

RESEARCH ON KOMBUCHA FERMENTATION OF VIETNAM GREEN TEA (*CAMELLIA SINENSIS*), APPLIED ON FACIAL TONER FORMULATION

NGHIÊN CỨU QUY TRÌNH LÊN MEN KOMBUCHA TỪ TRÀ XANH VIỆT NAM, ỨNG DỤNG TRONG CÔNG THỨC NƯỚC CÂN BẰNG DA

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ABSTRACT

Kombucha is a fermented sweetened tea that is abundant in organic acids such as acetic acid, lactic acid, gluconic acid, etc., antioxidants such as flavonoids, polyphenol, etc., and vitamins such as B1, B2, B6, B12 and C which are promising to be applied on skin care products. Fermentation of Vietnamese green tea was commonly applied in beverage but their application in skin care cosmetics in Vietnam is surprisingly mediocre. This study was conducted to optimize the Kombucha fermentation of Vietnamese green tea (*Camellia sinensis*) aimed at applying in facial toner. The result showed that the optimized fermentation condition was green tea 100g, SCOBY 50g, sucrose 100g, pH 4.5 in water 1000mL for 14 days at room temperature. Toner formulation using 15% of the Kombucha fermentation has a pH of 6.0, viscosity of 11.5mm²/s and being able to maintain good texture after 28 days in acceleration test at 50°C. The toner was certified for standards such as skin irritation, heavy metal content, and microbial limit by National Institute of Drug Quality Control (NIDQC). A preliminary sensory survey was conducted and gave excellent results, which is promising in further commercial applications.

Keywords: Kombucha, fermentation, Vietnamese green tea, gluconic acid, cosmetics, facial toner.

TÓM TẮT

Kombucha là dịch trà ngọt lên men có chứa nhiều các axit hữu cơ như axit acetic, axit lactic, axit gluconic..., các chất chống oxy hóa như flavonoids, polyphenol..., và các vitamin như B1, B2, B6, B12 và C, có tiềm năng ứng dụng trong các sản phẩm chăm sóc da nói chung. Dịch lên men trà xanh của Việt Nam thường được ứng dụng phổ biến trong đồ uống nhưng lại hiếm khi thấy ứng dụng trong mỹ phẩm chăm sóc da ở Việt Nam. Nghiên cứu này được tiến hành nhằm tối ưu hóa quy trình lên men trà Kombucha từ trà xanh Việt Nam nhằm ứng dụng trong công thức toner. Kết quả chỉ ra điều kiện lên men tối ưu là sử dụng 100g lá trà, 50g SCOBY, 25g đường, pH ban đầu là 4,5 trong 1000mL nước kéo dài 14 ngày ở nhiệt độ phòng. Công thức toner sử dụng 15% dịch trà Kombucha có pH được điều chỉnh ở 6,0 có độ nhớt 11,5mm²/s và có thể bảo quản tốt sau 28 ngày trong thử nghiệm gia tốc. Sản phẩm được kiểm nghiệm về các chỉ tiêu như kích ứng da, hàm lượng kim loại nặng và giới hạn nhiễm khuẩn bởi Viện Kiểm nghiệm Thuốc Trung ương - Bộ Y tế. Khảo sát cảm quan sơ bộ được tiến hành và thu được kết quả đánh giá cao, hứa hẹn có thể ứng dụng trong thương mại sau này.

Keywords: Kombucha, lên men, trà xanh Việt Nam, axit gluconic acid, mỹ phẩm, nước cân bằng da.

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1. INTRODUCTION

Kombucha is a naturally fermented beverage made by inoculating a sweetened tea base typically derived from *Camellia sinensis* (green or black tea) with a

symbiotic culture of bacteria and yeast (SCOBY). Kombucha comes in two forms, solid and liquid. Of these two forms, liquid Kombucha is the most popular product.

The active ingredients in Kombucha that have potential benefits for human health and skin are derived from tea polyphenols, especially epigallocatechin gallate [1, 2], vitamins B1, B2, B6, B12 and C [3, 4], and organic acids such as gluconic acid, glucuronic acid [5, 6] or D-saccharic-1,4-lactone acid [7] by SCOBY.

During fermentation, the microbial activity breaks down sucrose into glucose and fructose, which are further metabolized into ethanol, acetic acid, gluconic acid, and other beneficial metabolites. The resulting kombucha tea is characterized by a slightly acidic pH, mild effervescence, and a complex chemical profile with potential antioxidant, antimicrobial, and anti-inflammatory effects making it a promising active ingredient in cosmetic applications.

