

Decaffeination of Coffee Bean Using Fermentation Process: Effect of Starter Concentration and Varieties on The Reduction of Caffeine and Antioxidant Activity

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ABSTRACT

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Coffee is a popular drink due to its special aroma and taste. Coffee contains caffeine that contributes a distinctive aroma to coffee, which can also be used as a quality parameter. However, if consumed excessively, it can have a negative impact on health. The decaffeination process using fermentation is one of the alternative methods to reduce the caffeine content without altering the characteristic taste and aroma of coffee. The objective of the study was to determine the influence of starter concentration (0-15%) and variety of coffee (Arabica and Robusta) in the decaffeination process using fermentation on reducing caffeine and the antioxidant activity of coffee products. The parameters were investigated regarding the chemical characteristics, including the concentration of caffeine, total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity tests using the DPPH free radical and FRAP methods. The results showed that the starter concentration and variety of coffee beans had a significant effect on the chemical characteristics (TPC and TFC) and antioxidant activity of decaffeinated coffee (p -value < 0.05). At a starting starter concentration of 5%, caffeine concentration was higher than a starter rate of 1% and did not significantly increase as stater up. Robusta coffee showed a higher decrease in caffeine compared to Arabica coffee.

Keywords: DPPH radical scavenging; decaffeinated coffee; caffeine; antioxidant

1. Introduction

Coffee contains the bioactive compound caffeine [1]. It is one of the compounds of xanthine derivatives that has benefits as a central nervous system stimulant, cardiac muscle stimulator, smooth muscle relaxation, and increasing diuresis. Besides caffeine, there is a high content of organic acids (chlorogenic acid), an antioxidant compound. Coffee has many benefits, such as antioxidants, stimulating brain performance, and anti-cancer agents. However, coffee also has a negative impact on health if consumed excessively due to its high caffeine and acidity. It can lead to heartbeat, hypertension, stomach disorders, anxiety, and insomnia[2], [3]. The caffeine content in coffee beans varies depending on the type and geographical location of the coffee grown[4]. Arabica coffee contains 0.4-2.4% of the total dry weight of caffeine; robusta coffee includes 1-2% of caffeine and 10.4% of acids[1], [4]. Since coffee has caffeine that is limited to consumed by the human

body, caffeine consumption should not be too high for everyday [5]. Therefore, there are low-caffeine coffee products that are made by reducing the level of caffeine in coffee, that is, using the decaffeination process. Low-caffeine coffee is a diversified product that can increase the added value of coffee[6], [7].

Decaffeination is a process that aims to decrease the level of caffeine in coffee[8]. Decaffeination often takes place to reduce the caffeine levels in coffee so that the taste of coffee is not too bitter[9]. Decaffeination is also used to suppress the side effects of caffeine and its activity in the body[10]. Coffee lovers often consume caffeinated coffee so that it enters the body and does not exaggerate and cause health side effects[5]. Coffee decaffeination using a process of microbial fermentation and involving enzymatic processes has the advantages of being relatively cheaper, non-toxic, and maintaining the quality of coffee[11]. The caffeine reduction level in

Arabica coffee with fermentation has been done previously[4]. In this study, the decrease in caffeine levels goes hand in hand with increased fermentation. In this process, the effect of microbial activity not only reduces caffeine levels but can also increase the acidity of coffee. The method of fermentation on a caffeine medium using one g/L of caffeine has been done by a previous study. The results showed that the reduction in caffeine reaches 20% in 33 hours, and the longer the fermenting time (more than 12 hours), the more degraded content will be [4], [12]. According to previous research, this caffeine reduction decreases coffee's antioxidant capacity [4]. Then, this study will study the influence of the starter concentration and type of coffee used in reducing caffeine and the quality of coffee in terms of antioxidant activity and content of phenolic and flavonoid compounds. EM4 (Effective Microorganism 4) was used as a starter that included a mixture of several types of microorganisms, such as photosynthetic bacteria (*Rhodopseudomonas* sp.), *lactobacillus* sp., *Streptomyces* sp., Actinomycetes, yeast.

2. Materials and Methods

2.1 Materials

Coffee beans (Robusta and Arabica), starter (microorganism EM4), green beans, NPK fertilizer (nitrogen source), and tapioca flour were purchased from the local market. DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, ferric chloride, Folin-Ciocalteu reagent, gallic acid, hydrochloric acid, quercetin, sodium hydroxide, sodium acetate, aluminum chloride, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), and Trolox were acquired from Sigma-Aldrich (USA).

