is swallowed. Salt/acid is an attribute of robusta coffee that expresses the level of

salinity, namely the sensation of salt in the coffee, while bitter/sweet is the bitter or sweet impression created in the coffee. Attribute balance is about the overall balance of coffee attribute assessments from several coffee samples tested. Mouthfeel is assessed using two aspects, namely the weight of the coffee drink and the texture of the coffee. Uniformity is an assessment of the uniformity of each sample cup of coffee. The more uniform each cup of coffee is, the higher the score obtained. The clean cup attribute is a coffee assessment regarding the negative impressions that may be found in the coffee when sipped. Overall is the total assessment felt by the panelists towards coffee drinks.

Coffee taste assessment was carried out in four stages. The first stage in the fragrance attribute was to smell the aroma of the coffee before brewing, then the coffee is brewed to smell the aroma 3 min after brewing. The second stage was carried out on the flavor, aftertaste, salt/acid, bitter/sweet and mouthfeel attributes by sipping and drinking the coffee drink after the coffee had been left to rest for 8-10 min and then assessing each of these attributes. The third stage of assessment was carried out on the balance, uniform, and clean cup attributes by drinking coffee that had reached a temperature of 37 °C. The final assessment was carried out on the overall attribute and total score which was carried out at a coffee temperature of around 16°C. The scale for assessing the taste of robusta coffee is: 6 ≤ 6.75 = good; 7 ≤ 7.75 = very good; 8 ≤ 8.75 = excellent; 9 ≤ 9.75 = excellent. The robusta coffee taste assessment was carried out by 15 panelists trained in the field of coffee.

3. RESULTS AND DISCUSSION

3.1. Coffee Fermentation Characteristics

Temperature and pH measurements are carried out during the fermentation process to observe the characteristics of coffee fermentation. Temperature changes during coffee fermentation are presented graphically in Figure 1. Both types of coffee samples show temperature changes with similar patterns. The temperature for the first 12h of fermentation starts from 24.67°C which is obtained from the average temperature of the K1S3 sample, then the temperature increases after the next 12 h. The temperature decreases at the 36th h and and then increase at the end of fermentation. The coffee fermentation treatment in this study did not affect significantly on temperature changes during the fermentation process. Environmental temperature has a greater influence on temperature changes during fermentation, seen from the time when the temperature measurement takes place.