1.1. Green tea leaves

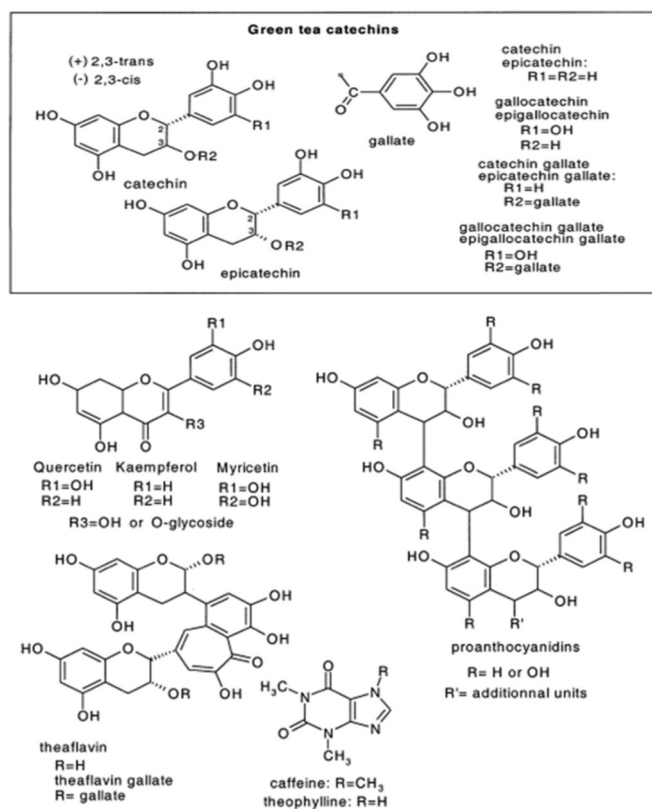


Figure 1. Chemical structure of chemical components in green tea

Green tea, derived from the leaves of *Camellia sinensis*, is a widely consumed beverage known for its rich composition of bioactive compounds, particularly polyphenols such as catechins. Unlike black tea, green tea leaves are not subjected to enzymatic oxidation during processing. Instead, they are quickly steamed or pan-fired after harvest, a method that preserves the integrity of their natural antioxidants especially epigallocatechin gallate (EGCG), which has been widely studied for its

beneficial effects on skin health, including anti-inflammatory, antimicrobial, and photoprotective properties.

Polyphenolic compounds are the main and most important components of tea leaves. They include flavonoids, tannins, and alkaloids. Flavonoids: include catechins and flavonols.

Catechins are colorless, easily oxidized compounds, in the form of flavanol-3. Its derivatives: (j)-epicatechin (EC), (j)-epicatechin gallate (ECG), (j)- epigallocatechin (EGC), (j)-epigallocatechin gallate (EGCG), (+)-catechin (C), and (+)-gallocatechin (GC) [8]. Catechins have the ability to reduce the absorption of cholesterol in the intestine [9]. Catechins have the strongest antioxidant capacity of all known plant phenols. EGCG is 20 times more powerful than vitamin C, 30 times more powerful than vitamin E, and 2-4 times more powerful than butylated hydroxyanisole (BHA) and hydroxytoluene (BHT) [8].

The main flavonol compounds are quercetin, kaempferol, myricetin and their glycoside derivatives. Quercetin and theanine reduce blood pressure, thereby reducing the development of cardiovascular diseases. Many scientific studies have shown that: Catechin and flavonol compounds of tea have very good antioxidant properties with the presence of morphological forms that can react with oxygen and free radicals under both hydrophilic and hydrophobic conditions [8].

As a fermentation substrate in kombucha production, green tea offers a unique chemical environment that influences both microbial dynamics and metabolite profiles. Studies have shown that green tea supports the growth of certain lactic acid bacteria, notably *Oenococcus oeni* and *Lactobacillus nagelii*, contributing to a higher production of lactic and acetic acids during fermentation. These organic acids, in combination with retained tea catechins, may enhance the skin-conditioning and protective potential of kombucha-based formulations when applied topically.

The combination of green tea's intrinsic phytochemicals and its ability to guide beneficial microbial activity during kombucha fermentation makes it an ideal candidate for developing natural, biofunctional ingredients in skincare products such as facial toners.

