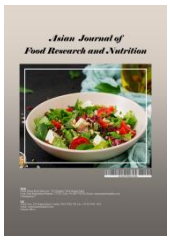

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Antioxidant Activity of Fermented Inferior Jember Robusta Coffee Beans Using *Saccharomyces cerevisiae* as a Starter in the Semi-carbonic Maceration Technique

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the antioxidant activity, total phenol compounds, total flavonoid compounds, and total tannin compounds of raw non fermented robusta coffee beans, the semi-carbonic maceration technique was employed at various concentrations of *Saccharomyces cerevisiae* starter and fermentation times.
Study Design: This study used a completely randomized design (CRD) consisting of 2 factors. The first factor was the starter concentration, and the second factor was the fermentation time.
Place and Duration of Study: This research was conducted in the laboratory of Faculty of Agricultural Technology from September 2022 to April 2023.

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Methodology: This research used a Completely Randomized Design (CRD) with two factors. The first factor was the concentration of yeast S. cerevisiae (A), which consisted of A1 at 20% and A2 at 30%. The second factor was the fermentation duration (B), which consisted of B1 for 24 hours, B2 for 48 hours, and B3 for 72 hours. Based on these two factors, a total of 6 treatment combinations were obtained, and each treatment combination was replicated 3 times. The testing includes the assessment of antioxidant activity, total phenol compounds, total flavonoid compounds, and total tannin. The data obtained will be analyzed using Analysis of Variance (ANOVA) through statistical software (SPSS) at a significance level of 5%. If significant differences are found in the results, the analysis will be followed by the Duncan New Multiple Range Test (DNMRT) to determine specific group differences and identify which treatments significantly differ from one another.
Results: The research results indicate that the highest antioxidant activity, phenolic compounds, flavonoids, and tannins in fermented inferior Jember robusta coffee beans using *Saccharomyces cerevisiae* were obtained from sample A2B2, which resulted from a starter concentration 30% and a fermentation time of 48 hours. Meanwhile, the lowest values for all parameters were found in sample A1B1, which resulted from a starter concentration of 20% and fermented time of 24 hours.
Conclusion: *Saccharomyces cerevisae* can enhance antioxidants in unfermented inferior robusta coffee beans from Jember.

Keywords: Inferior robusta coffe; Saccharomyces cerevisiae; semi carbonic maceration; antioxidant activity.

1. INTRODUCTION

Coffee is one of the most beloved beverages in society due to its unique taste and aroma. The most commonly grown coffee in Jember is robusta coffee, known for its distinctive bitter taste. Robusta coffee beans are known to contain alkaloids, tannins, saponins, and polyphenols. They have the largest content of active phenolic compounds, ranging from 200 to 550 mg per 100 grams of coffee, with chlorogenic acids (CGA) as the main group, constituting 4-14% and serving as antioxidants Rosyidi et al. [1]. The antioxidant activity in coffee beans involves the inactivation of oxidation reactions by providing 1 electron.

These coffee beans are dried seeds that have been separated from the pulp, parchment, and silverskin. Raw robusta coffee beans have acceptable quality but can undergo further processing to achieve even better quality. The semi-carbonic maceration technique is a fermentation method for coffee beans in a closed tank without the addition of CO^2. This technique is cost-effective as CO^2 gas is generated through fermentation itself. The use of CO2 in fermentation helps to limit the growth of undesirable microorganisms. The fermentation process continues until CO2 is evenly distributed throughout the tank [2).

