# The Effect of Fermentation Time on pH and Total Soluble Solids of Kelud Simplex Pineapple Peel Kombucha

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ABSTRACT

Kombucha is a functional fermented beverage produced through the symbiotic interaction of bacteria and yeasts, and pineapple peel can serve as an alternative substrate because of its fermentable sugar content. This study aimed to determine the effect of fermentation time on pH and Total Soluble Solids (TSS) in kombucha made from Kelud Simplex pineapple peel. The experiment used a completely randomized design with eight treatments and three replications over 14 days of fermentation, with observations taken every two days. pH was measured using digital pH meter, whereas TSS was measured using Brix refractometer. Data were analyzed using the Shapiro-Wilk normality test, Levene's homogeneity test, one-way ANOVA, and Tukey HSD post hoc analysis. The pH decreased progressively from 3.68 on day 0 to 2.76 on day 14, while TSS declined from approximately 11.0 °Brix to 8.0 °Brix. Statistical analysis showed that fermentation time significantly affected both pH and Total Soluble Solids ($p < 0.001$). These findings indicate that longer fermentation promotes greater acidification and gradual decline in TSS, with the 14 days of fermentation producing the lowest TSS and the highest acidity, indicating a more advance stage of microbial activity.



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## 1. Introduction

The increasing adoption of a healthy lifestyles in society has driven consumption toward functional food and beverages (Suharman et al., 2024). Kombucha is one of such functional beverages produced through the symbiotic activity of bacteria and yeasts in a SCOBY culture (Andrade et al., 2025). Kombucha fermentation generates various bioactive compounds such as minerals, vitamins, phenolic compounds, and organic acids that contribute to antioxidant and antimicrobial activity (Rezaldi et al., 2022; Pereira, 2026). In line with this potential, alternative non-tea substrates, such as tropical fruits has also been developed to diversify product characteristics. Fruit-based substrates are potential choice because their nutritional composition can support the fermentation process (Zubaidah et al., 2023).

Ngancar District is the main center for pineapple (*Ananas comosus*) production center in Kediri Regency, with a total production reaching 2,9 million quintals in 2023, including local varieties such as Kelud Simplex pineapple peel. This high yield consequently increases the accumulation of pineapple peel as a processing by-product. Although it is a by-product, pineapple peel still contains various nutritional component that may be used as a fermentation substrate, especially carbohydrates, water, protein, fat and minerals (Pratiwi et al., 2024; Wandhekar et al., 2025). The carbohydrate content of plant materials can function as a carbon source that supprots microbial growth during fermentation (Umaru et al., 2026). The use of Kelud pineapple in fermented products shows that this material has potential to be developed as a valued-added fermentation substrate (Devi et al., 2025).

The use of pineapple peel as a kombucha fermentation substrate may affect microbial activity during fermentation (Barros et al., 2024). The symbiotic interaction between bacteria and yeasts plays a role in converting substrate components into various metabolites, thereby gradually altering the chemical changes in the chemical characteristics of the product. These changes are influenced by fermentation conditions, including fermentation time, which determine the dynamics of microbial activity and the final characteristics of the product (Li et al., 2025).

During fermentation, sugars act as the primary carbon source for yeasts, which convert them into ethanol through glycolysis and alcoholic fermentation processes. The produced ethanol is subsequently oxidized by acetic acid bacteria into various organic acids, especially acetic acid, thereby increasing the acidity of the fermentation medium (Shofia et al., 2022). The accumulation of these organic acids causes a gradual decrease in pH during fermentation. In addition to pH, Total Soluble Solids (TSS), expressed in °Brix, is a parameter commonly used to monitor changes in dissolved compound during fermentation. Changes in TSS values indicates alterations in the soluble components of the medium that are associated with ongoing fermentation process (Tirado-Kulieva et al., 2024). Hartini et al. (2025) reported that decreases in pH and °Brix accompanied by an increase in reducing sugars indicate active fermentation. Although research on kombucha fermentation using various plant-based substrates has been widely conducted, information on the dynamics of pH and Total Soluble Solids (TSS) changes during fermentation of kombucha from Kelud Simplex pineapple peel, remains limited. Therefore, this study aimed to analyze the effect of fermentation time on pH and Total Soluble Solids (TSS) in kombucha made from Kelud Simplex pineapple peel.

