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Utilization of *Bacillus subtilis* Culture for Fermentation of Arabica Coffee (*Coffea arabica*) from Toraja Regency

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ABSTRACT

The aim of this research was to determine the effect of giving Bacillus subtilis concentrations on caffeine levels and the physicochemistry of Arabica coffee beans. This study used a Complete Randomized Design (RAL) where the concentration of B. subtilis was 0%, 1%, 2%, 3%, 4%, 5%. Variables observed in this study included total plate numbers (ALT), total titrated acid (TAT) and pH, as well as arabica coffee bean tests (water and caffeine content). The results of this study show that B. subtilis affects ALT, TAT, pH, water content and caffeine content of fermented liquid in arabica coffee beans. The lowest caffeine content of arabica coffee beans is made from arabica coffee beans added 5% B. subtilis during fermentation with a caffeine content of 1.34%.

INTRODUCTION

Arabica coffee (*Coffea arabica*) is a plantation crop with high economic value. This plant grows optimally in the tropics at an altitude of 1,000-2,000 meters above sea level. One of the arabica coffee producing areas in South Sulawesi is Toraja Regency. Toraja Arabica coffee is one of the most popular and high-quality coffee options in Indonesia. In addition to its distinctive flavor, Toraja coffee has its own uniqueness (Raharjo, 2012).

The unique characteristics and flavors of coffee are influenced by the place or places of coffee cultivation, harvesting and post-harvest processing of the coffee. Post-harvesting of coffee is an important process that must be considered carefully, because it can affect and determine the quality of the coffee beans produced. Arabica coffee bean processing in Toraja Regency is still largely traditional, namely with harvested coffee cherries going through a fermentation process. The purpose of fermenting coffee horns is to loosen the slime layer that is still attached to the coffee horn shell so that the horn shell is easier to remove during washing. In addition, it facilitates the drying and processing of coffee beans.

Fermentation is a process in which microorganisms act to produce a desired product. Spontaneous fermentation is known to be the best way to reduce the caffeine content in arabica coffee beans. Fermented coffee is of better quality than unfermented coffee. By adding microbes to the fermentation process, the quality of the coffee beans produced can be said to be better. One of the microbes that can be used in the spontaneous fermentation process of coffee beans is *B. subtilis* (Avallone et al., 2002).

Bacillus subtilis is a positive bacterium, rod-shaped and oval in the center of the cell, 2-3 µm long and 0.7-0.8 µm wide, chain-shaped, corporating and aerobic (Jawetz *et al*, 1996). *B.*

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subtilis has more favorable characteristics than other microorganisms because it can survive for a long time in environmental conditions that are unfavorable for its growth (Wong, 1994).

B. subtilis uses sodium citrate as a carbon source for metabolic processes and growth. This type of bacteria can produce protease, amylase and lipase enzymes as pathogen cell wall degrading enzymes (Hatmanti, 2000). In addition, *B. subtilis* can produce enzymes that can prevent the growth of other competing bacteria, giving it antagonistic properties against other species. Bacteria of this species are used in food fermentation, as a source of extracellular enzymes for industry and medicine, and for the production of peptide antibiotics (Schetzer, 2006).

The use of *B. subtilis* in coffee fermentation is not widespread among coffee farmers, developers or scientific researchers. The purpose of this study was to evaluate the effect of *B. subtilis* concentration on the fermentation process of arabica coffee beans. It is expected that this study will be able to improve the quality of fermented coffee beans with the addition of *B. subtilis*.

MATERIALS AND METHODS

This research is a type of experimental research with a completely randomized design (CRD). This study used six experimental units consisting of 1 control (without the addition of *B. subtilis*) and 5 treatments (addition of *B. subtilis* with concentrations of 1%, 2%, 3%, 4% and 5%). The experimental unit of this study was repeated three times so that 18 experimental units were obtained.

Time and Place

This research was conducted from December 2020 to July 2021. This research was conducted at the Laboratory of the Agricultural Technology Education Study Program, Faculty of Engineering, Makassar State University Jl. Dg. Tata Raya Parangtambung Makassar.