Saccharomyces cerevisiae is used in coffee fermentation to produce invertase enzymes that convert disaccharides into monosaccharides [3]). Invertase enzymes play a crucial role in breaking down disaccharides into monosaccharides. During fermentation, *Saccharomyces cerevisiae* produces and releases glutathione. Glutathione is a powerful antioxidant that protects cells from oxidative damage and helps to maintain antioxidant stability in the fermentation product. *Saccharomyces cerevisiae* requires a carbon source in the form of sugar to produce glutathione. In the context of coffee fermentation, these sugars come from the coffee fruit used as raw material. These sugars are fermented by yeast to produce glutathione. Glutathione can be extracted from natural biomass or chemically synthesized. However, currently, industrial production of glutathione is achieved through fermentation using *Saccharomyces cerevisiae*, which yields high concentrations of glutathione and is accepted as a food-producing microorganism. Junior et al. [4] have also conducted research on arabica coffee fermentation using the carbonic maceration technique. Rizwan et al. [5] found that the higher the concentration of *Saccharomyces cerevisiae*, the higher the bioethanol content produced. The highest bioethanol content in avocado seed fermentation was 32.65% obtained with a 30% yeast tape concentration. Ethanol concentration significantly affects the yield, total phenolic content, total flavonoids, total tannins, and antioxidant activity of rambusa leaf extract, as stated in the research by Koesnadi et al. [6]. They found that a 70% ethanol concentration produced rambusa leaf extract with the highest antioxidant activity. In the study by Larasati et al.

[7], it was stated that a 48-hour fermentation time produced the best coffee powder in terms of moisture and ash content. Based on the above description, this research aims to determine the antioxidant activity of fermented Jember robusta coffee beans using *Saccharomyces cerevisiae* as a starter in the semi-carbonic maceration technique.

2. METHODOLOGY

Local coffee, also known as "kopi asalan," refers to coffee beans produced using very simple methods and facilities, resulting in a mixture containing a relatively high amount of other materials [8]. Historically, a significant portion of coffee commodities has been processed into primary products (dried coffee beans). The traditional coffee processing methods used by farmers often yield low-quality coffee (grade 5 and 6) with relatively high moisture content (around 16%) due to inadequate processing techniques. Typically, the local coffee traded in the market is not sorted by the farmers, resulting in coffee that still contains some materials that can lower the quality of the coffee [9].

2.1 Tools and Materials

The tools used in this research includes a UV-VIS spectrophotometer (Genesys 10S UV-VIS Thermo Scientific), test tubes (IWAKI Pyrex), beaker glass (IWAKI Pyrex), Erlenmeyer flasks (IWAKI Pyrex), test tube rack, volumetric flasks (IWAKI Pyrex), measuring cylinders (IWAKI Pyrex), analytical balance (Scout Pro), pipette pump, volumetric pipettes, glass stirrers (IWAKI), syringes, Petri dishes (Pyrex), Bunsen burner, 1 kg capacity fermentor jar, micropipettes (Dragon Lab), spoons, magnetic stirrer, rotary evaporator (Buchi), and vortex (Thermolyne type 16700).

The main materials used in this research are raw unfermented inferior robusta coffee green beans obtained from coffee farmers in Sidomulyo Jember, rice flour (Rose Brand), granulated sugar (Gulaku brand), and pure culture of *Saccharomyces cerevisiae* obtained from the Laboratory of Universitas Gadjah Mada (UGM), along with the isolation medium for S. cerevisiae, which is Malt Extract Agar (MEA). The materials used for analysis include DPPH (2,2-diphenyl-1-picrylhydrazyl radical), ethanol p.a. solution, 96% ethanol solution, gallic acid, ascorbic acid, tissues, filter paper, aluminum foil, distilled water, tannic acid, quercetin, dark glass bottles, filter cloth, and Folin-Ciocalteau reagent.

2.2 METHODS

2.2.1 The making of Malt Extract Agar (MEA) media

The preparation of Malt Extract Agar media (MEA) was conducted based on the research conducted by Dewi et al. [10] with modifications. The first step involved preparing 3.36 grams of Malt Extract Agar and placing it into a 100 mL Erlenmeyer flask, followed by dissolving it in 70 milliliters of distilled water (aquades). The mixture was then heated on a hotplate until it reached boiling point and stirred until completely dissolved. Once homogenous, the solution was poured into test tubes, closed with cotton and aluminum foil, and then sterilized in an autoclave at 121°C for 15 minutes under 1 atm pressure. The test tubes were then tilted to allow the media to solidify. After cooling down, the media was ready to be used as a growth medium for the *Saccharomyces cerevisiae* culture.

