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Comparison of Quality and Ethanol Content of Liquid and Powder Kombucha from Jasmine Tea (*Jasminum sambac* L.)

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Abstract. *The fermentation process of kombucha has become a focus of halal product research due to its ethanol content. Processing kombucha into powder form can be an alternative to address the ethanol content in liquid kombucha, as it has undergone a drying process, making it a potential halal-certified functional drink. The objective of this study was to compare the quality of liquid and powdered kombucha. The research was conducted from March to September 2024 at the Biotechnology Laboratory, Muhammadiyah University of Bandung. The method used was the Complete Randomized Design (CRD) method. The parameters analyzed included ethanol content, total BAA (Bacterial Acetic Acid), pH, organic acid content, and sugar content. In the production of powdered kombucha, optimization of maltodextrin and gum arabic usage was also conducted to achieve the best solubility and taste. The best powdered kombucha was obtained with a maltodextrin-to-gum arabic ratio of 3:2. In the research, liquid kombucha contained ethanol at an average of 0.09%. In comparison, no ethanol was detected in the powdered kombucha (0%). Total BAA in powder decreased after the drying process ($p<0.05$). The pH value of kombucha in liquid form (4.4) also differed significantly from powdered (3.2) ($p<0.05$). However, the levels of organic acids and sugars in liquid and powdered kombucha did not differ significantly ($p>0.05$). The sugar content in liquid and powdered kombucha was lower than that in kombucha in general.*

Keywords: *ethanol, gum arabic, kombucha, maltodextrin, powder*

Citation
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INTRODUCTION

Kombucha is a beverage fermented from tea and sugar solution using Symbiotic Culture of Bacteria and Yeast (SCOBY) microbial starter incubated for 7-14 days (Nyhan et al., 2022). As a functional food product, kombucha can provide many health benefits because it contains probiotics, various organic acids, and other secondary metabolite compounds. However, kombucha contains ethanol produced by yeast during the fermentation process. The presence of ethanol in liquid kombucha raises significant concerns regarding the product's halal status. While the ethanol content in kombucha is typically below the allowable threshold of 0.5%, this is contingent upon an incubation period of 5 to 8 days (Ihsani et al., 2021). Likewise, in the storage process for a period of 21 days at a storage temperature of 4 ºC. The ethanol content remained no more than 0.5% (Ihsani et al., 2023).

Based on the Indonesian Ulema Council's Fatwa No.10/2018 on food and beverage products containing ethanol, as a product of the non-khamr fermentation industry, kombucha is permissible if it is medically harmless and the ethanol content at the end of the product is below 0.5% (Majidah et al., 2022). However, the concern of ethanol content in liquid kombucha remains a consideration for consumption by consumers, especially muslims. Therefore, making kombucha in powder form can be an alternative in producing ethanol-free kombucha because the manufacturing process involves a drying process (Rosida et al., 2021). In addition, powdered kombucha can facilitateproduct distribution and extend the shelf life.

Conventional drying via crystallization techniques can be used to convert the liquid product into a powder (Su et al., 2022). This method is commonly used in the production of instant powdered beverages because it requires a short manufacturing time, is inexpensive, and is easy to implement (Khan et al., 2016; Mursalin et al., 2019). In the research of Rosida et al. (2021), kombucha powder using ashitaba, kersen, and fillers such as maltodextrin and gum arabic with a ratio of 6:4 (Rosida et al., 2021).

Differences in substrate use during fermentation can result in final products with distinct characteristics (Wang et al., 2021). In addition, the use of jasmine tea as a substrate in the production of powdered kombucha has not been reported. Likewise, when the product is converted into a different form, optimization of the addition of fillers must be optimized. Therefore, making green tea-based kombucha with the addition of jasmine flowers (jasmine tea) also requires optimization process of the maltodextrin and gum arabic composition. In addition, the quality of kombucha processed into powder can differ form that of the liquid form. Therefore, this study optimized the preparation of kombucha powder from jasmine tea substrate with the addition of maltodextrin and gum arabic at a ratio of 3:2 and 6:4 (w/v). The optimum ratio of maltodextrin and gum arabic is expected to yield the best solubility and taste (aroma, color, flavor) in jasmine tea kombucha. The quality comparison analysis between liquid and powder kombucha was further conducted by comparing the products using ethanol, total Bacterial Acetic Acid (BAA) count, pH, organic acids, and sugar as parameters.