Tools and Materials

In this study the tools used were fermenters, analytical balances, petri dishes, alcohol, ose needles, *laminar air flow*, *autoclave*, measuring cups, erlenmeyer, bunsen, test tubes, tube racks, ovens, volume pipettes, pH meters, incubators, *object glass*, *bath*, stirring rod, *hot plate*, volumetric flask, dropper, uv-vis spectrophotometer, goblet, filter paper, separatory funnel, *water bath*, porcelain cup, desiccator, furnace, mortar, vortex, cuff, aluminum foil.

The materials used in this research are arabica coffee fruit, *B. subtilis*, PCA microbial growth media, NA agar media, spirtus, cotton, pH 7 and pH 4 *buffers*, *distilled* water, ascorbic acid, 0.1 N sodium hydroxide, 0.01N sodium hydroxide, PP indicator, standard caffeine, chloroform, nitric acid, 37% formaldehyde, 1% phenolphthalein indicator, anhydrous glucose, nelson *reagent*, arsenomolybdate, pure glucose solution, 5% phenol, sulfuric acid, gallic acid solution, *folin ciocalteu reagent*, 10% sodium carbonate, DPPH, methanol p. a and tissue.a and tissue.

Research Procedure

The procedure used in this research consists of several steps, namely:

Bacillus subtilis Culture Preparation

The procedure for preparing the *B. subtilis* culture is described as follows:

1. Making Tilted Agar

NA agar media (5 g) was dissolved in 100 mL of distilled water to 100 mL of media, then heated and mixed on a *hot plate* (*magnetic stirring*) until the dissolved media appeared light yellow, the media was placed in a *protube* covered with cotton and aluminum foil. The media was then sterilized using an autoclave at 121°C for 15 minutes. Sterilized media was placed in a *laminar air flow* with an oblique position so as to form an oblique media.

2. Culture Refresh

Refreshing is done to produce healthy and fresh *B. subtilis* cell cultures. NA medium is put in a test tube. Inoculate the agar medium aseptically with a wire *loop*, transfer a *loop of* pure culture from the existing growth medium, and then scratch it onto the surface of the plate on NA medium in a zigzag pattern. The mouth of the test tube was then burned and closed again and incubated for 48 hours.

3. *Starter* Making

The starter is taken obliquely from the nutrient agar, then 10 ml of sterile distilled water is added and the growing bacteria are stirred with an ose needle, then transferred to an erlenmeyer flask containing 360 ml of sterile culture medium. To prepare Water for starter and homogenized to 1440 ml.

Coffee Fruit Sorting

The coffee processed is Arabica coffee from the Toraja region. Before processing, the coffee cherries are first sorted. Sorting coffee cherries aims to distinguish good red coffee from beans from empty, rotten or damaged coffee. The method of separating coffee beans is based on specific gravity by soaking the cherries in water in a tub. In this soaking, young and powdered coffee cherries float while old cherries sink. Then the coffee cherries can be ground.

Coffee Fruit Skin Peeling

Peeling of the fruit skin is done with a fruit peeler (*pulper*). Along with the peeled fruit with the help of water flowing into the cylinder. The *pulping* process is carried out under running water so that the *sludge is* removed from the coffee husk. This process is called full washing.

Coffee Bean Fermentation

The fermentation process aims to release the slimy pulp that is still attached to the horn shell. The fermentation process carried out is a wet process, with the ratio of coffee done 1: 1 with water. In the fermentation process, *B. subtilis was* added as much as (0% control, 1%, 2%, 3%, 4%, and 5%) fermentation was carried out for 48 hours with observations of 0, 24 and 48 hours using plastic ziplo.

Coffee Bean Washing

The purpose of washing is to remove the remaining layer of mud and other contaminants that remain after fermentation or after leaving the *pulper*. Washing is done by hand in a tub or bucket, this is an important stage after the fermentation process so that the mucus removal process can be maximized, this washing must be maximized so that the mucus in the coffee beans can be removed.

Drying Coffee Beans

The washed coffee is dried to reduce the water content to 20%. With this water content, the coffee is not easily broken when peeled. Semi-wet drying of beans refers to the wet drying process when drying pumpkin coffee powder (coffee beans that still contain mucilage).

Coffee Bean Cooling

The dried coffee beans are then cooled so that the moisture content of the coffee is relatively the same. The purpose of this cooling is to minimize the risk of split coffee beans when *hulling*. Coffee beans that have been dried in the sun are allowed to stand for ± 24 hours at room temperature.

