

Development and Sensory Evaluation of Herbal Tea Products from Leaves and Fruits of Mulberry (*Morus alba*)

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Abstract. This study explores the development and evaluation of two innovative herbal beverages: mulberry tea and mulberry-flavored kombucha tea. Leveraging the therapeutic potential of mulberry leaves and fruits (*Morus alba*), the research aims to create affordable, nutritious, and consumer-accepted alternatives to conventional beverages. Mulberry is renowned for its health-promoting properties, including antioxidant, antidiabetic, and anti-inflammatory effects, while kombucha, a fermented tea, is celebrated for its probiotic and detoxifying benefits. An experimental research design assessed the beverages' sensory, microbial, and nutritional properties. Mulberry tea was prepared using sun-drying and air-drying methods, with air-dried samples showing superior sensory attributes. Kombucha was prepared by fermenting mulberry tea with a symbiotic culture of bacteria and yeast (SCOBY) over two fermentation phases. An analysis of sensory evaluations from 100 respondents, using the Mann-Whitney U test, highlighted the acceptability of air-dried mulberry tea and kombucha prepared with 10 tea bags, achieving a more balanced flavor profile than other formulations. Microbial analysis confirmed the safety of the beverages, with no detection of harmful bacteria such as *Salmonella* and *Escherichia coli*. Nutritional analysis revealed that the kombucha samples contained essential macronutrients and were free from fats and cholesterol. Sample 1 offered higher protein content and caloric value, making it suitable for energy-conscious consumers. The results demonstrated that mulberry and kombucha tea are viable health-oriented beverages with significant commercial potential. Both products received high overall acceptability scores, showcasing their ability to meet consumer preferences. The study underscores the potential of mulberry-based beverages to align with the growing global demand for functional and wellness-oriented drinks. In conclusion, this research highlights the versatility and economic value of mulberry leaves and fruits, paving the way for innovative, health-promoting beverage options suitable for large-scale production.

Keywords: Functional beverages; Kombucha; Mulberry tea; Sensory evaluation.

1.0 Introduction

The increasing popularity of herbal beverages is attributed to their health-promoting properties, including anticancer, antibacterial, antidiabetic, anti-inflammatory, and antioxidant effects. This trend is aligned with the growing global emphasis on wellness, further driving the expansion of the herbal tea market (Chandrasekara et al., 2018). Herbal beverages are prepared by boiling or steeping plant-based materials such as leaves, flowers, roots, and seeds. These ingredients can be utilized individually or in combination to create flavors and confer potential health benefits (Piljac-Žegarac et al., 2013).

Globally, tea ranks as the second most consumed beverage after water, with a per capita daily consumption of approximately 0.12 liters. Its regular consumption is recommended based on well-documented health benefits and demonstrated safety (Pastore et al., 2006). Among various plant-based teas, the mulberry (*Morus spp.*, Moraceae) stands out for its therapeutic properties. The primary species of interest include red mulberry (*Morus rubra*), black mulberry (*Morus nigra*), and white mulberry (*Morus alba*) (Yigit et al., 2010). Mulberries are cultivated worldwide, particularly in temperate, tropical, and subtropical regions, with significant concentrations in Asia, especially China, Japan, Korea, and India (Sanchez et al., 2000).

Historically, the mulberry tree has served diverse purposes. In sericulture, its leaves are an essential food source for silkworms. Mulberry fruits are consumed fresh or processed into products like jams and wines. Additionally, mulberry leaves have been widely used in traditional medicine, particularly in Asia, where mulberry tea is valued for its nutritional and medicinal benefits (Wang et al., 2018). The bioactive compounds present in mulberries are regarded as nutraceuticals due to their effectiveness in addressing various health conditions, including anemia and diabetes (Acharya et al., 2022). Beyond medicinal uses, mulberry is also incorporated into culinary applications, underscoring its versatility.

