Chemical Characteristics of Fermented Inferior Jember Robusta Coffee Beans Using Commercial Yeast Starter in Semi-carbonic Maceration Technique

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Abstract. This research aims to determine the effect of starter concentration and fermentation time on the chemical characteristics of robusta coffee fermented using the semi-carbonic maceration technique with commercial *Saccharomyces cerevisiae* starter. This research method uses a Randomized Block Design with two factors, namely A is the starter concentration (20% and 30%), and B is the fermentation time (24, 48 and 72 hours). The observation parameters carried out included the amount of yeast growth, pH, water content, reducing sugar, caffeine content and protein content. The research results showed that the amount of starter concentration and length of fermentation time using commercial *S. cerevisiae* starter with semi-carbonic maceration technique could reduce pH, water content, reducing sugar, caffeine content and protein content of coffee when compared to coffee without fermentation (control). Treatment for a long fermentation time of 72 hours with a starter concentration of 30% produce the lowest values for each test parameter (pH=4.43; water content=11.30; reducing sugar=0.75%; caffeine content=0.87% and protein content=10.96%). These data can be compared with samples of robusta coffee without fermentation (pH=6.03; water content=15.47; reducing sugar=1.92%; caffeine content=1.72% and protein content=14.80%). %). The results of the research also showed that the growth of yeast increased during the fermentation time from 9.11 log cfu/ml in coffee before fermentation to 10.32 log cfu/ml which was the highest value in fermented coffee at a fermentation time of 72 hours with a starter concentration of 30%.

Keywords: Robusta Coffee, S. cerevisiae, Semi-carbonic Maceration, Starter Concentration, Fermentation Time.

1 Introduction

Coffee as a plantation commodity plays an important role in Indonesia's economic sector. This commodity is an export mainstay in addition to palm oil, tea, rubber and tobacco. Coffee contributes to providing employment and a source of income for the community [1]. The types of coffee that are widely cultivated in Indonesia are robusta and arabica. East Java province is among the largest coffee producers in Indonesia, with

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with Malang Regency and Jember Regency as the largest coffee plantation areas. The type of coffee that is widely cultivated in Jember Regency is robusta. Coffee plantations in Jember Regency are spread in almost all subdistricts [2]. This shows that coffee is still a potential plantation commodity to be cultivated in Jember.

The quality of robusta coffee from smallholder coffee plantations is generally low. Processing robusta coffee beans in a dry way, or without going through the fermentation process, is the cause. Efforts to improve coffee processing methods through innovation continue to be made to create good quality coffee beans, and be able to compete in the market. Fermentation is important in determining the final quality of the coffee beans produced. The application of post-harvest processing of robusta coffee beans using the semi-carbonic maceration fermentation technique can be done to improve the quality of Jember robusta coffee beans. This technique is carried out by soaking dry processed robusta coffee beans first for 2 hours, then putting the beans in a plastic tank and removing O_2, as well as adding commercial yeast (fermipan). Carbonic maceration is the anaerobic fermentation of coffee fruits in a closed tank with the addition of CO_2 gas [3]. The semi-carbonic maceration technique is the anaerobic fermentation of coffee beans in a closed tank without the use of CO_2 gas. The difference between these two fermentation techniques is the use of CO_2 gas during fermentation. The carbonic maceration technique resembles the production process of alcoholic beverages from grapes. This technique was adopted in coffee processing because anatomically, coffee fruit and grapes have structural similarities. Coffee fermentation with carbonic maceration technique for 120 hours at 380C can produce excellent coffee with a score of 85[4].

The addition of commercial yeast in robusta coffee bean fermentation using the semi-carbonic maceration technique was carried out to obtain a microbial source of *Saccharomyces cerevisiae*. *S. cerevisiae* strains have high pectinolytic activity and good fermentation capacity. This yeast inoculant will give rise to several flavor attributes of the final product such as caramel, chocolate and almond [5]. Commercial yeast is readily available and does not require specific treatment. Adoption of the semi-carbonic maceration technique as a way of processing coffee beans has not been widely practiced. The success of the semi-carbonic maceration technique is determined by the starter concentration and fermentation duration used. The use of starter concentration and fermentation duration that can produce good quality robusta coffee beans is not yet known. Therefore, it is necessary to conduct research on how to ferment robusta coffee beans with different amounts of commercial yeast starter concentrations, and fermentation duration using the semi-carbonic maceration technique.

