Antioxidant activity kombucha coffee (Coffee spp) with variation concentration and type

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Abstract. Kombucha is a sweet tea solution fermented by utilizing the symbiotic growth of yeast and bacteria. Kombucha can be made from coffee which can be used as a growth medium for *Acetobacter xylinum.* Coffee has antioxidant compounds that can ward off free radicals and are expected to be used as functional drinks. This study aims to determine the antioxidant activity and chemical properties of kombucha coffee. This study used a completely randomized design (CRD) with two factors, namely coffee variety (Arabica, Robusta, Liberica) and coffee concentration (2.5 g/250 ml water; 5 g/250 ml water; and 7.5 g/250 ml water). Kombucha coffee with the highest antioxidant activity was used 2.5 g/250 ml liberica coffee. The antioxidant activity reaches 74.86 % of RSA DPPH and has 10.02 % of sugar, pH 3.14, 0.56% of total acid, total phenolic content 9.10 mg GAE/g, and tannin 0.04 %. Kombucha with liberica coffee had highest of antioxidant activity.

1 Introduction

Kombucha is a fermented tea which can used for medical purposes. Several studies show that kombucha has the health benefit like antimicrobial [1, 2], anti-diabetic [3], anti-cholesterol [4], antioxidant [5] and the other benefit. Kombucha is easy to find in commercial stores and can made at home. Kombucha consists of a mixture of yeast and bacteria and produces organic acids, enzymes, polyphenols and amino acids. Kombucha can used for detoxifying and can release toxins from the body through the liver and kidneys [4].

Kombucha is a sweet tea solution fermented by the symbiotic growth of yeast and bacteria. The microorganisms in kombucha convert the tea and sugar solution into various other compounds, namely various types of acids (lactic acid, acetic acid, folic acid, carbonic acid, gluconic acid, chondroitin sulfate, usnic acid, and hyaluronic acid), vitamins (B1, B2, B3, B6, B12, B15 and C) and polyphenols with strong antioxidant effects [6]. One of microorganisms in kombucha was *Acetobacter xylinum* and several types of yeast [7]. Kombucha is usually made from black tea (*Camellia sinensis*) but it can also be made from other plant extracts such as coffee leaves [3], yarrow (*Achillea millefolium*), leaf (guava, oak), snake fruit, vegetables, milk and juices [8].

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One use of coffee that is safe for consumption and has positive effects on health is making coffee kombucha. Coffee kombucha is safe for consumption because the caffeine content in coffee will decrease after it has been fermented for 12 days [9]. Kombucha made from Robusta coffee leaf tends to be potentially for anti-diabetic drink, which prevent pancreatic demaged [3].

Apart from that, the chlorogenic acid content in coffee increases with the length of fermentation time, where chlorogenic acid is one of the antioxidant compounds found in coffee which has a positive effect on body health. The fermentation process produces new compounds and also increases certain compounds such as phenolics. Antioxidant activity has increased which is possibly due to the presence of free phenolics produced during the fermentation process, so the higher the phenolic content produced, the higher the antioxidant activity [10].

There has not been much research related to coffee-based kombucha, for example research conducted by [11] regarding antioxidant activity, total phenolic content and caffeine content in robusta coffee kombucha fermentation in various sugar concentrations, the results showed that the highest antioxidant activity and total phenolic content occurred in robusta coffee kombucha fermentation on day 6, amounting to 26.316 ± 3.636 µek/ml and 26.316 ± 1,494 µek/ml, namely when adding sugar concentrations of 0% and 10%. The Antioxidant activity was lower when compared to the antioxidant activity of coffee kombucha with the highest activity being 74.86%. The fermentation process that occurs in coffee kombucha is thought to reduce the caffeine levels contained in coffee, so that coffee can be consumed safely and has positive benefits for human health.

So far, kombucha is usually made from tea, rosella and other ingredients. The antioxidants contained in coffee also come from chlorogenic acid. The amount of chlorogenic acid content is different for each type of coffee, apart from that, other factors that cause differences in the amount of chlorogenic acid content are the roasting process and the location of the area where the coffee plants are planted. Because of these factors, the antioxidant activity produced in coffee kombucha will also vary [12]. Chlorogenic acid, caffeine, fat, polysaccharides, phenolic compounds, melanoidins, dietary fibre water soluble, and minerals, as nutritional content in coffee. A major contributor that has a positive effect on the body, was caffeine. Caffeine has several functions most of which are related to the central nervous system, as the stimulant effect [13].

There has not been much research related to coffee-based kombucha. So far, kombucha is usually made from tea, rosella and other ingredients. This study aimed to determine the antioxidant activity of kombucha from variety of coffee and concentrations. Apart from that, researchers are conducting research on coffee kombucha which is expected to be used by the public as a functional beverage product to prevent various diseases, then it is hoped that it can be used as a source of information to the public about the use of coffee processed as kombucha. The core research aims to determine the antioxidant activity of kombucha from several types of coffee as well as the coffee concentration.

