

CHEMICAL AND BIOLOGICAL CHANGES IN KOMBUCHA FROM BLACK TEA, PINNEAPPLE AND MANGO

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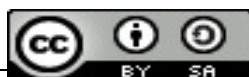
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ABSTRACT

Kombucha is a beverage made by fermenting tea or fruit with added sugar and a scoby. A scoby (Symbiotic Culture of Bacteria and Yeast) is a starter that breaks down sugar into acetic acid and alcohol. This study used tea, Indramayu mango, and Honi pineapple. This study was conducted with observations on days 0, 7, and 14 by measuring pH, reducing sugar, alcohol, and scoby weight. The purpose of this study was to observe chemical and biological changes using different media. The results showed that fermentation occurred during this period. The longer the fermentation, the lower the pH level in all media and the sugar content also decreased. The longer the fermentation, the higher the alcohol content as a result of the fermentation process. The highest alcohol content was in pineapple kombucha with 3.25% on day 14. Increasing the fermentation time showed an increase in scoby weight. The results indicate that the media can be used as a kombucha medium. The fermentation time and sugar addition affect the quality of the final product.

KEYWORDS

Chemical, Kombucha, Mango, Pinneapple, Tea



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INTRODUCTION

Kombucha is a product of tea and sugar fermentation by Symbiotic Culture of Yeast and Bacteria (SCOBY) fermented for 7-14 days. The yeast found in SCOBY is *Saccharomyces* sp., *Zygosaccharomyces* sp., *Dekkera/Brettanomyces* sp., and *Pichia* sp., while the bacteria found in SCOBY are *Acetobacter xylinus*, *Acetobacter xylinum*, *Acetobacter aceti*, *Gluconobacter xylinus*, *Gluconacetobacter xylinus*, *Lactobacillus* sp., *Leuconostoc* sp., *Lactococcus* sp., and *Oenococcus* sp (Laureys et al. 2020). This fermented drink from Asia has become popular in the West due to its therapeutic effects, such as antimicrobial, antioxidant, anticancer, antidiabetic, treatment for stomach ulcers and high cholesterol (Coelho, 2020). Microorganism activity during fermentation produces various

compounds that are beneficial to health, including vitamins, minerals, enzymes, and phenolic compounds. The higher the phenolic compounds produced, the higher the antioxidant activity (Khamidah and Antarlina, 2020).

Kombucha is usually made from tea leaf extract (*Camellia sinensis*). Black tea and white sugar are good substrates for making kombucha (Zubaidah et al., 2021). Currently, kombucha production has expanded widely by utilizing natural ingredients that can enrich food diversification. Fruits that can be used as ingredients for kombucha are pineapple and mango. Pineapple (*Ananas comosus*) is a fruit rich in various nutrients such as water (about 85%), carbohydrates (13-14%), vitamin C, and minerals such as calcium, phosphorus, and potassium. This fruit also contains bioactive compounds such as flavonoids, alkaloids, saponins, and tannins that act as antioxidants and support the body's health (Fauzi et al., 2023). The kombucha fermentation process using pineapple or pineapple juice produces a product with low pH and high organic acid content that is good for health and has a unique taste (Rahmi et al., 2020).

Mangoes (*Mangifera indica* L.) are one of the most popular fruits among Indonesians because they contain a complete range of vitamins and are affordable (Umam et al., 2021). According to Ayyun et al (2023), mango flesh contains various nutrients that are beneficial to health. Antioxidant content such as carotenoids (vitamin A) and vitamin C play a role in preventing cancer, while potassium and vitamin C content play a role in maintaining heart health. This fruit contains vitamin A, vitamin B1, vitamin B2, vitamin C, and minerals such as potassium, phosphorus, and iron, which are important for the body's health (Andriani et al., 2019; Samudi et al., 2024). Indramayu mango is a superior variety characterized by its sweet taste and strong aroma. The nutritional content of this mango contains various bioactive compounds such as vitamin C, carotenoids, and high antioxidants, so it has the potential to be used as a raw material for making high-quality kombucha (Putri et al., 2021). The addition of fruit juices to kombucha, such as pineapple, apple, and pomegranate juices, can increase metabolic components such as total phenolic content, total antioxidant activity, and organic acids, as well as improve the sensory and functional properties of kombucha (Osiripun & Apisittiwong, 2021).

This study aims to explore substrates as media for kombucha production by utilizing the potential of local fruits, as well as mapping changes in chemical composition (pH, sugar content, and alcohol content), microbiology (SCOBY weight), and sensory properties of various ingredients, namely black tea, Indramayu mango, and Honi pineapple.