2 Materials and Methods

2.1 Materials and Tools

The main materials used in this study were unfermented Jember robusta coffee beans, commercially obtained yeast with fermipan brand, rose brand rice flour and gulaku brand granulated sugar. Materials used for analysis were Malt Extract Agar (MEA) brand Merck, distilled water, physiological solution, NaCl, 70% alcohol, HgO, H_2SO_4,

K_2SO_4, anhydrous glucose, nelson reagent, arsenomolybdate solution, MgO, sulfuric acid, dichloromethane and KOH.

The tools used in this study include spoutpack with a capacity of 1 kg, analytical balance, autoclave, spatula, laminar air flow, incubator, micro pipette, test tube, bunsen, petri dish, waterbath, colony counter, spoon, erlenmeyer, vortex, kjeldahl flask, filter paper, volumetric flask, and spectrophotometer.

2.2 Research Methods

This research used an experimental method. The research design used was factorial Randomized Block Design with two factors, namely differences in starter concentration (A) and fermentation time (B). The starter concentration factor added consisted of A1 20% and A2 30%. The fermentation time factor consists of 24, 48 and 72 hours. The control used in this study was robusta coffee green bean without fermentation.

Table 1. Combination of starter concentration and duration of fermentation.

Duration of Fermentation (B)	Starter Concentration (A)	
	20% (A1)	30% (A2)
24 hours (B1)	A1B1	A2B1
48 hours (B2)	A1B2	A2B2
72 hours (B3)	A1B3	A2B3

The research was carried out through several stages including: making Malt Extract Agar (MEA) media, making yeast starter, fermenting robusta coffee green beans, and analysis consisting of calculating the number of bacteria, reducing sugar content test, protein content test and caffeine content test.

2.3 Observation Parameters

The variables observed in this study were the calculation of the number of bacteria [6], test of reducing sugar content Nelson-Somogiy method [7], test of protein content Kjeldahl method [7] and test of caffeine content Bailey method [7].

2.4 Data Analysis

The test results data that have been obtained are analyzed using ANOVA (Analysis of Variance) with a test level of 5% using SPSS software, if significantly different results are obtained, they will be further tested using DNMRT (Duncan New Multiple Range Test).

3 Results and Discussion

3.1 Calculation of Total Yeast

Saccharomyces cerevisiae has an important role in the coffee fermentation process. The addition of Saccharomyces starter in coffee fermentation is known to significantly increase ($P<0.05$) the production of compounds that play a role in the

development of coffee flavor [8]. The growth of *S. cerevisiae* in fermentation can be seen in Fig. 1.

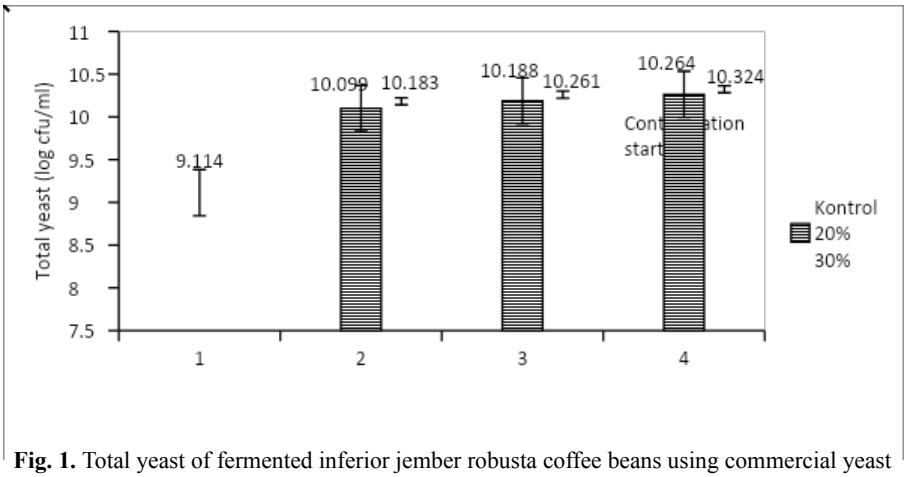

Fig. 1. Total yeast of fermented inferior jember robusta coffee beans using commercial yeast

