# Formulation of Tomato Fruit (*Lycopersicum esculentum Mill*) Kombucha Patch with Erosivity Test and Teeth Brightness Level

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**Abstract.** Kombucha is a probiotic fermented drink produced through a consortium of bacteria and yeast known as Scoby (Symbiotic Culture/Colony Bacteria & Yeast), serving as an initial culture that aids in the fermentation process. Kombucha also contains organic acids, including ascorbic acid, which is also found in tomatoes and can contribute to teeth whitening. This study aimed to assess the level of tooth brightness as a whitening agent and the degree of reduction in erosive effects on tooth specimens following the application of the tomato kombucha patch preparation. This study employed the methods of dependent and independent variables. The independent variable took the form of variations in the length of fermentation time for kombucha and tomato fruit kombucha in the preparation of teeth whitening patches. The dependent variable involved evaluations before the patch formation and evaluations after the patch formation. The results of the tomato kombucha patch formulation exhibit an increase in tooth brightness following the application process. Meanwhile, in the erosivity test, the tomato patch formulation exhibits a smaller erosion effect compared to the tomato kombucha patch formulation, as observed in the results of the Scanning Electron Microscope (SEM) characteristics test. The tomato kombucha patch formulation shows potential in enhancing tooth brightness due to the significantly increased content of ascorbic acid produced during the combination process of fermenting kombucha tea with tomatoes.

**Keywords:** Tomato, Patch formulation, Kombucha tea

## 1 Introduction

White and clean teeth are among the most coveted aesthetic aspirations, significantly influencing an individual's self-confidence during social interactions. In this modern era, appearance holds considerable importance and garners heightened attention. However, alterations in tooth color can diminish one's visual appeal, leading to discomfort in smiling and communication.

According to Riskesdas (Basic Health Research) (2022), dental and oral issues in Indonesia have surged to 58% of the population since the onset of the Covid pandemic. Contributing factors identified in Riskesdas during the pandemic include imbalances in


Z. B. Pambuko et al. (eds.), *Proceedings of 5th Borobudur International Symposium on Humanities and Social Science (BISHSS 2023)*, Advances in Social Science, Education and Humanities Research 856,
https://doi.org/10.2991/978-2-38476-273-6_125

personal hygiene practices, such as inadequate teeth brushing compared to handwashing, and a prevalent use of hand sanitizers without concurrent usage of mouthwash. Among the dental concerns faced by Indonesians, tooth discoloration is noteworthy, a condition often addressed through dental bleaching [1].

Nurwiyana [2] distinguishes between two types of teeth whitening methods: bleaching and whitening, utilizing either chemical or natural ingredients. Frequently employed chemical agents include hydrogen peroxide, carbamide peroxide, or non-hydrogen peroxide systems containing sodium chloride, oxygen, and sodium.

In Indonesia, teeth whitening preparations are primarily limited to commonly used products such as toothpaste and mouthwash. However, recent trends have introduced novel preparations gaining popularity, including teeth whitening pens, radiation technology, and instant teeth whitening gel.

A patch is a dosage form comprising one or more layers or polymer films containing drugs and/or other excipients. It may incorporate a mucoadhesive polymer layer for controlled drug release onto the oral mucosa, gingiva, or teeth [3].

The chosen patch for this study is a dry patch, demonstrating non-stickiness upon contact with hands or skin, with no release of active substances. Dry patches exhibit enhanced adhesive strength against teeth, remaining attached for prolonged periods, yielding improved whitening effects with lower concentrations of active substances [4].

Researchers have sought cost-effective and anti-erosion alternatives for natural dental bleaching materials. Tomatoes, containing peroxide compounds (Fauziah, Fitriyani, and Diansari [5]), have emerged as a potential natural teeth whitening agent. Lumuhu et al. [6] and Berliana [7] have investigated tomatoes' efficacy in teeth whitening, with both studies indicating that tomato juice is more effective and induces less erosion.

Kombucha is a probiotic fermented drink produced through a consortium of bacteria and yeast known as Scoby (Symbiotic Culture/Colony Bacteria & Yeast) as an initial culture that helps in the fermentation process [8]. Kombucha also contains organic acids, one of which is ascorbic acid, which is also present in tomatoes and known for its teeth-whitening properties. In previous research, tomatoes were used, vegetable tomatoes which are often circulated on the market and different types of patches, whereas in this study tomatoes were used, a type of fruit which was later combined or fermented with kombucha as the basic ingredient in making patches to prove and discover the latest research on dental and oral health.