2.2.2 Isolation of pure culture of *Saccharomyces cerevisiae*

Isolation of pure culture of *Saccharomyces cerevisiae* was conducted based on the research by Rubiati [11] with modifications. The first step of the isolation process involved transferring the yeast isolate source aseptically using the streak plate method with an inoculating loop. The loop was then streaked onto Malt Extract Agar (MEA) media contained in a test tube. Before each streaking, the inoculating loop was heated over a Bunsen burner. The streaking process was performed inside a Laminar Air Flow (LAF) to maintain sterility. Afterward, the test tubes were incubated at 30°C for 48 hours. Subsequently, these pure yeast isolates could be used as yeast cultures in the fermentation process.

2.2.3 The making of *Saccharomyces cerevisiae* starter

The making of the starter was carried out based on the research by Noerdinna and Rizky [12] with modifications. The process of making the starter began by dissolving 5 grams of granulated sugar and 5 grams of rice flour in 250 ml of distilled water (aquades). Then, 2 test tubes containing S. cerevisiae were added to the mixture. Next, the mixture was incubated for 48 hours, and the *Saccharomyces cerevisiae* starter was ready for use.

2.2.4 Processing of raw unfermented robusta coffee beans using the semi-carbonic maceration technique

The processing stages of raw unfermented robusta coffee beans using the semi-carbonic maceration technique were conducted based on the research by Riyansyah [13] and Hernani and Kaliza [14] with modifications. In this stage, the following materials were used: 1000 grams of dried green robusta coffee beans. The coffee beans were soaked in 2 liters of clean water for 2 hours. After soaking, the coffee beans were drained to obtain wet green robusta coffee beans. The wet coffee beans were placed into fermentor jars, and *Saccharomyces cerevisiae* yeast starter with concentrations of 20% and 30% was added. After adding the yeast culture, mixing (homogenization) was performed, and the jars were closed to create anaerobic conditions. Fermentation was carried out for 24, 48, and 72 hours. Once the incubation period was completed, the fermented green robusta coffee beans were washed with flowing water until no mucilage was left. The cleaned coffee beans were then dried until they reached a moisture content of 12%. The dried fermented green robusta coffee beans were subsequently crushed using a milling machine and sifted using a 60-mesh sieve. The result was fermented coffee powder.

2.2.5 The extraction of fermented coffee powder

The extraction of coffee powder was conducted based on the research by Pastiniasih [15] and Rosmainar [16] with modifications. The first stage began by dissolving 20 g of coffee powder in 600 ml of ethanol and 400 ml of water in an Erlenmeyer flask. The mixture was then homogenized for 3 hours. After allowing the sample to settle, it was filtered using filter paper and cloth to separate the residue from the filtrate. Once the filtrate was obtained, it underwent evaporation using a rotary evaporator at 60°C for 2 hours to obtain a concentrated extract. The resulting extract was then stored in dark glass bottles and placed in the freezer.

2.3 The Observation Parameters

2.3.1 DPPH method for antioxidant activity test

The antioxidant activity test using the DPPH method was conducted following the procedure described by Kiromah et al. [17] with ascorbic acid used as the standard.

2.3.1.1 Preparation of DPPH blank

The DPPH solution was prepared by dissolving 0.015 grams of DPPH powder in 30 ml of 96% ethanol gradually, then the volume was adjusted to 100 ml. For the preparation of the blank, 2 ml of 96% ethanol was pipetted and mixed with 2 ml of DPPH. The mixture was vortexed and covered with aluminum foil. After 30 minutes of incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Genesys 10S UV-VIS Thermo Scientific).

2.3.1.2 Sample absorbance

The sample was first diluted to 0.2x10-1 using 96% ethanol. Then, 50µL of the diluted sample was pipetted and mixed with 1950 µL of 96% ethanol to obtain a total volume of 2 ml. Subsequently, 2 ml of the DPPH solution was added, and the mixture was vortexed. The sample was then covered with aluminum foil and incubated for 30 minutes. The absorbance of the sample was measured at 517 nm using the spectrophotometer.

2.3.2 Total phenolic compounds

The testing of total phenolic compounds refers to the method used by Sam et al. [18] with gallic acid used as the standard.

