# Standardization, Fermentation, and pH-Based Stability Assessment of Kombucha from Gambyong Kemuning Green Tea

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***Abstract***

This study aims to conduct the standardization, fermentation, and stability assessment based on pH of kombucha made from Gambyong Kemuning green tea (Camellia sinensis L). Kombucha is widely known for its health benefits; however, the standardization of herbal kombucha products still requires further research. This study employed methods including determination, simplicia preparation, simplicia standardization, extraction using the infusion method, and the preparation and standardization of kombucha. The results of the simplicia standardization test showed a drying loss of 4.6%, moisture content of 6.12%, and total ash content of 3.61%, all of which meet the recommended standards to prevent microbial growth and contamination. In addition, heavy metal and ethanol tests indicated that both the infusion and kombucha are safe for consumption. The pH test conducted over 14 days of fermentation showed values within the safe range, between 4.2 and 2.5, during the first 10 days. However, on days 11 to 14, the pH dropped below the safe limit, indicating an excessive acetic acid content that could pose a health risk. In conclusion, Gambyong Kemuning green tea kombucha demonstrated promising results in terms of standardization and safety, but strict monitoring of fermentation duration is necessary to maintain a stable and safe pH for consumers.

## 1. Introduction

Tea (Camellia sinensis L.) has long been known and consumed worldwide, not only as a refreshing beverage but also for its significant health benefits (KEMENPAREKRAF, 2024). These benefits largely stem from its content of secondary metabolites, particularly polyphenols such as flavonoids, catechins, and theaflavins, which possess potent antioxidant activity to combat free radicals in the human body (Rahmatillah et al., 2025; Yasmin, 2016). Regular tea consumption is associated with a reduced risk of heart disease, cancer, and improved blood sugar management (KEMENPAREKRAF, 2024; Yasmin, 2016).

Gamyong Kemuning green tea, a superior product from Kemuning Village, Karanganyar, Indonesia, is a tea variety rich in natural antioxidants (KEMENPAREKRAF, 2024). The use of this green tea in the development of functional food products is of interest, one of which is through the fermentation process to produce kombucha.

Kombucha is a fermented beverage made from sweet tea using a symbiotic culture of bacteria and yeast (SCOBY) (Kristianingtyas, 2015). This fermentation process converts sugar into various organic acids, such as acetic acid and gluconic acid, as well as other bioactive compounds that contribute to the beverage's health benefits (Kristianingtyas, 2015; Putri & Fitranti, 2016). Scientific studies have demonstrated the potential of kombucha, both black and green tea, as an effective hypoglycemic agent in lowering blood sugar levels (Putri & Fitranti, 2016; Zubaidah et al., 2019). The Acetobacter sp. bacteria in kombucha cultures play a crucial role in producing gluconic acid, which aids calcium absorption and insulin production by pancreatic beta cells (Kristianingtyas, 2015).

Despite the rapid rise in popularity of kombucha, standardization of industrial herbal products still requires further research (Sulistiawaty & Solihat, 2022). A key characteristic of kombucha is its acidity (pH), which determines its microbiological safety and stability. The ideal pH value during fermentation ranges from 2.5 to 4.2 (Sulistiawaty & Solihat, 2022). Values above 4.2 pose a risk of contamination by pathogenic microbes, while values below 2.5 indicate excessively high acetic acid content and can pose a health risk to consumers (Sulistiawaty & Solihat, 2022). These pH fluctuations are highly dependent on the fermentation duration, which also affects the alcohol content in the final product (Sulistiawaty & Solihat, 2022).

Therefore, this study focuses on standardizing the kombucha production process from Gambyong Kemuning green tea, starting from the preparation of the herbal extract, standardization of the extract (infusion), and the fermentation process. This study aims to assess product stability based on pH parameters during a 14-day fermentation period to identify the optimal and safe fermentation duration for consumption. The results of this study are expected to provide scientific guidance for the production of Gambyong Kemuning green tea kombucha that is standardized and safe for consumers.