The results of yeast growth analysis showed that the more starter addition and the longer the fermentation time, the number of yeast in the sample increased (see Fig. 1). The lowest total yeast count was found in the robusta coffee sample without fermentation, which was 9.114 log cfu/ml. Coffee treated with 24-hour fermentation and 20% and 30% starter addition resulted in total yeast counts of 10.009 log cfu/ml and 10.183 log cfu/ml, respectively. The number of yeast further increased in coffee fermented for 48 hours with the addition of 20% and 30% starter, namely 10.188 log cfu/ml and 10.261 log cfu/ml, respectively. The increase in yeast count continued to occur in samples with 72 hours of fermentation time at the addition of 20% and 30% starter, namely 10, 264 log cfu/ml and 10, 324 log cfu/ml.

Growth of yeast continued until 72 hours of fermentation, at a temperature of 300 C. This condition is due to the availability of nutrients from the remaining coffee layers and additional nutrients from sugar and rice flour so that the yeast continues to grow. Anaerobic fermentation environmental conditions can support yeast growth, and inhibit aerobic microbial contamination. Total microbial growth is due to an increase in the number of microbes, and is characterized by gas formation during fermentation. During fermentation there is microorganism activity, especially yeast, which breaks down the mucus layer into organic acids [9].

3.2 Test of pH

The pH test aims to determine the acidity level of coffee after fermentation. The acidity of the product is influenced by the degradation of sugar into organic acids during fermentation [10]. The results of statistical analysis with ANOVA showed that differences in fermentation time and starter concentration did not significantly affect the pH of coffee after fermentation ($p>0.05$). The results showed that coffee after fermentation had a pH value between 4.88-5.85 (see Fig. 2).

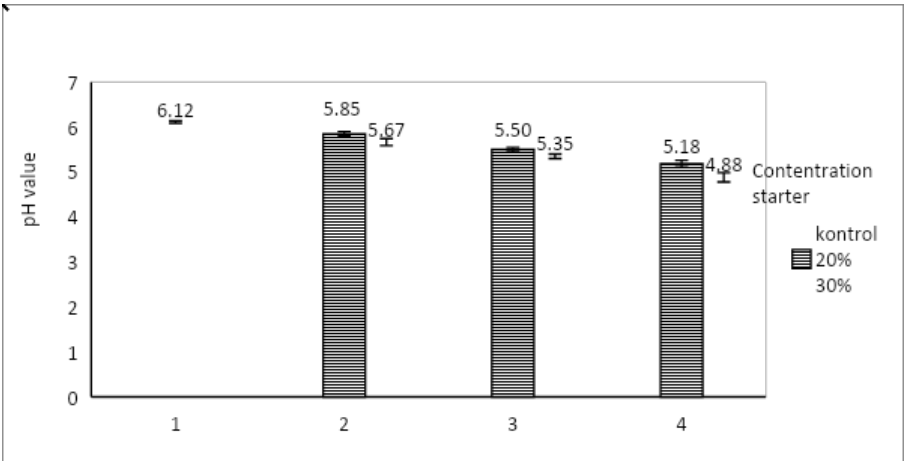

Fig. 2. pH value of fermented inferior jember robusta coffee beans using commercial yeast.

The pH value of commercial yeast-fermented Jember robusta coffee shows that the increase in starter addition and fermentation duration can reduce the pH of the sample. The sample with the highest pH was robusta coffee without fermentation with a pH of 6.03. Coffee with 24 hours fermentation time and 20% and 30% starter addition resulted in pH values of 5.85 and 5.67, respectively. The pH value of coffee with a fermentation time of 48 hours and the addition of 20% and 30% starter is 5.50 and 5.35, respectively. The pH value of coffee continues to decrease in samples with a fermentation time of 72 hours with the addition of 20% and 30% starter, namely 5.18 and 4.88.

