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# EFFECT OF KOMBUCHA CULTURE ON CAFFEINE AND CHLOROGENIC ACID CONTENT IN FERMENTATION OF ROBUSTA GREEN COFFEE BEANS (*Coffea canephora* L.)

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**ABSTRACT**

Caffeine and chlorogenic acid are two substances that are usually contained in coffee as a stimulant and an antioxidant. Green coffee has been reported to contain higher amounts of caffeine and chlorogenic acid compared to black coffee. In Indonesia, green coffee highly produced in Garut, Soreang, Ciwidey, and Aceh. The purpose of this research is to determine the content of caffeine and chlorogenic acid and acidity (pH) from Robusta green coffee beans, by adding kombucha culture at 0, 6, 12 and 18 days of their fermentation. The analysis of pH was carried out using a pH meter, while caffeine and chlorogenic acid were carried out using High-Performance Liquid Chromatography (HPLC). The results showed a decrease in pH in Garut, Soreang, Aceh and Ciwidey green coffee beans, the pHs obtained on the 18th day were 65.33; 66.67; 64.00; 64.86 % respectively, and the content of caffeine decreasing concentration at the 18th day were 34.47; 39.06; 36.93 and 35.69 % respectively, while the highest contents of chlorogenic acid increasing concentration on the 18th day were 13.17; 10.15; 14.02 and 11.13 %, respectively. We also recommended testing the fermentation of Arabica green coffee beans to obtain a comparison of the results of Robusta coffee beans.

**Keywords:** Caffeine, chlorogenic acid, green coffee beans, kombucha



## INTRODUCTION

The world community, including Indonesia, processes coffee into a quality drink that has a high selling price. Indonesia is one of the largest producers of Robusta coffee in the world (674.000 ton/year) such as from West Java and Sumatra. Coffee ranks second in all food commodities consumed and traded throughout the world[1].

There are four types of coffee beans classified by species, namely arabica coffee (*Coffea arabica*), robusta coffee (*Coffea canephora*), liberika coffee (*Coffea liberica*), and excelsa coffee (*Coffea excelsa*)[2]. A group of coffee beans that are known to have economic value and are traded commercially are arabica and robusta coffee. Arabica coffee has a high taste and low caffeine content, so it is recognized as having the best quality and valued at a high price. Whereas Robusta coffee has a lower quality than Arabica, so it is valued 30% - 40% lower than Arabica. Liberica coffee and excelsa are less desirable because the results of the commodities which are included are rare and arguably rare. Indonesian people are already familiar with the aroma of Robusta and Arabica coffee, which is better for coffee. Two coffee species that provide economic value are Arabica coffee and Robusta coffee. Arabica and Robusta coffee have several differences in their ideal climates used for their growth, phenotypic aspects, and chemical composition[3]. Robusta coffee in Indonesia is a very high commodity and dominates the National market, but only controls 30% of the world market. Based on existing conditions in Indonesia, Robusta coffee production is more than Arabica coffee, but the coffee that is widely used is Arabica coffee, where the caffeine content of Robusta is higher than Arabica Compared to Arabica coffee commodities which control 70% of the world market. Robusta Coffee beans can be easily planted on terrain with a low variation of temperatures[1]

The chemical contents of coffee are caffeine, phenolic acid, and chlorogenic acid. Caffeine has a clinically beneficial pharmacological effect by stimulating the central nervous system as an adenosine


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receptor antagonist[3]. Chlorogenic acid in human health is widely reported as an antioxidant, antiviral, hepatoprotective and antispasmodic[4]

Bicho et al.[5] reported that the caffeine level of Robusta coffee is higher than Arabica, 2.38% compared to 1.45 %. The content of chlorogenic acid in Robusta coffee is around 7.0-10.0%. Arabica coffee caffeine content is 0.9-1.3 g / 100 g, while robusta coffee is 1.5-2.5 g / 100 g. The chlorogenic acid content of Arabica coffee is 4.1-7.9 g / 100 g, while Robusta coffee is 6.1-11.3 g / 100 g[4]. Chlorogenic acid is found in many coffee and tea drinks[6].