2.3.2.1 Preparation of gallic acid solution

To prepare the gallic acid solution, 5.4 mg of gallic acid was added to 5 mL of distilled water (aquades) and made up to a total volume of 10 mL with aquades. The gallic acid solution was pipetted in various volumes, i.e., 0, 50, 75, 100, and 150 µl, and then made up to 5 mL with aquades. The solutions were homogenized with 0.5 mL of Folin-Ciocalteau reagent and left to stand for 5 minutes. After that, 1 mL of 7% Na_2CO_3 was added to each solution, and the mixtures were homogenized again. The solutions were then left covered for 1 hour. The absorbance of each solution was measured at 765 nm using the UV-Vis spectrophotometer.

2.3.2.2 Absorbance of the extract

The sample was first diluted to 0.2x10-1 using aquades. Then, 50 µl of the diluted sample was pipetted and made up to 5 mL with aquades. To

this solution, 0.5 mL of Folin-Ciocalteau reagent was added and left to stand for 5 minutes. Subsequently, 1 mL of 7% Na_2CO_3 was added to the solution, and the mixture was homogenized and left to stand for 1 hour. The absorbance of the solution was measured at 765 nm.

2.3.3 Total flavonoids compounds

The testing of total flavonoid compounds refers to the method used by Pujiastuti et al. [19] with quercetin used as the standard.

2.3.3.1 Preparation of quercetin solution

To prepare the quercetin solution, 20 mg of quercetin was dissolved in 10 mL of distilled water (aquades). The quercetin solution was then pipetted in various volumes, i.e., 0, 50, 100, 150, 200, 250, 300, and 400 µl, and then made up to 2.4 mL with aquades. The solutions were then homogenized with 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$, and left to stand for 5 minutes. After that, 2 mL of 1M NaOH was added to each solution and homogenized again, then left to stand for 1 min. The absorbance of each solution was measured at 510 nm using the UV-Vis spectrophotometer.

2.3.3.2 Absorbance of the extract

The sample was first diluted to 0.2x10-1 using aquades. Then, the solution was homogenized with 0.3 mL of 5% NaNO2 and 0.3 mL of 10% AlCl3, and left to stand for 5 minutes. After that, 2 mL of 1M NaOH was added to the solution and homogenized again, then left to stand for 1 min. The absorbance of the solution was measured at 510 nm using the UV-Vis spectrophotometer.

2.3.4 Total tannins compounds

The testing of total tannins refers to the method used by Malangngi et al. [20] with tannic acid used as the standard.

2.3.4.1 Preparation of tannic acid solution

To prepare the tannic acid solution, 0.1 g of tannic acid was dissolved in distilled water (aquades) up to 100 mL, resulting in a concentration of 1000 ppm. From this stock solution, concentrations of 20, 40, 60, 80, and 100 ppm were prepared. Each concentration solution was pipetted with 1 mL, then added with 7.5 mL of aquades and 0.5 mL of Folin-Ciocalteau reagent. The mixture was homogenized and incubated for 3 minutes. Next, 1 mL of 15% Na_2CO_3 was added to each solution, and the mixture was homogenized and incubated for 15 min. The absorbance was measured at 740 nm using the UV-Vis spectrophotometer.

2.3.4.2 Absorbance of the extract

The sample was first diluted to 0.2x10-1 using aquades. Then, 1 mL of the diluted sample was pipetted and added with 0.1 mL of Folin-Ciocalteau reagent. The mixture was homogenized and left to stand for 3 minutes. After that, 1 mL of 15% Na2CO3 was added to the solution, and it was left to stand for 15 min. The absorbance was measured at 740 nm using the UV-Vis spectrophotometer.

2.4 Data Analysis

The data obtained from the testing results were analyzed using Analysis of Variance (ANOVA) with the SPSS software at a significance level of 5%. If the data results show significant differences, further analysis will be conducted using Duncan New Multiple Range Test (DNMRT).

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

The antioxidant activity of coffee is associated with its content of chlorogenic acid, ferulic acid, caffeic acid, and n-coumaric acid. In some publications, caffeine and trigonelline are also considered antioxidants. Phenylalanine formed during the roasting process exhibits high antioxidant activity, as well as heterocyclic compounds (Hasanah et al. [21]. Based on the conducted testing results, the antioxidant activity of fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae* ranged from 15.94 ± 1.8% to 37.68 ± 1.04%. ANOVA calculations showed that variations in starter concentration and fermentation time significantly influenced the antioxidant activity. The values of the tested antioxidant activity of fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* can be seen in Fig. 1.

