

PHYSICOCHEMICAL AND SENSORY ANALYSIS OF MONK FRUIT (*Siraitia grosvenorii*) SWEETENER AS SUCROSE SUBSTITUTE IN BLACK TEA KOMBUCHA

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ABSTRACT

Sugar is an essential component in kombucha production to support microbial fermentation, yet a considerable amount often remains in the final product to maintain taste, raising concerns over excessive sugar intake. Monk fruit (*Siraitia grosvenorii*), a natural, low-calorie sweetener rich in mogrosides, offers sweetness 150-200 times greater than sucrose, making it a promising sugar substitute in kombucha production. This study aimed to evaluate the effect of replacing sucrose with monk fruit sweetener on the physicochemical and sensory properties of black tea kombucha. Kombucha was prepared with three sucrose-to-monk fruit ratios (0%, 50%, 100% monk fruit sweetener). Physicochemical analyses found that after 7 days of fermentation, kombucha with 100% monk fruit sweetener had significantly higher pH compared to the control (3.40 to 2.53, $p < 0.05$), but remained within the safe consumption range. However, no significant effect on °Brix values was found. Sensory evaluation assessed color, sweetness, sourness, flavor, and overall liking using a hedonic scale, with average overall liking scores ranging from 6.63 to 7.11 and no significant differences ($p > 0.05$) between formulations. However, the preference ranking test indicated that most panelists favored the 50:50 formulation the most, suggesting positive potential for partial sucrose substitution with monk fruit sweetener in black tea kombucha production.

Keywords: affective sensory evaluation; kombucha; monk fruit; physicochemical analysis

ABSTRAK

Gula merupakan komponen esensial dalam produksi kombucha untuk mendukung proses fermentasi mikroba, namun sebagian besar gula masih tersisa pada produk akhir untuk mempertahankan cita rasa, sehingga menimbulkan kekhawatiran terkait asupan gula yang berlebih. Buah monk (*Siraitia grosvenorii*) adalah pemanis alami rendah kalori yang kaya akan mogrosida, yang memiliki tingkat kemanisan 150-200 kali lebih tinggi dibandingkan sukrosa, sehingga berpotensi menjadi pengganti gula dalam produksi kombucha. Penelitian ini bertujuan untuk mengevaluasi pengaruh substitusi sukrosa dengan pemanis buah monk terhadap sifat fisikokimia dan sensori kombucha teh hitam. Kombucha dibuat dengan tiga rasio sukrosa terhadap buah monk (0%, 50%, 100% pemanis buah monk). Analisis fisikokimia setelah 7 hari fermentasi menunjukkan bahwa kombucha dengan 100% pemanis buah monk memiliki pH yang secara signifikan lebih tinggi dibandingkan kontrol (3,40 vs 2,53; $p < 0,05$), namun tetap berada dalam kisaran aman untuk dikonsumsi. Sementara itu, nilai °Brix tidak menunjukkan perbedaan yang signifikan antar formulasi. Evaluasi sensori yang menilai warna, tingkat kemanisan, keasaman, rasa, dan kesukaan secara keseluruhan menggunakan skala hedonik menunjukkan skor kesukaan keseluruhan rata-rata berkisar antara 6,63 hingga 7,11, tanpa perbedaan signifikan ($p > 0,05$) antara formulasi. Namun, uji pemeringkatan preferensi menunjukkan bahwa sebagian besar panelis paling menyukai formulasi 50:50, mengindikasikan potensi positif substitusi sebagian sukrosa dengan pemanis monk fruit dalam produksi kombucha teh hitam.