Green coffee is coffee beans from the Coffee fruit that has not been roasted. The roasting process of coffee beans can reduce the amount of chlorogenic acid[7]. Studies on the efficacy of green coffee bean extract containing caffeine and chlorogenic acid added to the nutraceutical component are reported to be effective in controlling weight management[8,9].

The addition of kombucha to green coffee beans consisting of bacteria and yeast, among others, *Acetobacter xylinum* and several types of yeast, namely *Brettanomyces intermedius*, *Candida fatama*, *Saccharomyces cerevisiae*, Mycoderma, Mycotorula, Torulopsis, *Pichia membrane*, *Zygosaccharomyces* sp., *Torulaspora delbrueckii*[10] is intended to determine its effect on caffeine and chlorogenic acid levels because it is usually added to tea. Kombucha is also reported to have various health effects, such as antibiotics, digestion, antioxidants, and antibacterial[11]. Green coffee beans have higher level of chlorogenic acid compared to ordinary coffee (roasted coffee beans).

Kombucha coffee (KC) is now famously consumed by people around the world. Kombucha is a liquid fermented by coffee bean extract using kombucha culture. It has been used for treatment therapy for thousands of years ago in some countries such as China, Russia, and Germany[12].

Kombucha is usually made from sweet tea that has been fermented with the combination and symbiotic bacteria and yeast[13]. It is a combination of bacteria and yeast which has a matrix symbiosis of mycelium like fibers[14], and *Acetobacter xylinum* is usually the first bacteria in the Kombucha culture[15].

The variations of the Kombucha composition may be due to geographical, climate and cultural conditions as well as the diversity of local species from yeast and bacteria[16]. The main components of the fermentation are acetic acid, ethanol, and glucuronic acid, while the minor components which are produced are lactic acid, phenolic acid, B vitamins, and enzymes. Kombucha has various health effects, such as antibiotics, digestive, antioxidants and antibacterial[11].

This study aimed to analyze the pH after the fermentation process and determine the content of caffeine and chlorogenic acid from 4 types of Indonesian robusta green coffee beans from West Java, i.e. Garut, Soreang, Ciwidey, and Aceh from Sumatra, by adding kombucha culture at 0, 6, 12 and 18 days of their fermentation. The duration of fermentation carried out for 18 days was intended to determine the changes that occur in the pH and concentration of caffeine and chlorogenic acid, kombucha fermentation is usually carried out for 8-14 days using a tea medium.

## EXPERIMENTAL

### Material and Methods

The materials used were Robusta green coffee beans (from Garut, Soreang, Aceh and Ciwidey, West Java, Indonesia) (Determination of plants by SITH ITB No. 2833 / I1.CO2.2. / PL / 2017), kombucha (Indokombucha), sucrose and water. Chemicals such as methanol (Merck), acetic acid (Merck) and aquadest were used for the analysis of caffeine and chlorogenic acid, as well as a filter membrane 0.45 μm. The instruments used were: a pH meter (Mettler Toledo), High-Performance Liquid Chromatography (HPLC) (Shimadzu) C-18 column, 150 mm length and 4.6 mm with a UV detecting system.

### General Procedure

### Kombucha Coffee Fermentation

0.375 g Robusta green coffee beans in 300 mL of boiling water were mixed with 30 g of sucrose and stirred until homogeneous. The suspension was chilled and filtered, then 30 mL of vinegar kombucha was added and was stirred until homogeneous. The suspension was then inoculated with 20 g of kombucha culture. A sterile porous cloth then covered the suspension containing jar and placed in the incubator. The cultures were kept incubated at room temperature ± 25 ° C. The duration of fermentation carried out for

18 days was intended to determine the changes that occur in the pH and concentration of caffeine and chlorogenic acid, for 18 days.

