ANTIOXIDANT CONTENT AND VITAMIN C IN MANGO LEAF KOMBUCHA WITH STEVIA SWEETENER AND DIFFERENT FERMENTATION DURATIONS

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ABSTRACT

Kombucha is made by the symbiotic fermentation of acetic acid bacteria and a species of yeast known as a SCOBY. Sucrose is converted by bacteria and yeast into organic acids such as acetic acid and glucuronic acid, amino acids, antibiotics, and various micronutrients during the fermentation process. The aim of this research was to determine the physical properties, pH, antioxidant content, and vitamin C and mango leaf Kombucha with stevia sweetener and variations in fermentation time. This research used a Completely Randomized Design (CRD), kombucha used stevia sweetener and used two variations of fermentation time, namely 11 and 13 days. The results showed that the physical properties of mango leaf kombucha showed a brownish color, sweet taste and became very sour. The pH of kombucha at 11 days of fermentation was 4 and 13 days was 4. The antioxidant content at 11 days of fermentation was 79.02% while at 13 days it was 84.97%. The Vitamin C content at 11 days of fermentation was 63.42%, while at 13 days it was 67.96%. Thus, it can be concluded that the fermentation time affects the antioxidant and Vitamin C content of mango leaf kombucha.

Keywords: Antioxidants, Vitamin C, Kombucha, Long Fermentation Time

INTRODUCTION

Kombucha is known for its high antioxidant content, exhibiting antitumor, anticarcinogenic, and mutagenic inhibitory properties (Shahbazi et al., 2018). Kombucha functions as an antioxidant and antibacterial, improves intestinal microflora, increases body resistance, and lowers blood pressure (Wistiana dan Zubaidah, 2015). According to Suhardini and Zubaidah (2016), kombucha has many health benefits, including increasing resistance to flu and colds, eliminating constipation, improving body condition, maintaining balanced body weight, and changing sleep patterns. According to research conducted by Sukmawati (2013), an atmosphere that is too acidic in kombucha tea causes phenolic compounds to become stable. As a result, vitamin C is oxidized and inhaled to form L-dehydroascorbic acid, which is then converted to L-diketogulanic acid, which lacks the activity of vitamin C. Compounds such as flavonoids and cateins, identified as phenolic compounds, contribute to the antioxidant properties of kombucha (Cardoso et al., 2020). The fermentation process significantly impacts the enhancement of antioxidant properties, with effects varying based on fermentation duration. Conversely, the fermentation process may lead to a reduction in reductive properties. Polyphenols in kombucha are responsible for its antioxidant activity, and during fermentation, polyphenols and flavonoids increase, while thearubigin transforms into theaflavin, giving kombucha a brighter appearance over time. Theaflavin is an advanced oxidation product of thearubigin, which serves as a colorant and imparts a slight acidity (Jakubczyk et al., 2020).

Kombucha symbiosis can transform sugar under aerobic conditions within 7–10 days, resulting in a carbonated, slightly acidic, and refreshing beverage rich in acids, 14 amino acids, vitamins, and hydrolytic enzymes (Villarreal-Soto et al., 2018). Kombucha is fermented at room temperature to maximize fermentation time. The substrate used for kombucha fermentation (10 g/L mixed with sucrose) serves as the primary carbon source in concentrations of 5-8%. Subsequently, media and nutrients required for microbial growth are introduced (Kaewkod et al., 2019). Kombucha cultures are stored at room temperature 30 degrees Celsius and not exposed to direct sunlight (Avenus, 2013). Symbiotic Cultures of Bacteria and Yeasts, or SCOBY, created at a concentration of 10% from the previous fermentation, can be used as a starter culture for subsequent fermentations (Martínez Leal et al., 2018).

