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Water Content, Total Dissolved Solids and Antioxidant Activity of Robusta Coffee Powder Produced by Fermenting Beans in Whey Kefir

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Abstract

Coffee is one of the plantation commodities that is usually processed into beverage products. The most widely cultivated coffee in Indonesia is Robusta coffee. However, Robusta coffee has a lower quality compared to other types of coffee. Fermentation of coffee beans during wet processing can improve coffee quality. The purpose of this study was to determine the best fermentation time for robusta coffee beans in whey kefir so that quality robusta coffee powder was obtained from the water content, total dissolved solids (TDS) and antioxidant activity. This study used a completely randomized design (CRD) with different fermentation time treatments. Parametric data were analysed by ANOVA and followed by DMRT test. Antioxidant activity data were analysed descriptively. Longer fermentation times reduced the total dissolved solids (TDS) of the coffee beans. However, prolonged fermentation can also increase the water content and reduce antioxidant activity. The best treatment was found to be a fermentation time of 18 hours, which resulted in a coffee bean with a good balance of TDS (3.25°Brix), water content (4.67%), and antioxidant activity (16.09 ppm).

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Introduction

Coffee is one of the plantation commodities in Indonesia which is generally processed into beverage products that are favoured by the community. Coffee consists of several types, namely robusta coffee (Coffea canephore), arabica coffee (*Coffea arabica*), liberica coffee (*Coffea liberica*) and excelsa coffee (*Coffea dewevrei*) (Artho *et al*., 2015). The most widely cultivated coffee in Indonesia is Robusta coffee. Robusta coffee production in Indonesia reaches 90% and the remaining 10% is Arabica coffee (Mutmainah, 2020). However, Robusta coffee has a lower quality than other types of coffee. Robusta coffee quality can be improved through the processing of coffee beans. Processing of coffee beans consists of two methods, namely dry processing (dry processing) without the bean fermentation stage and wet processing (wet processing) through the bean fermentation stage (Prayuginingsih *et al*., 2012).

Fermentation in coffee processing is generally done by soaking coffee beans in water. Fermentation in the coffee bean processing process can be developed by fermenting the right ripe coffee beans in whey kefir which contains probiotic bacteria in the form of lactic acid bacteria. Kefir is a functional product produced from the milk fermentation process using kefir seeds containing yeast, acetic acid-producing bacteria and lactic acid bacteria in the form of *Lactobacillus lactis*, *Lactobacillus kefir*, *Lactobacillus acidophilus*, *Lactobacillus kefirgranum*, *Lactobacillus kefiranofaciens*, *Lactobacillus parakefir* and *Lactobacillus brevis* (Sinurat *et al*., 2018).

Whey kefir is a clear liquid that is separated from the solid or curd in the kefir fermentation process (Sari et al., 2020). The content of lactic acid bacteria in whey kefir has the potential to improve the physical, chemical and microbiological quality of coffee beans through the fermentation process. Lactic acid bacteria can help maximizing the release of mucus layer from the coffee beans so the drying process can be more optimal, the

growth of spoilage bacteria can be suppressed, and the quality of the coffee is better. Reducing the water content of coffee can be more effective because of the removal of the coffee mucilage layer during bean fermentation. The water content of coffee grounds must below 7% because it can affect the shelf life and organoleptic properties of coffee (Lestari *et al*., 2017). The duration of fermentation can also affect the total dissolved solids, especially the content of sugar compounds where too long fermentation will produce less sugar content because the sugar has been broken down into organic acids. Coffee antioxidant levels can also be influenced by the length of fermentation where the longer the fermentation, the more caffeine compounds that are broken down into chlorogenic acid so that the antioxidant levels increase. If the fermentation time goes too long, it would decrease in the quality of the coffee product. The aimed of this study was to determine the best fermentation time of robusta coffee beans in whey kefir to obtain quality coffee powder based on water content, total dissolved solids and antioxidant activity.

Materials and methods

Robusta coffee beans obtained from Pulosari Village, Pemalang, milk from the Lumintu Ungaran Livestock Farmers Group, kefir grains from laboratory. The chemicals used in this research were DPPH solution, methanol, and aquadest.

