Characteristics of Kombucha from Various Herbal Tea Leaves

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ABSTRACT

Kombucha has been well-known as functional food in Asia. Regular kombucha is produced using Camellia sinensis tea leaves. However, kombucha can also be produced from other substrates. This study aimed to investigate characteristics of kombucha from various herbal tea by determine flavonoid, phenolic and antioxidant content as well as sensory profile. This study was conducted using Randomized Block Design with one factor and two duplicates of each treatment. Based on 8 days of fermentation, buasbuas (Premna pubescens Blume) has the highest phenolic content (926.19 µg/ml GAE), flavonoid content (391.81 µg/ml), and antioxidant activity IC50 values were 18.16 µg/ml. Based on the sensory evaluation, panelists preferred indian jujube, buas buas, moringa, regular tea, and fig for aroma, while for taste panelist preferred indian jujube, ambarella, and breadfruit. While based on color, they preferred tea.

Keywords: *Antioxidant; Flavonoid; Kombucha; Phenol; Sensory Evaluation*

INTRODUCTION

According to Balitbangkes (2018), degenerative disease cases have increased every year in all regions of Indonesia. Degenerative diseases are diseases caused by changes in the function and structure of tissues in the body, such as cardiovascular disease, diabetes, hypertension, cancer, and osteoporosis. One of the causes of degenerative diseases is unhealthy consumption patterns. To prevent degenerative diseases, people need to change the consumption pattern of more healthy food, such as consumption of functional food.

Kombucha has been well-known as a functional food in Asia. Functional food is a food product that plays a role in providing health effects when consumed. Kombucha has health effects such as increasing the immune system, as antibacterial, antioxidant, anti-inflammatory, anticarcinogenic, and antihyperglycemic. These health effects might be due to the presence of phytochemical compounds produced by acetic acid bacteria, lactic acid bacteria, and yeast during fermentation (Villarreal-Soto et al., 2018).

Several factors influence kombucha fermentation, one of them is a substrate. Generally, substrate used in making kombucha comes from *Camellia sinensis* tea leaves. However, kombucha can also be produced from other substrates, such as various herbs commonly consumed by Indonesian people. Herbal tea consists of ambarella (*Polyscias fruticosa*), mango (*Mangifera indica*), Chinese teak (*Alexandrian senna*), buasbuas (*Premna pubescens Blume*), moringa (*Moringa oleifera*), breadfruit (*Artocarpus altilis*), indian jujube (*Ziziphus mauritiana*), fig (*Ficus carica*), and star gooseberry leaves (*Sauropus androgynus*). Therefore, this research aimed to analyze characteristics of kombucha from herbal tea leaves as functional food.

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METHODS

Materials

Materials used in this experiment were kombucha starter which bought from indokombucha Bandung and various herbal tea leaves consisting of ambarella (*Polyscias fruticosa*), mango (*Mangifera indica*), chinese teak (*Alexandrian senna*), buasbuas (*Premna pubescens Blume*), moringa (*Moringa oleifera*), breadfruit (*Artocarpus altilis*), Indian jujube (*Ziziphus mauritiana*), fig (*Ficus carica)*, and star gooseberry (*Sauropus androgynus*). Meanwhile, regular tea (*Camellia sinensis*) leaves was used as control. For analysis, materials used in this experiment were Na_2CO_3, Folin-Ciocalteau, aquadest, $NaNO_2$, $AlCl_3$, NaOH, DPPH, and methanol.

Tools

Spectrophotometer (Spectro 20 D Plus), volumetric flask, beaker glass, measuring cylinder, graduated pipette, rubber bulb, Erlenmeyer flask, burette, and test tubes.

Procedure of Making Kombucha Starter

Store-bought kombucha was poured into 1L of sweatened tea and fermented for 8 days at room temperature.

Procedure of Making Kombucha from Various Herbal Tea

Water was boiled at 80-90°C for 15 minutes, added 2% of herbal tea and 10% of sucrose, then stirred and transferred to a bottle jar. Once the tea was cool, added 10% of kombucha starter and covered bottle jar with a cloth. Kombucha was fermented for eight days at room temperature.

Procedure of Analysis Total Phenolic Content

Analysis of total phenolic content was using Folin Ciaocalteu assay described by Zofia et al., (2020). Briefly, 0.1 ml sample was mixed with 0.75 ml Na_2CO_3 7%. After incubation for 5 minutes, 0.75 ml Folin Ciocalteu 10% was added and incubated again for 15 minutes at room temperature. Absorbance of sample was measured at ʎ=735 nm.