Kata kunci: analisis fisikokimia; analisis sensori afektif; buah monk; kombucha

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 7 Issue 1

Manuscript ID
V7N1657-1

Received Date
30 Jul 2024

Accepted Date
20 Aug 2025

Published Date
30 Aug 2025

DOI:
10.33555/jffn.v7i1.8

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Citation:

Tan, D., Eldyjo A.G., Josephine S.T., Sastra T. & Dewi D.P.A.P. 2025. Physicochemical and Sensory Analysis of Monk Fruit (*Siraitia grosvenorii*) Sweetener as Sucrose Substitute in Black Tea Kombucha. J. Functional Food & Nutraceutical, 7(1), pp. 1-11

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INTRODUCTION

Kombucha is a traditional fermented drink made from tea extract, sugars, and a symbiotic culture of yeast and bacteria (SCOBY), which is microbiologically active. It is characterized by a refreshing, acidic, and slightly sweet flavor similar to carbonated cider (Jayabalan et al., 2014). The critical aspect of kombucha production is the fermentation process when the sucrose is hydrolyzed by the yeast into glucose and fructose, in which the end product results in beneficial compounds including polyphenols, amino acids, and organic acids, which offer health benefits like antioxidant, antibacterial, anticancer, anti-inflammatory, and antimicrobial properties (Kitwetcharoen et al., 2023). This health-boosting drink is believed to enhance immunity, aid in intestinal detoxification, and reduce blood pressure. Given the purported health benefits, kombucha has gained popularity, leading to increased demand (Laureys et al., 2020).

Another observed trend is that as consumers become more concerned about excessive sugar intake and its health risks, there is a growing interest in finding healthier sweeteners (Rodriguez-Castro et al., 2024). Particularly, natural sweeteners derived from fruits are currently rising in interest. One of the examples is monk fruit (*Siraitia grosvenorii*) sweetener, which is a natural, low-calorie sweetener that has been shown to alleviate symptoms of asthma and diabetes, prevent oxidation and cancer, enhance immune regulation, and lower glucose levels (Ban et al., 2020). Its application is especially relevant in products like kombucha, which typically require a considerable amount of sugar to support microbial fermentation. Although some of the sugar is consumed during the fermentation process, a significant portion often remains in the final product to retain its pleasant taste (Sinamo et al., 2022). Hence, replacing part of this sugar with monk fruit sweetener could help reduce the total sugar content

without compromising sweetness, aligning with consumer demand for healthier, functional beverages (Hallak et al., 2022). This is because the extract of monk fruit contains mogrosides (particularly mogroside 5) which are a natural sweetening agent which has the ability to show a high level of sweetness through taste that is estimated to be 150-200 times sweeter than sucrose (Xia et al, 2018). Other mogrosides present in monk fruit extract contribute to its overall sweetness profile, with some having a delayed sweetness onset and lingering aftertaste (Hodge et al, 2018).

With the potential functional benefits of monk fruit as sweeteners, several studies have explored the use of monk fruit sweeteners in yogurt and milk products, finding similar physicochemical properties compared to sucrose as the control (Buchilina & Aryana, 2021). Despite that, no research has yet investigated their application in kombucha. With that, this paper aims to explore the use of monk fruit erythritol sweetener as an alternative sweetener to sucrose in black tea kombucha through physicochemical (Brix and pH) as well as affective sensory evaluation to evaluate the acceptance.

MATERIALS AND METHOD

Materials

The materials used were purchased from a local supermarket, which include sucrose, monk fruit erythritol sweetener (Allaire, Indonesia), black tea (Sosro, Indonesia), drinking water (AQUA, Indonesia), and 9 cm diameter SCOBY i.e., kombucha starter (Kombucha Forever, Indonesia).

Kombucha Preparation

A total of three formulations were made, namely 0% monk fruit sweetener (MFS), 50% MFS, and 100% MFS, with each formulation having three biological replications. The production method of kombucha is in accordance with the study by Ivanišová et al. (2020) with minor modifications. Furthermore, the amount of each ingredient used for a batch of kombucha is listed in Table 1 below. Water was heated to 90°C, then the black tea was steeped for 10 minutes before the sugar was added. From there, the sweetened tea was transferred to a sterile glass jar before being cooled to 30°C. After the desired temperature was reached, inoculation with SCOBY was done. The jar was then covered with a muslin cloth tightened by a rubber band, and the tea was fermented for 7 days at room temperature.

pH Analysis

pH assessment protocol was in accordance with Sinamo et al. (2022) by utilizing a pH meter (Eutech Instruments Eutech™ Spear pH Meter, Singapore). Triplicate analysis was performed for each batch of kombucha on day 0 and day 7.