Peeling the Antler Skin

Hulling when the coffee beans are still relatively moist (pumpkin coffee), *hulling* can be done in 2 ways, dry *huller* and wet *huller*, but what is used is dry *huller* because the moisture content of the coffee to be *hulled* is 12%.

Green Bean Sorting

Sorting is done to separate coffee beans based on size and defects. The measurement can be done with a mechanical *screener* (*screen reader*) or manually. This is done so that the size of the coffee is the same and to separate defective coffee beans when *hulled*.

Drying Stage

The drying stage is carried out again to reduce the moisture content of the hulled coffee beans by drying at 40-50°C until the moisture content is 11-12%.

Data Collection Technique

Observation and laboratory analysis were used to collect data in this study. Observation technique was used to collect data by directly observing the object. Analysis was used to determine the TAT, ALT and pH values of fermentation liquid, moisture content and caffeine content of arabica coffee beans. The laboratory analysis used is described as follows:

Total Plate Count (ALT)

The total plate count (ALT) analysis procedure in arabica coffee bean fermentation liquid according to SNI 01-3642-2004 is as follows, Petri dish filled with 1 ml of sample mixing results and dilutions. 10^{-1}, 10^{-2} Pour PCA media as much as 12-15 ml into the last 2 dilutions, then rotate the Petri dish left, right, front and back so that the media is mixed homogeneously. Let the Petri dish stand until the media is solid, then wrap the Petri dish using paper. Incubate for 48 hours at room temperature, calculate the total plate count value with the condition that the number of colonies is 25-250 colonies per Petri dish. Calculate the total plate count using the following formula:

$$ALT = \frac{\sum C}{((1\ x\ n1) + (0.1\ x\ n2)\ x\ d))}$$

Description:

C	Number of colonies from each petri dish
n1	The number of petri dishes from the first dilution is counted
n2	Second dilution petri dish
d	The first dilution is calculated

Total acid titration (TAT)

The procedure for analyzing total titratable acid (TAT) in Arabica coffee bean fermentation liquid according to AOAC (2005) is as follows:

Fermentation liquid as much as 10 ml is placed in a 250 ml volumetric flask, add distilled water to the limit and homogenize, take 25 ml of sample and put it in a 250 ml erlenmeyer, add 3 drops of *phenolphthalein* indicator.

After that, titrate the sample with 0.1 N NaOH solution until the color turns pink. Calculate the total value of titratable acid. using the following formula:

$$TAT\ (\%) = \frac{V\ x\ N\ x\ FP\ x\ BM}{BS\ x\ 1000}\ x\ 100\%$$

Description:

V	Titration Volume (start - end)
N	Normality (0.1)
FP	Dilution Factor (4)
BM	Molecular weight (60)
BS	Sample weight (ml)

Acidity (pH)

The procedure for analyzing pH in Arabica coffee bean fermentation liquid according to AOAC (2005) is as follows:

The pH meter is turned on and calibration is carried out using pH 7 *buffer*, insert the pH meter tip to the limit into the coffee bean fermentation liquid and wait a while, record the number printed on the pH meter screen, rinse the pH meter using distilled water and then dry it using a tissue.

Water Content

The procedure for analyzing the moisture content value of Arabica coffee beans after fermentation according to SNI 01-2907-2008 is as follows:

10 ml fermentation liquid is placed in a 250 ml volumetric flask, add distilled water to the limit and homogenize. Calculate the total titratable acid value. using the following formula:

$$TAT\ (\%) = \frac{V\ x\ N\ x\ FP\ x\ BM}{BS\ x\ 1000}\ x\ 100\%$$

Description:

V	=	Titration Volume (start - end)
N	=	Normality (0.1)
FP	=	Dilution Factor (4)
BM	=	Molecular weight (60)
BS	=	Sample weight (ml)

Caffeine Content

How to analyze the caffeine value of Arabica coffee beans after fermentation according to Fitri, (2009) as follows:

1. Preparation of Caffeine Standard Solution

Weigh pure caffeine as much as 0.01 grams and then put it into a 100 ml volumetric flask and then add distilled water to the limit to make a standard solution of 1000 ppm, then homogenize the solution, the previously prepared standard solution is transferred into a 10 ml volumetric flask to obtain a standard solution concentration of 0; 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5 and 5 ppm Add distilled water to the limit and then homogenize, the absorbance value is measured using a wavelength of 290 nanometers on a spectrophotometer. The relationship between the concentration of the standard solution and the resulting absorbance value is then made a calibration curve

2. Calculation of Caffeine Content

Coffee beans that have been finely then weighed to close to 1 gram and then placed in a 100 ml beaker, hot distilled *water* with a temperature of 70^0 C is put into the beaker and then stirred until homogeneous, then the homogeneous sample is put into a *waterbath* at a temperature of 70^0 C for 1 hour while stirring the sample, the sample is then filtered using filter paper into an Erlenmeyer, 0.1 ml of the sample is taken and placed in a 10 ml volumetric flask. Then added distilled water to the limit of tera, The sample that has dissolved is then measured using a spectrophotometer with a wavelength of 290 nanometers, Calculate the value of caffeine content using the following formula:

$$Kafein\ (\%) = \frac{k\ \left(\frac{mg}{L}\right)\ x\ v\ (L)\ x\ fp}{mg\ sampel}\ x\ 100\%$$

Description:

V	=	Volume
Mg	=	Sample weight/number of samples
K	=	Concentration
FP	=	Dilution factor

Data Analysis Technique

The data analysis technique used in this research is ANOVA (*Analysis of Variant*) analysis of variance using the SPSS version 22 application to determine the effect of the addition of *B. subtilis* on the caffeine content of arabica coffee beans. The analysis requirement test used normality and homogeneity tests. If there is a significant difference between treatments, then it is continued with Duncan*'s Multiple Range test*.

RESULTS AND DISCUSSION

The results showed that the addition of *B. subtilis* concentration influenced the ALT, TAT and pH values of the fermentation liquid as well as the water content and caffeine content of Arabica coffee beans.

Total Plate Count (ALT)

Total Plate Count (ALT) is a number or value that refers to the number of microbes in 1 ml of the fermentation liquid sample tested. The principle of ALT testing is one of the methods used to determine the bacteria that grow in the sample tested (Oktadina et al., 2013). The effect of the addition of *B. subtilis* on Arabica coffee bean fermentation liquid can be seen in Figure 1.

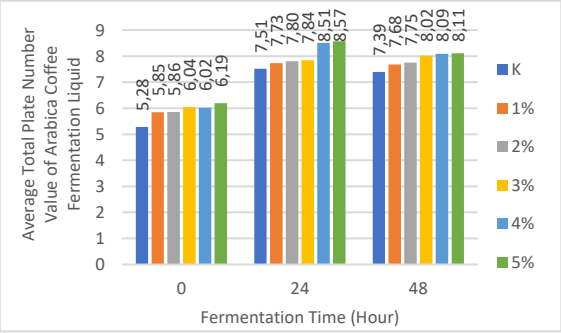

Figure 1. Average Total Plate Count (ALT) Value of Arabica Coffee Fermentation Liquid

The results of Anova analysis of variance showed that the length of fermentation and increased levels of *B. subtilis had* an effect on the 48-hour ALT value, while 0 and 24 hours of fermentation had no effect on the ALT value. The results of Duncan's further test with 48 hours of fermentation showed that the addition of *B. subtilis* concentration was significantly different from the control.

The results of the analysis showed that the value of the 0-hour time interval did not significantly affect the ALT of coffee bean fermentation liquid due to the initial phase (*lag phase*) of microbial growth. At the interval of 24 hours the fermentation process took place, the amount of ALT in coffee bean fermentation liquid increased. The increase in the number of *B. subtilis is* due to the growth in the number of cells by utilizing nutrients that are broken down into simple sugars which are used as energy sources so that *B. subtilis* undergoes maximum division and produces high enough cells. At the 48th hour there was a decrease in *B. subtilis*, where it entered the death phase caused by nutrients in the medium being exhausted (Suprihatin, 2010).