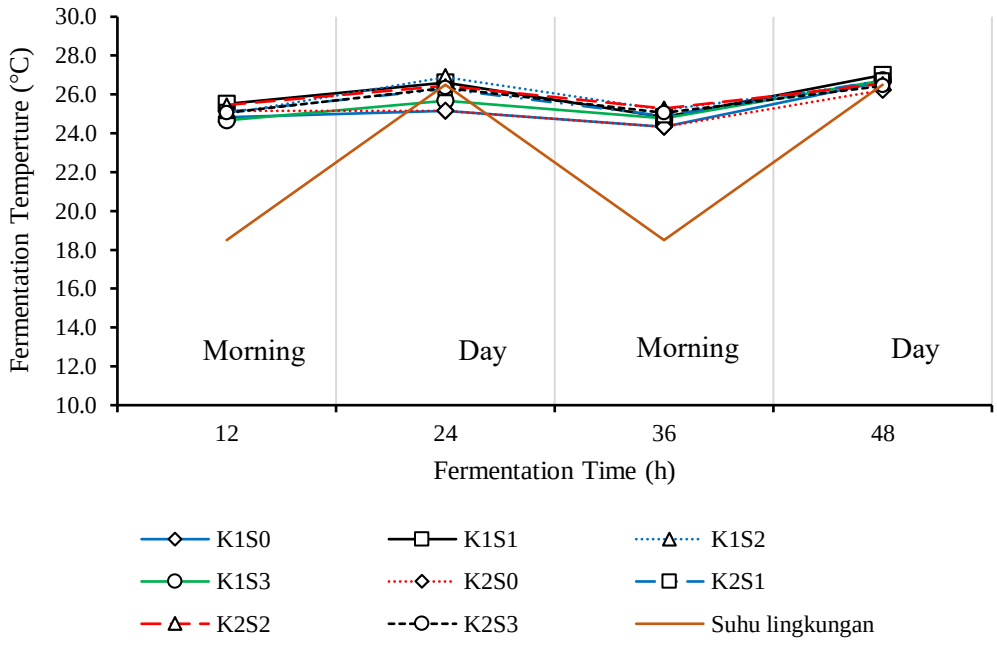

Figure 1. Temperature changes during fermentation

Temperature fluctuations during coffee fermentation are influenced by the environmental temperature at the time the measurements take place. The lowest environmental temperature with an average of 18.5°C was recorded at the 12th and 36th h of fermentation in the morning and the highest with an average of 26.5°C was recorded at the 24th and 48th h of fermentation in the midday. Environmental factors are the main intervention in temperature changes during fermentation, followed by the presence of humidity (Evangelista *et al*., 2015). Apart from that, fermentation microbial metabolism also plays a role in temperature changes (Bressani *et al*., 2018).

These temperature changes are known to be due to the influence of environmental temperature during fermentation, however, the type of starter culture and different types of coffee maturity samples play a role in influencing the fermentation temperature. The temperature measured at the first hour of fermentation (12th h) was influenced by both K (maturity level) and S (starter type) treatments as well as their interaction. This appears from the initial stage of fermentation which was quickly influenced by these two treatments. The S treatment plays a major role in influencing the temperature increase at the 24th h, as well as the interaction of the K and S treatments. These results show that the longer the fermentation process take places, the use of starter culture shows its influence on the fermentation temperature which can be observed from the fermentation temperature at 36 and 48 h. This influence is followed by the interaction of K and S treatments, but not with the influence of K treatment. The type of K treatment is not different between red harvest coffee and rainbow coffee at fermentation temperature, it is suspected that rainbow coffee still has a few percent of red coffee so this is not different away from red harvest coffee.

The fermentation process of coffee berries actually takes place at a low pH of around 4–5.5 and consistently decreases during the fermentation process (Lee *et al*., 2015). The type of starter culture treatment had a significant effect on the pH value at the 24th and 48th h ($P<0.05$ with Tukey's test). The pH value of the first 12 h of fermentation process started from 5.74 – 6.05, the lowest is in the K1S3 sample and the highest is in the K2S0 sample (Table 1). The consistently decreasing pH value continues until the fermentation time ends. The lowest final pH value of the fermentation of coffee berries was 4.24 in sample K1S2 and 4.67 was the highest value found in sample K2S0.

The use of starter culture in coffee fermentation influences the pH in the 12 and 48 h fermentation process. Starter cultures or fermentation microorganisms work with their metabolic processes which can produce acidic secondary metabolites and the breakdown of the mucus layer of coffee berries which produces organic acids, so that as the fermentation process takes longer the pH will decrease (de Melo Pereira *et al*., 2016; Larassati *et al*., 2021). These results show that starter type (S) is more influential than the maturity (K) of coffee beans or the interaction of the two factors. Coffee berries harvested selectively with red beans or rainbow conditions have the same effect on changes in pH during fermentation.

The number of microorganisms present in the coffee fermentation process continues to increase as the duration of fermentation increases. The results of calculating the growth activity of each type of starter culture are presented in Table 2, which shows the growth of the starter culture colony during the coffee fermentation process. The number of cell colonies in the first 12 h ranged from 5 to 7 log CFU/mL. Sample K1S3 had the highest number of log CFU/mL until the end of the coffee fermentation period. The highest number of colonies at the 12th, 24th, 36th and 48th hour was 7.47; 7.92; 8.45; and 8.89 log CFU/mL, respectively all obtained from the K1S3 sample. The lowest amount of starter