MATERIALS AND METHODS

Place and Time of Research

The research took place at the Biotechnology Laboratory of the University of Muhammadiyah Bandung. The study was conducted from March to September 2024.

Tools and Materials

The tools used in this research are a incluide are titration set, Erlenmeyer flask, distillation set, pH meter, Falcon tube, autoclave, pycnometer, measuring flask, petri dish, refractometer, drop pipette, and micropipette. Meanwhile, the materials used in this study are jasmine tea obtained from PT Gunung Slamat, sugar, seven-day-old SCOBY, maltodextrin from Heilongjiang Haotian Corn Development Co., Ltd. (MUI halal no.: 00220076960516), gum arabic from Texture Innovation Center Gums (IFANCA-USA halal no.: 4514.12864.111900566), distilled water, phenolphthalein, peptone, yeast extract, glucose, agar powder, $CaCO_3$, NaOH and oxalic acid.

Methods

The Preparation of Kombucha

The preparation of kombucha follows the method Putu (2018), Ihsani et al. (2021) and Rosida et al. (2021), with modifications to the incubation time. 1 L of water was heated to a boil in a pot. Then 15 g of jasmine tea and 107 g of granulated sugar were added to the water to produce a tea solution. The tea solution was stirred for five minutes. The solution temperature was allowed to decrease to 25°C. After the solution reached 25°C, the leaves were filtered, and 30% SCOBY water was inoculated along with a 3.8 g (w/v) SCOBY layer. The container was covered with sterile cotton cloth and tied with rubber. Fermentation lasted for ten days in the dark.

Optimization of Powdered Kombucha Preparation

Preparation of kombucha powder follows to the method of Rosida et al. (2021) with modifications to the drying temperature and duration. In the preparation of kombucha powder, optimization of the addition of fillers was carried out using maltodextrin and gum arabic with a ratio of 3:2 and 6:4. Maltodextrin and gum arabic with a ratio of 3:2 (90 g: 60 g); and 6:4 (60 g: 40 g) (w/v) respectively, were mixed with the harvested kombucha solution. Then, the solution was stirred until homogeneous (for five minutes) and formed into a thick kombucha paste. The kombucha paste was dried at 40°C for two hours. The dried kombucha paste powder was blended for two minutes. Kombucha powder was weighed for three repetitions.

Solubility and Taste Testing (Organoleptic) Kombucha Powder

The tools Solubility testing of kombucha powder was performed by weighing 10 g of the 3:2 and 6:4 ratios. Then, each was added 4 g of jasmine aroma flavor, 4 g of sugar, 0.25 g of citric acid, and dissolved in 100 mL of distilled water. This solubility test was conducted to see the presence of lumps during brewing.

Organoleptic testing of the kombucha powder solution follows the method described by Naibaho et al. (2019) with modifications. Organoleptic testing parameters include taste, aroma, and color. Powdered kombucha solutions in the 3:2 and 6:4 ratios were poured into 25 mL plastic cups. The 25 people who participated in the organoleptic testing of the kombucha powder solution. Ranging in age from 17 to 60 years old and residing in the city of Bandung. The test was conducted by distributing questionnaires with a Likert scale 1-5 (1=strong dislike; 2: dislike; 3: neutral; 4:

like; 5: strong like) to all respondents.

Measurement of Ethanol Content

The determination of ethanol content follows the method described by Ihsani et al. (2023), using a distillation device. Each sample of liquid kombucha and powdered kombucha solution, up to 25 mL, was placed into the distillation flask, and 50 mL of distilled water was added. The distillate was collected and placed in a vial. The ethanol concentration was measured using a pycnometer using a standard curve of absolute ethanol concentrations of 0%, 0.005%, 0.01%, 0.02%, 0.04%, 0.08%, 0.16%, and 0.32%.

Determination of Bacterial Acetic Acid (BAA) Population Number by Technique: Total Plate Count (TPC)

The determination of BAA follows the method of Rizki et al. (2022) with modifications. BAA determination was performed using Glucose-Yeast Extract-Calcium Carbonate media (GYC) (10% glucose, 1% yeast extract, 0.4% peptone, 2% $CaCO_3$, and 1.5% agar powder). Each liquid kombucha sample and powder solution was diluted with sterile 0.9% NaCl at 10^2 to 10^{-2}. Determination of BAA was performed using the sowing method, with three aseptic replicates. The culture was incubated at 30°C for 2-3 days. Data on total BAA across various treatments were analyzed using SPSS 25.0. Data normality was assessed using the Shapiro–Wilk test before performing the dependent t-test at the 95% confidence level.