The ALT value of coffee bean fermentation liquid decreased from 24 to 48 hours of fermentation. Coffee fermentation for 48 hours causes an increase in the total organic acid value as a result of the metabolism of *B. subtilis* involved during the fermentation process and causes a decrease in pH value. This has an impact on the viability of *B. subtilis* during the coffee bean fermentation process. *B. subtilis* is one of the bacteria that cannot tolerate acidic conditions, so when organic acid increases, the amount of *B. subtilis* involved in coffee bean fermentation will be reduced and have an impact on reducing the total microbial count (ALT) in the coffee fermentation liquid. According to Surti *et al.* (2016) *B. subtilis* is unstable in an acidic atmosphere and has stability at neutral pH.

The decrease in total bacteria in the fermentation process is also caused by *B. subtilis* bacteria converting carbohydrates into energy sources for growth. This will cause changes in acidic liquids which result in a decrease in pH value, so that bacteria that are not resistant to acidic conditions can be inhibited from growing. Increasing the value of *B. subtilis* can reduce the pH value and increase the acidity of the fermentation liquid this occurs due to the fermentation process.

Total acid titration (TAT)

Total titratable acid (TAT) is the amount of acid produced during the fermentation process. In this study, the TAT value focuses on

the total percentage of acid value produced by bacteria during the fermentation process. The TAT value is the value resulting from the acids formed during the fermentation process (Prastuti *et al.*, 2018). The effect of the addition of *B. subtilis* on the total titratable acid (TAT) of Arabica coffee bean fermentation liquid can be seen in Figure 2.

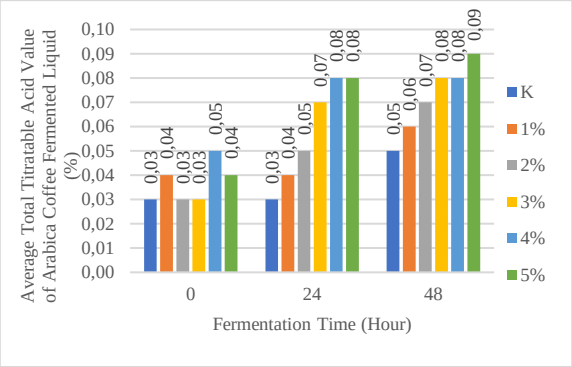

Figure 2. Average Total Titratable Acid (TAT) Value in Arabica Coffee Fermentation Liquid

The results of Anova analysis of variance showed that the length of fermentation and increased levels of B. subtilis had an effect on the TAT value at 24 hours, while for 0 and 48 hours fermentation had no effect on the TAT value. The results of Duncan's further test with 24 hours of fermentation showed that the addition of B. subtilis concentration was significantly different from the control.

The increase in TAT is due to an increase in the amount of organic acids produced by B. subtilis involved in fermentation. At the 48-hour interval, the TAT increased significantly, this increase was due to the activity of the B. subtilis culture using the available carbon source as an energy source. During this metabolic process, changes in chemical compounds occur, namely changes in reducing sugar compounds into organic acid compounds. According to Hadipernata and Nugraha (2012), the result of the sugar breakdown process is lactic acid and other acids, namely ethanol, butyric acid, and propionate.

According to Aditiwati and Kusnadi (2003), bacterial activity will produce acetic acid compounds, organic acids and also alcohol so that the longer the fermentation time, the higher the concentration of acids produced by bacteria involved in the fermentation process. B. subtilis in the fermentation liquid can also cause the process of breaking down complex compounds in the fermentation liquid into organic acids. B. subtilis also produces protease enzymes that degrade proteins in coffee bean mucilage during the fermentation process into amino acids, peptides and ammonia (Chukeatirote, 2015).

During the fermentation process, the acidity of the coffee and fermentation liquid increases. This is due to the formation of aliphatic acids during the fermentation process. The acids formed are released into the environment, causing changes in acidity (Sulistyowati & Sumartono 2002).

pH value

The pH value is a number that refers to an acidic or alkaline condition of a solution, pH is measured on a scale of 0-14 (Febrianti et al., 2019). The pH value contained in a solution will affect the metabolism of microorganisms contained in the solution. The effect of the addition of B. subtilis on the pH value of Arabica coffee bean fermentation liquid can be seen in Figure 3.