Table 1. Amount of Ingredients Used for Each Kombucha Formulation

Sample	Sucrose (g)	Monk Fruit Sweetener (g)	Black Tea (g)	Drinking Water (mL)
0% MFS	100	0	5	1000
50% MFS	50	50	5	1000
100% MFS	0	100	5	1000

Total Soluble Solids (Brix Value)

Total soluble solid determination of each kombucha sample was carried out using a refractometer (Atago™ MASTER-53 Refractometer, Japan) according to Sinamo et al. (2022) with some modifications. Triplicate analysis was performed for each batch of kombucha.

Affective Sensory Evaluation

To further assess the consumers preference and likeability of the kombucha products, the 9-point hedonic scale followed by a preference ranking sensory evaluation was employed. The method itself was in accordance with the study conducted by Ulusoy and Tamer (2019). Panelists were recruited via Google Forms together with the evaluation consent form. From there, a total of 38 panelists took part in the evaluation, fulfilling the inclusion criteria encompassing healthy people who are familiar with the sensory properties of kombucha, otherwise considered as the exclusion criteria. The parameters tested for the samples including color, flavor, sweetness, sourness, and overall liking were given in the form of a questionnaire via Google Forms, where the panelists gave their hedonic ratings ranging from 1 (extreme dislike) to 9 (extreme like) for each sample. Following that, a ranking test was conducted to allow the panelists to list their most to least preferred sample.

Data Analysis

The results obtained were presented in the form of mean ± standard deviation, and to be further analyzed statistically using JASP software version 0.17.2.0. For the physical analysis, a normality test using Shapiro-Wilk ($n < 50$) was performed to determine the subsequent test, which was ANOVA test or Kruskal-Wallis test, followed by Tukey post-hoc analysis. Furthermore, a paired T-test or Wilcoxon test was used to compare the results before and after the fermentation process, depending on the distribution of the data. As for the 9-point hedonic scale, the Kruskal-Wallis test with a Tukey post-hoc would be conducted to analyze the statistical significance between the non-parametric results, where statistically significant difference results were presented by $p < 0.05$.

RESULTS AND DISCUSSION

Acidity (pH Value)

One of the most prominent characteristics of kombucha is its acidity, which is expressed as pH level. Not only that, but pH is also an indicator of quality and safety. According to Table 2, there was a significant decrease ($p < 0.05$) in pH in all kombucha samples. These results, where the pH of kombucha is lower than tea, were expected, as the pathway of fermentation results in the production of acids. Specifically, acetic acid bacteria as a part of the SCOBY metabolize sugar and ethanol into gluconic and acetic acid, respectively (Villarreal-Soto et al., 2018). Other than that, malic, citric, tartaric, and lactic acid are also present in kombucha. All these acids produced contribute to the decrease of pH over time and overall give the characteristic sourness and tartness (Cohen et al., 2023). Despite that, there were also significant differences ($p < 0.05$) between the pH of different treatment samples, both before and after fermentation. On day 0, there were also significant differences ($p < 0.05$) among the kombucha samples, with the highest pH obtained by the 50% MFS sample (6.67), followed by the 0% MFS (6.61), then 100% MFS (6.51) kombucha. It is said that the irregular trend is due to the unique interaction of erythritol when in combination with sucrose, where a more neutral pH can be achieved (Runnel et al., 2013). As for day 7, the 0% MFS kombucha has the lowest pH, followed by the 50% MFS and 100% MFS kombucha samples. This outcome was aligned with the study by

Kruger (2017), who mentioned that sugar alcohols, including erythritol, have a different chemical structure compared to sucrose. Sucrose is readily hydrolyzed, while erythritol with its four-carbon compound and less reducing sugar ends makes it more difficult to metabolize, hence a slower fermentation rate. A kombucha is safe to consume if its pH is between 2.5-4.2. Consumption of kombucha lower than pH 2.5 may cause effects such as digestive discomfort. Additionally, it is also an indicator of contamination or improper fermentation, hence negatively affecting its safety (Hammel et al., 2016). Furthermore, a pH above 4.2 might compromise the microbiological safety of the product, or indicate an incomplete fermentation process (Tejedor-Calvo & Morales, 2023). Hence, it is crucial to measure the pH value of kombucha as a quality indicator. Overall, the pH is aligned with the standard which was between 2.5-4.2.