## 2. Methods

### 2.1. Research design

This study employed a Completely Randomized Design with eight treatments and three replications. The experiment was conducted at the Microbiology Laboratory of Universitas Nusantara PGRI Kediri from January to February 2026.

### 2.2. Tools and materials

Kombucha was prepared using glass jars, a double layer 100-mesh filter, a blender, a pan, a digital scale, a stove, a knife, a sterile cover cloth, a cutting board, thin-walled containers, and labeling paper. pH and Total Soluble Solids analyses were performed using glass pipettes, beakers, a pH meter, and a Brix refractometer. The raw materials consisted of pineapple peel from Kelud pineapple (Simplex variety) obtained from Ngancar District, Kediri Regency, granulated sugar (Rose Brand Premium), and a commercial SCOBY starter consisting of a cellulose layer (pellicle) and fermented kombucha liquid. The materials used for analysis included distilled water and buffer solutions at pH 4.01 and pH 6.86.

### 2.3. Preparation of pineapple peel extract

The kombucha preparation followed the method described by Budiandari et al. (2023). Pineapple peel was separated from the fruit flesh, thoroughly washed with running water, and drained. The peel was cut into small pieces (1 × 2 cm), and 250 g was mixed with 750 mL of water. The mixture was blended at medium speed until smooth, then filtered through a double layer of 100-mesh filter to obtain the filtrate. The filtrate was then pasteurized at 70°C for 10 minutes.

### 2.4. Preparation of pineapple peel kombucha

The pasteurized extract was supplemented with 75 g of sugar and stirred until completely dissolved. The solution was then cooled to 30–35°C before inoculation. The cooled substrate was inoculated with 75 mL of starter SCOBY, equivalent to 10% (v/v) of the fermentation medium volume. Fermentation was carried out for 14 days at room temperature in sterile glass jars covered with sterile breathable cloth to allow gas exchange while prevent microbial contamination.

### 2.5. pH measurement

pH was measured according to method of Ismaini et al. (2023) using a digital pH meter. Before use, the pH meter was calibrated with standard buffer solutions of pH 4,01 and 6,86. The electrode tip was rinsed with distilled water and gently dried before use. The electrode was then immersed in the sample until the sensor fully submerged and left until stable reading was obtained.

### 2.6. Total Soluble Solids measurement

Total Soluble Solids was measured according to (Rysha & Mulaj, 2026). The kombucha sample was measuring using a Brix refractometer calibrated with distilled water. The sample was placed on the refractometer prism until the entire surface fully covered, and the cover plate was gently closed to minimize air bubble formation that could affect measurement accuracy. After measurement, the refractometer prism was rinsed with distilled water and dried before use on the next sample.

### 2.7. Data analysis

pH and Total Soluble Solids were measured over 14 days at 2-day intervals. Data were analyzed using SPSS version 31. Normality was tested using the Shapiro–Wilk test, and homogeneity of variance w using Levene's test. Data were subsequently analyzed using one-way ANOVA, followed by Tukey's HSD post hoc test at a significance level of 95%.

## 3. Results and Discussion

### 3.1. pH value

Changes in the pH value of pineapple peel kombucha during fermentation are presented in Figure 1, which shows variations in pH value with increasing fermentation time.