Table 1. pH values during fermentation of Robusta coffee

Sample	pH			
	12 h	24 h	36 h	48 h
Red coffee berries (K1)				
Spontan (K1S0)	5.87^{a} ± 0.04	5.49^{ab} ± 0.13	5.10^{a} ± 0.21	4.62^{a} ± 0.26
S. cerevisiare (K1S1)	5.81^{a} ± 0.14	5.36^{ab} ± 0.17	4.97^{a} ± 0.16	4.40^{a} ± 0.12
C. parapsilosis (K1S2)	5.78^{a} ± 0.26	5.13^{b} ± 0.19	4.69^{a} ± 0.24	4.24a ± 0.03
T. delbrueckii (K1S3)	5.74^{a} ± 0.23	5.19^{ab} ± 0.12	4.87^{a} ± 0.21	4.36^{a} ± 0.08
Rainbow coffee berries (K2)				
Spontan (K2S0)	6.05^{a} ± 0.12	5.60^{a} ± 0.15	5.19^{a} ± 0.16	4.67^{a} ± 0.22
S. cerevisiare (K2S1)	5.96^{a} ± 0.14	5.42^{ab} ± 0.09	5.07^{a} ± 0.25	4.44^{a} ± 0.20
C. parapsilosis (K2S2)	5.84^{a} ± 0.33	5.41^{ab} ± 0.12	5.04^{a} ± 0.23	4.33^{a} ± 0.08
T. delbrueckii (K2S3)	5.93^{a} ± 0.21	5.43^{ab} ± 0.09	5.00^{a} ± 0.23	4.54^{a} ± 0.16

Note: The number after the ± sign is the standard deviation. Different letters indicate significant effects ($p < 0.05$) in Tukey's test.

Table 2. Growth of starter culture colonies during coffee fermentation

Sample	Log CFU/mL			
	12 h	24 h	36 h	48 h
Red coffee berries (K1)				
Spontan (K1S0)	5.45 ± 0.11 ^{d}	6.52 ± 0.11 ^{cd}	7.26 ± 0.08 ^{de}	7.36 ± 0.11 ^{de}
S. cerevisiare (K1S1)	6.70 ± 0.15 ^{b}	7.20 ± 0.13 ^{bc}	8.09 ± 0.21 ^{abc}	8.52 ± 0.25 ^{ab}
C. parapsilosis (K1S2)	6.92 ± 0.18 ^{b}	7.56 ± 0.06 ^{ab}	8.29 ± 0.19 ^{ab}	8.34 ± 0.17 ^{abc}
T. delbrueckii (K1S3)	7.47 ± 0.16 ^{a}	7.92 ± 0.19 ^{a}	8.45 ± 0.16 ^{a}	8.89 ± 0.24 ^{a}
Rainbow coffee berries (K2)				
Spontan (K2S0)	5.43 ± 0.28 ^{d}	6.31 ± 0.52 ^{d}	7.09 ± 0.31 ^{e}	7.22 ± 0.40 ^{e}
S. cerevisiare (K2S1)	6.12 ± 0.16 ^{c}	6.79 ± 0.14 ^{cd}	7.68 ± 0.14 ^{cd}	7.95 ± 0.15 ^{bcd}
C. parapsilosis (K2S2)	6.56 ± 0.10 ^{bc}	7.13 ± 0.24 ^{bc}	7.80 ± 0.11 ^{bcd}	7.74 ± 0.14 ^{cde}
T. delbrueckii (K2S3)	6.90 ± 0.21 ^{b}	7.59 ± 0.27 ^{ab}	8.18 ± 0.24 ^{abc}	8.36 ± 0.23 ^{abc}

Note: The number after the ± sign is the standard deviation. Different letters indicate significant effects ($p < 0.05$) in Tukey's test.

culture was consistently obtained from coffee fruit samples that fermented spontaneously (without starter culture) from the 12th to the 48th hour. The growth of microbial colonies during spontaneous fermentation is influenced by the presence of the initial number of microbial cells from the time fermentation begins (Bressani *et al*., 2018).

