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The Effect of Saccharomyces cerevisiae Addition on the Antioxidant Activity of Arabica Coffee Beans (Coffea arabica) using Natural Fermentation Method

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A B S T R A C T

Coffee is not only a regular consumption but can also be said to be part of today's lifestyle. However, despite the popularity of coffee products among the public, many people do not know the contents of coffee which are beneficial for the body. There are many antioxidant compounds in coffee, and antioxidants are an antidote to free radicals, inhibiting oxidation. The post-harvest process of coffee and the fermentation process during post-harvest coffee can affect the content and compounds contained in coffee. Fermentation generally uses bacteria, fungi, and yeast. The type of yeast used in research is Saccharomyces cerevisiae; its metabolic products can influence the antioxidant content of coffee beans. This research aims to determine the effect of Saccharomyces cerevisiae concentration on antioxidant activity in coffee beans. This research treatment consisted of without the adding starter (A), fermentation with a concentration of 1.5% (B), fermentation with a concentration of 2% (C), fermentation with a concentration of 2.5% (D), fermentation with a concentration of 3% (E). The data obtained were analyzed statistically using ANOVA and continued with DNMRT at the 5% level. Chlorogenic acid and caffeine were tested using High-Performance Liquid Chromatography (HPLC) to determine how much of these compounds are contained in the coffee beans. The results of this research show that differences in Saccharomyces cerevisiae concentrations significantly affect the 5% level of the degree of acidity (pH) and antioxidant activity. The best treatment was obtained in treatment E, with a pH value of 5.9 and antioxidant activity of 54.72%.

1. INTRODUCTION

1.1. Research Background

Coffee is one of the longstanding plantation crops that has been cultivated for a considerable time and holds significant economic value. There are four main species of coffee: Arabica, Robusta, Liberica, and Excelsa. Among these, Arabica and Robusta are the most economically valuable and are typically traded commercially [1]. Arabica coffee is renowned for its superior flavor and aroma compared to Robusta. Post-harvest processing is a crucial method for supporting coffee development, as it greatly influences the physical quality, chemical composition, and flavor profile of coffee beans [2].

Coffee processing can also enhance the quality and flavor of coffee. One post-harvest handling method is the fermentation of coffee cherries. Fermentation induces chemical reactions crucial in developing flavor precursors in coffee beans. This process combines biological and chemical activities, where bacteria, fungi, and yeast degrade complex mucilage molecules into simpler compounds, producing liquids and volatile compounds. [3]. Microorganisms such as yeast make the mucus layer a source of nutrients, as it is rich in pectin and sugars [4].

The post-harvest processing process of coffee has been innovative, and few coffee fermentations have been carried out to find new flavors in coffee beans. One of the fermentations in coffee uses the yeast *Saccharomyces cerevisiae*. Fermentation carried out by *Saccharomyces cerevisiae* will produce alcohol. The fermented metabolites will later react with the acid compounds in the coffee beans to affect antioxidant activity.

This researcher used *Saccharomyces cerevisiae* as a fermentation starter because the researcher wanted the results of the yeast metabolite in the form of alcohol that can react with compounds in the coffee beans. Fermented alcohol can react with the organic acids contained in coffee beans to form compounds

that are antioxidants, such as chlorogenic acid. Alcohol can also be converted to acid by other microbes during coffee fermentation. The parameters analyzed were the number of yeast, pH, antioxidant activity, chlorogenic acid levels, caffeine levels, and *cupping test*.

1.2. Research Objective

This study aimed to see the effect of coffee fermentation using *Saccharomyces cerevisiae* yeast starter on coffee beans' antioxidant activity and coffee taste.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The main materials used in this study are Arabica coffee fruit from KPSU Solok Radjo, Fermipan, methanol, HPLC special methanol, aquades, Acetonitrile, Formic Acid, 2,2-Diphenyl-1-Pikrilhydrazile, PTFE 0.45 µm filter paper and PDA. The tools used for the research are pulper machine, huller machine, grinder, an analytical scale, roaster machine, plastic, mortar, beaker, 10 ml flask, 5 ml flask, 50 mesh sieve, spatula, funnel, cuvette, vial, test tube, drop pipette, a micropipette, test tube rack, gobble, measuring cup, Petridish, an ultrasonic bath, pH meter, moisture analyzer, spectrophotometer and set of HPLC of the Agilent brand.