1.2. Tea fungus (SCOBY)

SCOBY, an acronym for Symbiotic Culture of Bacteria and Yeast, is the functional microbial consortium responsible for fermenting sweetened tea into

kombucha. This cellulose-based biofilm, often referred to as the “tea fungus,” floats on the surface of the tea and provides a structured matrix for microbial growth under aerobic conditions. The primary microbial members include acetic acid bacteria (AAB), such as *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, and *Komagataeibacter* [10-13], and yeasts such as *Zygosaccharomyces*, *Candida*, *Lachancea*, *Kloeckera/Hanseniaspora*, *Torulaspora*, *Rhodotorulaspora*, *Pichia*, *Brettanomyces/Dekkera*, *Saccharomyces*, *Schizosaccharomyces* and *Saccharomycoide* [10-16].

SCOBY initiates fermentation by metabolizing sucrose into glucose and fructose, which are further converted into a complex array of bioactive compounds. These include organic acids (acetic, gluconic, glucuronic, and lactic acid), ethanol, CO₂, vitamins (notably B-complex and C), and polyphenol derivatives. These metabolites are not only responsible for kombucha’s unique taste and acidity but also contribute potential antimicrobial, antioxidant, and anti-inflammatory effects—attributes of high interest for topical skincare formulations.

As both a physical structure and a living microbial engine, the SCOBY plays a central role in determining the chemical profile and functional quality of kombucha, especially when green tea is used as a fermentation base.

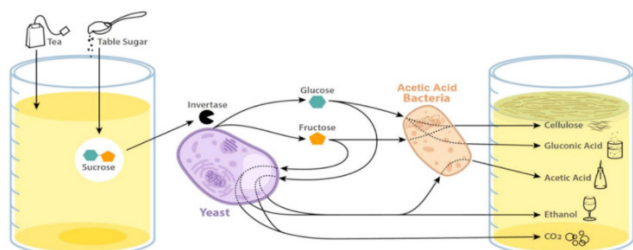


Figure 2. Mechanism of action of Yeasts and Microorganisms in SCOBY [17]

1.3. Factors affecting fermentation process

Effect of Substrate Concentration

The main substrates for kombucha fermentation are tea leaves and sugar solution. In tea leaves, the primary components are carbohydrate compounds, which are key factors influencing the fermentation process of kombucha. The content of sugars and starches in tea leaves is quite low, while pectin appears in relatively high amounts (sugar: 0.73 - 1.41%; pectin: 6.1%; starch: 0.82 - 2.96%). Therefore, additional sugar must be added during kombucha fermentation to provide sufficient nutrients for microbial activity.

The composition of the sugar solution plays an important role in the fermentation performance of

kombucha. However, this composition must be within an appropriate structural range and ratio to support the growth and development of the microbial culture. This structure typically falls within three thresholds: minimum, optimal, and maximum. At the optimal value, nutrients are supplied in a beneficial way that allows the kombucha culture to grow, develop, and metabolize normally with complete physiological characteristics thus producing the desired final product. On the other hand, if the nutritional composition is insufficient or excessive, it can inhibit microbial activity.

Effect of Temperature

Temperature directly affects the growth and metabolism of microorganisms. Therefore, identifying the optimal temperature range is essential for better control of the fermentation process. To determine this optimal range, laboratory experiments must be conducted using available fermentation equipment. From these experiments, solutions can be developed to stabilize the fermentation process such as temperature control using heat transfer plates or airflow regulation.

Effect of pH

The pH value of the environment influences microbial growth and metabolite production in different ways. There are pH levels at which microorganisms can still grow normally, but product formation may be limited or absent. Each microbial group has a specific optimal or minimum pH for growth. Many molds can thrive at pH 2 - 4.5, yeasts at pH 4 - 4.5, while pathogenic and spoilage bacteria often prefer neutral to alkaline environments. Therefore, modifying pH can be an effective method for product preservation and for establishing suitable conditions to produce the desired kombucha quality.

Effect of Fermentation Time

Fermentation time is also a key criterion in evaluating the effectiveness of the kombucha fermentation process. Typically, kombucha is fermented over a period of 5 to 14 days. A 14-day fermentation tends to yield a product with favorable qualities, although the acidity level can be relatively high, around 8g/L (based on acetic acid content). Therefore, research is needed to determine the optimal fermentation duration that ensures product quality while balancing economic feasibility for practical production.