The greater number of log CFU/mL found in red coffee fruit is due to the composition of nutritional sources for microbes being more complete (Elhalis *et al*., 2020; Silva *et al*., 2013). There are more polysaccharide and sucrose components in red coffee fruit than rainbow coffee fruit (Bastian *et al*., 2021). Rainbow coffee berries are half-ripe coffee, which tend to have a lower sugar component, which causes lower microbial growth in the coffee samples.

Each fermentation treatment factor, namely K and S, was found to have a significant influence on the growth of microorganisms during the coffee fermentation process. The effect of treatment K was due to the difference in composition between red harvested coffee and rainbow harvested coffee. Meanwhile, treatment S had an effect because of the different type of starter culture used, with the species *T. delbrueckii* obtained the highest quantity. The effect of this treatment was found in each microorganism count which was carried out every 12 h for 48 h of fermentation, but no effect was found from the interaction of K and S treatment factors on the number of microorganisms during fermentation.

3.2. Caffeine Content

Calculation of the caffeine content of coffee beans was carried out using a Thermo Scientific Genesys 10S UV-Vis spectrophotometer at a wavelength of 272 nm (Supriyanti *et al*., 2018). The caffeine content of coffee beans obtained from robusta coffee fermentation ranges from 1.39% to 2.70% (Figure 2). The caffeine content of coffee berries fermented spontaneously (no inoculum), both red and rainbow coffee beans, has a value below that of coffee samples with added inoculum. The highest caffeine was obtained from the K1S2 coffee sample at 2.70%, followed by the K1S1 coffee sample at 2.54%. Coffee with *T. delbrueckii* Y594 inoculum had a caffeine content of 2.24 ± 0.05% which was obtained from rainbow coffee berries. Red coffee fruit with a spontaneous fermentation process is the sample that has the lowest caffeine content, namely 1.39 ± 0.24%.

Caffeine found from spontaneous fermentation showed the highest results in rainbow coffee berries and the lowest in red coffee berries. The high level of caffeine in spontaneously fermented rainbow coffee is due to the secondary metabolite process being more active because it is growing towards maturity so that the formation of caffeine compounds is also involved in this process (Latunra *et al*., 2021). While, red coffee berries with low levels of caffeine is thought to have secondary metabolite activity was lower because the growth focus on reaching the maturity level had passed. Based on analysis of variation (ANOVA) this result did not cause any significant influence on caffeine content due to differences in coffee maturity.

A significant effect was obtained from the type of starter culture (S) used in fermentation on the caffeine content, where spontaneously fermented coffee had different caffeine levels to the results of coffee fermented with starter culture. The high caffeine in robusta coffee is also caused by the dominant expression of certain genes, such as CaXMT1,

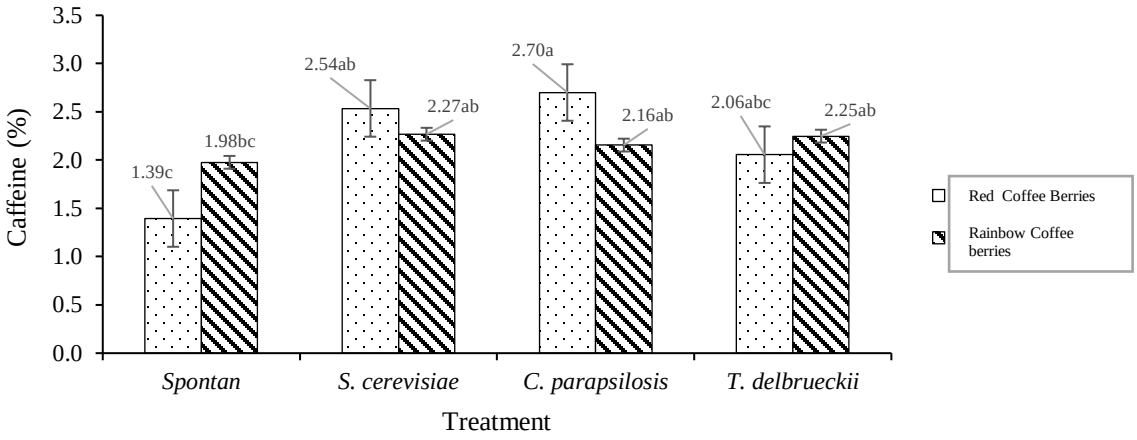

Figure 2. Effect of coffee maturity level and starter culture on robusta coffee caffeine content