Consequently, based on the aforementioned background, our study will focus on the 'Formulation of Tomato Fruit (Lycopersicum esculentum Mill) Kombucha Patch as an anti-erosion and teeth whitener.

## 2 Methods

### 2.1 Time and place of research

The research was conducted at the Laboratory of Natural Materials Technology and Microbiology, Universitas Buana Perjuangan Karawang. The research duration spanned approximately 5 months.

## 2.2 Tools and materials

The tools utilized in this research encompassed implements for fermentation, extracts, and preparation-making. These included 3-liter glass jars, knives, blenders, scales, stainless steel pans, funnels, filters, jute rope, napkins, aluminum foil, analytical scales, mortar, stamper, spatula, glass beaker, hot plate, film mold, micropipette, oven, weighing bottle, desiccator, measuring cup, silica, patch packaging holder, viscosester, thermometer.

The materials utilized in this research comprised tomatoes, granulated sugar, kombucha starter culture along with liquid scoby, HPMC, PVP, Glycerin, distilled water, tegaderm thin film, tooth specimens, and artificial saliva.

## 2.3 Process of making tomato fruit kombucha

Weigh the tomatoes, thoroughly wash them in running water. Cut the tomatoes into small pieces and blend them to a puree without adding water. Strain the tomato juice and retain the liquid. Boil the fruit juice until it reaches a boiling point, turn off the heat, add sugar, and stir until it dissolves. Allow the boiled tomato juice to cool to a temperature of 30°C. Place the mixture in a jar and add kombucha aged for 7 days. Seal the jar tightly and ferment for up to 7 days. After fermenting the tomato kombucha, measure the pH using a pH meter.

## 2.4 Film-forming liquid preparation process.

Accurately weigh all ingredients. Dissolve HPMC in a portion of distilled water (marked M1) in one beaker glass. Dissolve PVP in another portion of distilled water (marked M2) in a separate beaker glass. Mix M2 into M1 while stirring with a magnetic stirrer until homogenous. Add glycerin and methyl paraben to the solution and stir until homogenous.

## 2.5 Patch creation process

Pour the film-forming liquid into a calibrated mold and dry at 40°C for 18 hours to form a film. Separate the film from the mold and place it in an airtight container filled with silica. Measure the film's weight once constant, and cut it to size. Coat part of the film with a Tegaderm backing membrane to form a patch. Conduct an evaluation test for the level of brightness and erosivity of the tooth specimen.

## 2.6 Afnor method of salivary fluid preparation

The preparation of saliva fluid utilized the Afnor method in a 1-liter solution: Place 500 mL of distilled water into an Erlenmeyer flask. Add 0.26 g Na2HPO4, 0.33 g KSCN, 6.00 g NaCl, 0.20 g KH2PO4, and NaHCO3 to the flask. Stir the mixture using a magnetic stirrer. Add another 500 mL of distilled water, then check the pH using a pH meter.

### 2.7 Film and patch evaluation tests

1. Macroscopic evaluation

Physical visual observation of the film, including color and surface texture.

2. Measurement of film weight

Weighing 10 patches with a size of 6 $cm^2$. 3. Patch surface pH measurement allowing a 2 x 1 $cm^2$ patch to expand in 1 mL of distilled water for 2 hours at room temperature, then measuring the surface using a pH meter.

3. Test for Patch Swelling Degree

Immersing the patch in a petri dish containing 25 mL of artificial saliva solution. Weights are recorded every 5 minutes, with measurements carried out until the 30th minute.

### 2.8 Dental specimen preparation

The tooth specimen is cleaned, smeared with white nail polish, and the tooth color is measured using a shade guide (Vitapan Classical). Afterward, the tooth is soaked in the tea solution for 12 days, changing the solution daily.

### 2.9 Test for brightness level of teeth using the immersion method

The prepared tooth specimens are placed in a sealed container containing tomato fruit kombucha, tomatoes, and kombucha. After seaing, the teeth are soaked for 42 hours. Post-soaking, the teeth are washed with running water, and the tooth color is measured again using shade guides (Vitapan Classical).