1.4. Facial Toner (Toner)

Facial toner, also known simply as toner, is a liquid skincare cosmetic. Toners help cleanse dirt from areas

that facial cleansers may not fully clean, remove excess oil, support better absorption of nutrients from moisturizers, and simultaneously prevent acne, tighten pores, reduce the risk of environmental damage, and balance the skin's pH level [20]. Common ingredients in facial toner include:

Table 1. Common ingredients in facial toner

No.	Ingredient	Function
1	Solvent	Dissolve and dilute other ingredients
2	Thickener	Modify, adjust, and control viscosity and consistency
3	Humectant	Retain moisture of the product
4	Emollient	Enhance skin softness and moisturization
5	Preservative	Prevent product spoilage
6	Extract	Add functionality to the product
7	Fragrance	Provide scent to enhance product experience
8	Additives	Adjust physical and chemical properties of the product

Facial toner is a commonly used cosmetic product in skincare routines, formulated to cleanse the skin, remove residual impurities, balance pH levels, and prepare the skin for subsequent treatments. As consumer expectations for skincare products continue to rise, the evaluation of toner quality has become increasingly important to ensure both efficacy and safety. Toner evaluation standards are typically based on a combination of **physicochemical**, **microbiological**, and **sensory** parameters. Key physicochemical criteria include pH, viscosity, stability (under various temperature and light conditions), and solubility. These attributes ensure the product is compatible with the skin, maintains consistency over time, and remains effective during use. **Microbiological testing** is essential to ensure the product is free from harmful microorganisms and complies with cosmetic safety regulations. **Sensory evaluation** such as texture, scent, skin feel, and absorption plays a crucial role in determining consumer acceptance and overall product experience.

2. MATERIALS AND METHODS

2.1. Method of fermenting Kombucha tea from *Camellia sinensis* green tea

2.1.1. Determining the Effect of Sugar Concentration on the Fermentation Process

To investigate the influence of sugar concentration on the fermentation process of kombucha, 100 grams of tea leaves were infused in 1 liter of water and boiled for 20 minutes. The resulting tea infusion was sterilized at 121°C

for 20 minutes. Preselected sugar was then added to the infusion at varying concentrations: 0, 80, 90, 100, 110, and 120g/L. After the sugar addition, the solutions were further sterilized at 121°C for 20 minutes. Once cooled to room temperature, the tea infusions were transferred to fermentation vessels. Each fermentation batch was inoculated with 0.25 liters of pre-fermented kombucha solution and 50 grams of SCOBY under identical conditions. The fermentation was carried out at 24 - 26°C for a duration of 14 days.

2.1.2. Determination of Sugar Content Using Bertrand Method

Preparation of Fehling's Solutions:

- **Fehling A:** 34.64 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were dissolved in distilled water and diluted to 500mL.
- **Fehling B:** 173 grams of potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) and 50 grams of NaOH were dissolved in distilled water and diluted to 500mL.
- 5mL of Fehling A and 5mL of Fehling B were mixed in a conical flask.

A measured amount of kombucha extract was diluted with distilled water to a defined volume. 10mL of the diluted extract was added to the Fehling's mixture. The solution was heated in a water bath at 70 - 80°C for 10 minutes until a brick-red precipitate formed. The precipitate was filtered and washed with distilled water. The precipitate was dissolved in 10mL of 25% H_2SO_4 and titrated with 0.02N KMnO_4 until a persistent pale pink color appeared for 30 seconds. The volume of KMnO_4 consumed was used to calculate the sugar content of the sample.

2.1.3. Determination of Glucuronic Acid Content via Colorimetric Method

According to the studies of Robert E. Mosher, a colorimetric method was established based on the correlation between absorbance and glucuronic acid concentration in kombucha. A standard calibration curve was used for relative quantification.

Procedure:

2mL of the fermented kombucha sample was added to a 15mL centrifuge tube, followed by 6mL of distilled water and 1mL of sodium tungstate solution. The mixture was shaken for 10 minutes using a shaker, then centrifuged. 5mL of the supernatant was transferred into a rectangular glass test tube (25 × 200mm). 4mL of naphthoresorcinol reagent and 3mL of HCl were added,

mixed thoroughly, and incubated in a thermal block for 60 minutes. The solution was then cooled for 10 minutes, followed by the addition of 5mL of β,β' -dichloroethyl ether. The mixture was shaken vigorously for 30 seconds and allowed to separate into two phases. The aqueous phase was removed, and the organic layer was transferred to a 1cm pathlength cuvette. Absorbance was measured at 580nm. The glucuronic acid concentration was determined using the pre-established calibration curve.