2.2. Research Design

This study employed a Completely Randomized Design (CRD) to investigate the effects of various concentrations of Saccharomyces cerevisiae on the antioxidant activity in Arabica coffee beans. The experiment consisted of five treatments, each with three replicates. Data were analyzed using fingerprint analysis (ANOVA), and significant differences were further examined with Duncan's New Multiple Range Test (DNMRT) at the 5% significance level. The CRD was specifically used for testing moisture content, acidity (pH), and antioxidant activity. The starter concentration was based on the wet weight of the coffee cherries. The treatments with different concentrations of Saccharomyces cerevisiae applied in this study were as follows:
A = No Addition *of Saccharomyces cerevisiae*
B = *Saccharomyces cerevisiae* concentration 1.5%
C = *Saccharomyces cerevisiae* concentration of 2%
D = *Saccharomyces cerevisiae* concentration of 2.5%
E = *Saccharomyces cerevisiae* Concentration of 3%

2.3. Research Implementation

2.3.1. Preparation of Raw Materials

The raw materials used in this study include ripe coffee cherries, identified by their predominantly red skin, sourced from KPSU Solok Radjo in Aie Dingin Village, Lembah Gumanti District, Solok Regency. The fermentation starter consisted of Saccharomyces cerevisiae yeast, specifically Fermipan brand instant yeast, purchased from a bakery in Padang City.

2.3.2. Starter Preparation

In the first stage, fermipan is dissolved in water at a temperature of 40ºC according to the predetermined concentration. Second, sugar is added to ascertain whether the microbes work, and if the solution produces gas, the microbes work. Starters are added to coffee grounds for fermentation without added sugar.

2.3.3. Coffee Fermentation

Arabica coffee cherries were harvested and sorted. The cherries were then divided into five groups for each treatment, with three replicates per group. A natural post-harvest processing method was employed, which does not involve peeling the coffee skins. The cherries were lightly beaten to slightly expose the coffee husk, facilitating the penetration of the fermentation starter into the coffee beans. Each batch of 4 kg of beaten coffee cherries was placed into plastic bags designated for fermentation. The starter, previously dissolved, was added to the bags, which were then securely tied. The starter solution was evenly distributed by rotating the plastic bags to ensure uniform mixing with the cherries. The bags containing the cherries and starter were wrapped with an additional plastic layer to maintain fermentation temperature better. They were placed inside larger black plastic bags to prevent light exposure during fermentation. The bags were then placed on wooden platforms. Fermentation was carried out in a dry, dark room for 40 hours.

2.3.4. Coffee Processing

Cherries fermented for 40 hours are removed from the plastic and then dried for approximately 40 days until a moisture content of less than 12% is obtained in the dry parchment. Coffee is dried on a terrace in the UV house so it is not directly exposed to sunlight. After drying, a hulling process is carried out to separate the horn skin, and green beans are obtained. The green beans obtained are then graded or sorted to separate good coffee beans and damaged or defective coffee beans.

2.3.5. Observation Procedure

Observations made on the coffee's raw materials and green beans produced are yeast mold number, acidity degree (pH), antioxidant activity, caffeine level, and chlorogenic acid level. Organoleptic tests are carried out on coffee that has gone through the roasting stage.

3. RESULT AND DISCUSSION

3.1. Number of Mold and Yeast (AKK)

The number of mold and yeast is used to determine the amount of mold and yeast in food and beverages [5]. The principle of the AKK test is the calculation of mold or yeast colonies with PDA media at a temperature of 25oC after 5 days of incubation[5]. The raw materials used in this study are dry starter *Saccharomyces cerevisiae* in the form of fermipan brand instant yeast and freshly harvested coffee cherries. The results of the observation of the Yeast Mold Number on raw materials are in coffee cherries 4.7 x 10^6 and in fermipan 2.0 x 1010

The purpose of testing the Yeast Mold Number on raw materials is to see if what will play a big role during the fermentation of coffee cherries is the starter added or microbes from the coffee cherries themselves. During the fermentation process, the number of fungi will be more than bacteria, but the bacterial community is more diverse than the fungal community [3] It is expected that the starter *Saccharomyces cerevisiae* added for fermentation has a greater influence than the natural microbes found in coffee cherries.