## 1.1 Literature Review

A. Gambyong Kemuning green tea (*Camellia sinensis* L.)

Gambyong Kemuning green tea is a trademarked product from Kemuning Village, Ngargoyoso, Karanganyar, known for its high antioxidant content (KEMENPAREKRAF, 2024). Antioxidants are essential for fighting free radicals (Rahmatillah et al., 2025). Regular consumption of this tea is associated with a reduced risk of heart disease and cancer, and is believed to lower blood sugar (KEMENPAREKRAF, 2024).

The tea plant (Camellia sinensis L.) is rich in secondary metabolites, including polyphenols, alkaloids, polysaccharides, amino acids, and vitamins (Yasmin, 2016). Polyphenols are the most abundant component, consisting of flavonoids (flavanols, proanthocyanidins, flavonols), theaflavins, thearubigins, and gallic acid (Yasmin, 2016). Research by Wang et al. (2000) identified kaempferol, quercetin, and myricetin as the main flavonoids, which are present primarily as glycosides. The catechin and theaflavin content in tea extracts also exhibits hypoglycemic activity by increasing insulin activity and protecting pancreatic β cells (Yasmin, 2016).

B. Infundation Extraction Method

The infundation method is an extraction process carried out by steeping the herbal medicine in hot water at 90°C for 15 minutes. This method is commonly used to extract water-soluble active substances from plant materials. However, the extract obtained through infundation tends to be unstable and susceptible to microbial contamination. Therefore, the resulting extract should not be stored for more than 24 hours (Si et al., 2021)

C. Kombucha

Definition of Kombucha

Kombucha tea is a fermented tea solution mixed with sugar and combined with a kombucha culture (SCOBY). Fermentation is usually carried out in a shaded area for approximately 8-12 days (Kristianingtyas, 2015).

Benefits of Kombucha

Several studies have demonstrated significant efficacy of kombucha. Putri and Fitranti (2016) reported that 150 mg of black tea kombucha in mice for 14 days reduced blood sugar levels by 56.4%. Another study by Zubaidah et al. (2019) found that snake fruit kombucha and black tea kombucha were effective as diabetes therapeutic agents, similar to metformin, in a STZ-induced mouse model by reducing FPG, improving oxidative stress status, and improving lipid profiles.

Kombucha cultures contain Acetobacter sp., bacteria that oxidize sugars into gluconic acid and vitamins. Gluconic acid plays a role in protecting cell membranes, strengthening the intestinal walls, helping calcium absorption, and lowering blood sugar levels through the formation of insulin by pancreatic beta cells (Kristianingtyas, 2015).

Kombucha Quality Standards

a. pH Value

During fermentation, the pH value decreases over time due to an increase in organic acids (Sulistiawaty & Solihat, 2022). A safe pH value for consumption ranges from 4.2 to 2.5. A pH below 2.5 poses a health risk due to the excessively high acetic acid content, while a pH above 4.2 poses a risk to microbiological safety (Sulistiawaty & Solihat, 2022).

b. Alcohol Content and Halal Certification

The alcohol content in kombucha is directly proportional to the length of fermentation, during which the yeast breaks down sugars into alcohol. The longer the fermentation, the higher the alcohol content. An alcohol content of 0.48% was found after 12 days of fermentation, approaching the limit for halal consumption. However, the bacteria Acetobacter aceti can subsequently convert the alcohol into acetic acid (Sulistiawaty & Solihat, 2022).

