International Journal of Recent Engineering Research and Development (IJRERD)
ISSN: 2455-8761
www.ijrerd.com || Volume 08 – Issue 06 || June 2023 || PP. 06-12

# Investigation of fermentation conditions and production methods of mulberry Kombucha tea

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**Abstract:** The increasing demand for healthy beverages worldwide has led to the development of new varieties of kombucha drinks using different plants and fruits. As a result, kombucha tea has become a popular and trendy health drink in recent times. This beverage is produced by fermenting tea leaves infused with a combination of bacteria and yeast in a symbiotic manner. Kombucha tea, also called tea fungus, is a complex mixture of bacteria and yeast that grows on a sugary tea broth to produce a slightly acidic and cider-like taste. To determine the factors affecting the fermentation of Kombucha, this study measured sugar content, amount of scoby starter culture, pH conditions and fermentation time. The study also investigated the ratio between tea juice and juice to ensure the visual satisfaction of consumers in beverage products from fermented tea combined with fruit. The results showed that the best condition in this study was 5g tea/liter water, 70g sugar, 2.5% scoby supplement, with pH ranged around 3.5 and fermentation time for 120 hours at room temperature room (30±5ºC).Mulberry Kombucha tea is produced by adding mulberry syrup with a concentration of 0.07%, then stored at 4±2ºC for 10 days. After 10 days, there was a significant change in pH, total soluble solids, strong acidity but stronger alcohol smell.

**Keywords:** Kombucha tea, healthy beverages, tea juice, scoby, mulberry.

## 1. Introduction

Kombucha tea is a non-alcoholic or low alcoholic, functional beverage obtained in the fermentation of sweetened green or black tea by symbiotic culture of bacteria and yeats [1]. All over the world, Kombucha is produced both at homes, handcrafted in small enterprises, and on large, commercial scales. Kombucha Brewers International (KBI) is global non-profit organization of commercial Kombucha companies involved in the global promotion of the drink and protection of producers [1, 2]. The kombucha industry has reported rapid growth in recent years and the research related to kombucha is flourishing[2] .

Kombucha is a probiotic, soft, functional and carbonated fermented beverage that obtained from fermenting tea with sugar by a consortium of microbes[3]. This beverages is fermented by co-cultures of bacteria (often Acetobacteria) and osmophilic yeasts in the cellulosic layer called SCOBY (symbiotic culture of bacteria and yeast)[3]. Kombucha has already been reported in the literature with antimicrobial activity against Gram-positive and Gram-negative bacteria, the effect being attributed to the acetic acid content of the beverage[4]. The yeast and bacteria composition of kombucha can differ significantly depending on the starter culture and growthconditions. The most commonly reported yeasts include *Saccharomyces, Schizosaccharomyces, Brettanomyces* and *Zygosaccharomyces*. The majority of bacteria in kombucha are acetic acid bacteria, i.e. *Acetobacter, Gluconobacter,and Komagataeibacter*, which metabolize glucose or ethanol to acetic acid [5]. Growth patterns of these microorganisms during the fermentation process of Kombucha are not well documented. Cellulose produced during the fermentation by *Ac. xylinum* appears as a thin film on top of the tea where the cell mass of bacteria and yeasts is attached [6].

The compounds that are produced during the production processes of different types of tea markedly affect the composition and total phenolic content that are found in kombucha tea. The major polyphenolic components, catechin and epicatechin, are known to possess antioxidant activity[7]. During the early stage of fermentation (0–3 days), the yeast converts cereal-derived sugars into ethanol and CO2. Subsequently, the acetic acid bacteria convert glucose and ethanol into several organic acids, including gluconic, acetic, critic, succinic, and malic acids[8] Traditionally, kombucha tea is prepared by fermenting sweetened black tea or green tea with bacteria and yeast embedded within the cellulosic mat which present on top of the broth. Kombucha fermentation can be done using kombucha starter cultures or previously fermented broth or fermenting tea with the cellulosic mat [9].

Actual food trends toward minimally processed products, without additives, high nutritional value and with health benefits have increased with consumer awareness. In this context, the traditional Kombucha tea has recently captured the attention of researchers and consumers because of its probiotic characteristics.[10]. Today, kombucha is sold worldwide in retail food stores in different flavors and kombucha culture is sold in several online shopping websites[11].