Fresh milks were fermented with kefir grains for 48 h and filtered using mori cloth to obtain whey kefir (Firdaus *et al*., 2019). Robusta coffee beans were fermented in whey kefir at room temperature for about 0 h (T_0), 6 h (T_1), 12 h (T_2), 18 h (T_3), and 24 h (T_4) h (Thalia *et al*., 2020). Fermented robusta coffee beans were dried at 50°C for 2 days by using cabinet dryer. Grinder and sieve 20 mesh were used to convert dried coffee beans into powder (Pratiwi *et al*., 2020). Water content was analysed by using oven drying method (AOAC, 1995). Refractometer was used to determine total dissolved solids (Nurhayati, 2017). Antioxidant activity was analysed using DPPH method (Rodina *et al*., 2016). Fermentation of robusta coffee beans in whey kefir was conducted using the following description. Robusta coffee beans were fermented in kefir whey with ratio of seeds: kefir whey which is 1:2. Incubation was done for 0, 6, 12, 18, and 24 h at room temperature (25°C) in anaerobic conditions.

The obtained data were statistically analysed using one-way ANOVA and Duncan's Multiple Range Test using SPSS 26.0. The level significance was set at $\alpha = 0.05$.

Results and Discussion

Water content is a parameter that shows the amount of water contained in food ingredients. The water content in coffee beans greatly determines the quality and durability of the coffee beans, the lower water content of the beans, the longer the shelf life of the coffee (Sundari *et al*., i2020). The water content of the resulting coffee powder ranged from 4.23 – 4.71% and decreased and increased along with the length of fermentation carried out. The decrease in water content in coffee grounds is caused by the release of the mucus layer on the coffee beans during fermentation in kefir whey so that coffee drying is maximized and the water content of coffee decreases. The mucus layer on the coffee beans will be easier to remove through the fermentation of the coffee beans after the peeling process (Poerwanty and Halid, 2019). During the coffee bean fermentation process, the ambient temperature, and the activity of lactic acid bacteria from kefir whey will increase and cause enzyme activity in coffee beans to become more active so that the mucus layer of the coffee beans becomes softer. The decrease in the water content of robusta coffee powder is also caused by the lactic acid bacteria from kefir whey utilizing the free water contained in the mucilage layer of the coffee beans to grow and develop. According to (Anam *et al*., 2021) microbes in the coffee bean fermentation process utilize the free water content in the mucus layer of the seeds so the more free water used, will decrease cause the water content of the coffee.

Table 1. Water Content, Total Dissolved Solids, and IC50 of robusta coffee powder from beans fermented in kefir whey.

Fermentation Duration (h)	Parameter		
	Water Content (%)	Total Dissolved Solids (%)	IC50 (ppm)
0	5.37±0.43^{c}	3.77±0.22^{b}	20.14
6	4.23±0.14^{a}	3.50±0.14^{ab}	20.32
12	4.65±0.30^{b}	3.42±0.26^{a}	16.95
18	4.67±0.05^{b}	3.25±0.17^{a}	16.09
24	4.71±0.05^{b}	3.22±0.09^{a}	16.27

$^{a-c}$ Superscript not sharing letters indicate significant differences ($p < 0.05$).

The increase in water content occurred in the T2, T3, and T4 treatments but the value did not exceed the water content in the P0 treatment without fermentation. The increase in water content in treatments T2, T3, and T4 was due to the absorption of water content from kefir whey through the open pores of the coffee beans. According to Tawali et al. (2018) fermentation of coffee beans for too long causes the water content of coffee to be higher due to more water being absorbed through the pores of the coffee beans during fermentation. Robusta coffee powder fermented in whey kefir has met the established standards for coffee powder. This is in accordance with SNI 01-3542-2004 which states that the water content of ground coffee products is a maximum of 7%. Water content can affect the shelf life of robusta coffee powder products, where the lower the water content, the longer the shelf life of the product. The low water content of coffee causes the absorption of moisture from the air to take longer so that it can maintain product resistance from damage by microbes

(Purnamayanti *et al*., 2017). Total dissolved solids is the total amount of solids in the product from all its constituent elements that can be dissolved. Sucrose, fructose, and glucose are the main substances that affect the total dissolved solids value of a product. Based on Table 1. the total dissolved solids produced ranged from 3.22 – 3.77°Brix and decreased with the length of fermentation carried out. The decrease in total dissolved solids was caused by lactic acid bacteria originating from kefir whey which degraded the sugar content in coffee for metabolic processes so that the sugar content in coffee decreased and the total soluble solids of robusta coffee powder decreased. According to Napitupulu *et al*. (2015) microbes in the fermentation process will consume substrates in the form of sugar and other substances from the product for their metabolism, causing the total soluble solids of the product to decrease along with the length of fermentation.