Kombucha, a beverage produced by fermenting sugar through the symbiosis of acetic acid bacteria and yeast, is gaining popularity as a fermented drink. Mango leaves, known for their antimicrobial and antioxidant compounds, including anthocyanidins that may help prevent cancer, can be used to make kombucha. Tea that has been brewed in hot water is used to make kombucha which is then added with sugar as a sweetener and nutrient to help the growth of the desired bacteria (Falahuddin et al., 2017). Research by (Sarah Bellarani Kania Dewi, 2018) indicates that kombucha fermented for 12-13 days exhibits the highest antioxidant levels. Sugar is a crucial ingredient in kombucha production as it is essential for the fermentation process. Overall, the antioxidant content of mango leaf kombucha suggests the potential health benefits of mango leaves.

Fermentation of kombucha typically involves brewing with sugar and adding a symbiotic colony of bacteria and yeast called SCOBY to the sugar mixture. SCOBY ferments the sugar mixture, producing a tangy and slightly effervescent beverage. In addition to tea leaves, kombucha can be made from various plant leaves, including mango leaves. The source of glucose, a sugar, serves as a substrate for cell growth and the formation of acetic acid products. This substrate is also used by microbes to reproduce and metabolize (Marwati et al., 2013). Research by (Villarreal-Soto et al., 2018) Kombucha are rich in antioxidants and contain essential vitamins such as B1, B2, B6, and B12, vital for immune system function, wound healing, and collagen production. Kombucha made from mango leaves has higher vitamin C content and antioxidant activity, beneficial for health (Roy et al., 2019). Mango leaf kombucha has the potential to contain more vitamin C and antioxidants with specific fermentation times, making it a potential health beverage.

According to research (Ulfa et al., 2022) Mango leaves offer numerous benefits, including antibacterial, anti-inflammatory, antitumor, antioxidant, and antimicrobial properties. These qualities have led to various initiatives to develop functional food products from mango shoots without compromising their nutritional value, particularly in kombucha production. Different types of leaves, including mango leaves, can be used to make kombucha. One type of mango leaf, brewed in hot water, is rich in antioxidants and contains several vitamins, such as vitamin B1, B2, B6, and B12, essential for immune system function, wound healing, and collagen production. Kombucha made from various tea types, including mango leaves, exhibits higher vitamin C content and antioxidant activity. Kombucha is one of the most commonly consumed beverages worldwide, with fresh leaves containing numerous polyphenols (Roy et al., 2019).

During kombucha fermentation, microorganisms produce organic acids that can affect the taste and aroma of kombucha. There is a relationship between antioxidant activity and vitamin C content in kombucha. Phenolic-free substances produced during fermentation enhance antioxidant activity (Fibrianto et al., 2020). Kombucha can be made from various leaves, such as bay leaves, guava leaves, betel leaves, soursop leaves, coffee leaves, and mango leaves (Purnami et al., 2018).

Naturally obtained sugars such as stevia are alternative sugars that are considered safe for consumption by people with diabetes and other diseases in the long term (Limanto, 2017). Stevia rebaudiana leaves are known to have many benefits and are low in calories and carbohydrates. Apart from its ability to control blood sugar levels, the sweet taste produced when consumed is much higher, reaching 200-300 times compared to sucrose (Ulfa et al., 2022).

The aim of this research is to determine the antioxidant and Vitamin C content, as well as organoleptic testing, of mango leaf kombucha with stevia sweetener and various fermentation durations. Fermentation of sugar results in kombucha, made with a mixture of starter kombucha culture called SCOBY (Symbiotic Cultures of Bacteria and Yeasts). In Russia and China, kombucha has long been known as a health drink, with a taste resembling apple juice. Bacteria and microorganisms from the yeast group are utilized for kombucha fermentation.

Acetobacter, also known as *Acetobacter xylinum*, and several types of yeast, such as *Brettanomyces*, *Zygosaccharomyces*, and *Sacharomyces*, are symbiotic of kombucha culture (Simanjuntak et al., 2016). These bacteria and yeast possess the ability to prevent microbial contamination (Villarreal-Soto et al., 2018).