Various parameters influence the properties and content of kombucha, including the type of tea, fermentation time, the content of SCOBY colonies, and temperature[12]. Although Kombucha tea has become increasingly popular, there is still a lack of comprehensive information on the effects of various parameters and types of tea on its properties and composition.

Despite the fact that kombucha has been studied in detail in terms of microbiological content and antibacterial properties, there is not enough research on different fruits after fermentation to produce finished products with diverse flavors. This is why our study used mulberry syrup, a fruit with many biological properties, distinctive taste and beautiful color.

Therefore, Kombucha fermented tea is often carbonated with a light aroma, a little sweet and sour taste. The distinctive flavor of a Kombucha beverage depends on how long the Kombucha is fermented, the type of tea used, and the addition of some other ingredients such as fruit juices, herbs, or fresh fruit. Vietnam is a tropical country with year-round heat and abundant fruit. The development of a beverage product that uses fruit for cooling and health benefits is suitable for use and enriches the healthy beverage products on the market.

## 2. Materials and methods

### 2.1. Starter Culture of Kombucha Tea

To make kombucha tea, we used tea leaves sourced from Tan Cuong in Thai Nguyen, Viet Nam. We also obtained a starter culture from the Faculty of Environmental Chemical Technology at the University of Technology and Education in Danang. The tea was prepared using the same method as the industry standard, with a formulation that included 1% tea leaves and 10% sucrose.

Mulberry syrup is prepared with a 1:1 ratio of mulberry and sugar, mixed well, soaked for 2 hours, then added 500ml of water and boiled for 1 hour. Filter the residue with a filter to obtain mulberry syrup for use.

### 2.2. Preparation of kombucha drink

To make the drink, we used black tea obtained from Tan Cuong in Thai Nguyen, Viet Nam as the substrate and white sugar from Bien Hoa Company, Viet Nam as the carbon source. We autoclaved the containers at 100 °C, and then added 5g of tea to 1 L of boiled distilled water in each container. After allowing the tea to infuse for 20 minutes, we removed the excess tea leaves using sterile filter paper. We added sugar at varying levels (70 g/l, 80 g/l, 90 g/l, 100 g/l, 110 g/l) to the brewing tea solution, dissolved all the sugar, and cooled the mixture before adding the starter solution. We then introduced the SCOBY into the mix and fermented it at room temperature (30±5 ℃) for 14 days. Finally, we checked the pH, soluble solids content, and conducted sensory evaluation to determine the optimal parameters for the finished product.

### 2.3. pH

The pH of kombucha tea was measured using a WalkLab pH meter, model: TI 9000, manufactured in Singapore.

### 2.4. Total Soluble Solids (ºBrix)

The total soluble solids (ºBrix) were measured usingAtago brand refractometer, made in Japan, has a measuring range of 0-32°Bx.

### 2.5. Methods of sensory evaluation of products

Use the taste scoring method to determine consumer acceptance. Then determine the priority of those products indirectly from the scores obtained.

## 3. Results and Discussion

### 3.1. Investigate the initial seed rate in the fermentation of Kombucha tea with black tea.

We studied the effect of different initial seed rates on the fermentation of Kombucha tea made with black tea. We added inoculum from previously cultivated Kombucha tea at varying rates (2.5%, 5%, 7.5%, and 10%) to the cooled tea solution to initiate the fermentation process.

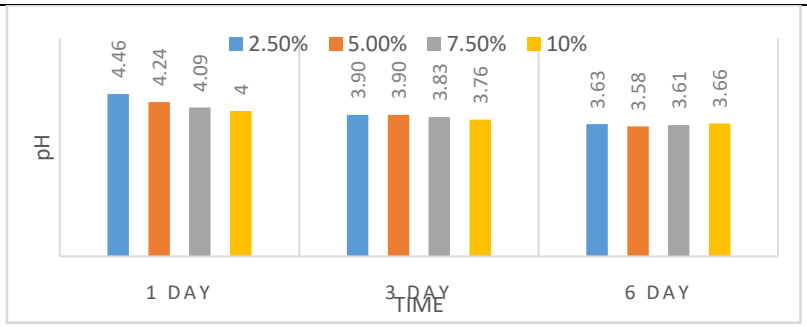


Figure 1: Investigate the initial seed rate in the fermentation of Kombucha tea with black tea.

We monitored the pH of the solution on day 1, day 3, and day 6 of the fermentation process. The results of our study are shown in the table below, with pH values recorded at each time point for each inoculum rate.