CaMXMT1 and CaDXMT2 (Olechno *et al*., 2021), which are difficult to degrade simply by the fermentation process with starter culture inoculation. Caffeine itself is formed from the degradation of xanthosine into caffeine with the help of several enzymes that degrade this compound, such as 7-methylxanthosine synthase, 7-methylxanthine nucleosidase, and caffeine synthase (Jeszka-Skowron *et al*., 2016).

The use of yeast in fermentation in research by Bressani *et al*., (2018) found that caffeine levels did not decrease in Arabica coffee. The low interval of caffeine levels in Arabica coffee is supposed as one reason. Coffee fermented with starter culture isolates, both red and rainbow, in this study turned out to have similar results, caffeine levels did not decrease or were still within the interval of Robusta coffee caffeine levels in two of the three samples. The type of starter culture in this research treatment was found to have the same effect on caffeine levels in Robusta coffee, when compared with Arabica coffee in previous research.

Caffeine does not dissolve easily or its amount is difficult to decrease during the wet fermentation process, this is because caffeine is strongly attached to alkaloid bonds and other coffee components (Wamuyu *et al*., 2017). The caffeine levels in the research samples were still within the Robusta coffee caffeine interval level, possibly because the yeast is not a caffeine-degrading microorganism, this happened both in the Arabica coffee in the study of (Bressani *et al*., 2018) and in the Robusta coffee samples in this study. Types of microorganisms known to degrade caffeine include *Alcaligenes*, *Brevibacterium*, *Klebsiella*, *Pseudomonas*, *Rhodococcus*, *Serratia*, and *Stenotrophomonas* (Arimurti *et al*., 2021; Summers *et al*., 2015). The caffeine found in rainbow harvested robusta coffee is no different from coffee from red harvested coffee. The use of yeast in the coffee fermentation process, however, affects the taste of Robusta coffee compared to spontaneously treated coffee.

3.3. Taste of Robusta Coffee

The assessment of the taste of Robusta coffee is carried out by assessing the attributes including aroma, flavor, after taste, salt/acid, bitter/sweet, balance, mouthfeel, uniform, clean cup, overall, defects, and total score. The quality of the taste of Robusta coffee based on the fragrance/aroma, flavor, after taste attributes is presented in Figure 3. The assessment of the fragrance/aroma attribute in Robusta coffee obtained a score in the very good category with a score of 7.53–7.72 with the highest score obtained in *S. cerevisiae* Y612 (K2S1) fermented rainbow colored coffee sample.

The assessment of the coffee flavor attribute given by the panelists was in the very good category with a score of 7.78–7.47 with the highest score being the fermented red fruit sample *C. parapsilosis* Y207 (K1S2). The assessment score for the coffee aftertaste attribute is in the very good category, ranging from 7.72–7.42 with the highest scores obtained from two samples, namely red fruit fermented using *C. parapsilosis* Y207 (K1S2) and rainbow coffee fermented using *S. cerevisiae* Y612 (K2S1). Aroma, flavor, and aftertaste are coffee attributes that are mostly formed in the coffee roasting process which involves the Maillard reaction. This reaction is responsible for the formation of aroma in coffee because it involves the hydrolysis process of protein and sucrose which are important precursors in the formation of coffee aroma (Lee *et al*., 2015; 2017).

The other attribute assessment, namely salt/acid and bitter/sweet, are presented in Figure 4. The salt/acid and bitter/sweet attributes only found in the Robusta coffee assessment. Salt/acid is responsible for the smooth taste that comes from the acidity and sugar levels in coffee, as well as the low potassium content. Bitter/sweet in Robusta coffee comes from the potassium and caffeine components for the bitter attribute, while sweet comes from the chlorogenic acid and sugar content in the coffee (Bicho *et al*., 2013; Fibrianto *et al*., 2018).