3.2. Degree of Acidity (pH)

In this study, pH testing was carried out twice, the first time before and after fermentation in coffee *mucilage*, where the results were 5 (before) and 4 (after) fermentation. The second pH test is carried out when *the green bean product* has been obtained. The pH data from the fermentation of coffee cherries can be seen in Table 1.

Table 1. Average pH Green Bean

Starter Concentration	pH (Average ± SD)
E (3%)	5.90 ± 0.01^{a}
D (2.5%)	5.94 ± 0.01^{ab}
C (2%)	5.98 ± 0.01^{bc}
B (1.5%)	6.02 ± 0.02^{c}
A (0%)	6.14 ± 0.01^{d}
CV (%)	0.51

The decrease in pH is caused by the overhaul of sugars carried out by *Saccharomyces cerevisiae* in coffee *mucilage* into alcohol and the reshuffle of other compound compounds by microorganisms other than *Saccharomyces cerevisiae*. This is in accordance with research conducted by [6] showing the pH of coffee fermentation using *Saccharomyces cerevisiae* at concentrations of 1% to 3% ranging from 4.12 to 4.03.

This result is in line with the study [7] that the pH of Arabica coffee fermentation decreases with the increase in the concentration of *Saccharomyces cerevisiae*, obtained a pre-fermentation coffee pH of 5.61 and a post-fermentation pH with a concentration of 1% to 3% ranging from 4.30 to 4.10. According to [7], the higher the concentration of Saccharomyces cerevisiae in fermentation, the more enzyme production will increase, so the components in the coffee beans that are decomposed will be more numerous. During fermentation, the degradation of reducing sugars and pectin by *Saccharomyces cerevisiae* passes through a series of enzymes converted into ethanol and other organic acids. The glucose in mucilage is remodeled into ethanol by the enzyme zimase, and the enzyme pectinase converts pectin into organic acids. The organic acids produced are pyruvic acid, malic acid, succinic acid, and citric acid [8]

Microbes that play a role during coffee fermentation are Saccharomyces cerevisiae and lactic acid bacteria, which also play a role in coffee fermentation and come naturally from coffee cherries. According to (Anon, 2014), the more microbes that play a role in the fermentation of coffee cherries, the more carbohydrates are remodeled into glucose, alcohol, acetic acid, and other acidic compounds. The results of fermentation metabolites will affect the antioxidants in coffee *green beans*. However, the lack of this test using pH paper makes it impossible to obtain accurate pH. However, if you look at the test results, it is according to the literature because the pH has decreased from 5 to 4.

The results of the various fingerprints in Table 1 show that the higher the concentration of *Saccharomyces cerevisiae* used, the lower the pH of *the coffee green bean*. The highest average value was obtained in treatment A without the fermentation process of 6.14, and the lowest value was obtained in treatment E using the highest concentration of 3% of 5.90. The pH of green beans in Table 1 shows that the higher the concentration of *Saccharomyces cerevisiae* added, the lower the pH of coffee *green beans*. The amount of concentration added really affects the pH of *green beans*. There are various acids in coffee beans that are secondary metabolites and are bound in coffee bean cells, including types of carboxylic acid such as acetic acid, citric acid, malic acid, lactic acid, formic acid, oxalic acid, and quinic acid. Later, this acid will form the taste of coffee at the roasting stage, and the binding of metabolite compounds will open like glycoside bonds so that the acid is released into a free form [9]

The results obtained are in line with the research conducted by [10] by adding *Saccharomyces cerevisiae* to coffee using concentrations of 0%, 1%, and 3%, explained in the results of the study that the pH of coffee beans will decrease linearly as the percentage of inoculum used increases. Acids are important attributes of coffee, including citric acid, malic acid, chlorogenic acid, and quinic acid, which are the main acids in coffee beans, especially for taste characteristics [11]. During fermentation, the process of breaking down sugars occurs, producing organic acids that cause the coffee beans' pH to decrease at the moment. Compounds resulting from carbohydrate reshuffling have a significant influence on the sensory properties of a product. The concentration of each metabolite produced is greatly influenced by yeast, environmental factors in the form of oxygen availability, temperature, and the chemical composition of the growth medium [12]