D. Standardization Parameters for Natural Raw Materials and Extracts

Standardization of natural raw materials and extracts is essential to ensure the quality and safety of these materials.

a. Standardization of Natural Raw Materials

This includes a Drying Loss test to determine the weight loss of the material after drying at 105°C (Silverman et al., 2023). Moisture Content is used to determine the stability and shelf life of the material (Silverman et al., 2023). Total Ash Content is used to determine the amount of inorganic/mineral substances remaining after complete combustion (Silverman et al., 2023).

b. Extract Standardization

This includes a pH test to determine the acidity or alkalinity of the solution (Aprilia et al., 2023). Heavy Metal and Lead Free Tests are used to ensure safe consumption (Eka et al., 2024). Ethanol Free Tests are used to free the extract from antibacterial ethanol, preventing false results in subsequent testing (Silverman et al., 2023).

## 2. Research Methods

### Research Method Overview

This study aimed to standardize, ferment, and assess pH-based stability of kombucha made from Gambyong Kemuning green tea (Camellia sinensis L.). This research method was designed to ensure the safety and quality of kombucha products through a series of standardization tests on the herbal extract, herb, and final product (kombucha). This approach was chosen to provide a current contribution to solving the problem of standardization of herbal products in the kombucha industry, which still requires further research. All stages of the study, from sampling to data analysis, are described in detail to ensure the reproducibility of the study by other researchers and the validity of the findings.

This research methodology consists of several main subsections: sampling, data collection (through a series of laboratory tests), and measurement (including physical and chemical parameters). A flowchart of the overall research process can be seen in Figure 1.

### Sampling

#### a. Description of the Target Population, Research Context, and Unit of Analysis

The target population in this study was Gambyong Kemuning green tea leaves (Camellia sinensis L.). The context of this research was a laboratory study focused on the standardization of herbal raw materials and their fermented products. The main units of analysis were dried Gambyong Kemuning green tea herbal extracts, infusion (tea extract), and fermented kombucha solution.

#### b. Sample

The sample material used in this study was black Gambyong Kemuning green tea leaves (Camellia sinensis L.) obtained from Kemuning Village, Ngargoyoso District, Karanganyar Regency, Central Java Province. Sampling was conducted purposively from that location.

#### c. Respondent Profile

There were no human respondents in this study because the focus was on laboratory analysis of natural materials and their processed products.

### Data Collection

Data collection was conducted through a series of structured laboratory experiments. This process includes the preparation of medicinal herbs, standardization of medicinal herbs, preparation of infusion extracts, standardization of extracts, and production of kombucha, followed by pH stability testing during the fermentation period.

### Tools and Materials

**Materials:** Gambyong Kemuning green tea leaves (Camellia sinensis L.), distilled water, sugar, kombucha culture (SCOBY), reagents $K_2CrO_4$, NaOH, $H_2SO_4$, acetic acid.

**Tools:** Analytical Balance (Fujitsu FSR-B4000), Electric Stove (Maspion S3000), Moisture Balance, Infusion Pan, Glass Beaker (one two cups), Measuring Cup (HERMA), 1L Glass Container, Solution Bottle, Test Tube, Digital pH Meter, Digital Thermometer, Disposable Syringe (Onemed), Stirring Rod, Dropper.

## Research Procedures

### Natural Raw Material Preparation and Standardization

After collection, the green tea leaves were thoroughly washed and dried to obtain a dry powder. The dried natural raw materials were then ground and sieved using a 40-mesh sieve. The resulting natural raw material powder was then standardized through the following tests:

**Loss on Drying:** A clean and dry evaporating dish was weighed. Two grams of natural raw material powder was placed in the dish, weighed, and placed in an oven at 105°C for 30 minutes. A final weighing was performed to determine the loss on drying.

**Moisture Content Determination:** Using a calibrated and tare-tested moisture balance, approximately 1.8–2.0 grams of natural raw materials were placed in the dish, covered, and left for approximately 15 minutes until the moisture content reading was displayed and recorded.

**Determination of Total Ash Content:** 2-gram sample was weighed and placed in a silicate crucible that had been dried in a furnace. The crucible was heated in the furnace until the sample was reduced to ash, then cooled in a desiccator. The ash content was calculated against the air-dried material (Ningsih et al., 2024).