LITERATURE REVIEW

Antioxidant Content in Kombucha

Research has highlighted the antioxidant properties of kombucha, with the fermentation process contributing to the formation of bioactive compounds like polyphenols. These compounds play a crucial role in scavenging free radicals, thereby exhibiting potential health-promoting effects. Studies have shown that the antioxidant content in kombucha can vary based on the substrate used, fermentation conditions, and additional ingredients, providing a foundation for investigating the antioxidant potential of mango leaf kombucha.

Vitamin C in Kombucha

Vitamin C, or ascorbic acid, is an essential nutrient with antioxidant properties that contribute to overall health. Kombucha has been explored as a potential source of Vitamin C, with studies suggesting that the fermentation process may enhance its bioavailability. Examining the Vitamin C content in mango leaf kombucha can shed light on the beverage's nutritional profile and its potential as a natural source of this essential vitamin.

Stevia as a Sweetener

Stevia, derived from the leaves of the Stevia rebaudiana plant, is widely recognized as a natural sweetener with minimal impact on blood sugar levels. Its use in kombucha may provide a healthier alternative to traditional sweeteners, with potential implications for the overall nutritional quality of the beverage. Existing literature on Stevia's influence on the sensory characteristics and health benefits of kombucha will be explored in this review.

Effect of Fermentation Duration:

The duration of fermentation plays a crucial role in shaping the chemical composition and sensory attributes of kombucha. Investigating the impact of different fermentation durations on the antioxidant content and Vitamin C levels in mango leaf kombucha will provide insights into the optimal conditions for achieving a balance between taste and nutritional benefits.

RESEARCH METHODOLOGY

Materials and Equipment

This study utilized young and unaffected mango leaves, stevia sweetener, 3% citric acid, kombucha starter, tissue, aluminum foil, label paper, mineral water, distilled water (aquades), diphenyl picryl hydrazyl (DPPH), methanol, and 1% amylum. Equipment included a digital scale, spoon, strainer, funnel, tweezers, glass jar, measuring glass, scissors, bowl, tray, spatula, stove, pot, filter, basin, knife, test tube, Erlenmeyer flask, pH meter, vortex, volumetric flask, spectrophotometer, pipette, and writing tools.

Treatment and Parameters

Fermentation durations of 11 and 13 days at room temperature without direct sunlight exposure were implemented, following a Completely Randomized Design (CRD). The measured parameters included pH, vitamin C content, and antioxidant content, with each measurement repeated three times.

Kombucha Production

Steps Using Mango Leaves

1. Select 2-5 young and best-quality mango leaves.
2. Wash mango leaves with clean water and sun-dry them.
3. Roast the dried mango leaves.
4. Sterilize all equipment such as glass bottles or jars, spoons, measuring glasses, strainers, knives, cutting boards, and tweezers.
5. Add 7.5 grams of mango leaves to a container with 1.5 liters of boiling water.
6. Add 15 grams of stevia sweetener and stir until dissolved.
7. Filter the solution to separate mango leaves from the liquid.
8. Let the liquid cool to warm temperature. The kombucha

production process is illustrated in Figure 1.

A B C

Figure 1. Mango Leaf Kombucha Production Process

A. Weighing mango leaves, B. Adding the solution according to the measurements, C. Putting the solution in a container

Antioxidant Content Test Using DPPH Method

DPPH Solution Preparation

Antioxidant content was measured using the following steps:

1. Take DPPH with a spatula tip and place it in a 250 mL Erlenmeyer flask.
2. Add methanol solvent.
3. Measure the solution's absorbance at a wavelength of 517 nm. If the absorbance is above 0.9, dilute the DPPH solution with methanol until the absorbance reaches 0.8.

Sample Preparation

The steps for preparing the mango leaf kombucha sample for antioxidant content testing are as follows:

1. Place 1 ml of the sample into a 50 ml Erlenmeyer flask.
2. Add 20 ml of distilled water plus 3% citric acid.
3. Stir the mixture using a magnetic stirrer at 60˚C, 750 rpm, for 30 minutes.
4. After mixing, separate the sample and tightly close the container.