3.3. Antioxidant Activity (% Inhibition)

Antioxidant testing uses the DPPH method. The principle of the DPPH method is that DPPH, a free radical, will be reduced by antioxidants due to the donation of electrons from antioxidants to DPPH so that there is a change from purple to yellow. The change in color intensity is proportional to the number of electron donations, followed by a decrease in DPPH absorbance. The tested sample used a concentration of 100 ppm. The results obtained are displayed in percent inhibitions. The results of the fingerprint of the percentage of the exemption obtained can be seen in Table 2.

Table 2. Average Antioxidant Activity of Green Beans

Starter Concentration	Antioxidant (%) (Average± SD)
A (0%)	14.67 ± 2.47^{a}
B (1.5%)	30.58 ± 1.51^{b}
C (2%)	42.39 ± 2.02^{c}
D (2.5%)	49.21 ± 1.99^{d}
E (3%)	54.72 ± 2.38^{e}

The results in Table 2 showed that the percentage of antioxidant inhibition in green beans increased significantly along with the increase in the concentration of Saccharomyces cerevisiae used during fermentation. The difference in the concentration of Saccharomyces cerevisiae significantly affected the antioxidants of coffee green beans. This is because the concentration of Saccharomyces cerevisiae is higher. The more

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Saccharomyces cerevisiae plays a role in fermentation, the more sugar in coffee is remodeled into alcohol and organic acids. Alcohol and organic acids are what will later react with the compounds in the green beans of coffee. The reaction that occurs produces compounds that are antioxidants. one of which is chlorogenic acid. The results obtained are in accordance with research conducted by Ref. [13]. [14] the antioxidant activity of fermented coffee beans increased significantly compared to the control.

Based on Table 2 and the discussion above, it can be concluded that the higher the concentration of Saccharomyces cerevisiae. The more microbes work, the more sugars are remodeled into alcohols and organic acids. The more metabolites from fermentation. the more acidic the pH of the coffee beans. and the more esterification reactions occur. With more and more esterification reactions, plus the results of fermentation, the compounds with polyphenols are increasing. and the antioxidant content in coffee beans increases.

3.4. Chlorogenic Acid

The results shown in the chromatogram are isomer chlorogenic acid 5-Caffeoylquinic acid (5-CQA). The results obtained can be seen in Figures 1a – f and Table 3.

Table 3. Chlorogenic Acid Test Results on Chromatogram

Sample	**Name**	***Area***	***Height***	***Width***	***Tail Factor***	***Theoretical Plates EP***	***Time Rate***	**Concentration (ppm)**
Standard	CGA	1.247.24	65.69	0.26	0.67	25.693.83	17.59	
A	0	95.10	5.06	0.27	0.69	23.170.47	17.48	7.62
B	1.5	121.45	5.73	0.27	0.64	24.165.39	17.61	9.74
C	2	110.47	5.66	0.29	0.69	20.611.46	17.90	8.86
D	2.5	107.25	5.56	0.27	0.66	24.624.92	17.78	8.60
E	3	114.33	5.97	0.26	0.65	25.556.92	17.59	9.17