### Extract Preparation and Extract Standardization

**Preparation of Infusion Extract Solution (Basic Tea Solution):** Total of 30 grams of tea powder, 120 grams of sugar, and 1 liter of water were mixed. Heated at 90°C for 15 minutes until the sugar dissolved. The filtrate was filtered to obtain a clean infusion without dregs.

**Extract Standardization (Infusion):** The obtained extract was tested for standardization using the following methods:

**pH Test:** The pH meter electrode was rinsed with distilled water and dried, then calibrated. The electrode was immersed in the test extract until the reading stabilized, and the results were recorded (National Standardization Agency of Indonesia, 2004).

**Heavy Metal Free Test (Lead (Pb) and Cadmium (Cd)):** The Pb test is performed by adding $K_2CrO_4$ reagent to 5 ml of the sample; a yellow precipitate indicates a positive result. The Cd test is performed by adding NaOH reagent to 5 ml of the sample; a white precipitate indicates a positive result (Ningsih et al., 2024).

**Ethanol Free Test:** 2 drops of $H_2SO_4$ and 2 drops of acetic acid are added to 1 mL of the sample, then heated. The extract is declared ethanol-free if no ester odor is detected (Ningsih et al., 2024).

### Kombucha Preparation and Stability Testing

The kombucha solution is prepared using a previously prepared infusion extract solution, followed by the addition of 60 grams of SCOBY. Fermentation is carried out in a glass container covered with a tissue or clean cloth, kept away from sunlight, for 12 days. During the fermentation process, pH stability tests are conducted periodically for 14 days to monitor changes in acidity.

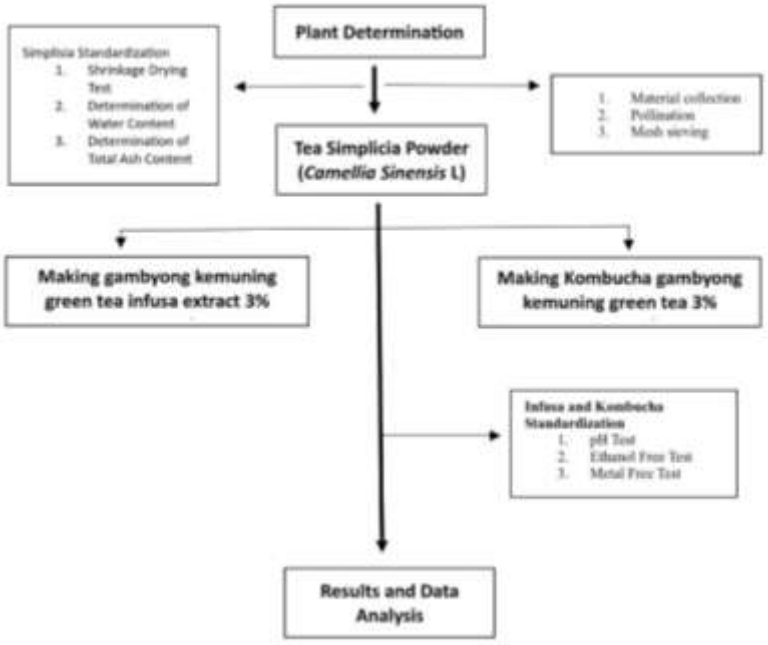


*Fig 1. Overall research process flow diagram.*

## 3. Result and Discussion

Identification of Gambyong Kemuning Green Tea Plants

Plant identification aims to ensure the correct and accurate identity of the Gambyong Kemuning green tea (Camellia sinensis L.) samples used for research, to avoid errors in material collection and to prevent contamination with other plants.

The identification results confirmed that the plants used were indeed green tea (Camellia sinensis L.). Identification of the Gambyong Kemuning green tea (Camellia sinensis L.) plants was conducted at the Research Center of the UPF YANKESTRAD Dr. Sardjito Tawangmangu General Hospital, Karanganyar Regency, Central Java.