In the Philippines, mulberries (*Morus spp.*) are widely cultivated for their versatile uses, mainly fruits and leaves. The fruits are consumed fresh or processed into various products, while the leaves are essential in sericulture, serving as the primary food source for silkworms (*Bombyx mori*). Additionally, in some provinces such as Pampanga, mulberry leaves are processed into tea (De leon, 2023). This study aims to explore the utilization of mulberry tea in kombucha production, incorporating mulberry fruit as a natural flavoring to enhance its appeal and value."

Kombucha, a fermented tea beverage, is produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY). Originating in China over 2000 years ago, kombucha has gained global recognition for its energizing properties and potential health benefits. It is referred to by various names, including red tea fungus (Malbaša et al., 2011), and is commonly prepared using black or green tea (Dubey et al., 2020). Green tea enhances fermentation, facilitating faster beverage production (Greenwalt et al., 2000). The fermentation process of kombucha involves a symbiotic interaction between yeast and bacteria. Yeasts hydrolyze sucrose into glucose and fructose, which are metabolized into ethanol. Acetic acid bacteria oxidize ethanol into acetic acid, imparting kombucha's tangy flavor while acting as a natural preservative (de Miranda et al., 2022). The final product is mildly acidic, effervescent, and slightly alcoholic, with a flavor profile evolving from fruity and sour to a mild vinegar-like taste over time (Goh et al., 2012). Kombucha is widely recognized for its benefits, including enhanced gut health, improved digestion, detoxification, and stress reduction. Additionally, it is linked to cardiovascular health and the prevention of oxidative stress-related illnesses such as cancer and neurological disorders (Martinez et al., 2018).

The rising popularity of kombucha as a health-oriented beverage has catalyzed significant market growth. The global tea market, valued at approximately \$207.1 billion in 2020, is projected to reach \$266.7 billion by 2025 (Statista, 2017). Within this context, the kombucha tea market is anticipated to grow at a compound annual growth rate (CAGR) of 12.3%, reaching \$1,655.6 million by 2032. Despite regulatory challenges, such as alcohol content restrictions, the industry continues flourishing, supported by over 350 producers and substantial investments. This study evaluated the potential of utilizing mulberry leaves for tea production and its fruits as a natural flavoring for kombucha, highlighting the plant's dual-purpose functionality and potential for value-added products. This creates affordable and nutritious alternatives to conventional soft drinks and commercial beverages while showcasing mulberry cultivation's health benefits and economic potential.

2.0 Methodology

2.1 Research Design

This study employed an experimental research design to systematically evaluate the acceptability and quality of mulberry and kombucha tea. Evaluation of these herbal teas followed procedures of Civile et al. (2024) for food sensory evaluation. Two preparation methods for mulberry tea (sun-dried and air-dried) were developed and compared to a commercial tea product to assess their sensory appeal and consumer preference. In the kombucha

product, varying numbers of mulberry tea bags were used during fermentation to determine their impact on flavor, aroma, and overall acceptability.

A preliminary assessment by trained panelists was conducted to refine the experimental formulations before a more considerable sensory evaluation was performed with 100 respondents (Civille et al., 2024). To ensure a robust evaluation, the study utilized sensory evaluation questionnaires to capture respondents' perceptions of the experimental products compared to commercial alternatives. Beyond sensory attributes, microbial contamination and nutrient content analyses were conducted to assess the beverages' safety, hygiene, and nutritional properties, further justifying the experimental approach. This comprehensive methodology underscores the scientific rigor of the study, aiming to explore the feasibility of mulberry tea and kombucha as innovative and health-oriented beverage options.

2.2 Research Locale

This study was conducted at Don Mariano Marcos Memorial State University, South La Union Campus, specifically at the College of Arts and Sciences in Agoo, La Union, and the College of Agriculture in Rosario, La Union. However, the mulberry leaves and fruits were handpicked from Imelda Naguilian, La Union. Only whole, unblemished leaves free from insect damage or brown spots were selected. The leaves were washed thoroughly with water before further processing.