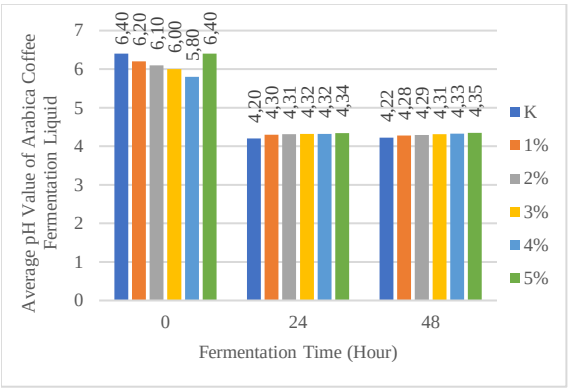

Figure 3. Average pH Value of Arabica Coffee Fermentation Liquid

The results of Anova analysis of variance showed that the length of fermentation and the increase in concentration of *B. subtilis had* an effect on the pH value of 0 hours, while 24 and 48 hours of fermentation had no effect on pH. The results of Duncan's further test at the fermentation time of 0 hours showed that the increase in concentration of *B. subtilis was* significantly different from the control treatment.

The results of the analysis showed that the value of time interval 0 hours to 24 hours fermentation decreased and was significantly different from the control, the decrease in pH was caused by an increase in organic acids formed during fermentation. The formation of organic acids occurs through the metabolic activity of the added microbes. The results of

the analysis of variance showed that the average pH value of the fermentation liquid decreased at 24 hours, indicating that there was an increase in activity. The pH value of coffee bean fermentation liquid during the 24-hour fermentation process decreased due to the culture of *B. subtilis* having the ability to produce cellulase and amylase enzymes whose role is to convert cellulose and amylose contained in coffee beans into simpler compounds. This resulted in *B. subtilis* involved in the fermentation of coffee beans to further utilize these simple components and convert them into organic acid compounds. The organic acids metabolized by *B. subtilis* during fermentation cause the pH value of the coffee bean fermentation liquid to become more acidic.

These organic acids such as malic acid, tartaric acid, citric acid, lactic acid, acetic acid, butyric acid and propionic acid as by-products, these acids lower the pH of the environment (Suprihatin, 2010). During the fermentation process, the amount of organic acids increases (Charalampopoulos et al., 2002).

Water Content

Moisture content is one of the factors that can affect the shelf life and quality of a product. High moisture content in a product will increase the growth rate of microorganisms contained in the product. The moisture content of coffee beans that have been fermented using the treatment of adding *B. subtilis* can be seen in Figure 4.

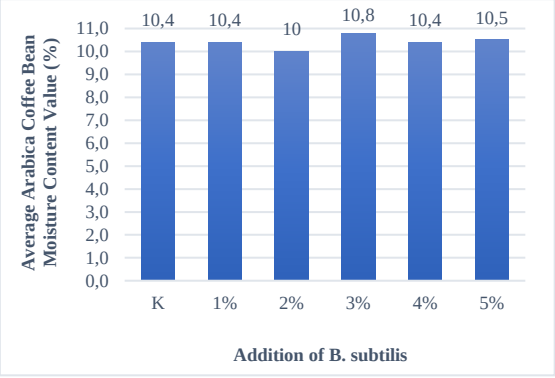

Figure 4. Average value of moisture content in Arabica coffee bean fermentation

The results of Anova analysis of variance showed that the concentration of *B. subtilis had* no effect on the moisture content of coffee beans. This can be seen from the calculated F value of 0.348> F Table 3.11 at the 0.05% level, based on this data, no further tests were carried out on water content. The moisture content of coffee beans produced is in the range of 9.98-10.81%. Based on the water content parameter, the water content of coffee beans produced from controlled fermentation using *B. subtilis is* classified as good quality. The moisture content of coffee beans obtained is still in accordance with the standard of coffee beans required by SNI, namely. the moisture content of coffee beans should not exceed 12%. Low moisture content can reduce or inhibit microbial attack on food ingredients (Winarno 2002).

According to Wibowo (1995), a moisture content of 12% with a tolerance of 1% is a limit that can ensure safety during storage. Enzymes in coffee beans undergo a relaxation phase when the moisture content is below 13%. Conversely, beans with a moisture content of less than 9% (too dry) damage the taste and color (Sivetz & Desrosier, 1999). To ensure the storage stability of coffee beans, it is best to dry them to a maximum moisture content of 11%.