Based on these results, we can see that the initial seed rate has a minor effect on the pH of the solution during the fermentation process. However, a higher initial seed rate of 2.5% led to a slightly faster decrease in pH over time compared to the other seed rates.

**3.2. Investigate the change of the initial added sugar ratio during the fermentation of Kombucha tea with black tea.**

Based on the data, it appears that the pH of the Kombucha decreases over time for all initial sugar ratios. This is likely due to the production of organic acids by the bacteria and yeast in the SCOBY during fermentation.

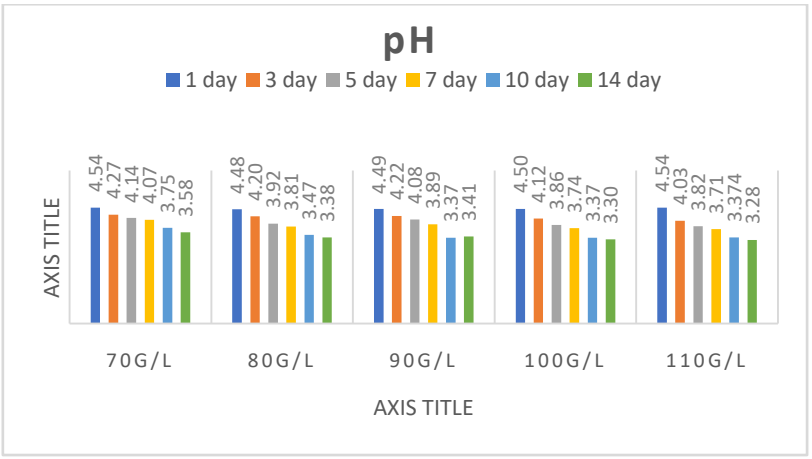


Figure 2: Changes in pH when changing the ratio of initial added sugars during the fermentation of Kombucha tea with black tea.

In general, it seems that higher initial sugar ratios result in a slower decrease in pH over time. For example, the Kombucha with an initial sugar ratio of 70g/l had a pH of 4.54 on day 1, which decreased to 3.58 by day 14. On the other hand, the Kombucha with an initial sugar ratio of 110g/l had a pH of 4.54 on day 1, but only decreased to 3.28 by day 14.

Sucrose is a common carbon source for kombucha fermentation that has used as an effective agent by researchers in various studies[3]The total soluble solids, which stands for degrees ºBrix, is a measure of the sugar content of a liquid. It indicates the percentage of sucrose by weight in a solution, and is commonly used in the food and beverage industry to measure the sweetness of a product.

The total soluble solids appears that initial sugar ratios have a significant impact on the ºBrix values of the Kombucha over time. In general, higher initial sugar ratios result in a higher ºBrix value at the beginning of fermentation, and a slower decrease in ºBrix over time.

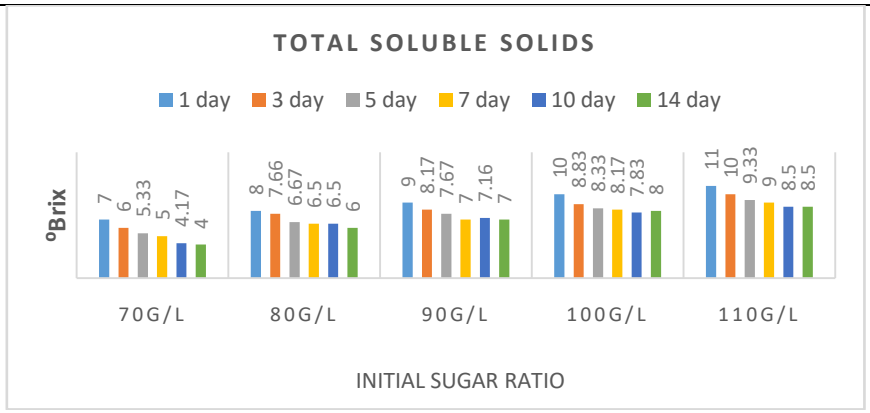


Figure 3: Comparison of invertase activity in Kombucha solutions on different days of fermentation

For example, the Kombucha with an initial sugar ratio of 70g/l had a ºBrix value of 7 on day 1, which decreased to 4 by day 14. On the other hand, the Kombucha with an initial sugar ratio of 110g/l had a ºBrix value of 11 on day 1, which only decreased to 8.5 by day 14.