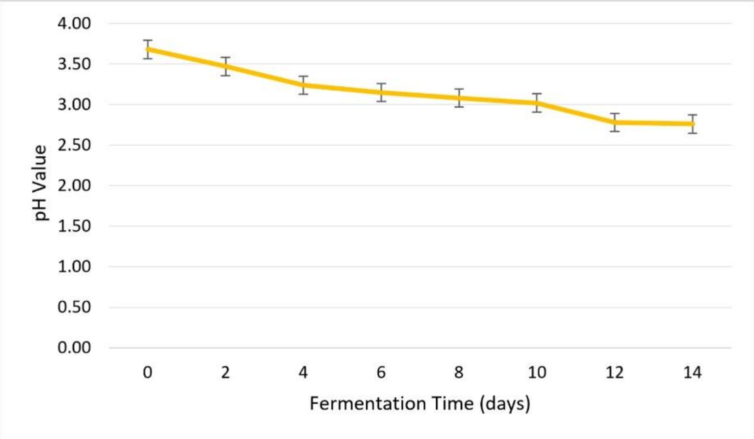


Figure 1. Changes in pH of pineapple peel kombucha during fermentation

Based on the figure 1, the pH of Kelud Simplex pineapple peel kombucha decreased as fermentation time increased. The highest pH value was recorded on day 0 at 3.68, then declined to 3.47 (day 2), 3.24 (day 4), 3.15 (day 6), 3.08 (day 8), 3.02 (day 10), 2.78 (day 12), and reached the lowest value of 2.74 on day 14. The initial pH value of 3.68 indicates that the fermentation medium was already acidic before fermentation began. This condition is presumed to be influenced by the natural organic acids in pineapple peel extract, such as citric acid and asorbic acid (Selvanathan & Masngut, 2023), as well as by the SCOBY starter liquid, which already contained acids from previous fermentation. The combination of these two factors contributed to the low initial pH of the substrate.

During fermentation, yeasts utilize sugar as a subtrate and convert it into ethanol, wich is subsequently oxsidized by acetic acid bacteria into various organic acids, especially acetic acids, thereby increasing the acidity of the fermentation medium. The decrease in pH in kombucha fermentation systems reflects the accumulation of organic acids formed during microbial metabolism. According to Managa et al. (2021), these organic acids are produced through acetic acid bacteria such as *Acetobacter aceti* and *Komagateibacter xylinus*, as well as through the activity of lactic acid bacteria, which also contribute a certain amount. This process takes place gradually as bacterial oxidative activity toward ethanol increases, leading to a decrease in pH. Mulyani et al. (2025) explained that the accumulation of organic acids is the main factor controlling pH changes in kombucha fermentation, especially in the phase when acetic acid bacreia begin to dominate.

The relatively slower decline in pH between days 4 and 10 suggests a shift from gthe alcoholic fermentation atge, dominated by yeast metabolism, to the acidification stage, characterized by enhanced oxidative activity of acetic acid bacteria that convert into organic acids (Li et al., 2025). This pattern indicates that organic acid formation was still ongoing, but at a relatively more stable rate. This is consistent with the characteristics of kombucha fermentation, which involves a gradual interaction between yeasts and acetic acid bacteria in producing various fermentation metabolites (Gunawan & Kurniasih, 2024). A more pronounced decline during the interval from day 10 to day 12 indicated an increased accumulation of acidic compounds during fermentation. This condition was presumably associated with enhanced microbial metabolic activity, which contributed to the formation of organic acids in the late fermentation phase (Khiabani et al., 2024; Kilmanoglu et al., 2024).

Based on the described changes in pH, variation across fermentation times indicates differences in the characteristics of the fermentation system at each observation phase. These conditions not only describe the dynamics of pH changes but also reflect a quantifiable trend that can be evaluated trough statistical analysis. Further interpretation of these differences relies on statistical test results to clarify the significance of changes among fermentation time treatments. Before further statistical analysis was conducted, the pH data for each fermentation time treatment group were subjected to a normality test to determine whether the data were normally distributed. The normality test was conducted using the Shapiro–Wilk method, and the results are presented in Table 1.