### 2.10 Test for brightness level of teeth after patch application

The patch formula will be applied to different groups of teeth. Tooth specimens are positioned upright by embedding tooth roots in plasticine. Each tooth specimen is moistened with 50 μL of artificial saliva, and the patch is applied with slight pressure until it remains in position. The application lasts for 3 hours, maintaining humidity by dripping artificial saliva (3 mL every 10 minutes). After application, the tooth specimen is washed, brushed, and dried at room temperature. The procedure is repeated 21 times, following the recommendations for using commercial products in terms of number and duration of patch application.

### 2.11 Tooth erosivity test after patch application

One sample of a tooth specimen is randomly selected from each group: untreated, hydrogen peroxide patch, tomato juice test, kombucha test, and tomato kombucha test. The sample will be examined for morphology using a Scanning Electron Microscope (SEM).

## 3 Result And Discussion

### 3.1 Results of the Kombucha Making Process

The fermentation process for kombucha tea was conducted over 14 days, utilizing two jars, each containing 2.5 liters. The first jar underwent fermentation for 14 days, while the second jar underwent fermentation for 7 days. In the second jar, fermentation on the 7th day was combined with tomato juice, followed by an additional 7 days of fermentation.

Kombucha tea fermentation results underwent organoleptic tests, assessing color, smell, taste, and pH. In the jar fermented for 14 days, the color produced is more intense compared to that fermented for 7 days. Longer fermentation times, as suggested by Akbar et al. [9], tend to yield a darker or brownish color due to increased microbial consortium activity during fermentation. Several studies attribute this phenomenon to the microbial consortium's ability to engage in symbiosis, leading to an increased population and consequently, a darker color.

In the organoleptic test results concerning taste and smell, it is observed that the longer the fermentation time, the more pronounced the sour taste and the stronger the sour smell. According to a study by Ningtyas [10], the total acid content increases as yeast and bacteria metabolize sucrose during the fermentation process, producing various organic acids, such as acetic acid and gluconic acid. This metabolic process leads to a higher total acid content.

At the initiation of fermentation, the tea retains a sweet taste, but this sweetness diminishes as sugar is broken down. Simultaneously, bacterial activity contributes to the emergence of a sour taste, initiating a transition from sweet to sour flavors. Adjusting the duration of fermentation allows for control over taste preferences; an early halt in fermentation results in a slightly sweet taste, while a more extended fermentation period produces a more pronounced sour taste. A study by Simanjutak [11] affirms that during fermentation, sugar transforms into alcohol, subsequently breaking down into acid, thereby causing an elevation in total acidity. This aligns with the findings from kombucha fermentation, where the total acid content was highest on the 14th day.

In the jar fermented for 7 days, a combination with tomato juice was introduced on the 7th day, followed by an additional 7 days of fermentation. The resulting tomato kombucha exhibits a more dominant color from the tomatoes, with a distinctive smell. The total acid content after fermenting with tomato juice presents a dominant sour taste from the tomatoes and a sour taste in the kombucha.

In this study, a dry-type tooth whitening patch was formulated, comprising two layers, namely the main layer containing a combination of HPMC and PVP polymers. The

main layer is composed with a polymer content of 10% w/w, maintaining a HPMC:PVP concentration ratio of 7:3 in each formula. The comparison is grounded in the optimization process conducted in preliminary tests, ensuring that the resulting film layer demonstrates resistance in film formation and exhibits adhesive properties to the teeth.

Hydrogen peroxide (H2O2) is an inorganic chemical widely used in the industrial sector, serving as a bleaching agent for paper, clothing, textiles, and as a disinfectant (germ killer) for furniture. However, it is a compound deemed unsafe for human use due to its oxidant properties, potentially inducing conditions in cells that shift from reductive to oxidative. Continuous consumption may lead to cancer [12]. The mucoadhesive characteristics of the patch necessitate support from appropriate materials. Previous studies have employed various synthetic, semi-synthetic, and natural polymers. Polyvinylpyrrolidone (PVP) acts as a developing agent, proving beneficial for increasing drug release, enhancing elasticity, and forming a film layer on the patch, with the hope that it will contribute to a fast (effective) and potentially impactful effect [13].