Fermentation causes the pH of coffee to decrease linearly. The lowest pH value was found in coffee fermented for 72 hours and the addition of 30% starter concentration. This is in accordance with the research on robusta coffee fermentation using *Saccharomyces cerevisiae* culture with different starter additions and fermentation times, it is known that the lowest pH is obtained in coffee with the longest fermentation time with a pH value of 4.16 [11]. The longer the fermentation time will produce more organic acids [11]. The more yeast during fermentation will cause the enzymes amylase, zymase and invertase to increase [12]. Yeast will break down sugar into ethanol and will be broken down into organic acids [13]. Where the sugar breakdown process will produce lactic acid and other acids such as ethanol, butyric acid and propionate [14]. The formation of acid will decrease the pH [15]. The higher the percentage of inoculum, the more *Saccharomyces cerevisiae* will synthesize sugar into acids that cause a decrease in pH in coffee beans.

3.3 Water Content

Water content is the amount of water contained in the material expressed in units of percent [10]. Water content testing is carried out to provide a range and limit of water content in the material. Based on the results of statistical analysis with ANOVA, it is known that differences in fermentation time and the amount of starter concentration

do not significantly affect the value of coffee moisture content after fermentation ($p>0.05$) (see Fig. 3).

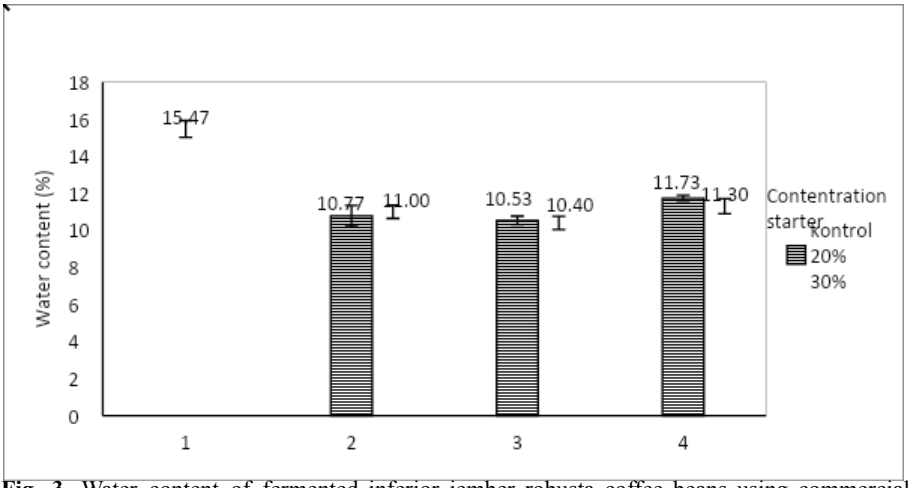

Fig. 3. Water content of fermented inferior jember robusta coffee beans using commercial yeast.

The results of water content analysis showed that the water content of commercial yeast-fermented Jember robusta coffee in all treatments after fermentation and drying had a lower water content than the control. The sample with the highest water content was the robusta coffee sample without fermentation, which was 15.47%. Coffee with a fermentation time of 24 hours and the addition of 20% and 30% starter produced a moisture content of 10.77% and 11.00%, respectively. The water content of 48-hour fermented coffee and the addition of 20% and 30% starter is 10.53% and 10.40%, respectively. While the water content in samples with 72 hours of fermentation time and the addition of 20% and 30% starter is 11.73% and 11.30%.

The data shows that the moisture content in all treatments is in accordance with the provisions of SNI 01-2907-2008 concerning coffee beans, the maximum moisture content requirement in coffee beans is 12.5%. Moisture content that exceeds 12.5% will make it easier for mold to grow and damage the quality of coffee beans. The use of airlock and *Saccharomyces cerevisiae* can reduce moisture content. Fermentation by *Saccharomyces cerevisiae* will cause microbial activity to increase and an increase in temperature that causes the water content in the beans to evaporate. Airlock will maximize the release of water vapor through the airlock hole. In addition, the increase in temperature and increased microbial activity will also cause enzyme activity to be more active and the pulp to become thinner. Heat will affect the destruction of the pulp and the pores of the seeds will open so that the water content evaporates faster [16].