Preparation of Natural Raw Materials for Gambyong Kemuning Green Tea (Camellia sinensis L.)

Sampel 600g of Gambyong Kemuning green tea (Camellia sinensis L.) samples were collected from Kemuning Village, Ngargoyoso District, Karanganyar Regency, Central Java Province. Total of 600g of dry Natural Raw Material was taken, then pollination was carried out to obtain Natural Raw Material powder, then sieving was carried out with a mesh sieve no. 40 to obtain powder with a uniform size (Ummah, 2019).

Standardization of Natural Raw Materials

Drying Loss Test

This test uses a gravimetric method, which essentially uses an oven at 105°C. This is because when the temperature reaches 105°C, water will evaporate, and compounds with lower boiling points than water will also evaporate (Wandira et al., 2023). The results of the drying loss determination for Gambyong Kemuning green tea powder (Camellia sinensis L.) are shown in Table 1.

*Table 1. Drying Loss Test Results for Gambyong Kemuning Green Tea Powder*

| Initial Weight (g) | Reference (FHI) (%) | Moisture Content (%) |
|---|---|---|
| 2 | < 10 | 4 |
| 2 | < 10 | 6 |
| 2 | < 10 | 4 |
| | | Average : 4,6 |

The drying loss for Gambyong Kemuning green tea powder (Camellia sinensis L.) was 4.6%. The drying shrinkage results of Gambyong Kemuning green tea (Camellia sinensis L) powder are in line with previous research (Silverman et al., 2023), which is no more than 10%.

Moisture Content Test

The moisture content test was conducted using a Moisture Balance at a temperature of 105°C, and the test was carried out until the device beeped automatically, indicating that the analysis was complete. The results of the moisture content test for Gambyong Kemuning green tea powder (Camellia sinensis L.) are shown in Table 2.

*Table 2. Moisture Content Test Results for Gambyong Kemuning Green Tea Powder*

| Initial Weight (g) | Reference Weight (FHI) (%) | Loss on Drying (%) |
|---|---|---|
| 2 | < 10 | 6.05 |
| 2 | < 10 | 6,20 |
| 2 | < 10 | 6,12 |
| | | Average : 6,12 |

The moisture content of the Gambyong Kemuning green tea powder (Camellia sinensis L.) was 6.12%. The results of the moisture content of Gambyong Kemuning green tea (Camellia sinensis L.) powder are consistent with previous research (Silverman et al., 2023), which is no more than 10%. This aims to prevent spoilage reactions in the powder caused by bacteria or fungi.

Total Ash Content Test

This test uses a furnace heating at approximately 900°C until the powder turns to ash (Silverman et al., 2023). The results of the total ash content determination for Gambyong Kemuning green tea (Camellia sinensis L.) powder are shown in Table 3.

*Table 3. Total Ash Content Test Results for Gambyong Kemuning Green Tea Powder*

| Initial Weight (g) | Reference (FHI) (%) | Total Ash Content (%) |
|---|---|---|
| 2 | < 5,6 | 2,79 |
| 2 | < 5,6 | 3,33 |
| 2 | < 5,6 | 4,75 |
| | | Average : 3,61 |

The total ash content test for Gambyong Kemuning green tea (Camellia sinensis L.) powder yielded 3.61%. The results of the total ash content of Gambyong Kemuning green tea (Camellia sinensis L.) powder are in line with previous research, which is not more than 5.6% (Silverman et al., 2023). This aims to assess the mineral nutrient content in the simplicia and indicate the quality of the material (the higher the total ash content of the simplicia indicates the presence of soil or sand contamination in the simplicia) (Utami et al., 2017).

Making Camellia sinensis L. Gambyong Kemuning Green Tea Infusion Extract

The infusion method is an extraction process carried out by brewing the herbal medicine with hot water at 90°C for 15 minutes. Powdering and sieving facilitate the extraction process because the smaller the powder, the greater the surface area, making the infusion more effective (Ummah, 2019). The calculation of the ingredients used is listed in Table 4.