2.2 Starter preparation

The starter is made by adding 40 grams of green bean seeds that have been smoothed using a blender, then adding 80 g of tapioca flour, then heating in a pot filled with 1 liter of water. After boiling, the water is first cooled. Then, 40 grams of NPK fertilizer is added, and EM4 is added, which has been fermented for 48 hours at a concentration of 0%, 1%, 5%, 10%, and 15%. Then, the mixture is added to the fermentation process.

2.3 Fermentation process

The fermentation process is conducted by preparing 70 grams of Arabica or Robusta coffee bean variety, dividing it into ten samples, and adding 100 ml

of starter into each sample (perfectly immersed). The fermentable medium is closed tightly, and the sample is taken after 48 hours. After that, the samples are washed with water flow until clear from the mucus, and the acid odor is reduced. The coffee beans are submerged in water for 3 hours, then water is changed every hour, and then the seeds are dried to reduce the water content by boiling at 60°C for 3-4 hours [12]. Then, the coffee beans were blended into powder for further analysis.

2.4 Analysis of caffeine

Caffeine analysis was conducted using UV-Vis Spectrophotometry. 0.1 grams of caffeine was dissolved into ethanol 99% and analyzed using the spectrophotometer UV-VIS at 273 nm. The standard caffeine curve was diluted at 1, 3, 6, 9, 12, and 14 mg/L[13].

2.5 Analysis of total phenolic content (TPC) and total flavonoid content (TFC)

Following the official procedure outlined in the Indonesian Herbal Pharmacopoeia (IHP)[14], the TFC and TPC of the polyherbal drink powder were determined. The TFC was reported as mg equivalent quercetin (QE)/g dry weight (DW) crude drugs, while the TPC was reported as mg equivalent gallic acid (GAE)/g DW crude drugs.

2.6 Determination of antioxidant activity

The powders' DPPH scavenging activity and FRAP were determined using a slightly modified standard method [15], [16]. DPPH scavenging activity and FRAP were expressed as M Trolox equivalent (TE)/g dry weight (DW) powder.

2.7 Data analysis

The two-way ANOVA and post-hoc Duncan's test were used to evaluate and compare, respectively, the effects of starter concentration and variety of coffee bean on caffeine compound, TPC, TFC, DPPH radical scavenging activity, and FRAP of the polyherbal drink powder. IBM SPSS Statistics version 26.0 (IBM, United States) designated the significance level $p < 0.05$.

3. Results and Discussion

3.1 Effect of starter concentrations and variety of coffee beans on caffeine concentrations of decaffeinated coffee.

The effect of starter concentrations and variety of coffee beans on the caffeine concentration of decaffeinated coffee beans is shown in **Figure 1**. The results showed that initial caffeine on the arabica and robusta bean were approximately the same (around 40 mg/g). After the bean was incubated into fermentation, the caffeine concentration of both arabica and robusta coffee significantly decreased, even for samples without starter added. Increasing the starter's concentration during fermentation decreased the caffeine content. Robusta coffee depicted significantly greater reduced caffeine content ($p < 0.05$). Increasing concentration up to 5% showed a substantially lower than 1%. After 5%, it showed the level of caffeine was constant. Adding a starter contributes to adding more microorganisms that degrade the bean walls. During the degradation of the wall of the bean, the caffeine also impairs or extracts and dilutes into the liquid medium. A previous study also showed that increasing starters during fermentation resulted in less caffeine content in decaffeinated coffee as more decomposed coffee beans [12].

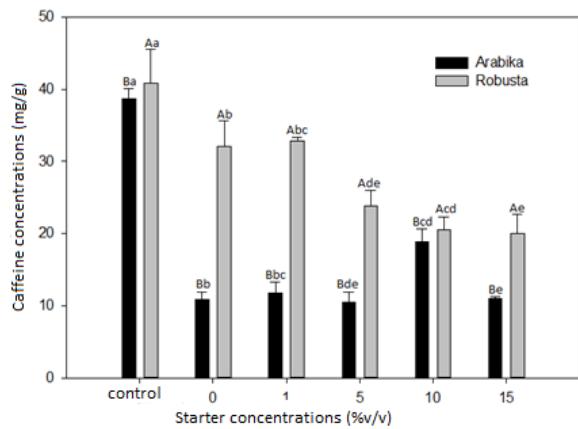


Figure 1. The caffeine concentration of decaffeinated coffee beans in different concentrations of starter in both different coffee varieties. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$.