Determination of pH

The pH of liquid kombucha samples and powdered kombucha solution was measured using a pH meter. The pH was determined three times. The pH measurement data with treatment variations were analyzed using SPSS 25.0. Data normality was assessed using the Shapiro–Wilk test before performing the dependent t-test at the 95% confidence level.

Determination of Organic Acid Content

The organic acid content of liquid kombucha samples and powdered kombucha solutions was determined by titration. Liquid kombucha samples were diluted 100 times. Meanwhile, powdered kombucha was diluted 10 times. Each dilution yielded liquid kombucha; 10 mL of the powder was pipetted, and two drops of phenolphthalein were added. Samples were titrated with NaOH until a pale pink color appeared. This organic acid measurement was carried out three times for each sample. Data on organic acid levels were analyzed using SPSS 25.0. Data normality was assessed using the Shapiro–Wilk test before performing the Wilcoxon Matched-Pairs test at a 95% confidence level.

Determination of Sugar Content

Measurement of sugar content in liquid kombucha samples and powdered kombucha solutions was carried out using a 0-32% Brix refractometer. Sugar content measurement data were analyzed using SPSS 25.0 to assess treatment variations. Data normality was assessed using the Shapiro–Wilk test before performing the Wilcoxon Matched-Pairs test at a 95% confidence level.

RESULTS AND DISCUSSION

Solubility and Taste Testing (Organoleptic) Kombucha Powder

Kombucha powder (10 g) from the process of adding maltodextrin and gum arabic at ratios of 3:2 and 6:4 can be dissolved in 100 mL of water. This solubility is characterized by the absence of lumps on the stirring rod during the dissolution process. However, based score on organoleptic test results, respondents prefer the 3:2 ratio over the 6:4 ratio because it produces the highest aroma, taste, and color values (Figure 1).

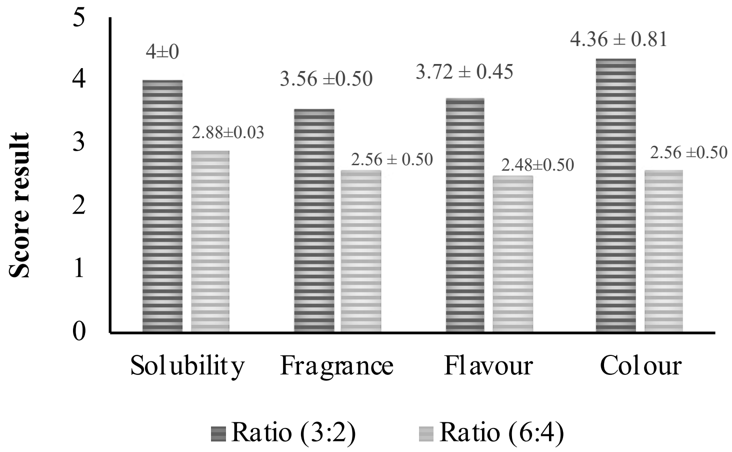

Figure 1. Ratio of organoleptic test results of kombucha powder

Kombucha powder with maltodextrin and gum arabic at a 3:2 ratio produces a jasmine tea aroma and a sour, sweet taste, and does not smell of gum arabic. At the same time, kombucha with the addition of maltodextrin and gum arabic at a ratio of (6:4) does not give rise to the aroma of jasmine tea, bland, not sweet, and sour. According to Rosida et al. (2021), the aroma and flavor produced in kombucha powder can be influenced by the content of organic acids from fermentation, along with the aroma arising from the type of substrate used (Rosida et al. 2021). In addition, the proportion of gum arabic used can affect the aroma and flavor produced by powdered kombucha (Yuliani and Maslahat, 2017). This is caused by gum arabic, which can coat flavor particles, thereby allowing flavonoid compounds involved in aroma formation to be retained during drying.

The addition of maltodextrin and gum arabic with a ratio of (3:2) also obtained the highest level of respondents' preference for the color of the powder kombucha solution. At this ratio, kombucha turns a bright yellow. This is due to a greater proportion of maltodextrin than gum arabic. Meanwhile, the ratio (6:4) produces a pale yellow color.