RESEARCH METHOD

Tools and Materials

The tools used in this study include Fermentation containers, cloth napkins, rubber bands, measuring cups, spoons, refractometers, pH meters, alcohol meters, and digital scales. The materials used in this study include SCOBY (*Symbiotic Culture of Bacteria and Yeast*), Sosro black tea, granulated sugar, Honi pineapple, and Indramayu mango obtained from the Los Buah Pasar Gedhe traditional market.

Method

This study is a qualitative study conducted using a laboratory experimental approach with two types of treatment, namely differences in fermentation duration, namely 7 and 14 days of fermentation. This method was chosen because it allows researchers to obtain descriptive data on pH value determination, sugar content determination, alcohol content determination, SCOBY (*Symbiotic Culture of Bacteria and Yeast*) weighing, and sensory evaluation. This method was divided into several stages, namely, making tea bag

kombucha, making pineapple and mango kombucha, the fermentation process, determining the pH value, determining the sugar content, determining the alcohol content, weighing the SCOBY, and sensory testing.

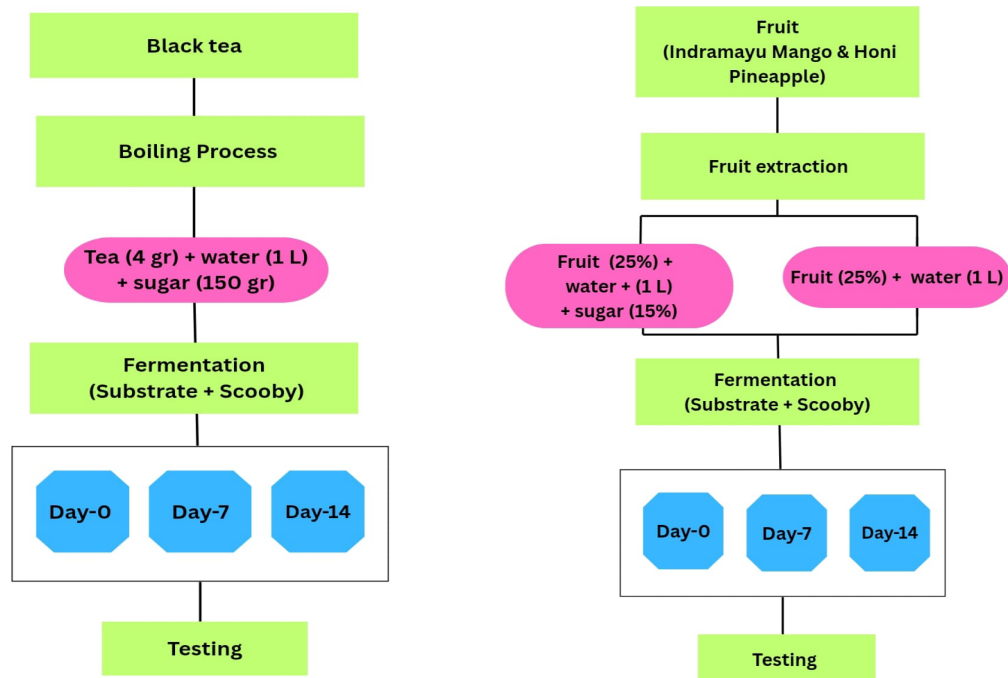


Figure 1. Research Methodology

Making Kombucha from Black Tea

The tea making process begins by boiling 1 liter of water, then adding 150 grams of sugar and 4 grams of black tea. The tea mixture is left to cool at room temperature. Rukman & Haerussan,(2023) state that in the production of Sosro kombucha tea bags, the tea and sugar solution is heated until the sugar is completely dissolved, then cooled to room temperature (20-25°C). After that, the tea solution, which has been left to stand for 15 minutes, is then poured into a 500 ml clear jar.

Table 1. Mango and Pineapple Kombucha Formulation

Composition	Sampel			
	Pineapple	Pineapple+sugar	Mango	Mango+sugar
Scoby	45 g	45 g	45 g	45 g
Water	1000 ml	1000 ml	1000 ml	1000 ml
Sugar	150 g	150 g	150 g	150 g
Pineapple	250 g	250 g	-	-
Mango	-	-	250 g	250 g

Making Kombucha from Mango and pineapple

This research method includes the process of making mango and pineapple substrate solutions, namely cleaning the fruit peel by peeling and washing the fruit. Next, the fruit is cut into small pieces to be blended with water to produce the substrate solution.