2.3 Tea Preparation

Two types of mulberry tea were prepared by modifying the methods of Raja et al. (2019). In the original method, leaves were blanched in hot water, thoroughly washed with cold distilled water, and then pat-dried. However, since mulberry leaves are thin, they were not blanched in this study but were thoroughly washed and pat-dried. Either sun-drying or air-drying subsequently processed the leaves. All materials were thoroughly cleaned, and tea bags were autoclaved to ensure sterility.

Sun-Dried Mulberry Tea

Ten kilograms of mulberry leaves were washed three times with distilled water, pat dried, and exposed to direct sunlight for three days, enclosed in a net cage to minimize contamination. Once brittle, the leaves were crushed using a blender at high speed. Approximately 1.5 grams of crushed leaves were placed into sterilized tea bags, which were then oven-dried at 120°C for 15 minutes.

Air-Dried Mulberry Tea

Another 10 kilograms of mulberry leaves were washed three times with distilled water, pat-dried, and air-dried for one day in the multi-commodity solar tunnel. The dried leaves were pulverized using a grinder, and 1.5 grams of the resulting powder were placed in sterilized tea bags and oven-dried at 120°C for 15 minutes.

2.4 Evaluation of Tea

Four trained panelists from the College of Agriculture at DMMMSU conducted a preliminary sensory evaluation. All panelists held bachelor's degrees in food technology, and two had master's degrees in food science. The evaluation focused on appearance, taste, tartness, and overall acceptability. The tea samples were labeled with randomly generated three-digit codes to minimize bias. The sun-dried tea was eliminated due to sensory issues, such as throat itchiness, while the air-dried and commercial green tea advanced to the final sensory evaluation. There were 100 randomly selected respondents from the DMMMSU community, including students, teachers, non-teaching personnel, and nearby residents. Among them, 34 respondents were between the ages of 18–30, 33 were between 31–50, and 33 were aged 51 and older. The acceptability of both products – air-dried mulberry and commercial tea – was evaluated using a 9-point Hedonic Scale. The scale, adapted from Su et al. (2022), ranged from 1 to 9, where: 1 = dislike extremely, 2 = dislike, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like, 8 = like very much, and 9 = like extremely. Respondents had to be free from cold or cough symptoms for at least a week before participation. Distilled water was provided as a palate cleanser and sensory rating sheets were used to record the data.

2.5 Preparation of Kombucha Tea

The preparation of kombucha tea followed the methods of Freitas (2020), where kombucha is produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY) in two stages. In Phase 1, or tea fermentation, sweet tea is prepared by dissolving sugar in boiling water, steeping tea, and cooling it to room temperature. Starter tea and the SCOBY are added, and the mixture is left to ferment in a covered jar at room temperature for 7–14 days. On Phase 2 or second fermentation, flavorings like fruit or herbs are added, and the bottles are sealed to allow carbonation to develop over 1–3 days at room temperature. Once fizzy, the kombucha is refrigerated to halt fermentation. Equipment and materials were sanitized using cost-effective home-based procedures. The SCOBY and starter tea were sourced from Cainta, Rizal, while mulberry fruits were procured from Wenceslao Caba, La Union.

2.6 Fermentation Process

Phase 1: Tea Fermentation

Sixteen liters of distilled water were boiled, and two cups of white sugar were dissolved in each pot (2 liters). Mulberry tea bags (10, 20, 30, and 40 bags per pot) were steeped for 15 minutes, removed, and the solutions cooled to room temperature. The mixtures were transferred to one-gallon glass jars, and a SCOBY pellicle and 250 mL of starter tea were added. The jars were covered with sterilized paper towels and secured with rubber bands. Fermentation was conducted at room temperature in a dark environment for 14 days.

Phase 2: Flavor Addition

After 14 days, the SCOBY was removed, and one cup (100 g) of chopped mulberry fruits was added to each jar. The jars were sealed and allowed to ferment for another 14 days. The final kombucha was filtered and bottled in 250 mL glass bottles, yielding 15 bottles per sample.