2.1.4. Method for determining appropriate fermentation time

After inoculating the tea fungus into the fermentation liquid, the liquid was fermented under sterile conditions for different periods. To evaluate the fermentation quality during the research process, 3 sensory standards were used: color, taste, and state, along with pH. The pH of the kombucha tea was tested using a pH meter (Hanna HI98107). Research formula for facial toner containing Kombucha

Table 2. Research formula for facial toner containing Kombucha

Phase	No.	Ingredient	Function	% w/w			
				F1	F2	F3	F4
A	1	Water	Solvent	Qs	Qs	Qs	Qs
	2	EDTA	Buffer	0.1	0.1	0.1	0.1
	3	Glycerin	Humectant	10	10	10	10
	4	Allantoin	Anti-irritant	0.3	0.3	0.3	0.3
	5	Geogard ECT	Preservative	0.5	0.5	0.5	0.5
B	6	1,2-Hexanediol	Solvent	1.5	1.5	1.5	1.5
	7	Kombucha tea	Active	15	20	25	0
C	8	Triethanolamine	pH adjuster	Qs	Qs	Qs	Qs

2.2. Evaluation of Kombucha Toner

2.2.1. Sensory Evaluation

The sensory evaluation included the assessment of appearance, odor, texture, and color. The appearance, texture, and color of the product were evaluated through direct visual observation. The fragrance of the toner was assessed through olfactory testing.

2.2.2. pH Measurement

The pH of the toner containing kombucha tea extract was measured using a digital pH meter (Hanna HI98107).

2.2.3. Viscosity Measurement

The viscosity of the kombucha-based toner was determined using a cone-and-plate viscometer (Anton Paar ViscoQC 100).

2.2.4. Accelerated Stability Testing (Thermal Cycling Test)

The thermal cycling test was carried out over three cycles spanning six days to simulate long-term stability under fluctuating temperature conditions. Each cycle consisted of 24 hours at $4 \pm 2^\circ\text{C}$, followed by 24 hours at $40 \pm 2^\circ\text{C}$. The product was observed after each cycle. Evaluated parameters included sensory characteristics, homogeneity, pH, and viscosity.

2.2.5. Volunteer-Based Consumer Survey

A consumer perception study was conducted with 100 volunteers aged 15 to 35. Prior to irritation testing, participants performed a patch test by applying a small amount of the product behind the ear. The volunteers then used the toner and completed a questionnaire assessing product attributes such as appearance, fragrance, viscosity, and perceived efficacy after use.

3. RESULT AND DISCUSSION

3.1. Research results on Kombucha tea fermentation process

3.1.1. Determine the effect of sugar on the fermentation process

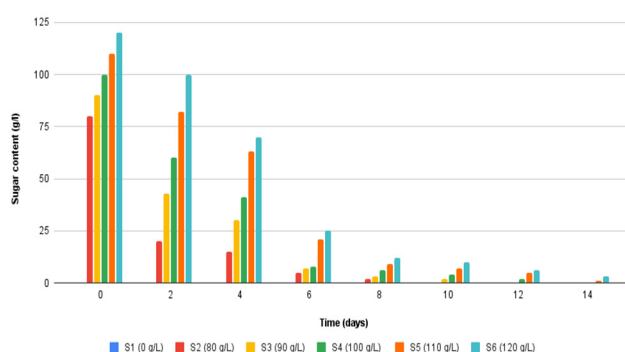


Figure 3. Sugar content of 6 Kombucha tea samples over 14 days fermentation

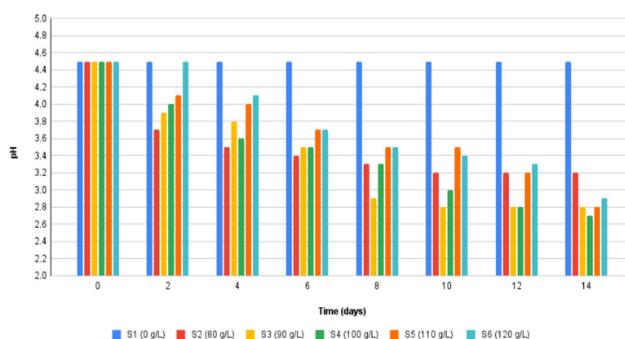


Figure 4. pH of 6 Kombucha tea samples over 14 days fermentation

To study the effect of sugar content on the fermentation process, sucrose was selected as the