Table 1. Result of normality test

**Tests of Normality**

| Time | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | df | Sig. | Statistic | df | Sig. |
| pH | 0 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 2 days | 0.253 | 3 | | 0.964 | 3 | 0.637 |
| | 4 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 6 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 8 days | 0.314 | 3 | | 0.893 | 3 | 0.363 |
| | 10 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 12 days | 0.253 | 3 | | 0.964 | 3 | 0.637 |
| | 14 days | 0.184 | 3 | | 0.999 | 3 | 0.927 |

a. Lilliefors Significance Correction

The Shapiro-Wilk normality test shows that the pH data in all fermentation-time groups, namely 0, 2, 4, 6, 8, 10, 12, and 14 days, each had p-values greater than the 0.05 significance level. A significance value greater than 0.05 ($p > 0.05$) indicates that the data in all group fermentation time are normally distributed. A homogeneity of variance test was conducted to assess similarity among treatment groups and to ensure that the homogeneity assumption was met before further statistical analysis. The results of the homogeneity test are presented in Table 2.

Table 2. Result of homogeneity variances test

**Tests of Homogeneity of Variances**

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| pH | Based on Mean | 2.598 | 7 | 16 | 0.054 |
| | Based on Median | 1.796 | 7 | 16 | 0.157 |
| | Based on Median and with adjusted df | 1.796 | 7 | 4.431 | 0.285 |
| | Based on trimmed mean | 2.551 | 7 | 16 | 0.057 |

The Levene's test for homogeneity of variances on the kombucha pH data yielded a significance value of 0.054 based on the mean, with degrees of freedom df1 = 7 and df2 = 16. This significance value is still greater than 0.05 ($p > 0.05$), indicating that the variance among groups is homogeneous. With the assumptions of normality and homogeneity fulfilled, the data are considered suitable for analysis using a parametric test, namely one-way ANOVA, to determine whether there are significant differences in the mean pH of kombucha across different fermentation times. The results of the ANOVA test are presented in Table 3.

Table 3. Result of one way ANOVA test

**ANOVA**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.128 | 7 | 0.304 | 307.846 | 0.000 |
| Within Groups | 0.016 | 16 | 0.001 | | |
| Total | 2.144 | 23 | | | |

The ANOVA results showed an F value of 307.846 with df1 = 7 and a significance value of $p < 0.001$. A significance value lower than 0.05 indicates that there are highly significant differences in kombucha pH based on different fermentation times. To identify more specifically which pairs of groups differed significantly, a post hoc test using Tukey's HSD was performed. This test was used because it can compare all pairwise means among treatments to determine which treatment groups show differences. The Tukey's HSD results are presented in Table 4.

Table 4. Result Post Hoc Tukey HSD test

**pH**

| Time | N | Subset for alpha = 0.05 | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 14 days | 3 | 2.756667 | | | | | |
| 12 days | 3 | 2.783333 | | | | | |
| 10 days | 3 | | 3.020000 | | | | |
| 8 days | 3 | | 3.050000 | | | | |
| 6 days | 3 | | | 3.150000 | | | |
| 4 days | 3 | | | | 3.240000 | | |
| 2 days | 3 | | | | | 3.473333 | |
| 0 days | 3 | | | | | | 3.680000 |
| Sig. | | 0.961 | 0.930 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Based on the results of the Tukey HSD post hoc test, the fermentation times were grouped into several homogeneous subsets. Groups within the same subset indicate no significant difference in mean pH values, whereas groups in different subsets indicate significant differences.

Fermentation on day 0 (mean 3.680) and day 2 (mean 3.473) were in separate subsets with the highest mean pH values, meaning that they differed significantly. This indicates that at the beginning of fermentation, microbial activity started to increase so that organic acid production became more intense and caused a decrease in pH.

The fermentation groups at 4, 6, 8, and 10 days are in different subsets but show a gradual decrease in pH. This condition indicates that changes in acidity occur progressively during the fermentation process. At this stage, microorganisms actively carry out metabolism so that the pH value continues to decrease over time.

Meanwhile, 14-day fermentation (mean 2.757) and 12-day fermentation (mean 2.783) were in the same group, indicating that they did not differ significantly. This condition occurred in the final phase of fermentation, when acid formation tended to stabilize, so pH changes at nearby time points did not differ significantly. Overall, the results of the Tukey HSD test on pH confirm that fermentation time has a significant effect on the pH value of kombucha.