3.4 Reducing Sugar Content

An important change that occurs during the coffee fermentation process is the degradation of the mucilage layer on the surface of the beans. Mucilage consists of reducing sugars, non-reducing sugars, pectin compounds, minerals and cellulose [17].

Mucilage consists of reducing sugar compounds, namely glucose and fructose as much as 30% [18]. The results of variance analysis with ANOVA showed that the treatment of differences in the addition of starter concentration and fermentation duration had a significant effect ($p<0.05$) on reducing sugar content in coffee (see Fig. 4).

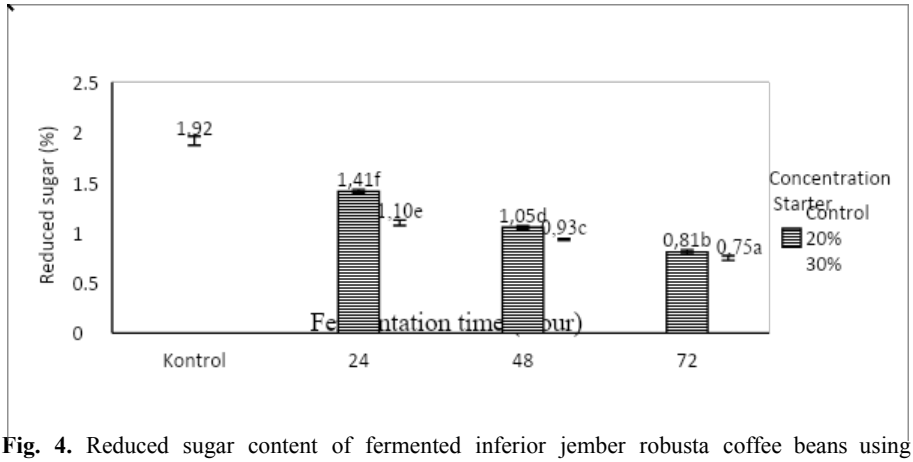

Fig. 4. Reduced sugar content of fermented inferior jember robusta coffee beans using commercial yeast.

The results of the analysis of reducing sugar content showed that increasing the addition of starter and the length of fermentation, caused a decrease in the reducing sugar content of commercial yast-fermented Jember robusta coffee. The sample with the highest reducing sugar content was found in robusta coffee without fermentation, which was 1.92%. Coffee with a fermentation time of 24 hours and the addition of starter as much as 20% and 30%, respectively, produced reducing sugar levels of 1.41% and 1.10%. Reducing sugar levels in coffee fermentation for 48 hours with the addition of 20% and 30% starter were 1.05% and 0.93%, respectively. Reducing sugar content continued to decrease in samples with 72 hours of fermentation time and the addition of 20% and 30% starter, namely 0.81% and 0.75%.

Coffee without fermentation had the highest reducing sugar content, while the lowest value was obtained by coffee fermented for 72 hours and the addition of 30% starter. This is because during the fermentation process, *S. cerevisiae* degrades reducing sugars and pectin contained in mucilage through enzymatic reactions into ethanol. *S. cerevisiae* produces zymase enzyme that converts glucose into ethanol, and pectinolytic enzyme will convert pectin into organic acids such as pectinic acid, galacturonic acid and pectic acid. Other organic acids produced during glucose metabolism include acetic acid, pyruvic acid, malic acid, succinic acid and citric acid. The more *S. cerevisiae* that is added causes more enzyme production so that more components will be broken down in coffee beans [19].

3.5 Caffeine Content

Caffeine (1,3,7-trimethylxanthine) is a type of alkaloid naturally contained in coffee beans. The form of caffeine in pure conditions is a white powder with a hexagonal

prism crystal shape, has a bitter taste, and has no odor. Caffeine ($C_8H_{10}N_4O_2$) is the most abundant secondary metabolite in coffee after chlorogenic acid [20].