2 Material and methods

2.1 Material

The main ingredients used in this research are Kerinci arabica coffee (from Mount Kerinci), kelir robusta (from the Mount Kelir area), and liwa liberica coffee (from the Lampung), granulated sugar (Gulaku), water, kombucha starter (*Acetobacter xylinum*) obtained from the Process Engineering Laboratory, Faculty of Technology and Food Industry, Slamet Riyadi

University, Surakarta, Nelson reagent, arsenic reagent, 40% NaOH solution, 30% HCl solution, anhydrous glucose, and distilled water. Also chemichal for analyses.

2.2 Experimental design

A completely randomized design (CRD) was employed with two factors: the ratio of type of coffee (J1 : arabica, J2: Robusta, J3:liberica) and concentrastion of coffee for 250 ml water (K1:2,5 g, K2:5 g, K3:7 g). Data were processed in SPSS Statistics v.25. ANOVA was used to analyze chemical parameter values, and if the results showed significant differences, a post-hoc Tukeys's test would be onducted at a significance level of 5%.

2.3 Preparation of kombucha coffee

Arabica, Robusta and Liberica coffee are weighed according to the treatment, then brewed in 250 ml boiling water for 5 minutes, then 10% (w/v) granulated sugar is added and dissolved, then filtered and cooled to a temperature of ±25ºC. After that, 10% starter was added and then fermented for 12 days at room temperature. The flow cart for making kombucha coffee can be seen in Fig. 1.

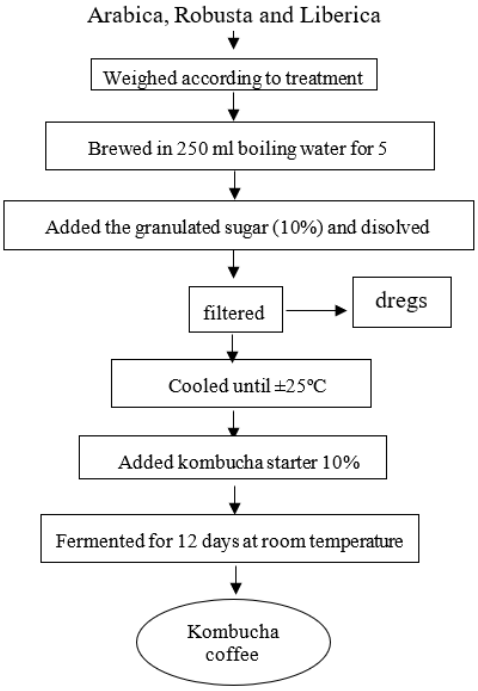

Fig. 1. Flow cart for making kombucha coffee [14].

2.4 Research paramaters

Chemical analysis included antioxidant activity with DPPH method [15], total carbohydrate with Nelson Somogyi method [16], pH with pH meter, total titrated acid with Mann's acid test [17], total fenol with Follin-Coicalteu [16], tannin content [18].

3 Results and discussion

3.1 Antioxidant activity

Table 1 showed that the highest antioxidant activity of kombucha coffee was 74.86% in the Liberica coffee type treatment with a coffee concentration of 2.5 g/ 250 ml. The lowest antioxidant activity was 47.43% in the Robusta coffee-type treatment with a coffee concentration of 7.5 g/ 250 ml. The results of this kombucha coffee research are different from the results of research conducted by Dewi et al [19] who stated that the antioxidant activity in kombucha coffee using robusta coffee which was fermented after 12 days experienced a significant decrease. The decrease in antioxidant activity in coffee kombucha using robusta coffee was caused by a decrease in nutrients needed for microbial growth and would be consumed by microbes so that its antioxidant activity decreases. Based on research by Sukamwati et al [20], that in acidic conditions antioxidant activity decreases due to phenolic compounds becoming more stable and having difficulty releasing protons that can bind to DPPH. The treatment of coffee type and coffee concentration had a significant effect on the antioxidant activity of coffee kombucha. Table 1 shows that the type of coffee and coffee concentration used influence the rise and fall of the antioxidant activity produced. Research by Wulandari [21, 22] the antioxidant activity was higher in kombucha from coffee leaf, namely 89.51%, which is of course greater when compared to this research on coffee kombucha with treatment of various types of coffee which had the highest antioxidant activity of 74.86%.

Table 1. Analysis of Kombucha coffee (antioxidant activity, total fenolic and tannin).