The difference in the color of the kombucha powder solution between ratios (3:2) and (6:4) can be caused by the Maillard reaction. The Maillard reaction is a reaction between reducing sugars and amino acids that occurs during heating. The Maillard reaction can produce a brown color in the solution. The presence of the Maillard reaction in kombucha powder may be due to the protein content of gum arabic (Pirestani et al., 2017). In contrast to the results of Rosida et al. (2021), kombucha with a ratio of (6:4) (w/v) produces the best kombucha flavor for kersen leaf substrate. In contrast, in this study, the 3:2 ratio produced the best flavor for jasmine tea. Furthermore, the analysis of bacterial acetic acid (BAA) count by Total Plate Count (TPC) technique, pH determination, and testing of sugar, ethanol, and organic acid content was carried out on kombucha powder with the addition of maltodextrin and gum arabic (3:2) because it had the best taste compared to the ratio (6:4).

Ethanol Content

Ethanol was detected in liquid kombucha at an average concentration of 0.09%, whereas in powder kombucha, no ethanol was detected (0%) (Figure 2).

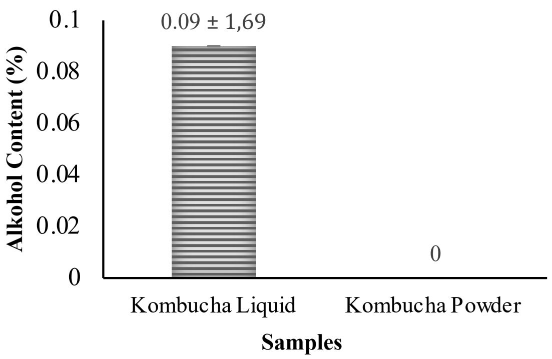

Figure 2. Ethanol content of liquid and powdered kombucha

The level of ethanol produced in this study is lower than the ethanol content of kombucha research in general. In the research by Neffe-Skocinska et al. (2017), which used the same incubation time and a SCOBY weight of 5 g (w/v), the resulting ethanol content was 1.1%. Likewise, with ethanol content from the results of Jakubczyk et al. (2020), which ranged from 0.2-3.5 % for kombucha with white tea, black tea, green tea, and red tea substrates, with the use of 10 g (w/v) % SCOBY weight. In addition, Jasman et al. (2015) noted that using sucrose as a substrate for yeast can yield lower ethanol levels than using glucose or fructose. The low alcohol content observed in this study may therefore be attributed to the smaller SCOBY weight used (3.8 g w/v) and the type of substrate utilized. Sugar serves as the primary substrate for SCOBY metabolism, which produces alcohol and organic acids. The greater the amount of sugar available, the more substrate SCOBY metabolizes to generate these metabolites, resulting in higher alcohol and organic.acid production (Villarreal-Soto et al., 2018).

In kombucha powder, ethanol content is undetectable (0%). This indicates that this kombucha powder product fully complies with the provisions of MUI Fatwa No. 10/2018 on halal beverages, as it does not contain alcohol exceeding the 0.5% (v/v) limit. The powder processing method plays a crucial role in stopping fermentation activity and preventing ethanol formation, while maintaining the stability and quality of bioactive compounds. Therefore, these results indicate that powder processing technology not only improves product quality but also ensures compliance with halal principles.

Total Population of Bacterial Acetic Acid (BAA) by Technique: Total Plate Count (TPC)

The results showed that the amount of Bacterial Acetic Acid (BAA) in kombucha decreased when it was in powder form. The amount of BAA produced from liquid kombucha was 1.35×10^3 CFU/mL, then decreased in kombucha powder to reach 1.19×10^3 CFU/mL (Figure 3). A decrease in the number of Acetic Acid Bacteria (BAA) during drying can cause some of the BAA in the powder to die. According to Holt (1994), acetic acid bacteria grow optimally at a temperature of 28-30°C (Holt, 1994), while the drying process carried out in this study used a temperature of 40°C.

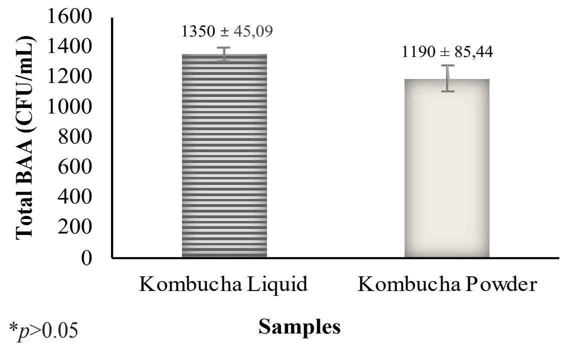

Figure 3. Total BAA kombucha liquid and powder

Acetic acid bacteria is one of the bacteria included in probiotic bacteria. Probiotics are live microorganisms that, when given in sufficient amounts, can have a healthful effect. Probiotics can improve the composition of the gut microbiome. The minimum amount of probiotics required to provide therapeutic effects in fermented products is 10^6 CFU/mL (Shah, 2007). Whereas in this study, the amount of Bacterial Acetic Acid (BAA) produced from liquid and powder kombucha was lower than 106 CFU/mL. Thus, liquid and powder kombucha prepared using the maltodextrin-gum arabic addition method in this study did not yield the required amount of BAA to meet the minimum probiotic standard. The low total BAA in this study may be due to the use of a 7-day-old kombucha starter (SCOBY).