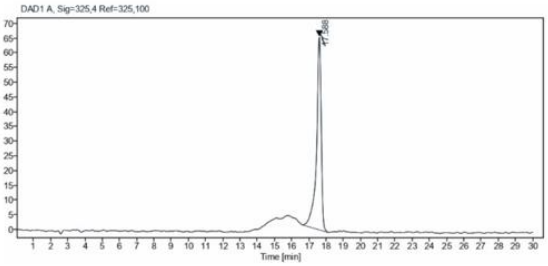

Figure 1a Chlorogenic Acid (5-CQA) Standard

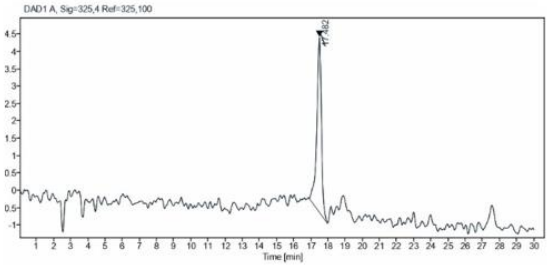

Figure 1b Treatment A of Chlorogenic Acid (5-CQA

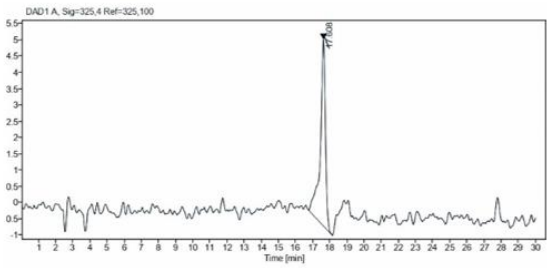

Figure 1c Treatment B of Chlorogenic Acid (5-CQA

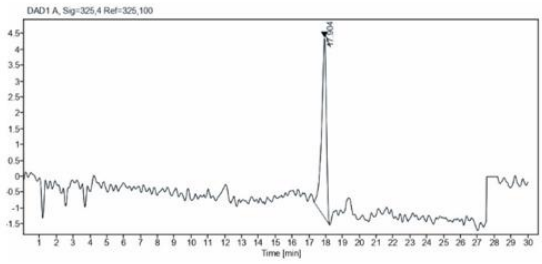

Figure 1 d. Treatment C of Chlorogenic Acid *(5-CQA)*

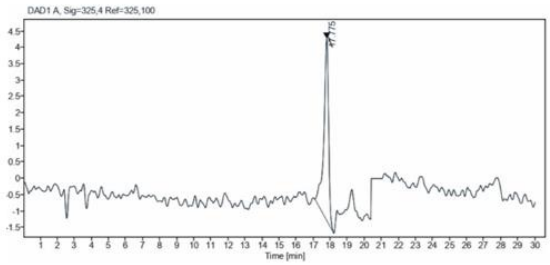

Figure 1 e. treatment D of Chlorogenic Acid *(5-CQA)*

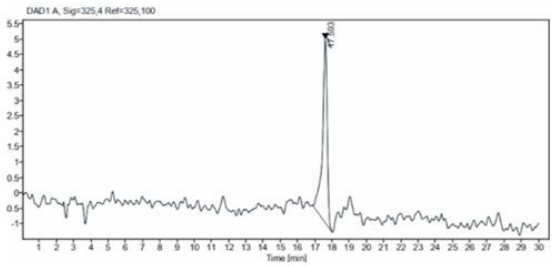

Figure 1 f Treatment E of Chlorogenic Acid *(5-CQA)*

Figure 1. Chromatogram of isomer chlorogenic acid 5-Caffeoylquinic acid (5-CQA).

Green beans of coffee that have completed the drying stage and are peeled off the coffee skin. Then chemical testing was carried out. one of which was the level of chlorogenic acid using HPLC. HPLC users use tools with the AGILENT 1220 Infinity II

brand. Using the YMC-Pack Pro C18 RS column (250 x 4.6mm x 5 µm). reverse phase. flow rate 1 ml/min. inject solution 20 µm. test time 30 minutes. wavelength used 325 nm. motion phase using formic acid and acetonitrile. The samples tested on HPLC are the samples with the best treatment and repeatability based on the results of previous tests.

Based on the data presented in Table 3, the results of chlorogenic acid testing using HPLC were obtained. The highest concentration was observed in treatment B, with an area of 121.45 and a concentration of 9.74 ppm. This was followed by treatments E, C, D, and A, in descending order of concentration. The lowest concentration was found in the control group (treatment A). The concentration increased in treatment B, decreased in treatments C and D, and then rose again in treatment E. There are nine isomers in the chlorogenic acid group. but only one isomer was obtained in this study. namely 5-O-Caffeoylquinic *acid*. while there are still 8 other isomers *3-O-caffeoylquinic acid. 4-O-caffeoylquinic acid. 3-O-feruloylquinic acid. 4-O-feruloylquinic acid. 5-O-feruloylquinic acid. 3.4-di-O-caffeoylquinic acid. 3.5-di-Ocaffeoylquinic acid. and 4.5-di-O-caffeoylquinic acid* [15]. There were eight isomers that were not obtained in this test. However, isomer 5-Caffeoylqunic acid is the largest isomer and cannot represent the total chlorogenic acid contained in coffee beans. This is because researchers only use one standard on HPLC. namely *5-O-Caffeoylquinic acid.* which causes other isomers to be unreadable.