The active substance employed as a teeth whitening agent was tomato juice, which possesses a pH range of 4.4-4.6. This pH measurement was conducted to ensure the stability and compatibility of the patch formula composition at an acidic pH. HPMC and PVP are polymers known for their stability in solutions with acidic pH levels. The HPMC solution maintains stability at pH levels of 3-11, while the PVP solution maintains stability at pH levels of 3-7 [7]. The active substances are diversified into three formulas with distinct tomato juice concentration ratios—55% (F1), 60% (F2), and 65% (F3). This variation aims to investigate the impact of tomato juice concentration on the resulting teeth whitening effect.

The inclusion of glycerin at 50% w/w of the total polymer weight is grounded in an optimization process, ensuring that the resulting film layer exhibits excellent flexibility.

The selection of distilled water as a solvent is based on the favorable solubility of PVP and HPMC in water, coupled with their compatibility with the active substance—hydrogen peroxide—contained in tomatoes [7].

In the film layer making process, a solvent casting technique is employed, utilizing distilled water as a solvent. This technique is selected for its simplicity in procedures and equipment. The mold, filled with film-forming liquid, is placed in an oven at 40°C for 18 hours until a film layer is formed. The selection of temperature and drying time is based on an optimization process. Subsequently, the film layer is cut into pieces measuring 6x1.5 cm.

The results obtained from macroscopic testing were utilized to observe organoleptics in longitudinal and cross sections. In F1, the results exhibit a reddish-orange color; in F2, they appear brownish-white and unclear; in F3, they exhibit a brownish-orange color; and in F4, they appear slightly brownish but clear. The texture of F1 is thin yet not brittle; F2 is not overly thin yet not brittle; F3 is thin, flexible, and non-brittle; and F4 is thin. The smell of F1 has a typical tomato smell, F2 has a typical kombucha smell, F3 has a typical tomato smell with a slightly strong kombucha smell, and F4 has a typical H2O2 smell.

**Table 1.** Table 1. Macroscopic Test Results

| | Macroscopic Testing | | | |
|---|---|---|---|---|
| | F1 (Tomato) | F2 (Kombucha) | F3 (Tomato Kombucha) | F4 (H2O2) |
| **Color** | Reddish-orange | Brownish-white and unclear | Brownish-orange | Slightly brownish but clear |
| **Texture** | Thin yet not brittle | Not overly thin yet not brittle | Thin, flexible, and non-brittle | Thin |
| **Smell** | Typical tomato smell | Typical kombucha smell | Typical tomato smell with a slightly strong kombucha smell | Typical h2o2 smell |

Based on the Smart Medical Journal, the results of the transdermal patch preparation show organoleptic characteristics of a brown color, smooth texture, and a jasmine smell. HPMC, as a polymer, also contributes to a physical appearance that avoids aeration or wrinkles, resulting in a smooth texture [14].

According to Novianita [7], observations of the hydrogen peroxide film layer reveal a clearer appearance compared to the tomato juice film layer, given that hydrogen peroxide is a clear, colorless liquid. The varying concentration of tomato juice in the three formulas does not yield different color intensities in the film. The film is thin, flexible, non-brittle, and has a distinct tomato smell.

The results obtained in testing patch weight uniformity were analyzed based on the average patch weight value and the standard deviation value. Standard deviation serves as a measure to gauge data deviation, and as per the literature, a good standard deviation is ≤0.05. The results for formulas 2, 3, and 4 meet these requirements. In line with other studies, weight uniformity is considered acceptable if there is no deviation >5%. However, in formula 1, there is a deviation exceeding 5%.

Based on the Pharmaceutical Journal of Indonesia, the results for all formulas meet the requirements. Weight uniformity is influenced by polymer components that possess more water-attracting properties. During the making process, water is easily retained in the patch, impacting the weight of the resulting patch. The making method itself can also play a role, as it may allow some of the patch solution to remain in the container.

Based on the Smart Medical Journal, weight uniformity testing aims to determine the similarity of the weight of each patch, evaluating the consistency of the making process in producing a uniform product. This is crucial in medicinal preparations, where the patch weight must be uniform, and the coefficient of variation (CV) value should be ≤ 5%. Measurement results indicate that formula 1 has an average weight of 0.27 g, formula 2 an average weight of 0.45 g, and formula 3 an average weight of 0.49 g. Table 2 shows that the CV value for weight uniformity meets the requirements, as it cannot exceed 5%.