Total Soluble Solids (Brix)

Total soluble solids, which is often indicated by Brix, is a simple indicator of sweetness level, proven by its high usage in the food industry for years. Specifically, Brix refractometer is a convenient method to analyze sugar content, with advantageous properties such as its high availability, short measurement time, and less sensitivity to variation due to temperature (Magwaza & Opara, 2015).

Table 2. pH and Brix Value Before and After Fermentation for 7 Days

Sample	pH		Brix	
	Day 0	Day 7	Day 0	Day 7
0% MFS	6.61 ± 0.02 ^{a*}	2.53 ± 0.08 ^{a**}	8.83 ± 0.35 ^{a*}	9.67 ± 0.75 ^{a**}
50% MFS	6.67 ± 0.02 ^{b*}	2.78 ± 0.02 ^{b**}	8.83 ± 0.35 ^{a*}	9.33 ± 0.26 ^{a**}
100% MFS	6.51 ± 0.02 ^{c*}	3.40 ± 0.01 ^{c**}	8.77 ± 0.26 ^{a*}	8.50 ± 1.44 ^{a**}

Note: Different alphabets in superscript within the same column indicate statistically significant differences ($p < 0.05$), while different numbers of asterisks in superscript within the same row indicate statistically significant differences ($p < 0.05$)

Theoretically, the Brix value should decrease after fermentation, since sugar is the source of nutrients for SCOBY. Furthermore, it is a part of the fermentation pathway, in which it is converted into ethanol by the yeast. The alcohol produced will then be converted into organic acids, particularly acetic acid (Gomes et al., 2018). However, the results in Table 2 did not reflect the aforementioned theory except for the 100% MFS kombucha formulation, showing a significant increase in total soluble solids content. It is noteworthy that the refractometer does not specifically measure only sugar content, instead, it also takes into account other solids that may be present, such as biomass and organic acids (Bunphan et al., 2015). Biomass production after fermentation was correlated with the carbon source in the system with the presence of sugar, especially in the form of sucrose, which will result in the yield of biomass after the process (Muhialdin et al., 2019). Furthermore, organic acids as products of fermentation are also detected by the refractometer, thus contributing to the increase of the Brix value (Zhou et al., 2023). Another trend shown in the results was the decrease in the kombucha's Brix value with increasing monk fruit sweetener concentration, despite no significant difference was observed ($p>0.05$). This was caused by erythritol being the main ingredient in monk fruit sugar which is not easily metabolized, whereas sucrose, which is a combination of glucose and fructose, is easily metabolized by the yeast strains in SCOBY (Seshadrinathan & Chakraborty, 2022).

Sensory Evaluation

The 9-point hedonic scale was used to determine the likability of the panelists towards a certain food or beverage product on a categorical scale from a list of nine terms (Ulusoy & Tamer, 2019). In this study, the color, flavor, sourness, sweetness, and overall liking were the sensory attributes evaluated for the three kombucha samples. Following that, the preference

ranking was carried out to assess the panelists' preference between the three formulations (Whelan et al., 2017). The results obtained with regards to the sensory-based on Table 3 showed no significant difference ($p>0.05$) between the different sample formulations. However, the 0% MFS kombucha was preferred in regards to its color and sourness. On the other hand, the 50% MFS had the highest likability in flavor, sweetness, and overall liking.

Color

Color and turbidity are the essential properties of fermenting beverages due to the basic effect on consumers such as quality and reflect the physical and chemical properties of the product, which could be influenced by raw materials, production process, and storage of the fermented beverages (Gabriel & Sigler, 2018). Based on the study conducted by Dartora et al. (2023), the kombucha that has a low concentration of sucrose has the highest amount of turbidity, regardless of the scoby weight and green:black tea ratio, which could be the reason for the lowest preference result for the kombucha with 100% MFS. The low clarity in the 100% MFS kombucha is attributed to the amount of cellulose that is produced by the acetic acid bacteria (AAB) in the biomass.