The significant differences in pH values among treatments were then considered in relation to the safe pH range of kombucha as an indicator of fermented product quality. According to Falasifah et al. (2025), who cited Nummer (2013), fermented beverages such as kombucha generally have a safe consumption pH range of 2,5-4,2, which helps inhibit the growth of pathogenic microorganisms. The results showed that all pH values remained within this range, indicating that the kombucha produced had an acidity level consistent with food safety standards based on the pH parameter.

### 3.2. Total Soluble Solids

Total Soluble Solids (TSS), expressed in °Brix, is one of the physicochemical parameters used to describe changes in soluble components during fermentation. Changes in TSS for each fermentation-time treatment are shown in Figure 2.

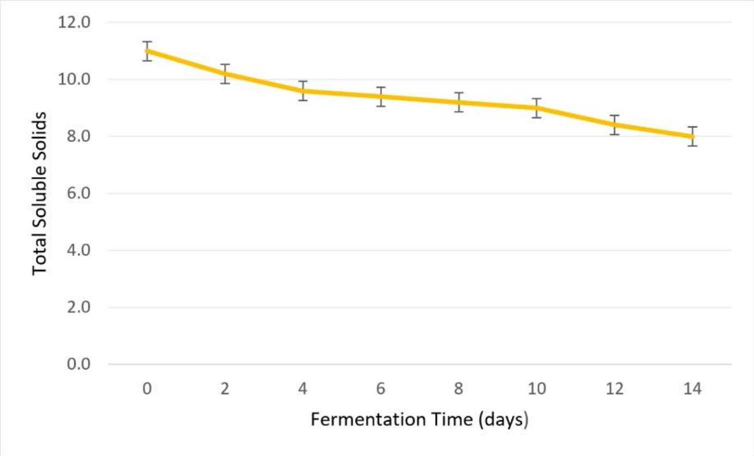


Figure 2. Changes in Total Soluble Solids of pineapple peel kombucha during fermentation

Based on figure 2, the TSS value of Kelud Simplex pineapple peel kombuccha showed a decreasing trend as a fermentation time increased. The highest TSS value was obtained on day 0 at 11.0 °Brix, then decreased to 10.2 °Brix on day 2 and 9.6 °Brix on day 4. The decline in TSS continued to 9.4 °Brix on day 6, 9.2 °Brix on day 8, and 9.0 °Brix on day 10. Subsequently, TSS decreased to 8.3 °Brix on day 12 and reached the lowest value of 8.0 °Brix on day 14. These results indicate that the longer the fermentation time, the lower the measured TSS in pineapple peel kombucha.

The decrease in Total Soluble Solids (TSS) during fermentation indicates changes in soluble components in the medium due to the metabolic activity of microorganisms in the SCOBY culture. TSS expressed in °Brix reflects the total soluble component in the fermentation medium, so its change can be used asa an indicator of changes in chemical characteristic during fermentation. In kombucha, reported soluble components include various sugars, proteins, vitamins, minerals, phenolic compounds, organic acids, and other fermentation metabolites whose compotition may change as fermentation progresses (Pereira, 2026).

The decline in Total Soluble Solids in this study did not occur at a constant rate. In the early fermentation phase (day 0 to day 4). The decline was relatively rapid because substrate availability was still high and the microorganisms were in an active growth phase. Entering the middle phase (day 4 to day 8), the rate of decline began to slow. This condition indicates changes in the composition of soluble substrate associated with changes in microbial activity during fermentation. In kombucha fermentation systems, these changes occur through the metabolic interaction between yeasts and acetic acid bacteria, which work synergistically during fermentation (Choi et al., 2025). This metabolic interaction contributes to changes in the chemical composition of the fermentation medium as fermentation time increases (Kumalawati et al., 2025). Subsequently, in the late fermentation phase (day 8 to day 14), TSS continued to decline, although at a lower rate than in the early phase. This pattern whows that fermentation continued until the end of observation, but with a reduced intensity of change in soluble components. Thus, the decrease in TSS in Kelud Simplex pineapple peel kombucha indicates changes in the chemical characteristics of the medium influenced by microbial activity and metabolite transformation during fermentation.