*Table 4. Calculation of Ingredients Used in Infusion*

| Water (ml) | Tea Powder (g) |
|---|---|
| 1000 | 30 |

Preparation of Gambyong Kemuning Green Tea Kombucha (Camellia sinensis L.)

The extraction method used to obtain Gambyong Kemuning green tea (Camellia sinensis L.) extract is the infusion method. This process involves brewing the herbal extract with hot water at 90°C for 15 minutes. During the brewing process, sugar is added as an energy source for the SCOBY (Symbiotic Culture of Bacteria and Yeast) to carry out the fermentation process. Then, cool the infusion and add the specified amount of starter. Place it in a glass container and cover with a clean cloth. The fermentation process takes up to 12 days. The calculation of ingredients used refers to previous research and is listed in Table 5.

*Table 5. Calculation of Ingredients in Making Kombucha Preparations*

| Water (ml) | Powder (g) | Sugar (g) | Starter (g) |
|---|---|---|---|
| 1000 | 30 | 120 | 60 |

Extract Standardization

Metal and Lead Free Test

Metal and lead free tests were conducted on Gambyong Kemuning green tea (Camellia sinensis L.) infusion and kombucha preparations using the tube method. This test aimed to determine the lead (Pb) content using two reagents: K2CrO4 solution and NaOH solution. The preparation reacted with K2CrO4 produced a white precipitate, namely lead hydroxide (Pbl2), while the preparation reacted with NaOH produced a white precipitate, namely lead hydroxide (PbOH2). Metal precipitation occurs due to the reaction between the metal with iodine and hydroxyl anions (Rahmadani et al., 2021). The results of the Metal and Lead Free Test on Gambyong Kemuning Green Tea (Camellia sinensis L.) Infusion and Kombucha Preparations are shown in Table 6.

*Table 6. Metal and Lead-Free Test Results for Gambyong Kemuning Green Tea (Camellia sinensis L.) Infusion and Kombucha Preparations*

| Identification | Procedure | References | Results | Description |
|---|---|---|---|---|
| Metal-Free Test | Infusion extract + NaOH | Positive for metal if a white precipitate appears. (Ningsih et al., 2024) | No white precipitate | No metal content |
| Metal-Free Test | Kombucha extract + NaOH | Positive for metal if a white precipitate appears. (Ningsih et al., 2024) | No white precipitate | No metal content |
| Lead-Free Test | Infusion extract + $K_2CrO_4$ reagent | Positive for lead if a yellow precipitate appears. (Ningsih et al., 2024) | No yellow precipitate | No lead content |
| Lead-Free Test | Kombucha extract + $K_2CrO_4$ reagent | Positive for lead if a yellow precipitate appears. (Ningsih et al., 2024) | No yellow precipitate | No lead content |

The metal- and lead-free tests are conducted to determine if the extract contains metals or lead. The extract used should be free from metals and lead so that it does not have a negative impact when administered orally to test animals (Rahmadani et al., 2021).

Ethanol-Free Test

The ethanol-free test was conducted on the infusion and kombucha preparations of Gambyong Kemuning green tea (***Camellia sinensis*** L) using a test tube method. This test aimed to determine the ethanol content in the preparations by using $H_2SO_4$ and $CH_3COOH$. The reaction indicating the absence of ethanol is marked by the lack of a fragrant ester odor. If an ester odor is present, it can be confirmed that ethanol, which has undergone an esterification reaction, is still present in the preparation (Priamsari & Yuniawati, 2019). The results of the ethanol-free test on the infusion and kombucha preparations of Gambyong Kemuning green tea (***Camellia sinensis*** L) can be seen in Table 7.