3.2 Effect of starter concentrations and variety of coffee beans on the total phenolic content (TPC) and total flavonoid content (TFC) of decaffeinated coffee.

Based on the research findings, as shown in **Figure 2**, the addition of starter concentration in the fermentation process of Arabica and Robusta coffee samples impacts the Total Phenolic Content (TPC). The TPC before fermentation (without fermentation) in the Arabica coffee sample was measured to be 20.90 mg GAE/g. After adding the starter, the TPC of decaffeinated coffee exhibited the highest decrease at a starter concentration of 15% at 11.92 mg GAE/g. Conversely, the lowest reduction was seen at a starter concentration of 5%, resulting in the value of 14.00 mg GAE/g. On the other hand, the pre-fermentation (non-fermented) sample of robusta coffee had a TPC of 18.26 mg GAE/g. Following the addition of the starter, the TPC of decaffeinated coffee exhibited the greatest decrease at a starter concentration of 15%, around 13.34 mg GAE/g.

Conversely, the lowest decrease was observed at a starter concentration of 10% at 15.26 mg GAE/g. The reduction in TPC is attributed to adding a starter. As it increases, it will reduce TPC. The fermentation process results in the degradation of the chemical composition of coffee beans. The reduction of TPC and TFC may result from the degradation of polyphenol compounds into small phenolic compounds due to the metabolism of microorganisms in the starter [17], [18].

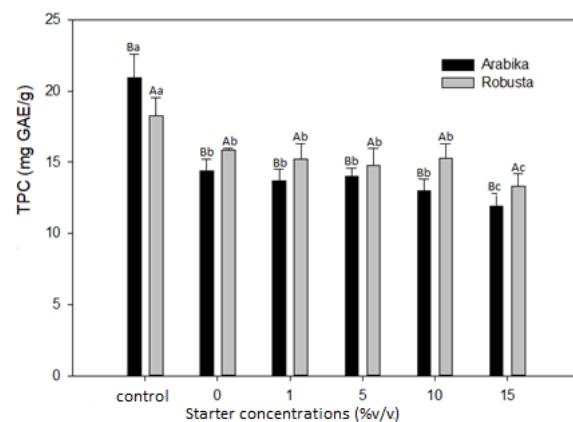


Figure 2. Total Phenolic Content (TPC) of decaffeinated coffee beans in different concentrations of starter in both coffee varieties. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$.

The Total Flavonoid Content (TFC) of decaffeinated coffee resulting from different starter concentration and various coffee both Arabica and Robusta were depicted in **Figure 3**. It can be seen that the addition of starter in the process of fermentation both Arabica and Robusta coffee samples has an influence on TFC results. The TFC before fermentation process (control) in the sample of Arabica coffee is 1.26 mg QE/g. After adding a starter, the TPC content has the greatest decrease at concentration of 15% at 0.72 mg QE/g. In comparison with the Robusta coffee sample before fermentation (control) at 2.39 mg QE/g. After fermentation process, TFC has had the most decreases with concentration of starter at 15% at around 1.88mg QE/g. The reduction of TFC of decaffeinated coffee using fermentation process is resulted from the microbial activity that uses coffee as a source of media. The degradation of

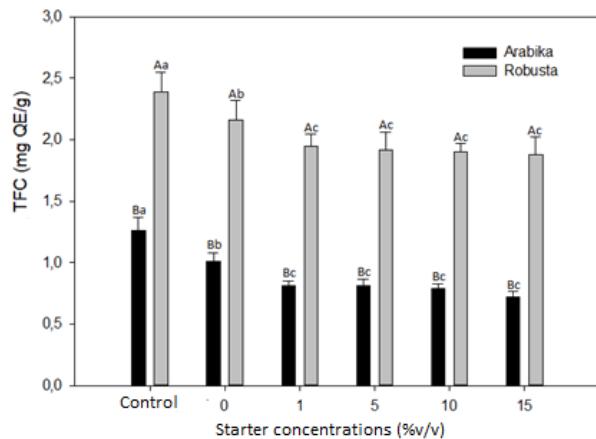


Figure 3. Total Flavonoid Content (TFC) of decaffeinated coffee beans in different concentrations of starter in both coffee varieties. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$.

3.3 Effect of starter concentrations and variety of coffee beans on the antioxidant activity of decaffeinated coffee using DPPH free radical scavenging and FRAP method.