It's important to note that the relationship between initial sugar ratio and ºBrix value is not linear. For example, the ºBrix values for the 80g/l and 90g/l concentrations do not follow a clear pattern over time. This could be due to a variety of factors, such as variations in the SCOBY or environmental conditions during fermentation.

**3.3 Sensory evaluation of mulberry kombucha tea.**

Black mulberry (Morus nigra L.) is a member of genus Morus of Moraceae family[13].Mulberry fruit is used as a fruit and wine in East, Central and South East Asia, including Vietnam. In this study, mulberry syrup was made from mulberry grown in DakLak province, Vietnam. After washing the mulberry fruit, it was mixed with saccharose sugar at a ratio of 1:1, soaked for 2 hours, then added 500ml of water and boiled. boil for 1 hour. Filter the residue with a filter to obtain mulberry syrup for use.

Mulberry Komobucha tea was surveyed with the following Kombucha: Mulberry syrup ratios: 0.03, 0.04, 0.05, 0.06, 0.07%.

Use the taste scoring method to determine consumer acceptance. Then determine the priority of those products indirectly from the scores obtained we have the results show in the graph below:


Figure 4: Sensory evaluation of the addition rate of mulberry syrup according to taste test.

**3.4 Investigation of changes after storage time of mulberry kombucha tea.**

Kombucha tea is fermented with the conditions investigated above: 5g tea/ 1L water, 70 g sugar, 2.5% scoby addition with a fermentation time of 120 hours at room temperature (30±5ºC). After being obtained, mulberry syrup was added with a concentration of 0.07%. Mulberry Kombucha tea is poured into 300ml bottles, stored in the refrigerator at 4±2ºC for 10 days. Every 5 days survey the change visible to the naked eye, the change in pH and dry matter concentration of the tea solution.

The **Figure 5** shows the changes in pH and total soluble solids during a 10-day storage period of mulberry kombucha tea. The measurements were taken on day 1, day 5, and day 10 of the storage period.

On day 1, the pH of the mulberry kombucha tea was 3.61, and the total soluble solids measured 12 °Brix. After 5 days of storage, the pH decreased to 3.53, and the total soluble solids decreased to 11.5 °Brix. By day 10, the pH had further decreased to 3.45, and the total soluble solids decreased to 11 °Brix.

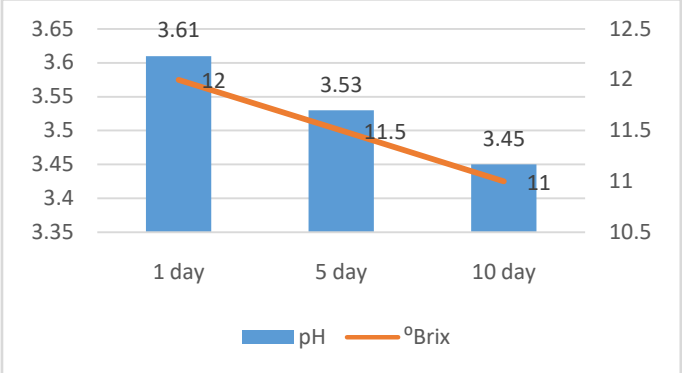


Figure 5: Changes of pH and total soluble solids during 10 days storage of mulberry kombucha tea.

The results indicate that the pH of the mulberry kombucha tea decreased over the 10-day storage period, while the total soluble solids also decreased slightly. These changes in pH and total soluble solids may have implications for the flavor and quality of the tea over time. Additional measurements and sensory evaluations may be needed to fully understand the changes in the mulberry kombucha tea during storage in table 2.

Table 2: Sensory changes after storage time of mulberry kombucha tea.

| | 1 day | 5 day | 10 day |
|---|---|---|---|
| Color | Deep red, transparent | Deep red, transparent, no cellulose film appears | Deep red, transparent, no cellulose film appears |
| Taste | Sweet and sour taste | Sweet and sour taste | Sweeter, more sour than day 1 |
| Smell | Light wine and mulberry scent | Light wine and mulberry scent | Stronger wine smell and mulberry scent |

## 4. Discussion

Kombucha is a fermented tea with strong antioxidant properties and a high content of polyphenols, especially flavonoids. There fore, it is favored to be consumed, especially by those who are exposed to oxidative stress[12]. It's important to note that pH and ºBrix is just one factor that can affect the quality and taste of Kombucha, and there may be other factors at play as well. For example, higher sugar ratios may result in a sweeter taste, while lower sugar ratios may result in a more tart or vinegary taste. Additionally, the specific strains of bacteria and yeast present in the SCOBY, as well as the temperature and other environmental conditions during fermentation, can all impact the final product.