**pH Measurement**

The addition of vinegar to the fermentation process is intended as an adaptation medium for kombucha so that it can affect the pH conditions during fermentation. Prepare 20 ml fermented kombucha coffee test solution taken on days 0, 6, 12 and 18 in a sample container, before testing, the pH meter is calibrated using a Buffer pH, then the sample is tested using a pH meter (Mettler Toledo) by inserting the electrode into the sample solution. Make a note of the stable results on the tool display, testing is done as much as triplo. The sample of pH measurements were performed on the fermented liquid after harvesting using pH meters (Mettler Toledo).

**Detection Method**

The caffeine and chlorogenic acid contents were determined and analyzed using HPLC from 10 mL of the sample. For the caffeine content, the system used in this study was C-18 columns with a five μm pore size, the mobile phase used was methanol: water (7:3) with a flow rate at 1.0 mL/minute and a UV detector at λ=273nm. For chlorogenic acid content, the HPLC system was used in this study, using a C-18 column with a 5 μm pore size, the mobile phase used was methanol:aquabidest with 1% acetic acid (40:60), the flow rate at 1.0 mL/minute and UV detector at λ=329 nm.

## RESULTS AND DISCUSSION

The results showed that the addition of kombucha to Indonesian Robusta green coffee beans affected pH, caffeine and chlorogenic acid as shown in Table 1.

Table-1: The Effect of Adding Kombucha Culture to Green Coffee Beans

| Item | Source of Green Robusta Coffee | Day of Fermentation | | | | Decreased of Increased (%) |
|---|---|---|---|---|---|---|
| | | 0 day | 6 day | 12 day | 18 day | |
| pH | Garut | 7.5±0.1 | 3.0±0.2 | 2.7±0.1 | 2.6±0.1 | 65.33 (-) |
| | Soreang | 7.5±0.1 | 3.0±0.1 | 2.8±0.2 | 2.5±0.2 | 66.67 (-) |
| | Aceh | 7.5±0.17 | 2.9±0.1 | 2.8±0.1 | 2.7±0.2 | 64.00 (-) |
| | Ciwidey | 7.4±0.1 | 2.9±0.17 | 2.8±0.17 | 2.6±0.1 | 64.86 (-) |
| Concentration of Caffeine (mg/L) | Garut | 36.84±1.08 | 29.15±1.57 | 23.45±1.16 | 24.14±1.87 | 34.47 (-) |
| | Soreang | 36.48±0.95 | 27.46±0.93 | 22.16±1.48 | 22.23±1.15 | 39.06 (-) |
| | Aceh | 35.22±0.96 | 26.79±1.60 | 22.18±1.65 | 22.21±1.36 | 36.93 (-) |
| | Ciwidey | 37.35±0.78 | 28.69±1.31 | 23.22±1.47 | 24.02±1.14 | 35.69 (-) |
| Concentration of Chlorogenic Acid (mg/L) | Garut | 131.58±0.67 | 136.72±2.68 | 142.46±1.30 | 151.53±0.50 | 13.17 (+) |
| | Soreang | 133.43±1.18 | 135.19±1.11 | 143.48±0.51 | 148.32±1.21 | 10.15 (+) |
| | Aceh | 125.73±2.03 | 129.34±1.52 | 138.37±0.87 | 146.24±1.38 | 14.02 (+) |
| | Ciwidey | 127.76±0.63 | 129.66±0.64 | 142.86±1.23 | 143.76±1.08 | 11.13 (+) |

**pH Measurement**

pH measurements were carried out to determine the extent of pH changes during the fermentation process after adding the kombucha vinegar culture. Kombucha vinegar culture is added to green coffee bean extract to adapt the initial conditions after the culture transfer process is carried out to maintain the acidity of the kombucha culture during the fermentation process.

From Table-1 it can be seen that the addition of kombucha culture to Indonesian Robusta green coffee beans gradually decreased acidity (pH) from day 0 to day 18. The decrease in acidity (pH) of Garut, Soreang, Aceh and Ciwidey green coffee beans obtained on the 18th day was 65.33; 66.77; 64.00; 64.86 % respectively.