DPPH Antiradical Activity Measurement

DPPH antiradical activity measurement involves the following steps:

1. Take 4 mL of the DPPH solution and place it in three capped test tubes.
2. Prepare two test tubes as blanks containing only DPPH solution. Add 100 µL of the sample to each test tube and start the timer.
3. Mix in the test tube and vortex for 10 seconds.
4. Keep the test tubes in the dark for 60 minutes after adding the extract.
5. Measure the absorbance with a spectrophotometer at a wavelength of 517 nm

Testing Vitamin C Content

Standardization Procedure

a. Standardize $Na_2S_2O_3$ ± 0.01 M with KIO_3 0.01 µ, using 10 milliliters of KIO_3: Add 2.5 milliliters of H_2SO_4 2µ and 2.5 milliliters of 20%, then close the mouth of the Erlenmeyer flask. Titrate with $Na_2S_2O_3$ until it turns light yellow. Add 2 drops of 1% amylum, and titrate again until the blue color disappears.
b. Standardize I ± 0.01µ with $Na_2S_2O_3$ ± 0.01µ: Pipette 10

milliliters of Na2S2O3 solution with ± 0.01µ and add 1% amylum. Close it with titration plastic with I3 solution with ± 0.01 µ until the blue color appears.

Sample Determination Procedure

The steps for determining the vitamin C content of mango leaf kombucha samples are as follows: 1). Pipette 5 milliliters of the sample into an Erlenmeyer flask. 2). Combine 20 milliliters of distilled water with 2 drops of 1% amylum. 3). Titrate with standard solution with I2 ± 0.01N until a blue color appears. 4). Calculate the average after repeating the process three times.

pH Measurement

pH measurement is carried out with the following steps: 1). Prepare pH paper. 2). Dip the pH paper into kombucha. 3). Compare the results on the pH paper with the pH indicator table.

RESEARCH RESULT

Antioxidant Content

The results of antioxidant content measurement in mango leaf kombucha are presented in Table 1 and Figure 2.

Table 1. Antioxidant Content in Mango Leaf Kombucha with Fermentation Duration Variations

Fermentation Time (Days)	Antioxidant Content in the 2nd Repeat (%)			Average (%)
	1	2	3	
11	78,58	79,02	79,46	79,02
13	86,82	82,74	85,34	84,97

Based on Table 1, the average antioxidant content in mango leaf kombucha fermented for 13 days (84.97%) is higher than in mango leaf kombucha fermented for 11 days (79.02%). This supports (Villarreal-Soto et al., 2018) research, concluding that phenolic compounds become more stable and less likely to release protons in an acidic environment due to enzymes produced by bacteria and yeast breaking down complex chemicals through biotransformation processes, utilizing plant cell enzymes to enhance specific biological activities, and the microorganism metabolism increases phenolic compound content. Mango leaf kombucha fermentation time can enhance its antioxidant content. The fermentation process impacts the increase in antioxidant properties, depending on the type of material and fermentation duration. On the other hand, the fermentation process affects the reduction in reductive properties. Polyphenols in kombucha are responsible for its antioxidant activity. During fermentation, polyphenols and flavonoids increase, while thearubigin transforms into theaflavin, making kombucha brighter over fermentation time. Theaflavin is an advanced oxidation product of thearubigin, with thearubigin providing color and a slight acidity.