According to the FDA (Food Drug Administration), the permitted dose of caffeine is 100-200mg/day, while according to SNI 01-7152-2006 the maximum limit of caffeine in food and beverages is 150 mg/day and 50 mg/serving. Coffee fermentation is an alternative method to reduce caffeine levels in coffee beans. Caffeine levels always decrease with the longer the coffee is fermented [21].

The results of variance showed that the treatment of different concentrations of starter and the length of fermentation time had a significant effect ($P < 0.05$) on the caffeine content of coffee. Coffee without fermentation had the highest caffeine content, while the lowest value was obtained by coffee fermented for 72 hours with the addition of starter as much as 30% (see Fig. 5).

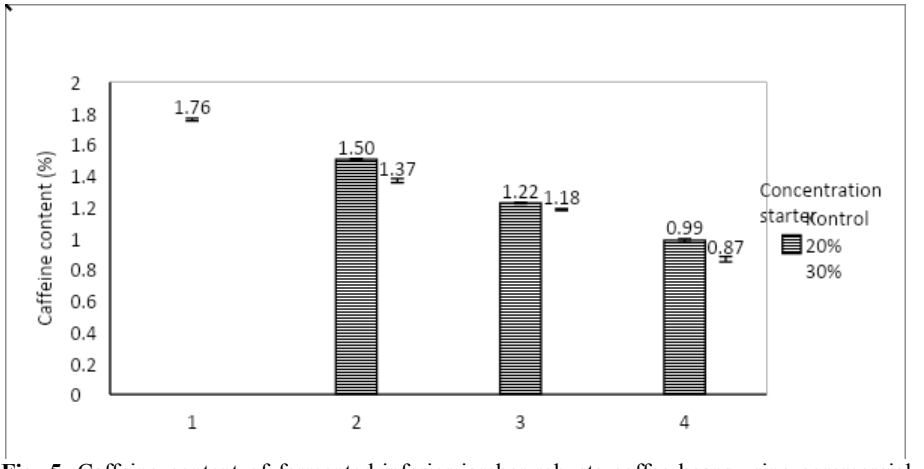

Fig. 5. Caffeine content of fermented inferior jember robusta coffee beans using commercial yeast

Based on the data in Figure 5, the sample with the highest caffeine content is robusta coffee without fermentation, which is 1.76%. Coffee with a fermentation time of 24 hours and the addition of starter as much as 20% and 30%, respectively, produced caffeine levels of 1.50% and 1.37%. Caffeine levels in coffee fermentation for 48 hours and the addition of 20% and 30% starter were 1.22% and 1.18%, respectively. Caffeine levels continued to decrease in samples with 72 hours of fermentation time and the addition of 20% and 30% starter, namely 0.99% and 0.87%. The lowest caffeine levels were found in samples with a fermentation time of 72 hours and the addition of 30% starter, this is in accordance with the research on the effect of *S. cerevisiae* fermentation on reducing robusta coffee caffeine levels with variations in fermentation duration of 24, 36, 48, 60, and 72 hours. The lowest robusta coffee caffeine content was obtained in 72-hour fermented coffee with a caffeine content of 1.54% (weight/weight) [22].

The decrease in caffeine content in coffee is caused by fermentation by commercial yeast (fermipan) that produces enzymes. Some of them are able to degrade caffeine, such as the enzymes caffeine demethylase, paraxanthine demethylase, theophylline

demethylase, theobromine demethylase, and heteroxanthine demethylase [23]. In coffee fermentation, the complex compound caffeine is decomposed into uric acid, 7-methylxanthine and xanthine [24].

The decomposition of caffeine compounds into simpler compounds will cause a decrease in the level of caffeine contained in coffee [25]. The breakdown of complex caffeine compounds that occurs results in the size and molecular weight of caffeine becoming smaller [26]. The small molecular weight of caffeine causes caffeine to easily diffuse through cell walls and will be dissolved in fermentation media so that caffeine levels in coffee will also decrease [27].