Caffeine Content

Caffeine is a xanthine alkaloid compound whose flavors act as a psychoactive sedative and mild diuretic. Caffeine is found in white alkaloids, whose chemical formula is 1,3,7-trimethylxanthine. The chemical structure of caffeine is similar to other alkaloid compounds such as xanthine, theophylline, and theobromine (Lelyana, 2008). The caffeine content of coffee beans that have been fermented using *B. subtilis* addition treatment can be seen in Figure 5.

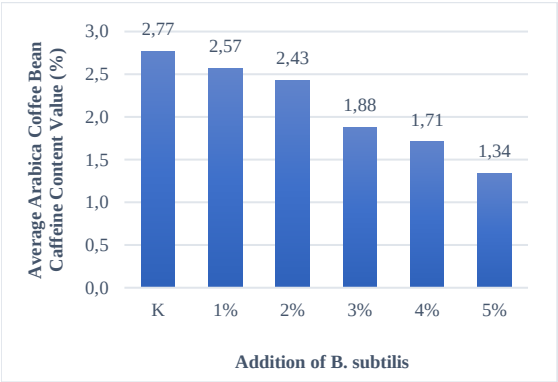

Figure 5. Average Value of Caffeine Content in Arabica Coffee Bean Fermentation

The results of the analysis of the caffeine content of Arabica coffee beans with the addition of *B. subtilis* showed different values in each treatment with the value of caffeine levels in the range of 1.34-2.77%. The results of

the analysis of variance of the provision of *B. subtilis* to the fermentation of coffee beans have a significant effect, the treatment with the highest caffeine content value is shown in the control treatment with a caffeine content value of 2.77%, while the lowest caffeine content value is shown in the treatment of 5% *B. subtilis* addition, which is 1.34% The decrease in caffeine content in coffee is inversely proportional to the concentration of B. *subtilis* added. With the addition of 1% *B. subtilis*, caffeine levels began to decrease compared to the control and continued to decrease until the addition of 5% *B. subtilis.*

The results of Anova analysis of variance treatment of *B. subtilis* on coffee bean caffeine has a real effect. This is known from the calculated F value of 506.483> F Table 3.11 at the 5% level. This indicates the need for further tests to determine the most influential treatment.

Duncan's further test results on caffeine content showed that the treatment of *B. subtilis* addition gave a significant difference with the control because each treatment occupied a different subset. Based on this, it can be assumed that the treatment of adding *B. subtilis is* able to reduce the value of caffeine content. The treatment with the lowest caffeine content value is the addition of 5% *B. subtilis* with a value of 1.34%.

The decrease in caffeine content of fermented arabica coffee beans is also caused by the activity of *B. subtilis* bacteria that are added by breaking down proteins in the outer shell of coffee beans to produce amino acids and ethanol. Based on research by Hadipermata & Nugraha (2012), sugar is broken down to produce lactic acid and other types of acids such as ethanol, butyric acid and propionic acid, while according to Usman & Suprihadi (2015), there is a layer of mucus outside the coffee beans consisting of 80% pectin and 20% sugar. This layer becomes a substrate for *B. subtilis*.

The addition of *B. subtilis* causes the decomposition of the mucus layer in the coffee skin area, so that water is absorbed by the coffee beans causing caffeine to dissolve, this is due to the nature of caffeine which is dissolved in water. This is explained by Ridwansyah (2003) who said that the solubility of caffeine in water is due to its nature that binds water molecules. In addition, the decrease in caffeine content is caused by the esterification process which causes the breakdown of complex caffeine compounds into chlorogenic acid. The caffeine compound is released with a smaller molecular size and weight and is easily dispersed so that it can reduce the electron receiver compound.

CONCLUSION

The conclusion based on the results of research using *B. subtilis* culture on fermented arabica coffee from Toraja Regency is that the addition of *B. subtilis* in different concentrations has a significant effect on ALT, TAT and pH of the liquid during fermentation and the caffeine content of arabica coffee beans after fermentation. Arabica coffee beans with the lowest caffeine content were obtained from the treatment with the addition of 5% *B. subtilis* which had a caffeine content of 1.34%.

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