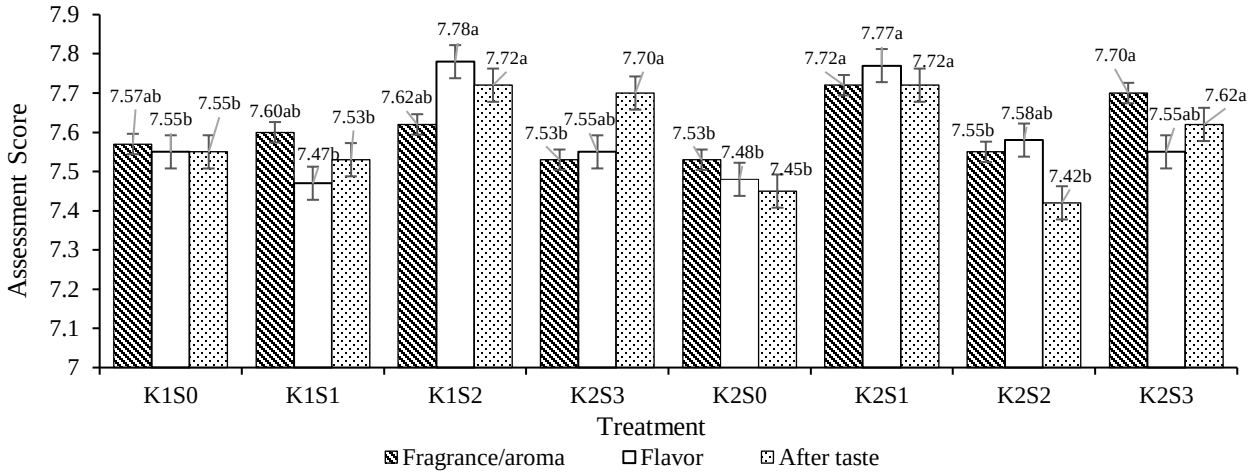

Figure 3. Coffee scoring scores for fragrance/aroma, flavor and after taste attributes