Based on the diagram in Fig. 1., it is evident that the highest antioxidant activity in fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* is observed in sample A2B2, which resulted from a starter

concentration of 30% and a fermentation time of 48 hours. On the other hand, the lowest value is seen in sample A1B1, obtained from a starter concentration of 20% and a fermentation time of 24 hours. This is because an increased addition of *Saccharomyces cerevisiae* enhances the antioxidant content in coffee beans. *Saccharomyces cerevisiae* contains glutathione, which is an antioxidant compound. According to Parhusip et al. [22], the addition of *Saccharomyces cerevisiae* can elevate the antioxidant activity in coffee kombucha.

In this study, the optimum fermentation time is found to be 48 hours. This result aligns with the findings of Leliqia et al. [23], which demonstrated that fermentation time can impact antioxidant activity. It was also confirmed that fermentation can enhance the antioxidant activity in a sample when the fermentation is performed at the optimum time. Beyond this optimum time, the antioxidant activity in the sample will significantly decrease (Hur et al., 2014).

3.2 Total Phenolic Compounds

Inside coffee beverages, there are numerous active compounds that belong to the group of phenolic compounds. Phenolic compounds and also flavonoid compounds have been reported to have positive effects on health and medical therapy. Based on the conducted testing, the phenolic compounds in fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* range from 0.638 mgGAE/ml to 0.4813 mgGAE/ml. The ANOVA test results indicate that the variations in sample concentration and fermentation time have a significant effect. The values of the antioxidant activity testing for fermented inferior Robusta coffee beans with *Saccharomyces cerevisiae* can be seen in Fig. 2.

Based on the diagram in Fig. 2., it shows that fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* at the highest phenolic content is sample A2B2, obtained from a starter concentration of 30% with a fermentation time of 48 hours, while the lowest value is in sample A1B1, obtained from a starter concentration of 20% with a fermentation time of 24 hours. The data on the diagram indicates that *Saccharomyces cerevisiae* can increase the phenolic content in coffee beans. According to Hartini et al. [24], the addition of *Saccharomyces cerevisiae* can increase the phytochemical content, including phenolic content, in local pineapple peels. Another study by Dewanjanti [25] stated that chlorogenic acid is one of the potent antioxidants from phenolic compounds that can inhibit oxidative damage and provide antioxidant effects. Chlorogenic acid belongs to the family of esters formed from the combination of quinic acid and several trans-cinnamic acids, usually caffeine, p-coumaric, and ferulic acid. Chlorogenic acid is a compound classified as a phenolic component and is soluble in water. The main subgroup of chlorogenic acid isomers in coffee is caffeoylquinic acid (CQA), feruloylquinic acid (FQA), dicaffeoylquinic acid (diCQA), and p-coumaroylquinic acid (pCQA) in smaller amounts.

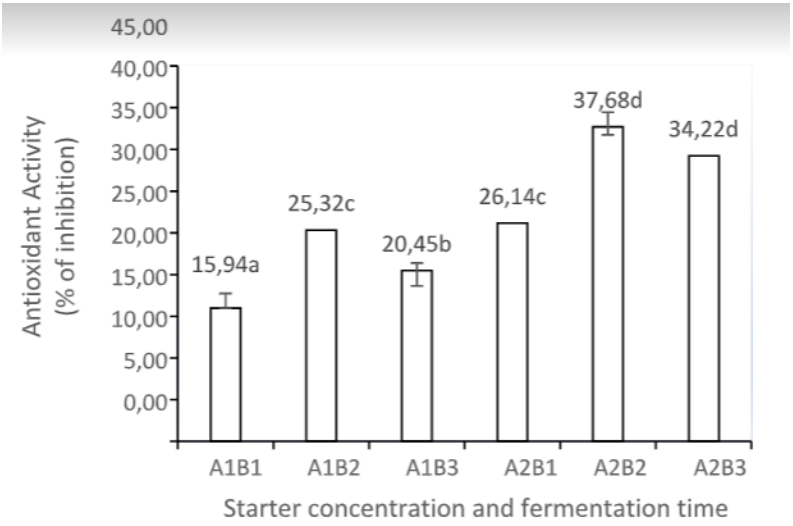

Fig. 1. Antioxidant activity of fermented inferior robusta coffee beans from Jember with *Saccharomyces cerevisiae* using semi-carbonic maceration