The results obtained in testing the pH of the tomato kombucha patch indicate the highest pH value, namely 5.75. The pH of the three patch formulations meets the critical pH limit requirements set for enamel etching (5.2-5.8) and for dentin (6.0-6.8). This can help reduce damage or lower the level of erosion on tooth enamel. Surface pH is

measured to determine the pH that will be exposed to the teeth when the patch is applied, allowing estimation of possible side effects.

Based on the Smart Medical Journal, pH testing aims to determine the safety of the preparation. The pH should not be excessively acidic as it may irritate the skin, and it should not be overly alkaline as it can lead to scaly skin. The results of the pH test show a pH value ranging from 5 to 7, meeting the safe pH for topical use, as the pH range for such use is between 4 and 8.18.

According to Novianita [7], the pH of the patch surface is measured using a universal pH indicator. The pH range of tomato juice in the study is 4.4-4.6, and each formula show the same pH of the patch preparation, falling within the pH range of 4-5. The critical pH limits established for enamel etching are 5.2-5.8, and for dentin, they are 6.0-6.8. Thus, the pH of the patch resulting from this study is still below the critical pH.

According to Inayah et al. [15], the ability of a patch to expand is one of the essential requirements for patch preparation. The expansion of the patch is linked to its ability to release the drug and its effectiveness in adhering to the mucosa. To measure the swelling ability of a patch, a parameter known as the swelling index is used, representing the percentage difference between the weight of the patch before and after treatment. There is an increase in weight after soaking for some time in a phosphate buffer medium with a pH of 6.8 due to water absorption by the patch. The observations of the ability to expand reveal that the highest swelling index value is found in the F1 formula, reaching 15.99%. Meanwhile, the swelling index value for F2 is 10.4%, and F3 is 8.7%. The HPMC polymer is part of the hydrophilic polymer group, where one of the characteristics of hydrophilic polymers is their ability to expand to an unlimited degree when in contact with water. However, a higher concentration of HPMC causes a longer swelling index time to reach the highest % swelling index. This is because the higher concentration makes the polymer composition denser, requiring more time for expansion when in contact with water. This aligns with previous studies, which also explain that patch preparations with a hydrophilic polymer composition have a swelling index value that increases with longer soaking time.

The results obtained in testing the degree of swelling show a significant increase in the degree of swelling at the 30th minute, after which the patch's ability to expand gradually decreased. The patch continue to expand until the 30th minute.

According to Novianita [7], the measurement results indicate a significant increase in the degree of expansion at the 5th minute, followed by a gradual decrease in the patch's ability to expand. The patch continue to expand until the 30th minute.

In tooth preparation, a tooth staining process aims to darken the tooth color, facilitating the observation of any increase in tooth brightness after immersing the tooth specimen in 100% tomato juice. Green tea is selected as the teeth staining factor in this study due to its empirical nature.

The staining process involves soaking the teeth in a tea solution for 12 days. After staining, the tooth specimens are divided into 5 test groups, each consisting of 6 tooth specimens. The results of the teeth staining process on 20 test tooth specimens indicate that tea can have a darkening effect on the color of the test tooth specimens, with an average of 79. However, the staining process's impact on the test tooth specimens varies, as evidenced by the standard deviation of the qualitative test (5.25) and the quantitative test (3.97) ($\geq$2.00).

The decrease in the brightness level of various tooth specimens may be attributed to differences in the porosity of each tooth specimen, with the amount of chromogen penetration depending on the level of porosity of each tooth specimen.

Following the tooth staining process, six teeth were soaked in three solutions, with two teeth in each solution, including tomato juice, tomato kombucha, and kombucha tea. The teeth exhibit a qualitative increase in color brightness in all three solutions, with tomato kombucha and tomato juice showing a more dominant increase. Subsequently, the initially smooth surface texture of the tooth specimen become rough after soaking in the third bath for 42 hours.

Testing the level of tooth erosivity after patch application was conducted by examining enamel morphology using a Scanning Electron Microscope (SEM) at magnifications of 1000 times and 5000 times on the samples. Samples were taken from tooth specimens that had been applied to tomato patches, tomato kombucha patches, and untreated kombucha patches. The results indicate that the tomato juice patch has a lower erosive effect compared to the tomato kombucha patch.