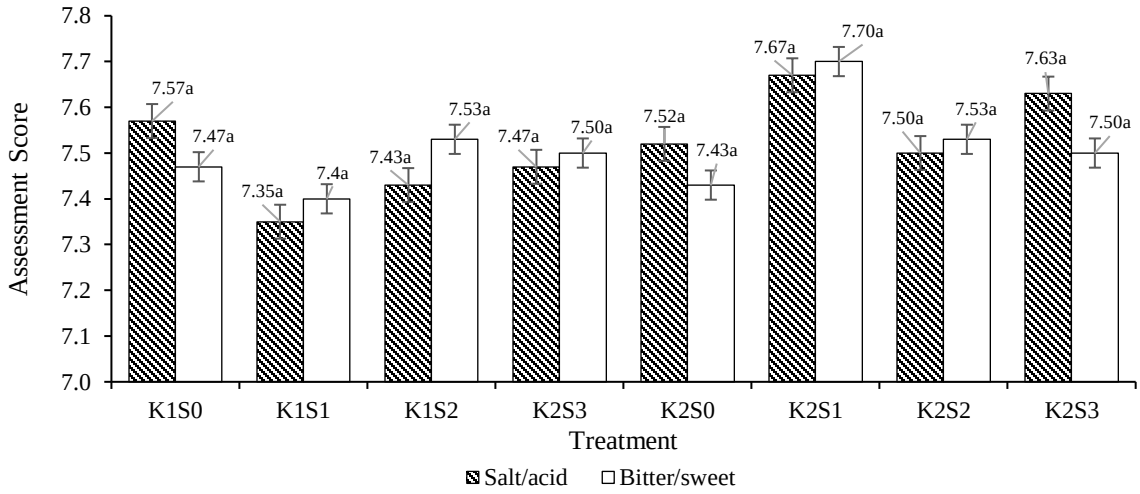

Figure 4. Coffee scoring scores for salt/acid and bitter/sweet attributes

Coffee with starter culture fermentation and wet fermentation methods tends to have a higher acidity level than coffee with spontaneous fermentation (Bressani *et al*., 2020; de Melo Pereira *et al*., 2019). The scores obtained for the two Robusta coffee attributes are in the very good category, with score values ranging from 7.35 to 7.67 for the salt/acid attribute and 7.4–7.7 for the bitter/sweet attribute. The highest score for the two Robusta coffee attributes was obtained for the *S. cerevisiae* Y612 fermented rainbow coffee sample, while the lowest score for the two coffee attributes was obtained for the *S. cerevisiae* Y612 fermented red coffee berries. The interaction of maturity level (K) and starter culture (S) is implied from the results of the ANOVA where no significant effect was found on the two coffee attributes. The type of starter culture (S), plays a role in influencing the composition of the compounds that form these two attributes, due to metabolism carried out by microorganisms which produce acidic products such as chlorogenic acid which can create higher sweet attributes than bitter (Fibrianto *et al*., 2018). The assessment of other attributes include balance, mouthfeel, and overall which can be seen in Figure 5. The assessment for the three attributes are classified in the very good category, with a score of 7.47–7.95 for the balance attribute, 7, 43–8.07 for the mouthfeel attribute, and a score value of 7.47–8.30 for the overall coffee attribute. Balance in coffee is an attribute

that describes the harmony between coffees; in other words, the higher the score, the more harmony is the coffee. The coffee sample with the highest balance score was found in the *S. cerevisiae* Y612 (K2S1) fermented rainbow coffee beans, namely 7.95. The mouthfeel attribute of coffee beans is the sensation felt when drinking coffee, the feeling of how the coffee drink covers the tongue and mouth. The highest score obtained by this attribute was found in the red beans fermented using *S. cerevisiae* Y612 (K1S1) of 8.07. Overall is the overall assessment of what the panelists feel, it can also be said to be the panelists' favorite value for the taste of coffee. This attribute obtained the highest score of 8.30 in the red coffee beans fermented using *S. cerevisiae* Y612.