2.7 Microbial and Nutritional Analysis

Twenty 250-mL kombucha tea samples were sent to the DOST-CAR Regional Standards and Testing Laboratory for microbial and nutritional analysis. The samples were tested for contaminants such as *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli*, as well as nutrient content, including total fat, carbohydrates, protein, sugar, and caloric content.

2.8 Evaluation of Kombucha Tea

As with the mulberry tea, four trained panelists conducted a preliminary evaluation of kombucha tea samples based on appearance, taste, flavor, tartness, and overall acceptability. If the intensity or quality of these attributes falls outside acceptable ranges or expectations, the product is rejected. Samples 1 and 3 met the acceptable ranges and expectations of the panel and proceeded to the final sensory evaluation. Sample 2, with 20 tea bags, was rejected due to its extreme sourness and bitterness, and sample 4, with 40 mulberry tea bags, was also rejected due to the absence of mulberry flavor. As with the mulberry tea, the one hundred respondents evaluated the qualified kombucha samples (1 and 3) using the 9-point Hedonic Scale. Data were collected to assess consumer preferences and product acceptability.

2.9 Data Analysis

Data collected from sensory evaluations were analyzed using the Shapiro-Wilk test to assess normality. Non-parametric analysis (Mann-Whitney U Test) was applied to identify significant differences among samples at a 0.05 significance level.

3.0 Results and Discussion

3.1 Sensory Evaluation of Mulberry Tea

The sensory properties of mulberry tea, including appearance, taste, tartness/texture, and overall acceptability, were evaluated to determine its quality and consumer satisfaction. A total of 100 respondents rated the air-dried mulberry tea against commercial tea. The sun-dried mulberry tea was excluded after being rejected by the trained panelist during preliminary screening. Sensory evaluation data were analyzed using the Mann-Whitney U test, as the Shapiro-Wilk test confirmed non-normal distribution. Table 1 summarizes the mean ranks for the acceptability of air-dried and commercial mulberry tea, with significant differences indicated. The higher mean ranks observed

for air-dried tea in taste, tartness, and overall acceptability suggest that evaluators found it more favorable in these attributes than the commercial product.

Table 1. Mean Ranks for the Acceptability of Mulberry Tea (n=100)

	Air dried	Commercial
Appearance	99.82	101.19
Taste	124.08*	76.92*
Tartness	124.03*	76.98*
Overall Acceptability	129.13*	71.88*

*Significant at $p < 0.05$

Table 1 shows that commercial tea and air-dried mulberry tea are comparable in terms of appearance, with no significant difference. This indicates that the visual appeal of the air-dried mulberry tea is comparable to that of commercial tea, suggesting that the air-drying process preserved the tea's visual quality. However, the air-dried mulberry tea scored significantly higher than the commercial tea in terms of taste, suggesting that respondents found the flavor profile of the mulberry tea to be more enjoyable than the commercial tea. The higher mean rank in tartness or texture of the air-dried tea contributed positively to its overall sensory evaluation. This result highlights the potential of air-dried mulberry tea as a favorable alternative to commercial teas.

3.2 Sensory Evaluation of Kombucha Tea

The sensory properties of kombucha tea, such as appearance, taste (sourness, sweetness, bitterness), flavor (vinegar and mulberry), tartness (acidity/texture), and overall acceptability, are critical to consumer preferences (Larasati et al., 2017). Appearance, including color and clarity, significantly influences consumer perceptions of quality, while flavor is a key determinant of satisfaction and preference. In this study, kombucha tea samples prepared with 10 mulberry tea bags (Sample 1) and 30 mulberry tea bags (Sample 3) were evaluated by 100 respondents following initial screening by trained panelists. The sensory evaluation results, analyzed using the Mann-Whitney U test, are presented in Table 2.