Based on the antioxidant testing, a different effect was observed compared to the chlorogenic acid testing, where no definitive conclusion was reached. Previous research indicates that fermentation can affect chlorogenic acid levels. During fermentation, ethanol reacts with organic acids in the coffee, including chlorogenic acid, forming new chlorogenic acid esters. These esters may be measured as chlorogenic acid. However, the current test did not include data on other esters. Consequently, no conclusions regarding chlorogenic acid levels can be drawn due to the lack of comprehensive quantitative data processing methods.

3.5. Caffeine

Green beans of coffee that have completed the drying stage and are peeled off the coffee skin. Then chemical testing was carried out. one of which was the caffeine level using HPLC. HPLC users use tools with the AGILENT brand. Using C18 column. reverse phase. flow rate 1 ml/min. inject 20 µm solution. test time30 minutes. wavelength used 275 nm. motion phase using formic acid and acetonitrile. The results are in Table 4.

Based on Table 4. data on caffeine testing results using HPLC were obtained. The highest result was obtained in treatment B, where the area obtained was 129.22 with a concentration of 1.21 ppm. Followed by treatment E. D. A and C. When viewed based on the test result data. the results of fermentation using *Saccharomyces cerevisiae* were obtained without trend data.

Table 4. Caffeine Test Results on Chromatogram.

Sample	Name	*Area*	*Height*	*Width*	*Tail Factor*	*Theoretical Plates EP*	*Time Rate*	Concentration (ppm)
Standard	Cafein	3.427.20	136.57	0.35	0.69	18.629.90	20.10	
A	0	83.18	3.22	0.40	0.95	11.150.57	17.94	1.21
B	1.5	129.22	4.21	0.49	1.11	7.721.22	18.12	1.89
C	2	65.85	3.45	0.34	1.24	15.984.82	18.22	0.96
D	2.5	91.19	4.15	0.36	1.26	14.085.03	18.15	1.33
E	3	100.81	4.28	0.39	1.21	11.998.31	18.00	1.47

This is because *Saccharomyces cerevisiae* does not remodel caffeine. Caffeine remodeling can be done by using Lactic Acid Bacteria. When at the time of fermentation. BAL also worked to overhaul the compounds in coffee cherries. BAAL comes from natural microbes found in coffee cherries. This study's results differ from the research conducted by Ref. [7], where the treatment of adding *Saccharomyces cerevisiae* affects caffeine levels. where the higher the concentration of caffeine levels decreases. The highest caffeine content was 1.18%, and the lowest was 1.01%.

3.6. Sensory Evaluation (Cupping test)

The results of *the cupping test* in the form of values can be seen in Table 5 and for the notes of taste and aroma that come out can be seen in Table 6.

Table 5. Cupping Score

Taste Attributes	A	B	C	D	E
Fragrance/aroma	7.75	8.25	8	7.75	8
Flavor	7.75	8	7.75	7.75	8
Aftertaste	7.75	7.75	7.75	7.75	7.75
Acidity	8	8	7.75	8	8
Body	7.75	8	7.75	7.75	7.75
Uniformity	10	10	10	10	10
Balance	7.75	7.75	7.75	7.75	7.75
Clean Cup	10	10	10	10	10
Sweetness	10	10	10	10	10
Overall	7.75	8	7.75	8	7.75
Defects	-				
Final Score	84.5	85.75	84.5	84.75	85