The results was conducted to determine the factors affecting the fermentation of Kombucha products such as sugar content, amount of scoby starter culture, pH conditions, and fermentation time.. From there, it is applied to produce beverage from fermented tea with fruit. The survey results showed that the most suitable conditions in this study were 5g tea/1 liter of water, 70g sugar, supplemented with 2.5% scoby with pH ranging from 3.5 and fermentation for 120 hours at temperature room (30±5ºC).

From the scoreboard of the members participating in sensory evaluation, we synthesized the average scores on color, smell and taste of tea samples with different concentrations of mulberry syrup in Table 1.

Table 1:Average score of evaluation of samples

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|---|
| Color | 5.43 | 8.17 | 5.57 | 7.78 | 7.65 |
| Taste | 6.74 | 6.13 | 7.04 | 7.13 | 7.83 |
| Smell | 5.74 | 6.96 | 6.17 | 7.30 | 6.96 |
| Average | 5.91 | 7.09 | 6.26 | 7.41 | 7.48 |

For color, Sample 2 has the highest average score (8.17), while Sample 1 has the lowest average score (5.43). Samples 4 and 5 have higher average scores (7.78 and 7.65, respectively) compared to Sample 1 and Sample 3 (5.57).

For taste, Sample 5 has the highest average score (7.83), while Sample 2 has the lowest average score (6.13). Samples 3 and 4 have slightly lower scores (7.04 and 7.13, respectively) compared to Sample 5.

For smell, Sample 4 has the highest average score (7.30), while Sample 1 has the lowest average score (5.74). Samples 2 and 3 have slightly higher scores (6.96 and 6.17, respectively) compared to Sample 1.

Overall, sample 5 has the highest mean score of 7.09, sample 1 has the lowest mean score of 5.91. Scores for color, taste, and odor varied between samples, indicating that different samples have different characteristics and qualities. Based on the rating of the tester's preferences, the sample with the highest average score was sample 5 with the addition rate of 0.07% mulberry syrup.

Mulberry Kombucha tea stored in the refrigerator at 4±2ºC for 10 days. Every 5 days survey the change visible to the naked eye, the change in pH and total soluble solids.We see a slight change in pH from 3.61 to 3.45 and total soluble solids decreased from 12 ºBrix to 11 ºBrix. This shows that during storage the microorganisms still grow but at a very slow rate.The yeast and bacteria composition of kombucha can differ significantly depending on the starter culture and growth conditions[5].The *Komagataeibacter xylinus*is best known for its ability to produce bacterial cellulose[5].With the naked eye, it is impossible to see the formed cellulose film, which proves that the cellulose-forming microorganisms areseems inactive.

The dominant bacteria of Kombucha tea culture are acetic acid bacteria, which are aerobic bacteria able to use alcohol as a substrate to form acetic acid. These bacteria, in contrast to yeast, require large amounts of oxygen for their growth and activity. The metabolic process is based on the conversion of acetaldehyde into ethanol and acetaldehyde hydrate into acetic acid by the enzyme acetaldehyde dehydrogenase[10]. Meanwhile, there was a change in the smell and taste of Kombucha tea after 10 days of storage, there was a slight sour taste and stronger alcohol smell. It can be said that the acetic bacteria are still weakly active during storage to create the alcohol taste of the product.

## 5. Conclusions

This study aimed to identify the factors that influence the fermentation of Kombucha by measuring the sugar content, the amount of scoby starter culture, the pH conditions, and the fermentation time. Moreover, the study examined the ratio between tea juice and fruit juice to ensure consumer satisfaction with the visual appearance of the resulting beverage products from the fermented tea combined with fruit. The findings revealed that the optimal conditions in this study were 5g of tea per liter of water, 70g of sugar, and 2.5% of scoby supplement, with a pH range of approximately 3.5 and a fermentation time of 120 hours at room temperature (30±5ºC).

To produce Mulberry Kombucha tea, the researchers added mulberry syrup with a concentration of 0.07% and stored it at 4±2ºC for 10 days. After 10 days of storage, there was a significant change in the pH, total soluble solids, and acidity of the tea. The tea also had a stronger alcohol smell, which may impact its sensory characteristics and overall quality. Further studies and sensory evaluations could be conducted to fully understand the changes in Mulberry Kombucha tea during storage and to optimize the production process.

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