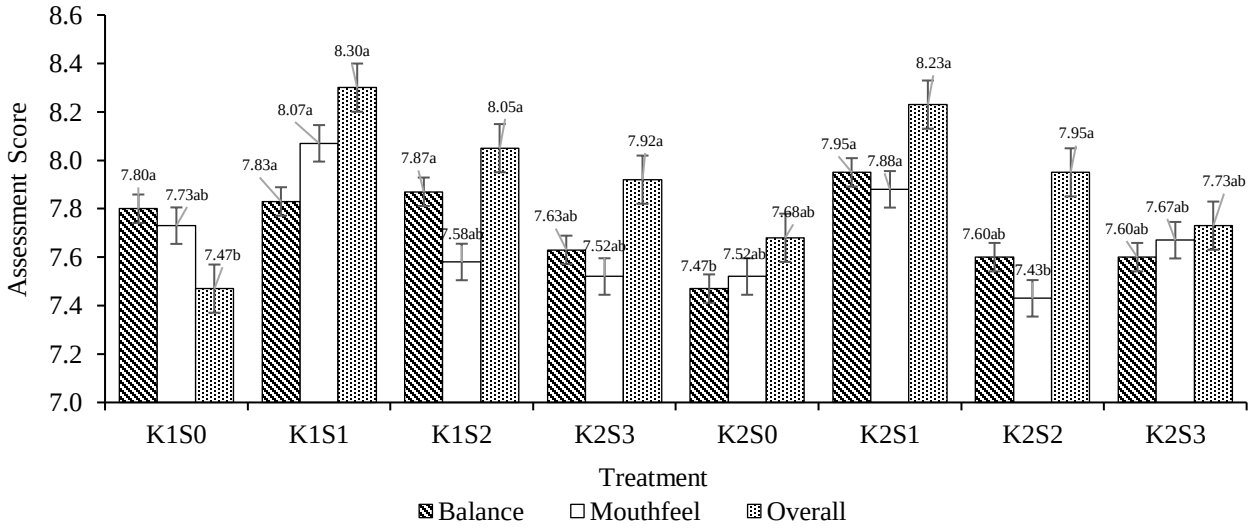

Figure 5. Coffee scoring scores for balance, mouthfeel, and overall attributes

The assessment of coffee taste attributes including the uniform, clean cup, defect, and total score are presented in Table 3. The uniform and clean cup attributes are in the extraordinary category, with uniform scores ranging from 9.73 to 9.87, while the clean cup scores range from 9.60 to 9.87. The defect assessment was not found, which indicates that the coffee does not have any negative disturbances that could interfere with the original taste of the coffee. The overall coffee taste assessment obtained ranged from 79.55 to 82.10. The lowest and highest total scores obtained from coffee berries were respectively found in samples of spontaneously fermented rainbow coffee (K2S0) and *S. cerevisiae* Y612 (K2S1). starter cultures of *S. cerevisiae* Y612 and *C. parapsilosis* Y207 are known to improve coffee quality by providing caramel, spice, and aroma components (Evangelista *et al*., 2015; Silva *et al*., 2013). The scores obtained from the uniform, clean cup, defect, and total score attributes found that there was no significant influence of the treatment. In other words, fermentation treatment using starter culture on red and rainbow coffee berries could cover the differences due to the level of maturity of the coffee beans.

Table 3. Coffee assessment scores for uniform, clean cup, defect, and total score attributes

Sampel	Uniform	Clean cup	Defect	Total score
Red coffee berries				
Spontan	9.73 ± 0.70 [a]	9.60 ± 0.83 [a]	0	80.03 ± 3.53 [a]
S. cerevisiare Y612	9.73 ± 0.70 [a]	9.87 ± 0.52 [a]	0	81.22 ± 2.54 [a]
C. parapsilosis Y207	9.73 ± 0.70 [a]	9.73 ± 0.70 [a]	0	81.05 ± 3.56 [a]
T. delbrueckii Y594	9.87 ± 0.52 [a]	9.60 ± 0.83 [a]	0	80.28 ± 3.07 [a]
Rainbow coffee berries				
Spontan	9.87 ± 0.52 [a]	9.60 ± 0.83 [a]	0	79.55 ± 2.42
S. cerevisiare Y612	9.87 ± 0.52 [a]	9.60 ± 0.83 [a]	0	82.10 ± 2.49
C. parapsilosis Y207	9.87 ± 0.52 [a]	9.73 ± 0.70 [a]	0	80.17 ± 3.09
T. delbrueckii Y594	9.87 ± 0.52 [a]	9.60 ± 0.83 [a]	0	80.47 ± 3.34

Note: The number after the ± sign is the standard deviation. Different letters indicate significant effects ($p < 0.05$) in Tukey's test.

4. CONCLUSIONS

Fermentation of Robusta coffee berries using the yeast starter culture S. cerevisiae Y612 on rainbow harvested coffee beans obtained the highest taste with a total score of 82.10 ± 2.49, while coffee berries fermented spontaneously received the lowest total score when compared with all fermented coffee samples using starter culture. The highest total score value is supported by good coffee attribute assessments, on average the highest score attributes assessment is supported by characteristics during fermentation that are better than spontaneous fermentation. Fermentation of Robusta coffee from different harvesting types and the addition of starter culture did not reduce the caffeine content in the coffee berries. The increase in taste was due to changes in the composition of the components of the fermented coffee beans. The *C. parapsilosis* Y207 inoculum produced the highest caffeine content in red harvested coffee berries (K1S2) at 2.7%, while the lowest was found in spontaneously fermented red harvested coffee samples (K1S0) at 1.39%. Caffeine levels that were still within the Robusta caffeine interval level in samples fermented with starter culture were probably due to the non-caffeine-degrading species microorganisms used in the experiment.

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