Procedure of Analysis Total Flavonoid Content

Total flavonoid content assay refer to study published by Christiani Dwiputri & Lauda Feroniasanti (2019). Briefly, 1 ml of sample was mixed with 4 ml aquadest and added with 0.3 ml $NaNO_2$ 5%. After incubation for 5 minutes, 0.3 ml $AlCl_3$ 10% and 2 ml NaOH 1 M was added and incubated again for 1 minute. Absorbance of sample was measured at ʎ=510 nm.

Procedure of Analysis Antioxidant Activity (IC50)

Antioxidant activity (IC50) methode that published by Pratama et al.(2015). 1 ml sample with different concentration (100, 200, 300, 400, 500 ppm) was mixed with 1 ml DPPH 0.2 M and 1 ml methanol. After incubation for 30 minutes, sample was measured at ʎ=515 nm.

Sensory Evaluation

Sensory evaluation was determined by using hedonic test described by Mardiana et al., (2021). Sensory evaluation was using 20 untrained panelist. Panelists were asked to give

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score from 1 until 5. The higher the score the higher the likeness of panelists for color, aroma, and tasteof kombucha.

Research Design

This research useda Randomized Block Design with one factor. This experiment was using 10 treatments with each group has different herbal tea leaves and it performed in two duplicates.

Test Parameters

Characteristic of kombucha from herbal tea was determined by measuring of total phenolic content (Zofia et al., 2020), total flavonoid content (Christiani Dwiputri & Lauda Feroniasanti, 2019), antioxidant activity (Pratama et al., 2015), and sensory evaluation (Mardiana et al., 2021).

Data Analysis

Data were analyzed using Friedman test with a 5% confidence range.

RESULT AND DISCUSSIONS

Total Flavonoid Content

Flavonoid compounds are part of a polyphenolic group commonly found in plant. Flavonoids are well-known has medicinal effects such as antiviral, antibacterial, anticancer, anti-inflammatory, anti ulcer, and anti-hepatotoxic. They also have ability as a scavenger of reactive O_2 species because of the presence of phenolic hydroxyl groups thus it has potential as antioxidants agent (Umamaheswari & Chatterjee, 2007).

Based on Figure 1, average total flavonoid of kombucha ranged from various types of leaves on 0^{th}-day before fermentation ranged from 58.94 to 128.13 µg/ml GAE. On the 4^{th} day of fermentation, total flavonoid content ranged from 131.48 to 228.85 µg/ml GAE, while on the 8^{th} day, it ranged from 191.64 to 391.81 µg/ml GAE. Total flavonoid content increased during fermentation from day 0 to 8. Jakubczyk et al., (2020) stated that fermentation time would increase polyphenol content, including flavonoid. The increase in flavonoid content during fermentation is mainly due to the action of enzymes produced during the growth of microorganisms. These enzymes are involved in releasing flavonoids, thus increasing flavonoid content the longer fermentation time.

Figure 1 also showed that kombucha from buasbuas (*Premna pubescens Blume*) leave has the highest flavonoid content (391.81 µg/ml GAE) compared to others leaves. It was noticed that each leaf has different flavonoid content. Buasbuas (*Premna pubescens Blume*) were rich in secondary metabolites such as terpenoid, flavonoid, polyphenols, saponin, alkaloid, steroid, and tannin (Daud et al., 2021). It also reported that plants flavonoid content is influenced by the environmental condition, such as humidity, light intensity, and harvesting period (Shukri et al., 2011).

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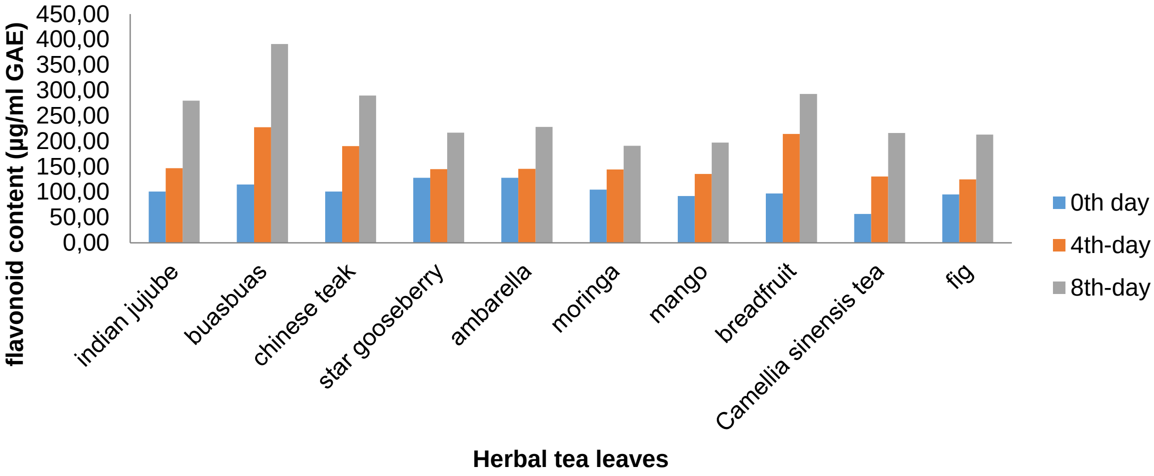