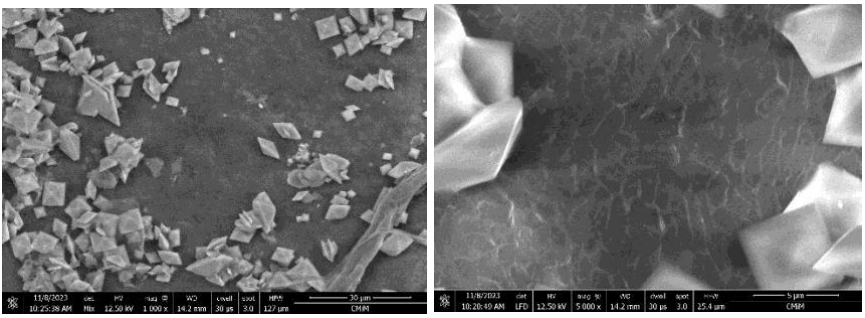

**Fig. 1.** Morphology of untreated tooth specimens; *right = 5000x and left = 1000x*

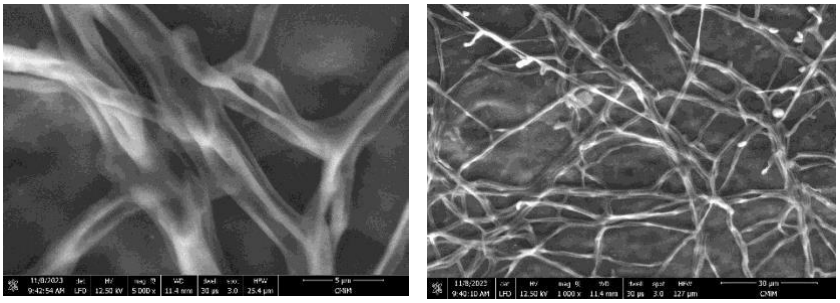

**Fig. 2.** Morphology of tooth specimens after application of the tomato kombucha patch; *right = 1000x and left = 5000x*

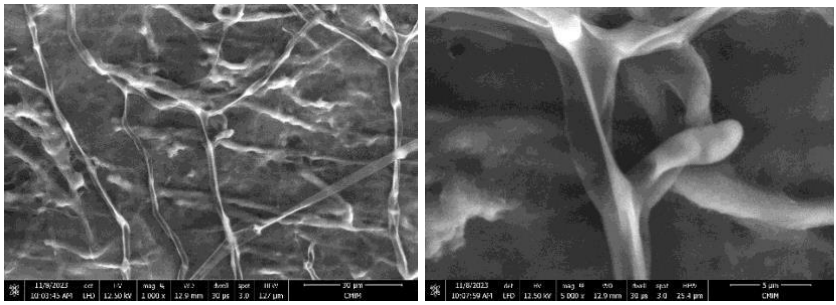

**Fig. 3.** Morphology of tooth specimens after application of the tomato patch; *left = 1000x and right = 5000x*

## 4 Conclusion

From the results of the research on the "Formulation of Tomato Fruit (Lycopersicum esculentum Mill) Kombucha Patch with Erosivity Test and Teeth Brightness Level," the following conclusions can be draw. The preparation of tomato kombucha patches significantly enhances the brightness of tooth specimens, similar to the tomato juice patch preparation. However, in terms of erosion, the tomato juice patch preparation exhibits a smaller erosive effect. Despite an increase in content leading to enhanced tooth brightness in tomato kombucha, it does not mitigate the occurring erosion effect. This is because the tomato patch formulation results in a smaller erosive impact. It is recommended for further research to carry out toxicity tests to optimize safety and a good formula and optimize the sugar content in kombucha tea before combining it with tomatoes to minimize damage to teeth.

**Acknowledgments.** The researchers wish to extend their gratitude to the Directorate of Learning and Student Affairs (BELMAWA) for funding the 2023 Student Creativity Program (PKM). Additionally, appreciation is conveyed to Universitas Buana Perjuangan Karawang, which has provided support in the form of facilities, funding, and consultants for the execution of this study.

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