hydrocarbon source for the fermentation. The study was conducted on 5 fermentation samples with the following concentrations: S1 = 0g/L (control sample without sucrose), S2 = 80g/L, S3 = 90g/L, S4 = 100g/L, S5 = 110g/L, and S6 = 120g/L. The remaining sugar content during the fermentation period was measured and showed the following results:

From the experimental results presented in Figures 3 and 4, the following observations were made:

In sample S1, which did not receive any sucrose supplementation, the sucrose concentration remained at zero throughout the fermentation period, indicating that no substrate was available for microbial conversion. In samples S2 and S3, the sucrose was rapidly depleted and completely converted within a short period. However, due to the insufficient initial sucrose concentration, these samples were unable to develop the characteristic organoleptic properties of kombucha, including its typical flavor profile.

Sample S4 demonstrated complete sucrose depletion by the end of the fermentation period, suggesting that the amount of added sucrose was just sufficient to support full fermentation and flavor development. The resulting product exhibited the typical sensory characteristics of kombucha, indicating optimal substrate utilization for this sample.

In contrast, samples S5 and S6 retained residual sucrose at the end of fermentation. This remaining sugar imparted an overly sweet aroma and taste that masked the distinctive kombucha flavor, suggesting an excess of substrate relative to microbial fermentation capacity.

In conclusion, a sucrose concentration of 100g/L was found to be optimal, as it allowed for complete substrate conversion while enabling full development of kombucha's characteristic sensory profile by the end of the fermentation process.

3.1.2. Changes in glucuronic acid concentration during fermentation compared to sucrose solution

When experiments were conducted on samples G1, G2, G3, G4, G5, and G6 with sucrose concentrations of 0, 80, 90, 100, 110, and 120g/L, the research results are shown in Table 3.

In sample G1, due to the inability of the microbial culture to initiate fermentation, signs of mold appeared on the surface after two days, and the kombucha culture showed signs of spoilage by day four. In contrast, samples G2 through G6 exhibited a rapid increase in glucuronic

acid concentration during the first six days of fermentation. From day 6 to day 14, the rate of increase slowed, and glucuronic acid levels peaked on day 14.

Table 3. The content of glucuronic acid of 06 Kombucha tea samples over 14 days fermentation

Time (days)	G1 (0 g/L)	G2 (80 g/L)	G3 (90 g/L)	G4 (100 g/L)	G5 (110 g/L)	G6 (120 g/L)
0	0.057	0.057	0.057	0.057	0.057	0.057
2	0.050	0.097	0.108	0.115	0.118	0.113
4	-	0.145	0.261	0.278	0.271	0.260
6	-	0.284	0.297	0.339	0.325	0.324
8	-	0.285	0.296	0.342	0.329	0.328
10	-	0.292	0.297	0.355	0.349	0.330
12	-	0.293	0.298	0.357	0.351	0.331
14	-	0.295	0.300	0.361	0.350	0.332

Among all samples, the highest glucuronic acid concentration was observed in sample G4, which corresponded to a sucrose concentration of 100g/L. This indicates that a sucrose concentration of 100g/L was optimal for kombucha fermentation, as it allowed for maximum production of fermentation metabolites while ensuring efficient substrate utilization. The highest glucuronic acid concentration recorded was 0.361g/L.

3.1.3. Determining the appropriate fermentation time

After studying the effects of sucrose concentration and glucuronic acid content, it was found that the fermentation process usually peaked on day 14 or later. Therefore, to clarify further, sensory testing was conducted on kombucha samples fermented at 26°C for 4 days, 6 days, 8 days, 12 days, 14 days, and 16 days to evaluate the optimal fermentation time for the product.

Table 4. pH and sugar content in Kombucha tea over 16 days

Time (day)	pH	Sugar content
4	3.6	41
6	3.5	8
8	3.3	6
10	3.0	4
12	2.8	2
14	2.7	0
16	2.7	0

The sensory evaluation showed that 14 days of fermentation resulted in the best pH and sugar content.