Fermentation Process

Each substrate solution was transferred into a 500 ml clear jar and 45 grams of SCOBY was added, then covered with a clean white cloth secured with a rubber band.

Next, each sample was repeated twice and fermented for 7 and 14 days to obtain comparison of the analysis results for kombucha.

Determination of Sugar Content

Sugar content testing is determined by dripping the sample onto a refractometer prisms, observing and recording the brix index value shown on the scale after ensuring there are no bubbles (Anwar and Khoirunnisa, 2024).

Determination of Alcohol Content

Alcohol content is measured using an alcohol meter by testing the kombucha water on the 7th and 14th days of fermentation.

Weighing the SCOBY

The weight of the SCOBY is measured after the fermentation process using digital scales.

Sensory Evaluation

Sensory evaluation includes aroma, taste, and color for each kombucha sample that is most preferred by the panelists. This test is based on the panelists' scores for aroma, taste, and color. The test was conducted on 13 panelists. The sensory analysis format is presented as follows.

RESULT AND DISCUSSION

Chemical Analysis

pH Testing

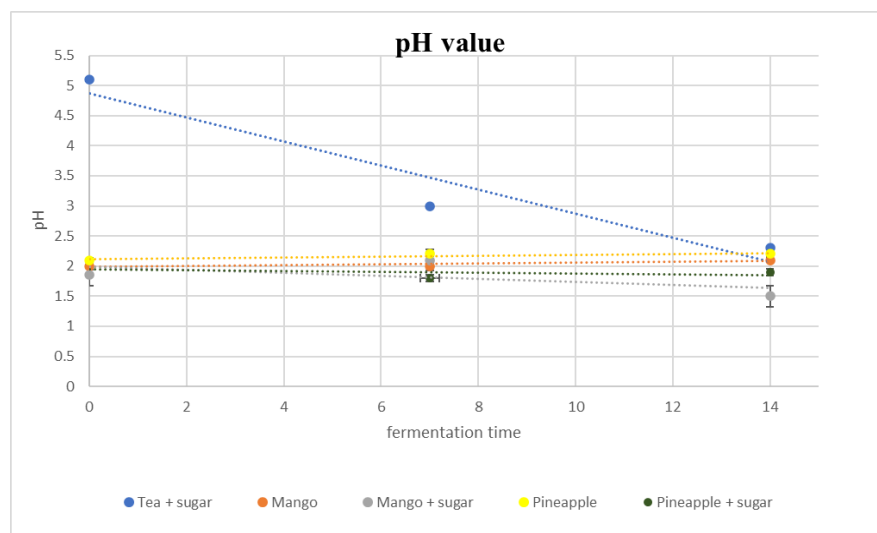


Figure 2. Graph of pH measurement results

In Kombucha, a pH test is conducted to determine the effect of fermentation on pH changes during the kombucha production process. The decrease in pH is caused by the transformation of glucose into alcohol and organic process, and acetic acid releases during the fermentation process, and acetic acid releases protons, which is thought to cause a

decrease in pH (Puspaningrum et al, 2022). This test aims to ensure that the beverage is safe for consumption and has the expected quality.

Figure 2 indicates that the pH value in tea samples with sugar showed a significant decrease, from 5,1 to 2,3. Meanwhile, the other samples showed an insignificant decrease. The addition of sugar to the samples caused fermentation to become more active, thereby further decreasing the pH of the kombucha and producing a sour taste. In the combination of mango with sugar and pineapple, there was an increase in the pH value and acidity of the kombucha, which tended to fluctuate because, in addition to being produced by acetic acid bacteria and lactic acid bacteria, organic acids were also utilized by yeast as a carbon source.

The longer the fermentation period, the lower the pH of the kombucha. This is due the metabolic process of microorganisms that transform sugar into organic acids, which increase over time, causing the pH to become low or acidic. However, at some point, the acid concentration may decrease if the sugar and ethanol sources are depleted because bacteria will use them as a carbon source ((Khamidah & Antarlina, 2020).