Figure 1. Flavonoid content

Total Phenolic Content

Based on Figure 2, average total phenolic content of kombucha from various types of leaves on 0^{th}-day before fermentation ranged from 224.90 to 297.56 µg/ml GAE. On the 4^{th} day of fermentation, total phenolic content ranged from 266.69 to 571.64 µg/ml GAE, while on 8^{th}-day fermentation, it ranged from 409.16 to 926.19 µg/ml GAE. Overall, the total phenolic content during 8-day fermentation was increased in all treatments. Microorganisms in kombucha play a significant role in the metabolism of phenolic compounds. Microorganisms provide enzymes such as glucosidase, esterase, dehydroxylase, and decarboxylase which helps biotransformation of phenolic compounds (Selma et al., 2009). The biotransformation process would convert complex phenolic compounds into less complex (Zubaidah et al., 2018).This process would increase phenolic compounds during fermentation.

Figure 2 also revealed that kombucha from buasbuas (*Premna pubescens Blume*) leave has the highest phenolic content (926.19 µg/ml GAE) compared to others leaves during 8 day fermentation. According to Uppin et al., (2017), buasbuas have high phenolic content, contributing to antioxidant activity.The previous study conducted by (Ruwali et al., 2019) revealed that in buasbuas leaves present of phytochemical compound such asphenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. These phytochemical compounds have physiological activities such as antibacterial, antioxidant, anti-inflammatory, and antiallergenic (Muñoz et al., 2017).

Antioxidant Activity

Evaluation of antioxidant activity of kombucha from various herbal tea was determined by Inihibitory Concentration 50 (IC50) test. IC50 test showed the number of kombucha that has ability as scavanger of 50% DPPH radicals. If the value of IC50 is low, it indicates high scavanger radical activity (Rivero-Cruz et al., 2020). Therefore, the lower IC50 value, the higher antioxidant activity of kombucha.

Based on Figure 3, IC50 values in kombucha from various herbal tea ranged from 78.78 to 162.22 on 0^{th}-day fermentation, while on 4^{th} day fermentation, it range from 36.99 to 144.90. During the last day fermentation, IC50 values ranged from 18.16 to 136.22. Overall, IC50 values of kombucha from various herbal tea decreased from day 0 to day 8. It showed that during kombucha fermentation, the antioxidant activity increased because the lower IC50

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values indicate higher antioxidant activity. Vohra et al. (2019) stated that during fermentantion of kombucha, biotransformation of phytochemical could increase phenolic and catechin compund which responsible for high antioxidant activity.

The highest antioxidant activity based on IC50 values was buasbuas (*Premna pubescens Blume)* (18.16µg/ml).The antioxidant activity comes from the nature of the herbal tea it self (Suhardini & Zubaidah, 2016). Based on phenolic and flavonoid values in this study, buasbuas has the highest value compared to other herbal teas. Phenolic has ability as antioxidant agent due to capabiltity to reduce free radical and chelating metal (Pereira et al., 2009).