3.6 **Protein Content**

The formation of coffee flavor is inseparable from the important role of protein contained in it. During the fermentation process, protease enzymes break down the mucilage layer containing protopectin and sugar in the coffee skin. This decomposition is expected to change the flavor of coffee [28]. The results of ANOVA statistical analysis showed that the use of different starter concentrations and fermentation duration significantly affected the protein content of coffee after fermentation ($p<0.05$) (see Fig. 6).

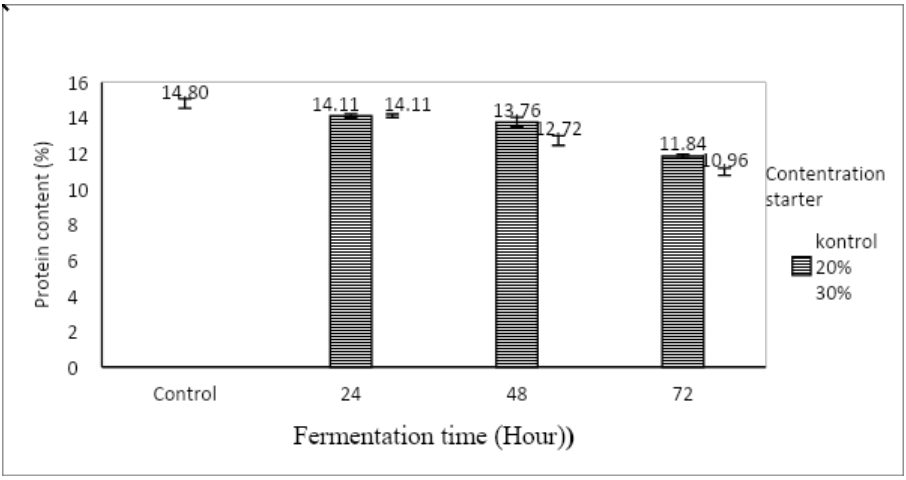

Fig. 6. Protein content of fermented inferior jember robusta coffee beans using commercial yeast

The results of protein content analysis showed that the more starter addition and the longer the fermentation time, the protein content in the sample decreased. The highest protein content value was found in the robusta coffee sample without fermentation, which was 14.80%. Coffee with 24 hours fermentation time and 20% and 30% starter addition each produced protein content with the same value of 14.11%. Protein levels in coffee fermented for 48 hours and the addition of 20% and 30% starter were 13.76% and 12.72%, respectively. Protein levels continued to

decrease in samples with 72 hours of fermentation and the addition of 20% and 30% starter, namely 11.84% and 10.96%.

The results of variance showed that the treatment of different concentrations of starter and the length of fermentation time had a significant effect ($P < 0.05$) on the protein content of coffee. Coffee without fermentation has the highest protein content value, while the lowest value is obtained by coffee fermented for 72 hours with the addition of starter as much as 30%. The decrease in protein content is due to *S. cerevisiae* producing various types of enzymes including proteolytic and amylolytic enzymes. The proteolytic enzymes produced are protease secretions that can degrade proteins. Protease is an enzyme that catalyzes the hydrolysis of peptide bonds in proteins in coffee so that the content of amino acids and peptides increases and produces a unique aroma and taste [29]. In addition to proteolytic enzymes, *S. cerevisiae* also produces amylolytic enzymes that will break down carbohydrates into acids. Then proteolytic enzymes will break down the coagulated protein so that it will accelerate the mucilage release process [30].

During fermentation, protein degradation will occur in coffee and form oligopeptides, dipeptides and become amino acids. The lower the protein content, the less bitter the coffee will taste. This protein breakdown will cause a savory taste in coffee. This savory taste is expected in fermentation so as to create a balanced taste [31].

Conclusions. Based on results of the study, it can concluded that the treatment of different lengths of fermentation and different starter concentrations had a significant effect on the reduction sugar content, caffeine content and protein content of robusta coffee. The control sample has the highest chemical compound content, while the lowest reduction sugar content is found in sample A2B3 with a value of 0.75%, the lowest caffeine content is obtained in sample A2B3 with a value of 0.87% and the lowest protein content is also found in sample A2B3 with a value of 10.96%. The length of fermentation and differences in starter concentration did not significantly affect the pH value and water content of robusta coffee.

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