Based on all the research parameters, including temperature, substrate concentration, and composition, the research determined that the optimal fermentation time at the laboratory scale was 14 days.

3.1.4. Optimal Fermentation Process of Kombucha Tea

Based on the experimental results from the study on kombucha tea fermentation, the following optimal fermentation process was proposed:

Tea Leaf Extraction: The extraction of green tea leaves was performed at a temperature of 85 - 100°C for 20 minutes. The ratio of tea leaves to water during the extraction process was 10 -12%.

Cooling: After extraction, the tea extract was cooled to a temperature of 40 - 50°C and mixed with 100g/L of sucrose in preparation for fermentation.

Fermentation: The fermentation temperature was maintained at 25 - 28°C, with a fermentation period of 14 days. The initial pH of the solution was set to 4.5. After the appropriate fermentation period, the kombucha solution was tested for acid content, including glucuronic acid, gluconic acid, lactic acid, acetic acid, and residual sugars. The fermentation process was then halted, and the product was considered complete.

Microbial Filtration: After boiling at 70°C, the fermented solution was filtered through a microbial filter to remove any microorganisms.

Storage: The kombucha tea solution was stored at 4°C before being used as an ingredient in cosmetic products.

3.2. Research Results and Evaluation of Kombucha Toner

3.2.1. Kombucha toner formulation

The formula for kombucha tea toner includes kombucha tea extract, EDTA, glycerin, allantoin, Geogard ECT, and TEA. Kombucha tea extract is an active ingredient rich in organic acids beneficial for the skin. Glycerin acts as a humectant, helping to absorb moisture from the air (with humidity > 80%) and from the stratum corneum. It is one of the most widely used humectants as it reduces water loss from the skin and provides hydration under conditions of high humidity. Geogard ECT is a preservative used in many personal care products, providing broad-spectrum protection against bacteria, yeasts, and molds. Furthermore, it is water-soluble and has a stable pH. TEA functions as an alkalizing agent to neutralize the acidic pH of kombucha.

3.2.2. Evaluation of Kombucha toner

- Sensory testing, pH, and Viscosity

Sensory testing was conducted by observing the fragrance, color, and texture of the formulation. The purpose of this test was to improve product quality. According to Table 5, the results of the sensory evaluation showed that the kombucha toner formulation had a light yellow color, which was due to the addition of kombucha to the product. The higher the concentration of kombucha, the darker the color became. The fragrance in all kombucha toner formulations produced a sour smell. This sour scent came from the fermented kombucha, with acetic acid being a key compound contributing to the vinegar-like aroma in kombucha. All kombucha toner formulations had a liquid texture, were non-sticky, and were easily absorbed by the skin.

Table 5. Result of sensory testing, pH and viscosity of Kombucha tea formulations

Parameter	F1	F2	F3	F4
Color	Light yellow	Yellowish brown	Yellowish brown	Colorless
Odor	Mild	Sour	Sour	Odorless
Texture	Liquid, non-sticky	Liquid, non-sticky	Liquid, non-sticky	Liquid, non-sticky
pH	5,5	5,5	5,5	7,0
Viscosity	11,5 cps	12,4 cps	12,75 cps	11,4 cps

- Cyclic Stability Testing

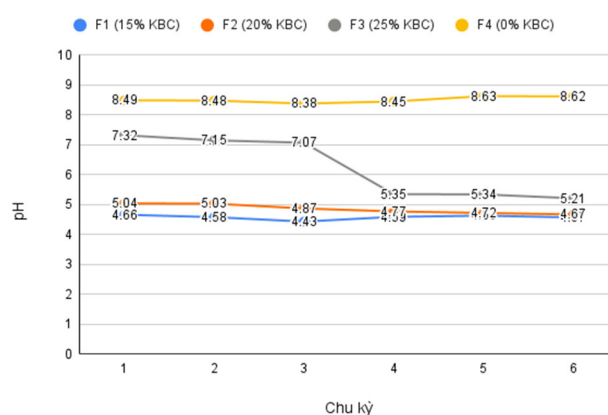


Figure 5. The change of pH of Kombucha toner formulations in cyclic stability testing

The cyclic stability of the tested formulation was evaluated by storing the product at temperatures of $\pm 4^{\circ}\text{C}$ and $\pm 40^{\circ}\text{C}$ for six cycles. Observed parameters included sensory characteristics, homogeneity, and pH. Sensory parameters indicated that no changes occurred in

fragrance, color, or texture in all kombucha turmeric toner formulations from cycle 1 to cycle 6. Homogeneity parameters showed that all kombucha turmeric toner formulations remained homogeneous from cycle 1 to cycle 6. The pH parameter in F3 started to decrease from cycle 4. In F2, the pH decreased, but to a lesser extent, while in F1 and F4, the pH remained relatively stable (Figure 5).