The antioxidant activity of decaffeinated coffee in different starter concentrations and varieties of coffee beans was measured using DPPH free radical scavenging (**Figure 4**) and FRAP method (**Figure 5**). All samples' DPPH free radical scavenging results were comparable ($p>0.05$) with the control. Also, there were no significant differences between

increasing starter concentrations and coffee bean varieties. As coffee beans contain starch that contains reducing sugar, it may affect the measurement of analysis of antioxidant activity using DPPH free radical scavenging. Since hydrogen atom transfer is one of the mechanisms of analyzing the DPPH free radical scavenging, reducing sugar contributes to the final results [2], [19].

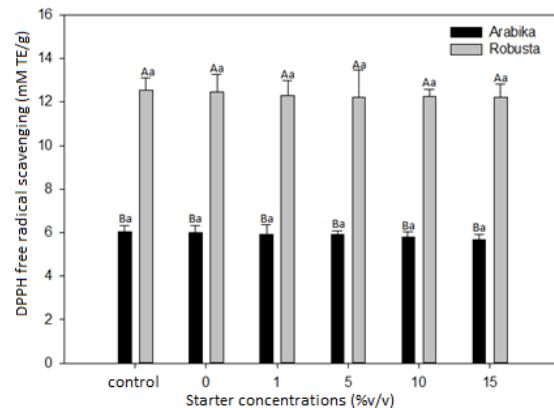


Figure 4. Antioxidant activity using DPPH free radical scavenging of decaffeinated coffee beans in different concentrations of starter in both different coffee varieties. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$

Meanwhile, the antioxidant activity of all decaffeinated coffee samples measured using the FRAP method showed significant differences from the control ($p<0.05$). The decreasing antioxidant activity of the decaffeinated coffee sample resulted from the reduction of TPC and TFC of the final sample. The decrease in FRAP results of decaffeinated samples from Arabica coffee was higher than that of Robusta coffee ($p<0.05$). The characteristic of Arabica coffee is that it has more organic acids that degrade during fermentation, reducing its bioactive compounds more [4], [20]. On the other hand, the increasing starter concentration was inconsistent with FRAP results. During fermentation, it may have been uncontrollable as there was no stirrer. Thus, the condition was not homogenous. Thus, it appears to result in the insignificance of FRAP results.

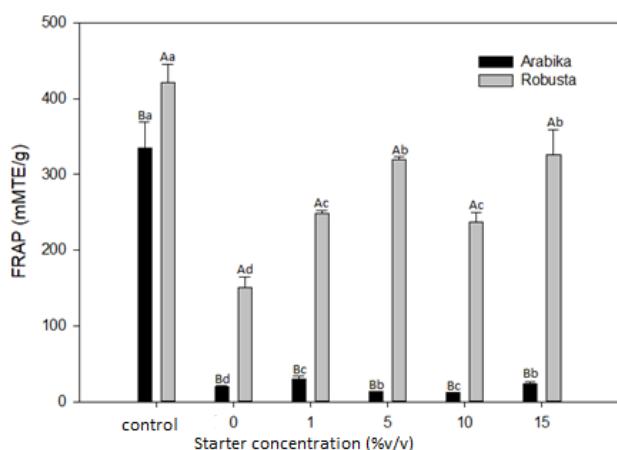


Figure 5. Antioxidant activity using Ferri Reducing Antioxidant Power (FRAP) of decaffeinated coffee beans in different concentrations of starter in both different coffee varieties. Different alphabets on each bar represented statistically different values, with $p \leq 0.05$.

4. CONCLUSION

The effect of starter concentrations and variety of coffee beans was investigated. The results showed that the concentrations of starter and type of coffee beans significantly reduced the caffeine content, TPC, TFC, and antioxidant activity from the FRAP method. The fermentation process using starter EM4 coffee beans reduced the caffeine content in the coffee beans, resulting in the best concentration of starter up to 5%. Arabica coffee had greater caffeine reduction and antioxidant activity than Robusta coffee beans. This result may help the coffee industry produce decaffeinated coffee beans to diversify coffee products.

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Conflict of Interest

No potential conflict of interest was reported by the author(s).

CRediT authorship contribution statement

Alwani Hamad: Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft, Resources.

Dwi Nugraheni: Data Curation, Investigation, Formal Analysis.

Bekti Wulan Sari: Validation, Writing Original Draft.

Mubshair Naveed: Validation, Writing Original Draft.

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