Substrates that are degraded by lactic acid bacteria from kefir whey during the fermentation process can be sucrose, fructose, glucose, protein, fat and caffeine. The longer the fermentation, the more sugar substrates and solutes in the product used by microbes for their metabolism, causing a decrease in the total dissolved solids of the product (Nurhayati *et al*., 2020). The decrease in total dissolved solids in coffee grounds can affect the taste of brewed coffee. Coffee steeping can be influenced by sugar and dissolved solids content in coffee (Figueiredo *et al*., 2012). Chemical components in coffee in the form of dissolved solids can optimize the taste of coffee.

IC50 antioxidant activity obtained results for T0, T1, T2, T3, and T4 sequentially are 20,14; 20.32; 16.95; 16.09; and 16.27. The IC50 value is inversely proportional to the antioxidant activity of robusta coffee powder where the smaller the IC50 value, the higher the antioxidant activity of robusta coffee powder. Robusta coffee powder treatment T0, T1, T2, T3, and T4 are classified as a very strong antioxidant because it has an IC50 value below 50 ppm (Hardoko *et al*., 2019).

Antioxidants can inhibit oxidation through the binding of free radicals and highly reactive molecules that can inhibit cell damage in the body. Antioxidant activity is proportional to the content of phenols such as chlorogenic acid in coffee. The higher the phenol content, the higher the antioxidant activity (Huliselan, 2015). Robusta coffee has a fairly high content of phenolic compounds, especially from the hydrocinnamic acid group which acts as an antioxidant. Coffee has antioxidants from polyphenolic compounds in the form of phenolic acids, especially from the hydro cinnamic acid group such as chlorogenic acid, caffeic acid, coumaric acid, ferulic acid and synaptic acid (Hecimovic *et al*., 2011). Chlorogenic acid is the main phenolic compound in robusta coffee beans that acts as an antioxidant. According to Suena and Antari (2020) Robusta coffee beans contain the most chlorogenic acid compared to other types of coffee where its function is as an antioxidant.

The antioxidant activity of robusta coffee powder started to increase at 12 hours of fermentation and 18 hours of fermentation is the optimum condition, this can be seen because of the decrease in antioxidants at 24 hours of fermentation. The increase in antioxidant activity during the fermentation process was caused by the lactic acid bacteria from kefir whey producing organic acids from their metabolism. Metabolism of microorganisms during the fermentation process can cause an increase in antioxidant activity in the product (Goh *et al*., 2012).

The increase in free phenolic during the coffee bean fermentation process in kefir whey causes an increase in the antioxidant activity of the product. The increase in polyphenolic compounds that act as antioxidants is due to a biotransformation process using enzymes from plant cells so that they can change the functional groups of chemical compounds in them. Yeast in the fermentation process has the ability to produce the enzyme vinyl phenol reductase and can synergize with the enzyme ferulic acid reductase to form phenol through the decarboxylation process of cinnamic acid and pyrulic acid (Suhardini and Zubaidah, 2015). Cinnamic acid and pyrulic acid are phenolic compounds that act as natural antioxidants in coffee beans.

The antioxidant activity of coffee grounds decreased at 24 hours of fermentation. This can be seen from the IC50 value of coffee powder which has increased from 16.09 ppm to 16.27 ppm. The decrease in antioxidant activity of robusta coffee powder was caused by prolonged fermentation causing the fermentation medium to become more acidic so that the phenolic compounds from coffee became more stable. According to Puspitasari *et al*. (2017) the acidic atmosphere in the fermentation process causes the phenolic compounds in the product to be more stable and difficult to release protons that can bind to DPPH so that the antioxidant activity decreases. Long fermentation can cause the polyphenol content of the product to become less due to biochemical modifications through complexation and polymerization with proteins, causing decreased antioxidant activity.

Conclusion

Longer fermentation times reduced the total dissolved solids (TDS) of the coffee beans. However, prolonged fermentation can also increase the water content and reduce antioxidant activity. The best treatment was found to be a fermentation time of 18 hours, which resulted in a coffee bean with a good balance of TDS, water content, and antioxidant activity.

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