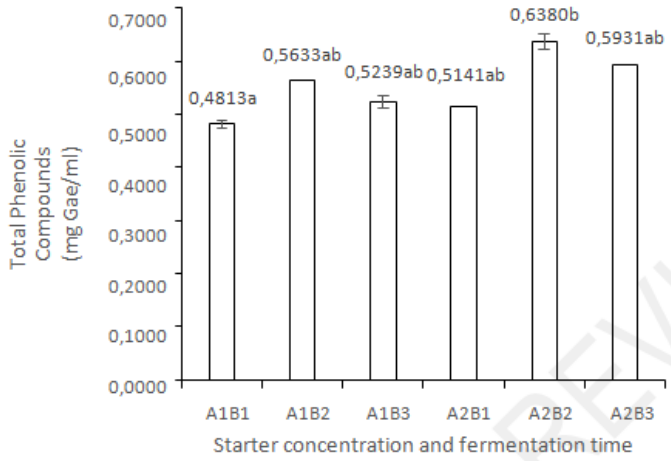

Fig. 2. Total phenolic compounds of fermented inferior robusta coffee beans from jember with *Saccharomyces cerevisiae* using semi-carbonic maceration

In the total phenolic compound testing, the optimum time is 48 hours. This fermentation effect is also consistent with the findings of Hapsari et al. (2018), which showed that the total phenolic content and antioxidant activity increase with fermentation time.

3.3 Total Flavonoid Compounds

Testing the total flavonoid compound is one of the parameters used to determine the flavonoid content, where the higher the flavonoid compound, the higher its ability to neutralize free radicals. Flavonoids are polyphenolic compounds with 15 carbon atoms that are neatly arranged in a C6-C3-C6 configuration, meaning that the carbon skeleton consists of two C6 groups connected by an aliphatic chain of three carbons (Wang et al. [26]. Based on the conducted testing, the flavonoid compounds in fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae* ranged from 0.708 mgQE/ml to 0.082 mgQE/ml. The ANOVA results indicate that variations in sample concentration and fermentation time have a significant effect. The values obtained from the testing of antioxidant activity in fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae* can be seen in Fig. 3.

Based on the diagram in Fig. 3., it shows that the highest flavonoid content in fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* is found in sample A2B2, which was produced from a starter concentration of 30% with a fermentation time of 48 hours, while the lowest value is in sample A1B1, produced from a starter concentration of 20% with a fermentation time of 24 hours. Previous research conducted by Dinh et al. [27] stated that at a fermentation time of 24 hours, there might be a decrease in flavonoids due to the emergence of high molecular weight polymerized compounds with limited water solubility. Extending the fermentation time to 48 hours can increase the flavonoid concentration due to depolymerization and the emergence of compounds with higher solubility. However, when the fermentation time is extended to 72 hours, flavonoid content decreases due to the action of flavonoid oxidase enzymes that cause phenolate diffusion in the cell fluid and oxidize it.

The optimal concentration of yeast should be used to provide optimal fermentation characteristics. Fermentation is an enzymatic process; therefore, low yeast concentration will result in slow fermentation Gunawan et al. [28]; Darwesh [29] also mentioned that the addition of *Saccharomyces cerevisiae* starter in cinnamon fermentation significantly increased the antioxidant content.

3.4 Total Tannin Compounds

Tannin is a phenolic compound with a large molecular weight that consists of hydroxyl groups and several related groups such as carboxyl, which form strong and effective complexes with proteins and some macromolecules [30]. Tannins are unique polyphenolic compounds that can have both positive and negative effects on health. They can influence the color, flavor, and nutritional quality of beans and the products

derived from them. Additionally, tannins can function as antioxidants by binding to free radicals, helping the body avoid cell damage and preventing various diseases.

Based on the results of the conducted analysis, the tannin content in the fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae* ranges from 0.0373 mgTAE/ml to 0.0302 mgTAE/ml. The ANOVA test indicates that the variation in sample concentration and fermentation time significantly affects the tannin content. The values of the antioxidant activity test for the fermented Robusta coffee beans with *Saccharomyces cerevisiae* can be seen in Fig. 4.