Based on these conditions, it indicates that the decrease in the pH of kombucha coffee is caused by an increase in the concentration of acids during the fermentation process. According to Sreeramulu et al.[17]

the decrease in pH occurred because the addition of vinegar at the beginning of fermentation is intended as an adaptation medium for kombucha so that it can affect the pH conditions during fermentation. During yeast fermentation, bacteria synthesize sucrose into organic acids, such as acetic acid and gluconate acid and several other organic acids, increasing the concentration of organic acids results in a decrease in pH in the fermentation medium.
In the kombucha coffee, which was composed of acetic acid-producing bacteria *Acetobacter* sp., the longer the fermentation performed, the lower the pH of the liquid will be. In addition to acetic acid, *Acetobacter* sp. also produced gluconic acid from the process of glucose oxidation. *Acetobacter xylinum* was "overoxidizers" converted acetic acid in the fermentation medium to $CO_2$ and $H_2O$. Sugar in the fermentation medium had been metabolized and depleted, the concentration of acetic acid in kombucha only increased to a certain extent, then it decreased[18].

**Determination Caffeine Content of Kombucha Culture Caffeine**
Caffeine has a clinically beneficial pharmacological effect by stimulating the central nervous system as an adenosine receptor antagonist[3]. While the benefits of chlorogenic acid for human health, namely as an antioxidant, antiviral, hepatoprotective, and play a role in antispasmodic activities[19]. Measurement of caffeine content was carried out on the fermented Kombucha Coffee (KC) using HPLC.
From Table 1 it can be seen that the addition of kombucha culture to Indonesian Robusta green coffee beans gradually decreased the caffeine content from days 6 to 18. Measurement of caffeine levels on the 18th day compared with caffeine levels on the 0th day to know the overall increase or decrease during the fermentation process. The results of the caffeine concentration of Garut, Soreang, Aceh and Ciwidey caffeine decreased on the 6th day of 20.87; 24.73; 23.94; 23.19 % respectively, then on the 12th day decreased by 19.55; 19.30; 17.21; 19.07 % respectively, while on the 18th day increased by 2.86; 0.31; 0.14; 3.33 % respectively, based on these conditions that caffeine concentration decreased on the 12th and 12th days and then increased on the 18th day. The decrease in the concentration during the fermentation process of the caffeine content of Garut, Soreang, Aceh and Ciwidey green coffee beans obtained on the 18th day was 34.47; 39.06; 36.93 and 35.69 %, respectively.
It indicated that kombucha converted caffeine to theophylline and further to methylxanthine, and finally ammonia and carbon dioxide. Initially, caffeine was converted into dimethylxanthine (theobromine, paraxanthine, and theophylline) by the caffeine enzyme demethylase. During the fermentation, caffeine in Robusta coffee converted into uric acid, 7-methylxanthine, and xanthine.
Active fermentation must begin within 12 hours. It might take longer for liquid yeast because of the lower cell count, around 24 hours. Fermentation activity can be strong or slow. Three important factors for successful fermentation are adequate yeast pitching, good wort nutrition, and maintaining a consistent temperature in the correct range[20]. Another study reported this happened between 12 to 36 hours fermentation[21]. The caffeine degradation pathway was due to the kombucha culture through the oxidation of carbon atoms in the C-8 position. It was similar to *Klebsiella* as well as *Acinetobacter* sp., where the enzyme that plays a role in the first stage of degradation in caffeine was oxidase caffeine with a product of 1,3,7-trimethylouric acid. Some reactions to caffeine levels decrease depending on the genus, species, and type of organism used. Bacteria *Psuedomonas putida* and *Serratia marcescens*, reduce caffeine levels with the main route is through theobromine to 7-methylxanthine to xanthine[22]. Whereas other studies have shown the degradation of methyl uric acid to become uric acid[23].