Type of coffee	Concentration (g)	Antioxidant activity (%)	Total Fenolic Content (mg GAE.ml)	Tannin (%)
Arabica	2.5	72.48 ± 0.719^{c}	6.67 ± 0.117^{a}	0.02 ± 0.000^{a}
	5	63.71 ± 0.517^{b}	6.59 ± 0.034^{a}	0.03 ± 0.000^{b}
	7	$68.95\pm0{,}719^{bc}$	6.72 ± 0.059^{a}	0.14 ± 0.000^{g}
Robusta	2.5	73.90 ± 3.081^{c}	8.87 ± 0.180^{dc}	0.04 ± 0.000^{c}
	5	71.05 ± 3.348^{c}	6.86 ± 0.068^{ab}	0.09 ± 0.000^{e}
	7	47.43 ± 3.969^{a}	7.02 ± 0.059^{bc}	0.14 ± 0.000^{h}
Liberica	2.5	74.86 ± 0.857^{c}	9.10 ± 0.090^{c}	0.05 ± 0.000^{d}
	5	69.52 ± 3.456^{bc}	8.67 ± 0.0117^{d}	0.14 ± 0.000^{f}
	7	73.05 ± 2.715^{c}	7.30 ± 0.068^{c}	0.16 ± 0.000^{i}

Note: Values followed by the same letter are not significantly different from one another according to Tukey's test at a 5%

3.2 Total phenol

The type of coffee and concentration of coffee, influence the total phenol produced. The highest total phenol yield was 9.10 mg GAE/ml in the Liberica coffee type treatment with a coffee concentration of 2.5 g/250 ml water, while the lowest result was 6.66 mg GAE/ml in the Arabica coffee treatment with a concentration of 2.5 g/250 ml water. Bhattacharya et al. [23] stated that the increase in total phenol content is due to enzymes released by bacteria and yeast in kombucha drinks which will degrade complex polyphenols into simple compounds during the fermentation process. Table 1 shows that different types of coffee and the addition of coffee concentration affect the rise and fall of the total phenols produced. This is caused by the differences in nutritional content contained in each coffee, then the greater the concentration of coffee given, the higher the total phenol results obtained and vice versa. Total phenols decreased during fermentation, possibly because the availability of substrate

and nutrients by the kombu fungus decreased so that the formation of phenolic compounds was hampered because the increasingly acidic substrate could inhibit the glycosidase enzyme which was less stable at low pH [24] This is in line with the results of total acid and pH analysis, where conditions of low pH and high total acid affect the total phenol produced.

3.3 Total tannin

The highest tannin content was 0.16% in the Liberica coffee-type treatment with a coffee concentration of 7.5 g/250 ml of water, while the lowest tannin content was 0.02% in the Arabica coffee-type treatment with a coffee concentration of 2.5 g/250 ml. water. The higher the coffee concentration, the higher the tannin content tends to be. The results of this research are higher compared to previous research conducted by Rahayu & Rahayu [25] where research on optimizing coffee liquid fermentation with kombucha culture inoculant produced the highest tannin content, namely 0.04%. This is likely due to the water-soluble nature of tannins. The type of coffee and coffee concentration influence the tannin levels produced. Apart from that, fermentation time and the use of sugar also influence the results of tannin levels in coffee kombucha. This is because the tannins contained in the coffee liquid as a fermentation medium are polymerized by the activity of the Acetobacter xylinum bacteria. During the fermentation process, the tannin compounds contained in coffee dissolve in water causing an astringent taste.

3.4 Total sugar

The highest total sugar content of kombucha coffee was 15.32% in the Arabica coffee treatment with a coffee concentration of 5 g/250 ml water, while the lowest total sugar content was 10.09% in the Robusta coffee treatment with a coffee concentration of 7.5 g/ 250 ml of water, and treatment of Liberica coffee with a coffee concentration of 5 g/250 ml of water. Research by Ningtyas [26], the highest total sugar, amounting to 3.44% in kombucha tea boiled with sweet corn, this result is lower when compared to the total sugar from coffee kombucha, namely 15.32%. According to research by Kusuma & Fibrianto [27], the highest total sugar in kombucha from old robusta coffee leaves was 61.92%. The treatment of coffee type and coffee concentration influences the total sugar content of kombucha coffee. Table 2 shows that there is a decrease in the total sugar content for each different type of coffee with different coffee concentrations. This occurs because each type of coffee has different nutritional content, as does the sugar content in each type of coffee. different. The sugar content in Arabica coffee beans is 6.0-9.0 g/100 g, for Robusta coffee beans the sugar content is 0.9-4.0 g/100 g, and Liberica coffee beans have a sugar content of 8 g/100 g, then in this study 10% sugar was added to each type of coffee at 25 g/250 ml of water [28]. Apart from that, the decrease in total sugar content was caused by the fermentation time, namely 12 days, where microorganisms used sugar as a nutrient and then converted it into alcohol and CO_2. Then, CO_2 gas reacts with water vapour to form carbonic acid. The decrease in total sugar content after fermentation is caused by the activity of Saccharomyces cerevisiae in synthesizing glucose by 2-3% and the remainder is utilized through the fermentation route. The activity of Acetobacter xylinum also causes sugar levels to decrease. Acetobacter xylinum oxidizes glucose to gluconic acid and other organic acids. Apart from that, Acetobacter xylinum can also synthesize glucose into polysaccharides or cellulose which looks like white fibers [22].