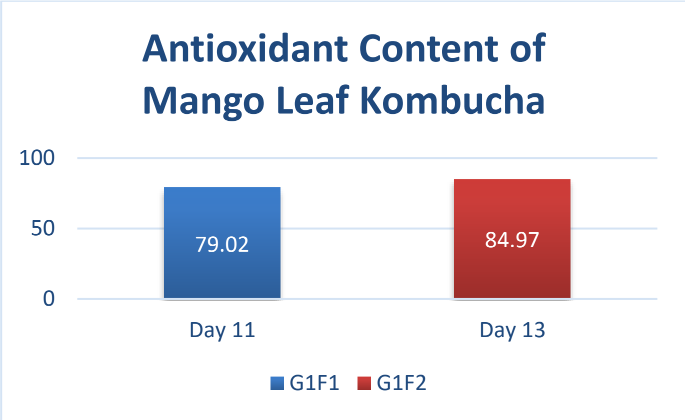

Figure 2. Antioxidant Content Graph of Mango Leaf Kombucha with Fermentation Duration Variations

During the fermentation process, free phenolics are produced, increasing the antioxidant content to 84.97% on the 13th day of fermentation. In other words, the more phenolics formed, the higher the antioxidant content. (Hapsari et al., 2021) found that during fermentation, kombucha phenol content increases. The presence of free phenolics produced during the fermentation process affects the increase in antioxidant levels during the 11 and 13-day fermentation periods. The metabolism of microorganisms during the fermentation process affects this increase in antioxidant content. With high antioxidant content, mango leaf kombucha can neutralize free radicals, prevent the flu, and enhance the immune system. Additionally, antioxidant content can be used to treat diseases such as diabetes and cancer (Wibawa, 2020).

Vitamin C Content

The results of vitamin C content measurement in mango leaf kombucha are presented in Table 2 and Figure 3.

Table 2. Results of Vitamin C Content Test

Treatment	Repeated of Vitamin C Content (%)			Average (%)
	1	2	3	
G1F1 Stevia Sweetener 15 g and fermentation duration 11 days	63,38	60,35	66,54	63,42
G1F2 Stevia Sweetener 15 g and fermentation duration 13 days	68,69	67,02	68,17	67,96

Based on Table 2, it is known that the average vitamin C content in mango leaf kombucha fermented for 13 days (67.96%) is higher than the vitamin C content in mango leaf kombucha fermented for 11 days (63.42%). The results of vitamin C content can be seen in the table, indicating a significant difference between the 11-day and 13-day fermentation periods.

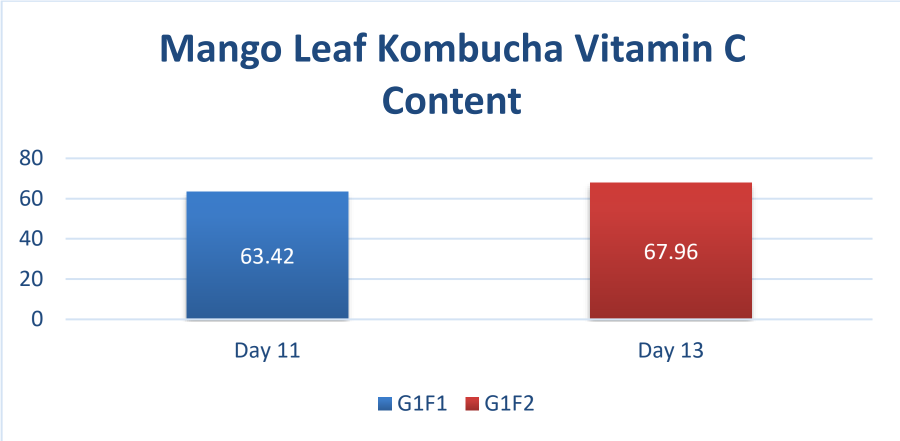

Figure 3. Vitamin C Content Test Results Graph of Mango Leaf Kombucha with Fermentation Duration Variations