*Table 7. Results of the Ethanol-Free Test on Infusion and Kombucha Preparations of Gambyong Kemuning Green Tea ( Camellia sinensis L)*

| Identification | Procedure | Reference | Results | Description |
|---|---|---|---|---|
| Ethanol-Free Test | Infusion extract + $H_2SO_4$ + $CH_3COOH$ + Heating | Ethanol-free if there is no ester odor. (Ningsih et al., 2024) | No ester odor | No ethanol content |
| Ethanol-Free Test | Kombucha extract + $H_2SO_4$ + $CH_3COOH$ + Heating | Ethanol-free if there is no ester odor. (Ningsih et al., 2024) | No ester odor | No ethanol content |

The ethanol-free test aims to determine whether the extract used contains ethanol. The extract used should ideally be free of ethanol so that it does not have a negative impact when administered orally to test animals (Priamsari & Yuniawati, 2019).

pH Test

The pH test is conducted to determine the acidity and alkalinity of the solution being tested. The test can be performed using a pH meter or pH paper (Aprilia et al., 2023). The pH meter should be calibrated first using a buffer solution. Then, the pH testing is performed on the kombucha for 14 days, from May 10th to May 23rd. The results of the pH test on the kombucha preparation of Gambyong Kemuning green tea (*Camellia sinensis* L) are listed in Table 8.

*Table 8. pH Test Results for the Kombucha Preparation of Gambyong Kemuning Green Tea*

| Identification | Test Date | Results | Description |
|---|---|---|---|
| pH Test | 10/05/25 | 3.33 | (+) |
| pH Test | 11/05/25 | 3.30 | (+) |
| pH Test | 12/05/25 | 3.23 | (+) |
| pH Test | 13/05/25 | 3.18 | (+) |
| pH Test | 14/05/25 | 3.12 | (+) |
| pH Test | 15/05/25 | 3.08 | (+) |
| pH Test | 16/05/25 | 2.84 | (+) |
| pH Test | 17/05/25 | 2.72 | (+) |
| pH Test | 18/05/25 | 2.65 | (+) |
| pH Test | 19/05/25 | 2.51 | (+) |
| pH Test | 20/05/25 | 2.41 | (-) |
| pH Test | 21/05/25 | 2.34 | (-) |
| pH Test | 22/05/25 | 2.30 | (-) |
| pH Test | 23/05/25 | 2.22 | (-) |

**Description:**

(+) : Meets the pH standard

(-) : Does not meet the pH standard

Based on Table 8, it can be concluded that the results of the kombucha pH testing from the 20th to the 23rd did not meet the pH standards if following the references from previous journals. During the fermentation process, the pH value ranges between 4.2 and 2.5; this range is still considered safe for consumption. A pH value below 2.5 poses a health risk due to excessively high acetic acid content (Sulistiawaty & Solihat, 2022). Meanwhile, the pH test results from the 10th to the 19th met the pH standard.

## 4. Conclusions

This study successfully standardized Gambyong Kemuning green tea (** *Camellia sinensis* ** L) simplicia, standardized the infusion and kombucha extracts, and assessed the pH stability of the kombucha product during 14 days of fermentation. The results of the simplicia standardization tests indicated that the raw material met the recommended standards, with safe limits for drying loss (4.6%), moisture content (6.12%), and total ash content (3.61%), effectively preventing microbial growth and contamination.

Furthermore, safety tests on both the infusion extract and the kombucha product showed negative results for heavy metal lead (Pb) content and were free from ethanol residue, indicating that the product is safe for oral consumption. The stability assessment based on pH over the 14-day fermentation period demonstrated that the kombucha product is safe for consumption until day 10, with a pH range between 4.2 and 2.5. However, from day 11 to day 14, the pH values dropped below the safe limit of 2.5, indicating an increase in acetic acid levels that could pose health risks.

Overall, Gambyong Kemuning green tea kombucha has the potential as a safe and standardized herbal product, provided that the fermentation duration is strictly monitored and does not exceed 10 days to maintain pH stability and consumer safety.

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