Table 2. Analysis of Kombucha coffee (total sugar, pH and titrated acid).

Type of coffee	Concentration (g)	Total Sugar (%)	pH	Titrated acid (%)
Arabica	2.5	14.15±0.0340^{b}	3.42±0.025^{g}	0.56±0.015^{a}
	5	15.32±0.0378^{c}	3.33±0.025^{f}	0.70±0.000^{b}
	7	10.49±0.296^{a}	3.25±0.025^{ef}	0.82±0.015^{d}
Robusta	2.5	10.96±0.0281^{a}	3.18±0.025^{de}	0.58±0.000^{a}
	5	10.43±0.020^{a}	2.95±0.025^{c}	0.60±0.000^{a}
	7	10.09±0.0304^{a}	2.84±0.036^{b}	0.77±0.030^{c}
Liberica	2.5	10.20±0.365^{a}	3.14±0.040^{d}	0.56±0.026^{a}
	5	10.09±0.232^{a}	2.88±0.032^{bc}	0.71±0.025^{b}
	7	10.54±0.538^{a}	2.74±0.035^{a}	0.98±0.015^{e}

Note: Values followed by the same letter are not significantly different from one another according to Tukey's test at a 5%

3.5 pH

The highest pH of Kombucha coffee was 3.42 in the Arabica coffee treatment with a coffee concentration of 2.5 g/250 ml of water, while the lowest pH was 2.74 in the Liberica coffee treatment and the coffee concentration was 7.5 g/250 ml of water. The type of coffee and coffee concentration have different pH, it can be concluded that the type of coffee and coffee concentration affect changes in pH. In reesearch conducted by Wistiana & Zubaidah [29], the pH of betel leaf kombucha was 2.30, this result is lower when compared to the pH of coffee kombucha, which is 2.74. The pH decreases as more and more coffee concentrations are used. The longer the fermentation time for kombucha, the higher the acid production [30]. Sreeramulu et al. [1] stated that during the fermentation process, yeast and bacteria metabolize sucrose and produce many organic acids, such as acetic acid and gluconic acid, so that organic acid levels increase during the fermentation process, and the pH value decreases from 5 to 2.5. Generally, the more acid content in the material, the lower the pH value. An increase in acid concentration during the fermentation process causes a decrease in pH in kombucha drinks [31].

3.6 Total Titrated acid

The type of coffee treatment and concentration had a significant influence on the total acid content of coffee kombucha. In several treatments, it showed that between treatments there was no real effect. This is possible because the increase in total acid in some treatments was not too large. Based on table 2, shows that the highest total acid content was 0.98% in the Liberica coffee-type treatment with a coffee concentration of 7.5 g/250 ml of water, while the lowest total acid content was 0.56% in the coffee-type treatment. arabica and liberica with a coffee concentration of 2.5 g/250 ml of water. This is directly proportional to the results of pH analysis, where the greater the total acid produced, the lower the pH produced. The increase or decrease in total acid levels that occur in coffee kombucha depends on the length of fermentation used and the amount of inoculum given during the fermentation process. This is because the longer the fermentation process takes, the more acetic acid is formed. Likewise, after adding inoculum, the more inoculum used, the faster the formation of acetic acid, so that in the same fermentation time high levels of acetic acid will be produced [26].

The increase in total acid content occurs along with the large concentration of coffee used. It can be concluded that the higher the concentration of coffee used, the higher the total acid content. In addition, an increase in total acid levels in kombucha is possible because the microorganisms in kombucha grow in the logarithmic growth phase. According to Aditiwati

& Kusnadi [30], the growth rate of yeast cells increases after two days. This is because the availability of substrate and pH of the medium is suitable for the growth of yeast cells to convert glucose into alcohol and organic acids. As with the growth of acetic acid bacteria, the substrate conditions are also suitable for the growth of acetic acid bacterial cells after the second day. This is because in the medium, yeast cell metabolism produces metabolites that convert sucrose into glucose and fructose with the help of the invertase enzyme.

4 Conclusion

Kombucha with liberica coffee and a concentration of 2.5 g/250 ml of water, was the highest of antioxidant activity than the other treatment. The type of coffee and concentration influence of the characteristics kombucha.

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