**Determination Chlorogenic Acid Content of Kombucha Culture Chlorogenic Acid**
A chlorogenic acid is a group of hydroxynamic acid[6], which are found in coffee and tea drinks. Measurement of chlorogenic acid content was carried out on the fermented Kombucha Coffee (KC) using HPLC.
From Table 1 it can be seen that the addition of kombucha culture to Indonesian Robusta green coffee beans increased the chlorogenic acid from day 6 to 18. The results of chlorogenic acid concentration of robusta coffee Garut, Soreang, Aceh and Ciwidey increased on the 6th day by 3.76; 1.30; 2.79; 1.47 % respectively, then on the 12th day there was an increase of 4.03; 5.78; 6.53; 9.24% respectively, while on

the 18th day increased by 5.99; 3.26; 5.38; 0.63 % respectively, based on these conditions that the concentration of chlorogenic acid increased with increasing fermentation time.
The increase in the concentration of chlorogenic acid in Garut, Soreang, Aceh and Ciwidey green coffee beans obtained on the 18th day was 13.17; 10.15; 14.02 and 11.13 % respectively. This indicates that the alcohol from glucose led to an increase in caffeic acid forming an ester with alcohol and quinic acid which is formed in the shikimate pathway, producing chlorogenic acid[24].
Chlorogenic acid (CGA) consisting of caffeoyl-quinic acid (CQA), dicaffeoyl-quinic acid (diCQA)[25] and feruloyl-quinic acid (FQA). hydroxycinnamoyl quinic acid (HQA), accumulates specifically in *Coffea canephora*[26]. Biosynthesis of 5-CQA can be catalyzed by the cytochrome P450 enzyme, from Arabidopsis. But only one can hydroxylate the chlorogenic acid precursor p-coumaroyl quinate[26].
The increase of chlorogenic acid is suspected to be due to kombucha bacteria grown in the logarithmic phase, at the same time bacteria synthesize alcohol into acid during the fermentation process, while yeast breaks sucrose into glucose and fructose, the glucose is further used for cell metabolism that produces ethanol and carbon dioxide. Chlorogenic acid in kombucha coffee is due to the metabolite results of fermentation in microorganisms[27].

**Statistical Test**
The results of the processing of pH, Caffeine and Chlorogenic acid using SPSS 16 can be seen in Table-2.

Table-2: Result of ANOVA Test with SPSS-Tukey HSD

| (I) Kombucha Culture | (J) Kombucha Culture | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| pH | Caffeine | -23.64500* | 4.34919 | .001 | -35.7880 | -11.5020 |
| | Chlorogenic Acid | -133.95000* | 4.34919 | .000 | -146.0930 | -121.8070 |
| Caffeine | pH | 23.64500* | 4.34919 | .001 | 11.5020 | 35.7880 |
| | Chlorogenic Acid | -110.30500* | 4.34919 | .000 | -122.4480 | -98.1620 |
| Chlorogenic Acid | pH | 133.95000* | 4.34919 | .000 | 121.8070 | 146.0930 |
| | Caffeine | 110.30500* | 4.34919 | .000 | 98.1620 | 122.4480 |

*The mean difference is significant at the 0.05 level.

Based on the results of statistical tests using SPSS 16 against the results of testing pH, Caffeine and Chlorogenic Acid using the ANOVA Tukey test showed that the sig value (0.000 - 0.001) is less than 0.05 so it can be concluded that there are differences between the test results of pH, Caffeine and Chlorogenic Acid obtained on fermentation from day 0 to day 18.

## CONCLUSION

The conclusion of this research is the kombucha culture fermentation in Indonesian Robusta green coffee beans caused a decrease in pH, a decrease in the content of caffeine and increased the content of chlorogenic acid in green coffee bean fermentation. The decreased pH from Garut, Soreang, Aceh, and Ciwidey on the 18th day was 65.33; 66.77; 64.00; 64.86 %, respectively. The decreased concentration of caffeine was decreased on the 18th day at 34.47; 39.06; 36.93 and 35.69 % respectively, while the increased concentration of chlorogenic acid was increased on the 18th day at 13.17; 10.15; 14.02 and 11.13 % respectively. We also recommended testing the fermentation of Arabica green coffee beans to obtain a comparison of the results of Robusta coffee beans.

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