Table 2. Mean Ranks for the Acceptability of Kombucha (n=100)

	Sample 1	Sample 3
Appearance	100.76	100.24
Sourness	118.47*	82.54*
Sweetness	118.36*	82.65*
Bitterness	114.01*	86.99*
Vinegar Flavor	117.81*	83.19*
Mulberry Flavor	95.07	105.93
Tartness	114.87*	86.14*
Overall Acceptability	118.49*	82.52*

*Significant difference at $p < 0.05$

As shown in Table 2, the Mann-Whitney U test revealed significant differences between Sample 1 and Sample 3 for most sensory attributes, including sourness, sweetness, bitterness, vinegar flavor, tartness, and overall acceptability. Sample 1 consistently scored higher mean ranks for these attributes, indicating a more favorable sensory profile. Respondents preferred the balanced sourness, sweetness, and tartness of Sample 1, while Sample 3 was perceived as overly sour and bitter. The vinegar flavor in Sample 1 was also rated more acceptable, contributing to its overall appeal.

Sample 1, made with 10 tea bags, achieved a more balanced and consumer-friendly flavor profile than Sample 3. This balance contributed to its higher overall acceptability, making it the preferred option among respondents. The results underscore the importance of optimizing ingredient concentrations in kombucha production to meet consumer expectations and preferences, positioning Sample 1 as a promising candidate for commercial success. In contrast, Sample 3 scored slightly higher in mulberry flavor, likely due to the increased concentration of tea bags. However, this did not result in higher overall acceptability, as the excessive intensity of other flavors detracted from the sensory experience. Additionally, there was no significant difference in appearance between the two samples, indicating that both kombucha tea formulations were equally visually appealing to respondents.

3.3 Microbial Analysis

Microbial safety is a critical consideration in the production of kombucha tea, as harmful bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* are common causes of foodborne illnesses ranging from mild

gastrointestinal infections to severe health conditions. Table 3 presents the results of microbiological analysis conducted on kombucha tea samples tested by the Department of Science and Technology-Cordillera Administrative Region (DOST-CAR) laboratory using standard methods.

Table 3. Microbiological Analysis Results

Bacterial Analysis	Sample 1 (CFU/g)	Sample 2 (CFU/g)	Sample 3 (CFU/g)	Sample 4 (CFU/g)
Total Coliform	<10	<10	<10	<10
<i>E. coli</i>	<10	<10	<10	<10
<i>Staphylococcus aureus</i>	45	<10	<10	<10
<i>Salmonella</i> Detection	Negative at 25g sample	Negative at 25g sample	Negative at 25g sample	Negative at 25g sample

The results in Table 3 indicate that all kombucha tea samples had total coliform and *E. coli* counts below 10 CFU/g, and *Salmonella* was not detected in any of the samples at a 25-gram detection limit. However, sample 1 exhibited a *Staphylococcus aureus* count of 45 CFU/g, which, although higher than the other samples, remains within the acceptable safety limit for human consumption as outlined by Gilbert et al. (2000). The acceptable limit for *Staphylococcus aureus* is less than 103 CFU/g, and values above 104 CFU/g indicate potential health risks. The microbial safety of kombucha can be attributed to the fermentation process, during which symbiotic cultures of bacteria and yeast (SCOBY) produce organic acids, ethanol, and antimicrobial compounds that inhibit the growth of harmful pathogens (Villarreal-Soto et al., 2018). Kombucha demonstrates antibacterial activity against gram-positive and gram-negative bacteria, including *E. coli*, *Shigella*, *Vibrio cholera*, and *Salmonella*. Studies by Kitwetcharoen et al. (2023) and Al-Mohammadi et al. (2021) confirm kombucha's efficacy as a preservative and its role in safeguarding against foodborne pathogens.

The low counts of total coliforms and *E. coli* (<10 CFU/g) across all samples suggest proper hygiene and sanitary practices during kombucha preparation, minimizing fecal contamination risk (Jnani et al., 2024). *E. coli* levels above 20-100 CFU/g are considered indicators of compromised hygiene and potential health risks (Gilbert et al., 2000). The absence of *Salmonella* in all samples further ensures product safety, as *Salmonella* contamination is a significant cause of foodborne illness globally (Hald et al., 2013). Kombucha's antimicrobial activity, driven by the metabolic by-products of acetic acid bacteria (e.g., *Komagataeibacter*, *Gluconobacter*) and lactic acid bacteria (e.g., *Lactobacillus*, *Lactococcus*), plays a critical role in inhibiting pathogens (Villarreal-Soto et al., 2018). Additionally, its acidic environment and ethanol content create unfavorable conditions for microbial growth, making kombucha a safe beverage when produced under controlled conditions.