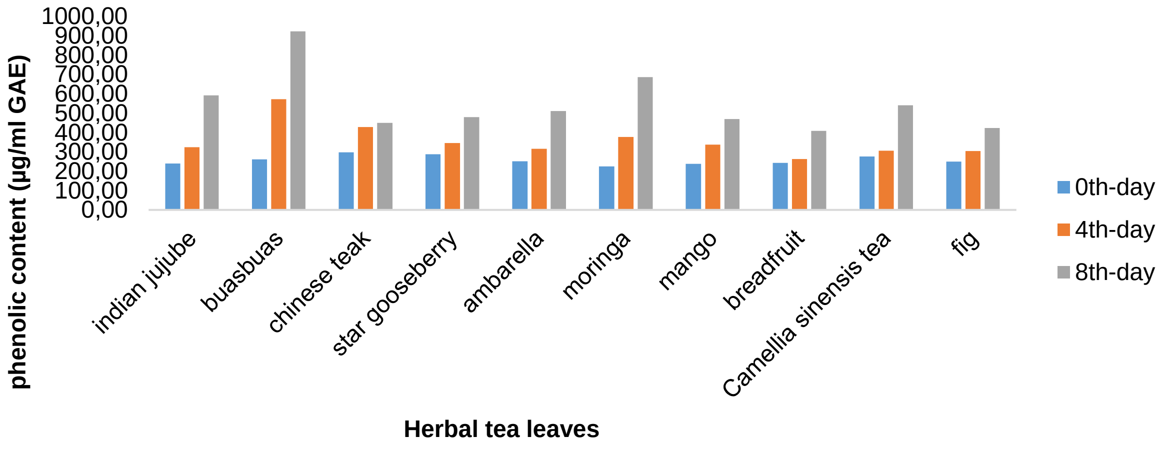

Figure 2. Phenolic content

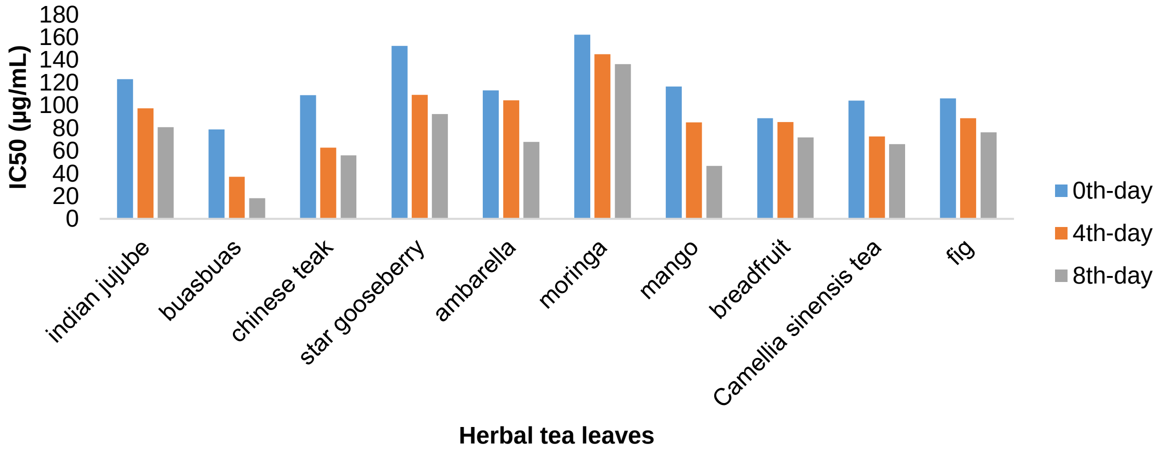

Figure 3. Antioxidant activity

Sensory Evaluation

On this study, the sensory evaluation was determined using 3 parameters (aroma, color, and taste). Panelist measured acceptance level by giving score 1 to 5. The result of sensory evaluation is shown in Table 1.

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Table 1. Sensory evaluation of kombucha from various herbal tea

Sample	Aroma	Color	Taste
Indian jujube	4.47 [a]	3.33 [c]	4.07 [a]
Buasbuas	4.47 [a]	2.20 [d]	3.40 [b]
Chinese teak	1.46 [e]	2.13 [d]	2.13 [d]
Star gooseberry	2.20 [d]	2.13 [d]	1.13 [f]
Ambarella	3.07 [c]	3.27 [c]	4.00 [a]
Moringa	4.47 [a]	4.00 [b]	2.40 [cd]
Mango	3.80 [b]	2.13 [d]	1.33 [ef]
Breadfruit	3.40 [c]	1.93 [d]	4.00 [a]
Regular tea (*Camellia sinensis)*	4.60 [a]	467 [a]	273 [c]
Fig	4.80 [a]	4.00 [b]	1.67 [e]

Note: Means with different letter in the same column are significantly different

In this study, we found that indian jujube, buas buas, moringa, regular tea, and fig has no significant different for aroma. This might have happened because panelists was having difficulty distinguishing between each treatment due to similar aroma. According to Zhao et al. (2018), the major components that contributed to kombucha aroma were alcohol, acid, ethyl ester, aldehyde, keton and others. As Smid & Kleerebezem (2014) reported, aroma production also depends on microorganism's enzyme activity.

The highest acceptance level for color is tea. Panelists preferred tea's color of kombucha because it looks like regular tea. In contrast, the color of kombucha from other herbal teas is lighter. The difference color of kombucha depends on the amount of chlorophyll and other pigments of leaves (Song et al., 2020). According to Nurikasari et al. (2017), color of kombucha becomes lighter during fermentation due to the degradation of pigments.

In addition, panelists preferred the taste of indian jujube, ambarella, and breadfruit compared to other treatments. Kombucha from indian jujube, ambarella, and breadfruit is less acid and less bitter than others. Microorganisms will use sugar as a nutrient to produce organic acid (Mardiana et al., 2020). The production of organic acid would change the taste of kombucha during fermentation from sweet to more acid (Neffe-Skocińska et al., 2017).

CONCLUSION

From the study, kombucha from various herbal teas significantly affects antioxidant activity and bioactive compounds such as phenols and flavonoids. It also demonstrated that kombucha from different herbal teas has unique characteristics on organoleptic.Therefore, further research to optimize bioactive compound and organoleptic should be conducted.

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