In addition, kombucha generally contains BAA of about 10^5 – 10^6 CFU/mL. In the research by Neffe-Skocinska et al. (2017), the number of acetic acid bacteria in liquid kombucha with a green tea substrate was 7.61×10^6. In addition, in the study by Rosida et al. (2021), the number of acetic acid bacteria in kersen leaf powder kombucha was as high as 5.32×10^5 CFU/mL. The use of young SCOBY was intended to minimize the ethanol produced by the kombucha inoculum after fermentation was complete. The amount of inoculum contained in kombucha can determine the concentration of ethanol formed. This is supported by Ningtyas's (2015) findings, which show that fermentation time can affect the amount of kombucha inoculum produced. The longer the fermentation process, the greater the amount of kombucha inoculum, leading to higher ethanol content (Ningtyas, 2015).

Determination of Organic Acid Content and pH Value

The results showed that organic acid levels were not significantly different between liquid and powder kombucha ($p>0.05$). The concentration of organic acid (acetic acid) in liquid kombucha was 0.0011 M, whereas in powder kombucha it was 0.0073 M (Figure 4). In this study, organic acid levels were much lower than those in kombucha in general, which ranged from 0.0102 to 0.0151 M (Puspaningrum et al., 2021). In the research of Rosida et al. (2021), which used a similar method using maltodextrin and gum arabic, the organic acid content produced was relatively high, namely 0.0041 M for kombucha with kersen leaf substrate (Rosida et al., 2021). This condition corresponds to the low BAA content in kombucha. The organic acids produced depend on the amount of BAA produced by the component (Puspaningrum et al., 2021). The young age of the kombucha starter (SCOBY) also affects the amount of BAA and organic acids produced.

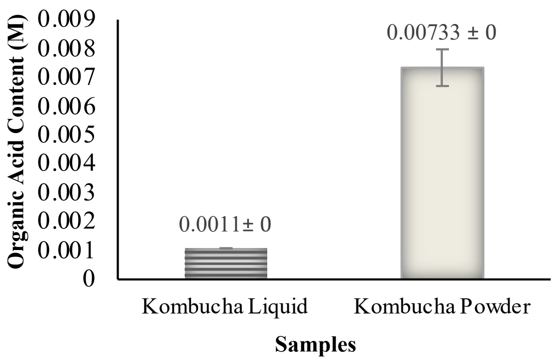

Figure 4. Organic acid content of kombucha liquid and powder

Converting kombucha to powder decreases the pH (Figure 5). The pH value of liquid kombucha obtained in this study was 4.4, while the pH value produced by kombucha powder was 3.2 ($p<0.05$). The pH of the liquid kombucha in this study was within the range reported for liquid kombucha, 3.43-4.02 (Savitri et al., 2024). This condition is also consistent with the pH value of 4.0 for moringa leaf powder kombucha reported by

Rosida et al. (2021) using the same filling material.

The decrease in pH of the kombucha powder in this study was due to the addition of 0.25 grams of citric acid per 100 mL during preparation. Adding citric acid to kombucha powder was intended to impart a sour taste. Dissolved citric acid will release protons, causing an increase in organic acids and lowering the degree of acidity (pH) (Rosida et al., 2021). The presence of H^+ or hydronium ion (H_3O^+) in citric acid can reduce pH (Munjal and Aakash, 2020); thus, when more citric acid is added, the pH decreases. FDA and food regulatory agencies around the world confirm that citric acid is safe for consumption if it is used in accordance with food production guidelines. The limit for citric acid as a solution flavor-balancing ingredient is 5%, or 500 mg per 100 mL of total beverage (Wati and Sutiadiningsih, 2016). The addition of citric acid to this kombucha powder was still within normal limits.