These findings align with studies emphasizing kombucha's role as a functional beverage with inherent antimicrobial properties (Kitwetcharoen et al., 2023; Al-Mohammadi et al., 2021). Although sample 1 had a higher *Staphylococcus aureus* count than the others, the results confirm that the kombucha tea samples meet microbiological safety standards and are suitable for human consumption. Kombucha tea prepared under appropriate sanitary conditions demonstrates low microbial risk, reinforcing its status as a safe and healthful beverage alternative. Regular monitoring and adherence to good manufacturing practices are essential to ensure consistent product safety and quality.

3.4 Nutrient Content

Table 4 provides the nutritional analysis of kombucha, sample 1 and sample 3, offering insights into their caloric and macronutrient composition. One serving (240 ml) of kombucha sample 1 contains 140 calories, 17 grams of sugar, 2 grams of protein, and 32 grams of carbohydrates. This sample has no detectable fat or cholesterol. In comparison, sample 3 has a lower caloric value of 120 calories, 26 grams of carbohydrates, and 19 grams of sugar but contains less than 1 gram of protein. The nutritional data reveal variations in calorie content, total carbohydrates, sugar, and protein levels between the two samples. Sample 1 offers slightly higher calorie and protein content, potentially more suitable for individuals seeking an energy-boosting beverage with moderate protein. In contrast, sample 2 has lower protein content but slightly higher sugar levels, which may appeal to consumers prioritizing lower caloric intake.

Table 4. Nutritional Analysis Results of Kombucha Tea

Amount per Serving (240 ml)	Sample 1	Sample 3
Calories (kcal)	140	120
Calories from Fat	0	0
Total Fat (g)	0	1
Total Carbohydrates (g)	32	26
Sugar (g)	17	19
Total Protein (g)	2	<1

The absence of fats and cholesterol in both samples highlights kombucha as a low-fat beverage option. Macronutrients like protein, fat, and carbohydrates perform vital functions in the body: proteins aid in tissue growth and repair, fats protect organs and contribute to cellular health, and carbohydrates serve as the primary energy source (Streit, 2021). Additionally, the calorie content reflects the energy the beverage provides, which can support daily energy requirements for adults engaging in moderate physical activity (Richards, 2020).

4.0 Conclusion

The study demonstrates that mulberry leaves can effectively produce mulberry tea, which is highly acceptable to consumers. Furthermore, adding mulberry fruits and the fermentation process to create kombucha tea offers an innovative beverage enriched with probiotics, enhancing its functional value. Sensory analysis of the kombucha tea confirmed its acceptability among consumers, highlighting the potential for further refinement and optimization of the formula for large-scale commercial production. Nutrient and microbiological analyses revealed that mulberry-flavored kombucha tea contains essential nutrients and meets safety standards for human consumption. These findings emphasize its viability as a health-promoting and safe beverage option. In conclusion, mulberry tea and kombucha tea derived from mulberry leaves and fruits offer affordable, nutritious, and consumer-accepted alternatives to traditional beverages. Their potential for commercial production aligns with growing consumer demand for functional and health-oriented drinks.

5.0 Contributions of Authors

Glennadi R Rualo – Conceptualization, Methodology, Validation, Formal Analysis, supervision, review, and editing
 Rose F. Cortez – Investigation, data curation, writing draft
 Crystal Monique J. Estipular – Investigation, Formal Analysis, writing draft
 Michelle V. Reyes – Investigation, resources
 Jervin S. Aquino – Investigation, resources

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7.0 Conflict of Interests

No conflict of interest.

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