Sugar Content

The sugar content in kombucha drinks decreases significantly as the fermentation time increases. This decrease is caused by the activity of microorganisms contained in kombucha, which consume sugar as an energy source to sustain their survival. Therefore, sufficient sugar availability is very important to ensure that the fermentation process runs optimally, considering that sugar is the main carbon source in fermentation (Putra et al., 2022)

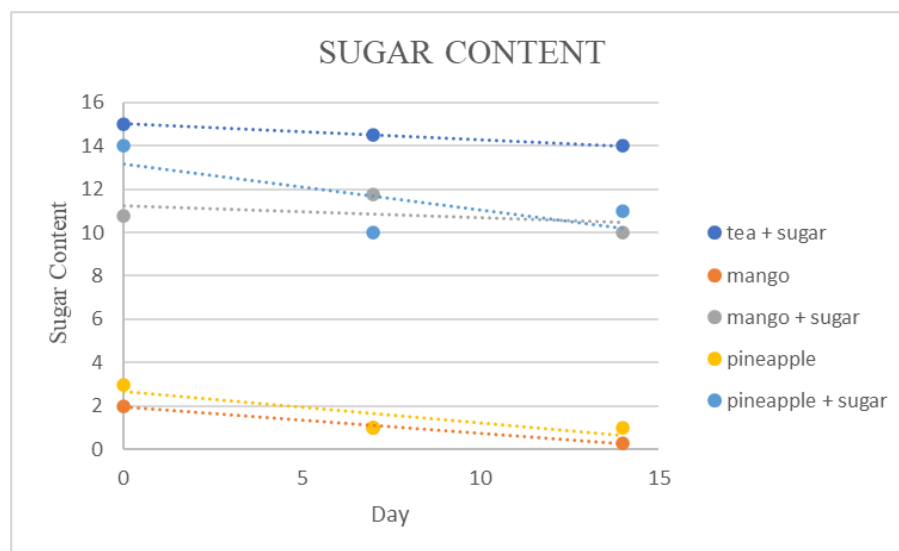


Figure 3. Graph Of Sugar Content Measurement Results

However, in the tea with sugar sample, there was a slow decrease in sugar content from day 0 to day 14, leaving 93% sugar. However, in the pineapple with sugar sample, 78% sugar remained, which decreased on day 7 from 14 to 10. Meanwhile, the mango with sugar sample retained the highest sugar content, namely 95%, because it increased on day 7 from 10,5 to 11,75 and changed on day 14 to 10

Sugar is a source of glucose that functions as a substrate for cell growth and the formation of acetic acid. The substrate is used by microbes to grow and metabolize. During

fermentation, sugar is consumed as a carbon source and converted into alcohol and CO₂ (Marwadi et al., 2016; Siregal et al 2023). It is concluded that the addition of sugar can essentially maintain higher sugar levels despite the decrease.

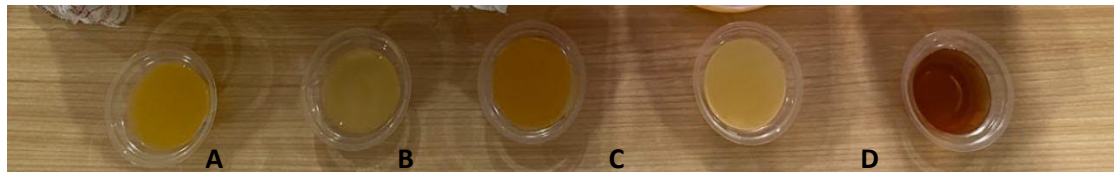


Figure 4. Kombucha on Day 7 (Description A : Mango, B: Pineapple, C: Mango+Sugar; D: Pinneapple+Sugar; E : tea+Sugar)

Alcohol content

Kombucha is a fermented beverage that contains a certain amount of alcohol (Sulistiawaty & Solihat, 2022). Kombucha can be a low-alcohol substitute for soda, wine, or soft drinks due to its high carbonation level, and as a healthier alternative (Coelho, 2020). Non-alcoholic and low-alcohol versions of kombucha (less than 0.5% (v/v) alcohol) can be found on the market, or even alcoholic versions (Nummer, 2013; Coelho, 2020). Alcohol content measurements were taken on days 7 and 14.