Based on the diagram in Fig. 4., it shows that the fermented inferior Robusta coffee beans from Jember with *Saccharomyces cerevisiae* have the highest tannin content in sample A2B2, which is produced from a starter concentration of 30% with a fermentation time of 48 hours, while the lowest value is in sample A1B1, which is produced from a starter concentration of 20% with a fermentation time of 24 hours. Increasing the concentration of *Saccharomyces cerevisiae* starter leads to an increase in tannin content.

This finding is consistent with a previous study conducted by Remok et al. [31], which stated that tannins have antioxidant properties, and Saccharomyces cerevisiae is capable of increasing tannin content in the extract of Salvia lavandulifolia plants.

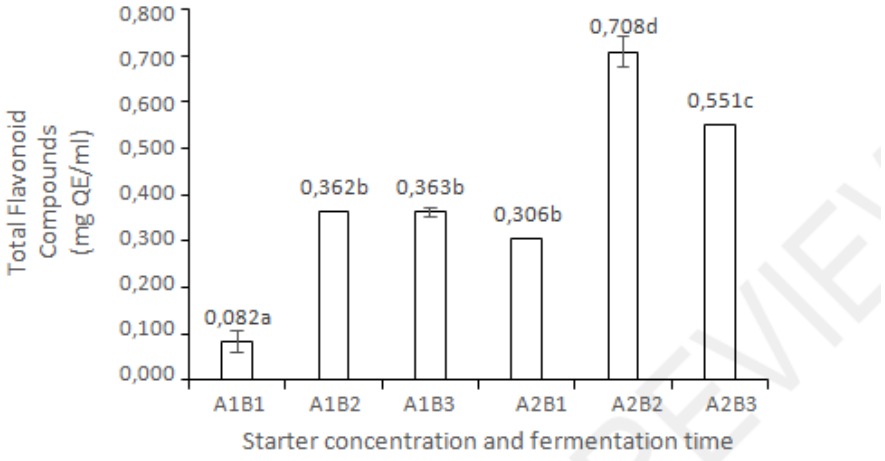

Fig. 3. Total flavonoid compounds of fermented inferior robusta coffee beans from jember with *Saccharomyces cerevisiae* using semi-carbonic maceration

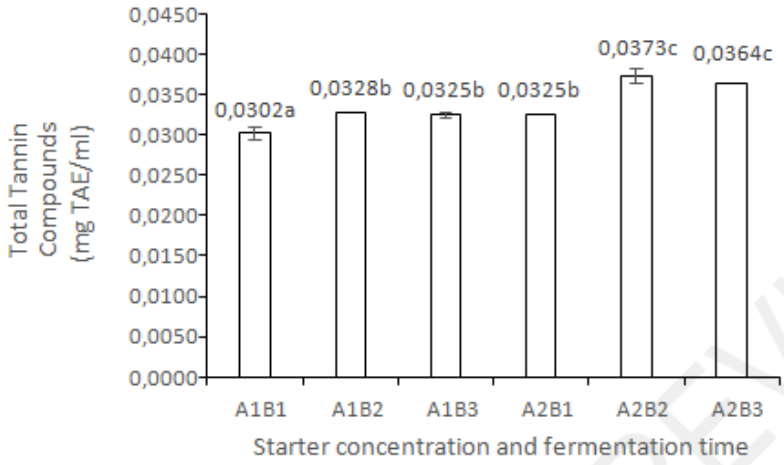

Fig. 4. Total tannin compounds of fermented inferior robusta coffee beans from jember with *Saccharomyces cerevisiae* using semi-carbonic maceration

4. CONCLUSION

The following conclusions that the variation in starter concentration and fermentation time significantly affects the antioxidant activity of fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae*. The highest activity is found in sample A2B2, which is produced from a starter concentration of 30% with a fermentation time of 48 hours. The variation in starter concentration and fermentation time also significantly affects the total phenolic, flavonoid, and tannin content of fermented Robusta coffee beans from Jember with *Saccharomyces cerevisiae*. Samples with the highest antioxidant, phenolic, and tannin content are those with a starter concentration of 30% and a fermentation time of 48 hours.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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