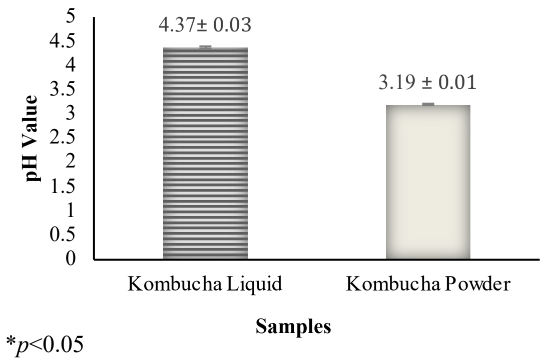

Figure 5. pH value of kombucha liquid and powder

Determination of Sugar Content

The sugar content of kombucha was not significantly different between liquid and powder forms (p>0.05). The sugar content in liquid kombucha was 3.2% (w/v), equivalent to 3.52 g of sugar per 110 mL of kombucha. The recommended daily kombucha consumption by U.S. Centers for Disease Control and Prevention (CDC) is 110 mL/day (Miranda et al., 2022). Meanwhile, the sugar content in kombucha powder is 4.4% (w/v), equivalent to 4.84 g in 110 mL of brewed kombucha (Figure 6). The addition of maltodextrin and gum arabic can increase the sugar content of kombucha powder. Maltodextrin contains 11.06% total sugar, while gum arabic contains less than 1% total sugar (Santoso et al., 2019).

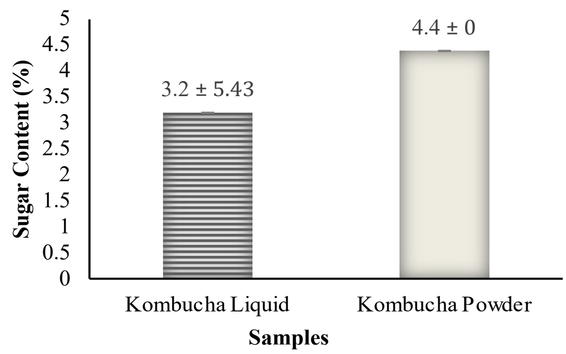

Figure 6. Sugar content of kombucha liquid and powder

The sugar content of kombucha produced in this study is lower than that in other studies. Rosida et al. (2021) reported that the sugar content in kersen leaf powder kombucha made in powder form was 12.57% (w/v) or equivalent to 13.83 g in 110 mL of kombucha. Puspaningrum et al. (2021) also reported that the sugar content of kombucha cascara arabica coffee is 6.15% (w/v), or equivalent to 6.77 g in 110 mL of kombucha. Although the sugar content in this research was low, maltodextrin possesses a high glycemic index (Keller et al., 2016). Therefore, it is necessary to replace this filler with another substance to convert liquid kombucha into powder form.

CONCLUSION

The best solubility and taste of kombucha powder are achieved with a 3:2 ratio of maltodextrin and gum arabic. Liquid kombucha contains 0.09% ethanol, while kombucha powder contains no ethanol (0%), thus complying with MUI Fatwa No. 10/2018 and potentially becoming a promising halal beverage innovation. The total BAA in kombucha powder decreased after the drying process ($p<0.05$). Therefore, further research is needed to explore microbial encapsulation techniques as a protective strategy during drying to maintain BAA viability without compromising the product's physical, chemical, and sensory quality. The pH value of jasmine tea kombucha in liquid form (4.4) was significantly different from powdered kombucha (3.2) ($p<0.05$). Meanwhile, the organic acid and sugar content in liquid and powdered kombucha did not differ significantly ($p>0.05$). The sugar content of liquid and powdered kombucha was lower than that of kombucha in general.

AUTHOR CONTRIBUTION

N.I. and **A.N.H.** conceived of the presented idea. **A.N.H.** developed the theory and performed the computations. **N.I.**, **M.R.M., A.G.S.,** and **O.R.A.** verified the analytical methods. **N.I.** encouraged **A.N.H.** to investigate and supervised the findings of this work. **A.N.H., A.N.N.A.,** and **M.F.M.** contributed to the fabrication and sample preparation. **A.N.H., A.N.N.A., T.S., L.P.** and **M.F.M.** carried out the experiment **A.N.H.** developed the theoretical formalism, performed the analytic calculations, and performed the numerical simulations. **M.R.M.** provided critical feedback and helped shape the research, analysis and manuscript. **A.N.H., A.N.N.A., T.S., L.P.,** and **M.F.M.** contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript. **N.I.** and **A.N.H.** contributed to the final version of the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest.

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