The increase in the turbidity in the increasing concentration of MFS may be correlated with the increase of cellulose production and other fibrous materials that are released into the kombucha broth and microbial growth in the fermented infusion (Amarasinghe et al., 2018; Zofia et al., 2020). The presence of these cellulose in the biomass affects the turbidity which can cause the less desirable color perceived by the panelists during the sensory.

Table 3. 9-Point Hedonic Scale Sensory Evaluation Results of Kombucha

Sample	Color	Flavor	Sweetness	Sourness	Overall Liking
0% MFS	7.50 ± 1.18 ^a	6.55 ± 1.81 ^a	6.16 ± 2.00 ^a	6.66 ± 1.88 ^a	6.63 ± 1.53 ^a
50% MFS	6.97 ± 1.53 ^a	6.76 ± 1.82 ^a	6.63 ± 1.78 ^a	6.34 ± 1.76 ^a	7.11 ± 1.59 ^a
100% MFS	6.71 ± 1.77 ^a	6.55 ± 1.80 ^a	6.47 ± 1.91 ^a	6.34 ± 1.94 ^a	6.76 ± 1.72 ^a

Note: Different alphabets in superscripts within the same column indicate statistically significant differences ($p < 0.05$).

Flavor

The other important attribute of food and beverage quality control also includes flavor, which is the combination of tastes such as non-volatile compounds, aromas such as volatile compounds, and chemesthetic sensations (Menis-Henrique, 2020). The study conducted by Frolova et al. (2023) suggested that higher organic acid content produced would result in a stronger flavor of kombucha. In accordance with the sweetness attribute, the organic acid content would depend on the glucose and fructose content from various sugars, and the expected highest organic acid content would be found in the 0% MFS kombucha as it utilized full sucrose for the fermentation process, resulting in stronger flavor than the other samples (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). Based on Table 3, the highest rating for the flavor attribute shows that the panelists prefer 50% MFS more than the other kombucha samples which may be attributed to the balanced flavor in comparison to the other samples which are not too strong or too weak at the same time. Furthermore, looking at the results of the sweetness and sourness, it could be depicted that sweetness likability played a more important role in the flavor acceptance.

Sweetness

Sweetness refers to the quality of being pleasant, tasting, or smelling sweet. The 0% MFS sample is made entirely of sucrose, which was broken down to simpler sugars such as glucose and fructose by the yeast invertase enzyme during the fermentation processes. Fructose was then converted to various organic acids, while glucose was converted to

gluconic and glucuronic acids by the bacteria in the SCOBY, hence the acidity from the organic acids depends on the amounts of glucose and fructose in the various sugar (Jayabalan et al., 2014; Villarreal-Soto et al., 2018). Whereas for the MFS kombucha, the kombucha is made the same as the 0% MFS but with the use of monk fruit sweetener instead of sucrose which is composed of only erythritol and monk fruit extract. Erythritol is a sugar alcohol used for sweeteners in food or beverage products and is known to be non-fermentable (Mazi & Stanhope, 2023; Shah et al., 2024). The other ingredients which is the monk fruit extract are made through water extraction from the fruit, which consists of cucurbitane glycosides and mogrosides as the main components. The mogrosides present in the monk fruit extract are found to be 250 times sweeter than sucrose (Younes et al., 2019), which could be the reason for the higher rating in the 50% MFS kombucha as there was a balance in the acidity from the organic acids by the fermentation process, and the sweetness addition from the erythritol and monk fruit extract in the MFS. Regardless, the absence of significant differences in the results was aligned with the instrumental results.

Sourness

Kombucha tastes altered to sparkling fruity sour with a slight vinegar-like taste from sweeter after the fermentation occurred (Kim & Adhikari, 2020). Based on the result shown above in Table 3, the sourness liking from all samples shows no significant difference ($p > 0.05$) which rated around 6 out of 9. The most preferred sourness was obtained by the 0% MFS sample 6.43 which is made with full sucrose, whereas the substitution of sucrose with 50% and

100% monk fruit sweetener showed the same degree of liking that is slightly lower than the 0% MFS sample, 6.12. The result of the hedonic sensory analysis of sourness was correlated with the produced organic acid that contributed to the perceived sour taste. In the fermentation process, pH changes occur altering the chemical structure of mogrosides and other compounds, resulting in variations in color intensity and flavor profile, including sourness. The monk fruit contains mogrosides which have a delayed sweetness onset and lingering aftertaste (Hodge et al, 2018). The fermentation process also occurs differently from the traditional kombucha made from sugar. Thus, the less sour might be perceived due to less acetic acid produced. The other possibility is the monk fruit is 300 times sweeter compared to sucrose, resulting in less sourness overall (Pandey & Chauhan, 2020).