The transformation of glucose in the solution into vitamin C by Acetobacter xylinum causes a significant increase in vitamin C levels during the fermentation periods of 11 and 13 days. D-glucose is reduced to D-sorbitol, which is then converted into L-sorbose by the enzyme produced by Acetobacter xylinum. L-sorbose is then oxidized to Ascorbic Acid 2-Ketol-L-Gulomat, which is further fermented into L-ascorbate. In the presence of oxygen, bacteria can oxidize the alcohol groups of sugar compounds. L-sorbose, which has undergone further fermentation, will be converted into vitamin C. The kombucha starter process by Saccharomyces cerevisiae oxidizes sucrose in the substrate into CO2 and H2O. The reaction between CO2 and H2O produces Vitamin C (Darmawan et al., 2018). The vitamin C content in mango leaf kombucha decreases on the 11th day of fermentation. This is due to the degradation process of vitamin C. Microorganisms stop producing vitamin C, leading to a decrease in food or sugar supply in kombucha, and microorganisms then produce other acidic compounds. Testing the vitamin C content of kombucha with variations in fermentation duration in treatment G1F2 on the 13th day of fermentation shows an increase of 67.96 mg/100 g. Fermentation time affects the increase in vitamin C content.

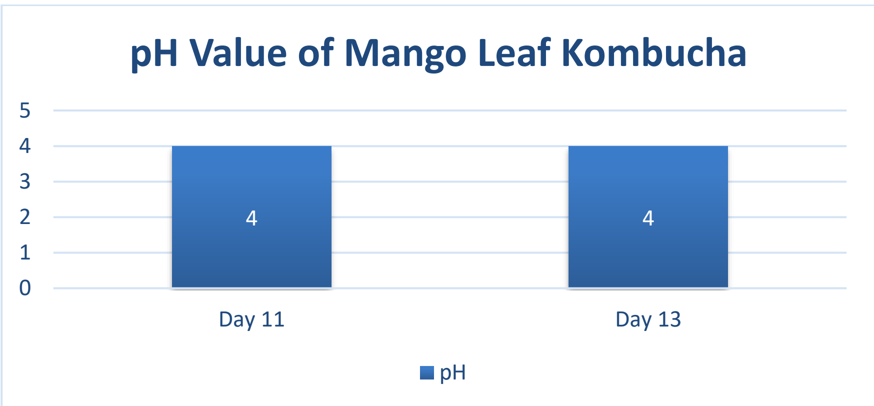

Figure 4. pH Value of Mango Leaf Kombucha

Observation of Physical Properties

Observation of physical properties indicates that mango leaf kombucha with stevia sweetener has a distinctive brownish color. The use of stevia sweetener in kombucha production may be the cause. For kombucha to have a sour taste, influenced by the fermentation process carried out by bacteria breaking down glucose into organic acids. The longer the fermentation, the more acidic the aroma.

Figure 5. Organoleptic Test Results on Day 11 of Mango Leaf Kombucha with Stevia

Mango leaf kombucha on the 11th day exhibits a sour taste, indicating an extended fermentation time resulting in an acidic taste.

Figure 6. Organoleptic Test Results on Day 13 of Mango Leaf Kombucha with Stevia

After the 13th day of fermentation, mango leaf kombucha has a taste that has changed from initially sweet before fermentation to very acidic. The addition of glucose or stevia sweetener when the ingredients are heated results in glucose degradation. The activity of Acetobacter xylinum bacteria reduces pH during fermentation, which is another factor that can affect this.

CONCLUSION

Based on the research results, it can be concluded that:

1. The antioxidant content in mango leaf kombucha fermented for 13 days (84.97%) is higher than fermentation for 11 days (79.02%).
2. The vitamin C content in mango leaf kombucha fermented for 13 days (67.96%) is higher than fermentation for 11 days (63.42%).
3. The pH level in mango leaf kombucha fermented for 11 and 13 days is pH 4.
4. The physical properties of mango leaf kombucha include a distinctive brownish color and a sour aroma and taste. The longer the fermentation, the browner the color, and the more acidic the aroma and taste.

Suggestion

1. Further research should be carried out using various types of leaves with various concentrations of stevia sweetener.
2. Further research must be carried out to examine other ingredients in kombucha tea sweetened with stevia, such as alcohol content or other ingredients that are good for humans.

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