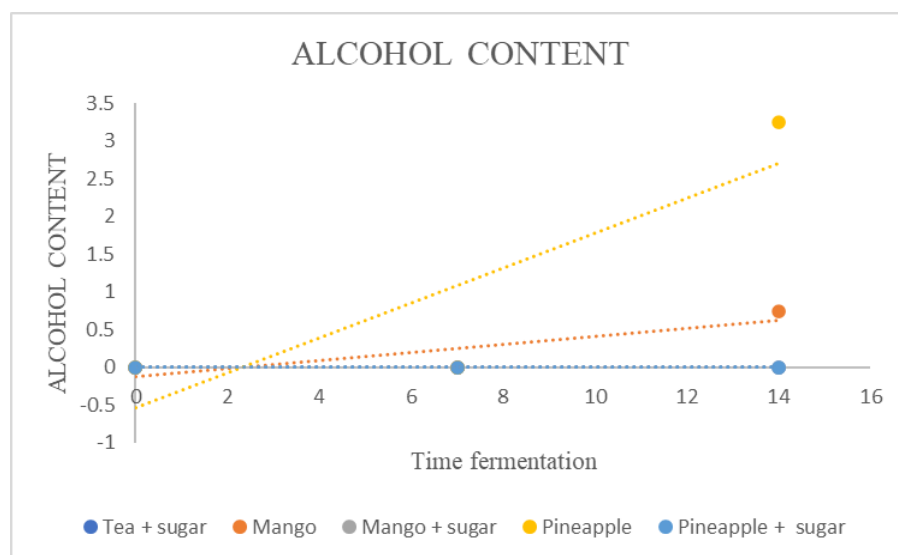


Figure 5. Graph of Alcohol Content Measurement Results

Based on Figure 5, the alcohol content measurements showed negative results in all samples on day 0 and day 7. Alcohol content measurements on day 14 showed undefined alcohol content in samples of tea with sugar, mango juice with sugar, and pineapple juice with sugar. Alcohol content measurements on the pineapple juice sample on day 14 showed an average alcohol content of 3.25.

To determine the alcohol content in all samples, a more accurate method is needed to determine the exact alcohol content in kombucha. Testing the alcohol content in kombucha is very important to ensure its halal status.

Microbiological Analysis (SCOBY Weight)

Microbiological analysis data on SCOBY weight on kombucha fermentation duration is presented in the scatter diagram below

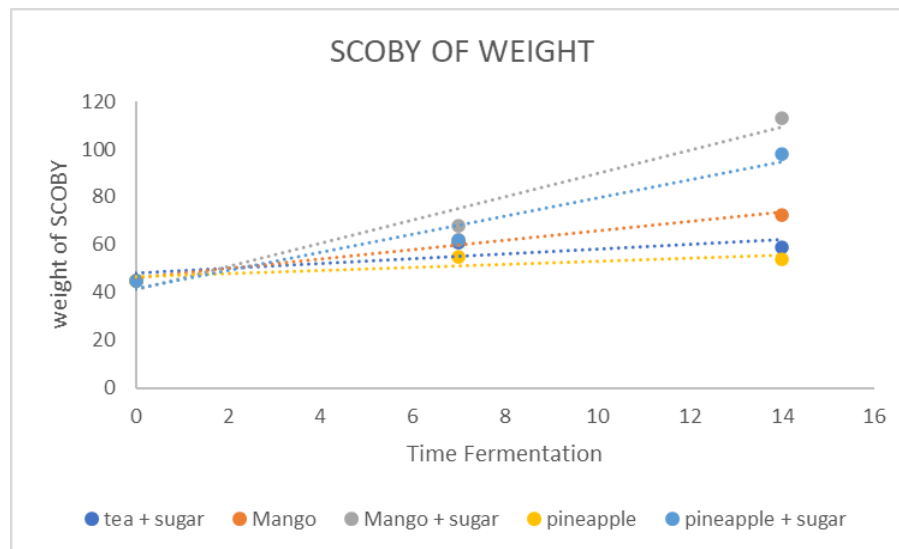


Figure 6. Graph of SCOBY Weight Measurement Results

In the Figure 6, the initial weight of the SCOBY on day 0 for each sample was 45 grams, where the SCOBY was weighed evenly and divided equally among all samples as the starting point for the fermentation process. On day 0, the SCOBY increase in kombucha weight on day 7, where the addition of sugar contributed to the increase in the kombucha weight, indicating the influence of fermentation power and nutritional content on SCOBY growth and kombucha production with different ingredient variations. As fermentation progressed over time, SCOBY continued to grow and increase in weight through the formation of new, thicker layers, indicating that the fermentation process was going well. However, there were samples that showed a decrease in SCOBY weight on day 14, namely the pineapple juice and tea kombucha with sugar samples

CONCLUSION

Kombucha is a fermented beverage made from tea or fruit juice mixed with sugar and a SCOBY as a microorganism culture. The addition of sugar encourages SCOBY growth, particularly in kombucha made from mango juice with sugar. A decrease in pH and sugar levels indicates optimal fermentation and quality. The SCOBY converts sugar to ethanol and then acetic acid, which produces a distinctive flavor. The longer the fermentation time, the lower the pH, the lower the reducing sugar content, the higher the alcohol content in some samples, and the higher the SCOBY weight.

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