Sample F1 was selected because its evaluation results met the technical specifications desired for a facial toner, in liquid form, homogeneous, yellow in color, with a characteristic tea fragrance. F1 also exhibited the best and most stable viscosity.

- Volunteer Survey

The evaluation scale used in the testing was: 0 (dislike), 1 (neutral), 2 (like), and 3 (very like), meaning the maximum possible score for each parameter was 300. As shown in Figure 3.5, the scores for each parameter were as follows: appearance (295), fragrance (290), thickness (291), after-feel effect / moisturizing effect after toner application (297), and irritation (300). Appearance, after-feel effect / moisturizing effect, and irritation received positive ratings, indicating that these parameters were rated favorably.

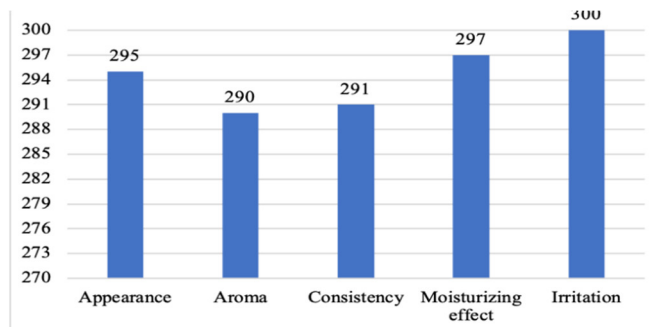
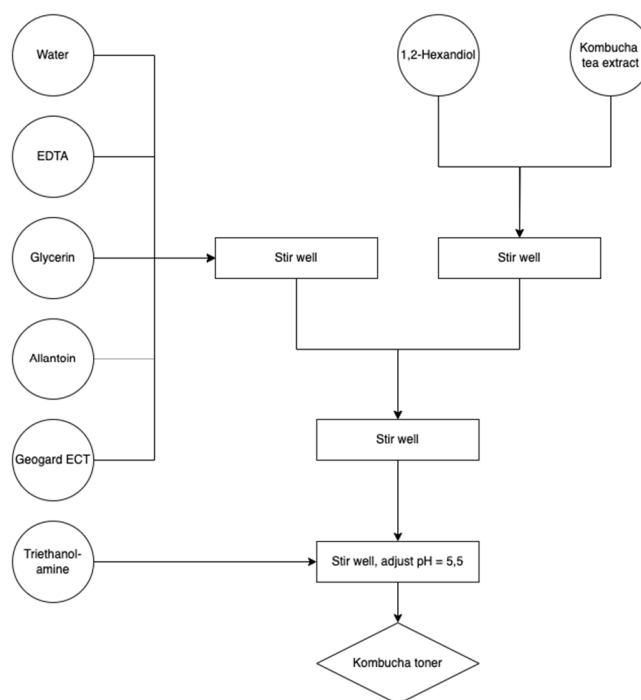


Figure 6. The results of volunteer survey

3.2.3. The optimize formulation of Kombucha toner

Table 6. The optimize formulation of Kombucha toner

No.	Ingredient	Function	% w/w
1	Water	Solvent	Qs
2	EDTA	Buffer	0.1
3	Glycerin	Humectant	10
4	Allantoin	Anti-irritant	0.3
5	Geogard ECT	Preservative	0.5
6	1,2-Hexanediol	Solvent	1.5
7	Kombucha tea extract	Active	15
8	Triethanolamine	pH adjuster	Qs



4. CONCLUSION

Based on the research results, the input factors (tea leaf to water ratio, type of sugar, sugar concentration, and fermentation time) for kombucha tea fermentation from *Camellia sinensis* green tea can be optimized. The optimal concentration of sugar used is 100g/L; and the fermentation period is 14 days. This process results in kombucha tea with a glucuronic acid content of 0.361g/L and a pH of 2.7.

From the research findings, it can be concluded that the effects of kombucha fermentation can be incorporated into kombucha toner products, meeting the required evaluation and stability testing criteria.

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