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After the coffee goes through the post-harvest stage, it enters the roasting stage before consumption. *The cupping test* aims to determine and see the quality of the processed coffee. *Cupping test* is a technique used to evaluate the quality of coffee and describe the taste that appears in coffee [16]. *The cupping test* in this test was carried out with *Q Grader* or what can be called a trained panelist in the coffee field. *Q Grader*, who conducts the cupping test has been internationally certified through SCAA. (*Specialty Coffee Association of America*). Based on the results of the cupping test that has been carried out. coffee with the highest score can be obtained in treatment B. In this cupping test. it is explained that concentration does not affect the cupping test value. Based on the SCAA assessment guidelines. coffee with a score above 80 is already classified as a specialty coffee group

Specialty coffee is the highest quality coffee that has been agreed upon by the World Coffee Association. Based on the data obtained and the SCAA guidelines. it can be concluded that all processed coffee treatments have reached *specialty* coffee and can be commercialized. The results of the cupping test obtained align with research conducted by [11] where coffee fermentation with the addition of yeast obtained a value of 84 and did not differ between fermenters in the *cupping test*.

Table 6. Taste Attributes in Coffee

Attribute	A	B	C	D	E
Fragrance	Banana. palm sugar. grapefruit	Banana. grapefruit. Chocolate jackfruit	Banana. sweet chocolate. Pineapple	Chocolate. Chocolate *Sugar*. Citrus. *Spicy*	Pineapple. Chocolate sweet. *Brown Sugar*
Aroma	Citrus. Chocolate	Pineapple. jackfruit. grapefruit	Chocolate Pineapple	Chocolate . *Brown Sugar*	pineapple. Banana
Flavor	Orange. Raspberry	Banana. Wine. Bali Orange	Brown Sugar. Pineapple	Raspberry. pineapple	honey. cherry. pineapple
Overall			*Long. Sweet. Clean*		

Based on Table 6. The results of the taste test that have been carried out can obtain a dominating coffee taste. In the fermentation treatment. a different taste and aroma are obtained without fermentation. In fermentation, it can be seen that there are notes of the taste and aroma of jackfruit and pineapple. The results obtained are in accordance with what was explained by Ref. [12], which is that fermentation produces isoamyl acetate (aroma-like bananas) and ethyl acetate (aroma-like pineapple). There are 14 volatile compounds produced during fermentation produce the aroma of fruits. *floral* and others. Volatile compounds include acetaldehyde. benzaldehyde. caprylic acid. ethanol. ethyl acetate. ethyl laurate. isoamyl acetate. 2.3-butanedione. 1-decanol. 3-methyl-1-butanol. 2-methyl-1-butanol. 2-hexanol. 2-octanol and 1-octanol. The most important volatile compounds include acetaldehyde. ethanol. isoamyl acetate and ethyl acetate [12]

The flavors produced by fermentation align with research conducted by [11] that Furfural (caramel. smell of baking). 2-heptanone (banana. fruit) and pyrazine-methyl (nuts. almonds. sweet) were identified in all samples but were perceived differently depending on the treatment. Banana aromas were only felt in CCMA 0543 B. while almond and caramel aromas were felt in other treatments. Fruity and *floral aromas* are felt when the coffee is fermented with yeast. This aroma note is related to the conversion of 2- and 3-methylbutanal into alcohol during fermentation

4. CONCLUSION

Based on the research that has been carried out. the following conclusions can be drawn: The addition of Saccharomyces cerevisiae concentration in the natural fermentation of Arabica coffee significantly affected the pH of green beans and their antioxidant levels. However. it had no significant effect on water content. chlorogenic acid levels. caffeine levels. or cupping test results. Thus. the null hypothesis (H0) that varying concentrations of Saccharomyces cerevisiae have no significant effect on the chlorogenic acid levels of naturally fermented Arabica coffee beans is accepted. The best treatment observed in this study was with a Saccharomyces cerevisiae concentration of 3% (treatment E). This conclusion was based on the highest antioxidant activity of 54.72% ± 2.38 and a green bean pH of 5.90 ± 0.01. These variables were used to determine the best treatment because moisture content and organoleptic test results did not differ significantly. and there was no quantitative data processing via HPLC. All treatments adhered to the Indonesian National Standard (SNI) for coffee and achieved the specialty coffee level

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