Overall Liking

For the overall liking, all samples obtain acceptable results which are higher than 6. The most preferred kombucha sample is 50% MFS, followed by 0% MFS and 100% MFS sample. The equal composition of sucrose and monk fruit sweetener formulation also showed as the first preferred for flavor and sweetness, and the second preferred for color and sourness, hence the 50% MFS formulation creates a balanced taste, perceived sweetness, and flavor complexity that makes it the most preferred by the panelists (Akesowan & Choonhahirun, 2021). The mixture of sucrose and monk fruit sweetener creates a unique flavor profile, avoiding overly intense sweetness that can occur with 100% sucrose and the potential bitterness or aftertaste associated with monk fruit sweetener when used alone (Massoud & Hashem, 2023). Additionally, the interaction between the sweeteners and the fermentation process might enhance the complexity of flavors, providing a richer and more satisfying taste experience (Bishop et al., 2022). That being said, the results signified that flavor

became the main driver of liking in kombucha among panelists. Furthermore, the combination may also improve other sensory attributes such as mouthfeel and aftertaste, making the 50% MFS formulation more appealing overall. Therefore, the preferred formulation likely strikes a harmonious balance that enhances the overall sensory experience, making it more favorable to consumers.

Preference Ranking Test

Based on the ranking test in Figure 1., the result reveals a clear preference for sample 50% MSF which is correlated with hedonic sensory analysis for the overall liking. Across all ranks, the 50% MSF sample received the highest number of selections, indicating its widespread appeal among participants. This preference suggests that the inclusion of monk fruit sweetener in combination with sucrose may have contributed to a more favorable taste profile, potentially balancing sweetness levels and enhancing overall flavor complexity (Pandey & Chauhan, 2020). While sample 2 emerged as the preferred option, it is important to acknowledge the variability in individual taste preferences, as evidenced by the equal number of votes for samples 1 and 3 in rank 3. Nonetheless, the consistent preference for sample 2 highlights its potential as a marketable product formulation.

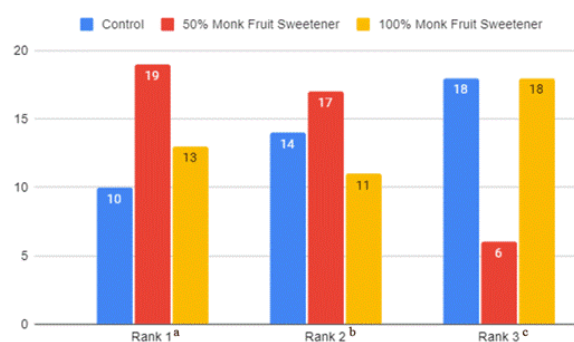


Figure 1. Preference Ranking Test Result. Different alphabets in superscripts between samples indicate statistically significant differences ($p < 0.05$).

CONCLUSION

Kombucha is a fermented beverage with a sweet-sour taste that has been increasing in popularity due to its health benefits. This experiment utilizes the monkfruit sweetener as a sugar alternative or substitute for sucrose in the production of kombucha. There was a significant ($p < 0.05$) reduction in the pH of kombucha from all of the formulations of sucrose : monk fruit sweetener, showing a successful fermentation process. However, results showed that there were no significant differences ($p > 0.05$) in the Brix value between the three kombucha formulations. Sensory likability analysis also showed no significant differences ($p > 0.05$) in the color, flavor, sweetness, sourness, and overall liking, which means panelists' likability towards all kombucha samples were indifferent. Despite that, the preference ranking test proved that the 50% monk fruit sweetener formula ranked first, followed by the control, then the 100% monk fruit sweetener kombucha. These results highlighted the potential of substituting sucrose with monk fruit sweetener, with comparable physical properties and sensory likability.

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