The pattern of TSS decline at each fermentation time shows variation among treatments that needs to be examined further through statistical analysis. Therefore, before variance analysis was performed, the data were first tested for normality using the Shaphiro-Wilk test to ensure that the assumptions for parametric analysis were met. The normality test result for kombucha TSS are presented in Table 5.

Table 5. Normality test result of Total Soluble Solids

**Tests of Normality**

| Time | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | df | Sig. | Statistic | df | Sig. |
| Total Soluble Solids | 0 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 2 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 4 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 6 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |
| | 8 days | 0.175 | 3 | | 1.000 | 3 | 1.000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 10 days | 0.175 | 3 | 1.000 | 3 | 1.000 |
| | 12 days | 0.175 | 3 | 1.000 | 3 | 1.000 |
| | 14 days | 0.175 | 3 | 1.000 | 3 | 1.000 |

a. Lilliefors Significance Correction

Based on the result of the Shapiro-Wilk test result presented in table 5, the significance values (Sig.) for all fermentation times, namely 0, 2, 4, 6, 8, 10, 12, and 14 days, were 1.000 (p > 0,05), indicating that the Total Soluble Solids data at each time point were normaly distributed. The data were tested for homogeneity using Levene's test. The homogeneity test results are presented in Table 6.

Table 6. Homogeneity test result of Total Soluble Solids data

**Tests of Homogeneity of Variances**

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| Total Soluble Solids | Based on Mean | 0.000 | 7 | 16 | 1.000 |
| | Based on Median | 0.000 | 7 | 16 | 1.000 |
| | Based on Median and with adjusted df | 0.000 | 7 | 16 | 1.000 |
| | Based on trimmed mean | 0.000 | 7 | 16 | 1.000 |

The homogeneity test showed that the significance value (sig.) under all approaches was 1.000 (p > 0.05). Since the significance value is greater than 0.05, it can be interpreted that there was no difference in variance among fermentation-time groups. Thus, the Total Soluble Solids data at different fermentation times had a homogeneous variance distribution. The data were subsequently analyzed using a one-way ANOVA test, as presented in Table 7.

Table 7. One Way ANOVA test result

**ANOVA**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 19.140 | 7 | 2.734 | 68.357 | 0.000 |
| Within Groups | 0.640 | 16 | 0.040 | | |
| Total | 19.780 | 23 | | | |

The data were then analyzed using one-way ANOVA, as presented in Table 7. Based on the ANOVA results for Total Soluble Solids, an F value of 68.357 was obtained with df1 = 7 and df2 = 16, along with a significance value of $p < 0.001$. A significance value lower than 0.05 ($p < 0.05$) indicates a significant difference in kombucha Total Soluble Solids among fermentation-time groups. The analysis was then continued with Tukey's HSD post hoc test, shown in Table 8.

Table 8. Tukey HSD test result

**Total Soluble Solids**

| Time | N | Subset for alpha = 0.05 | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 14 days | 3 | 8.000000 | | | | |
| 12 days | 3 | 8.400000 | | | | |
| 10 days | 3 | | 9.000000 | | | |
| 8 days | 3 | | 9.200000 | 9.200000 | | |
| 6 days | 3 | | 9.400000 | 9.400000 | | |
| 4 days | 3 | | | 9.600000 | | |
| 2 days | 3 | | | | 10.200000 | |
| 0 days | 3 | | | | | 11.000000 |
| Sig. | | 0.283 | 0.283 | 0.283 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Based on the Tukey HSD post hoc test, the fermentation times were divided into five homogeneous subsets. In the first subset, day 14 fermentation (mean 8.00°Brix) and day 12 fermentation (mean 8.40°Brix) were in the same group, so they did not show a significant difference. This result indicates that in the late fermentation phase, changes in TSS began to slow and tended to approach a more stable condition. This condition shows that most changes in soluble components had occurred in the earlier fermentation phase, so the TSS decline at the end of fermentation proceeded at a lower rate. In contrast, day 0 fermentation (mean 11.00 °Brix) and day 2 fermentation (mean 10.20 °Brix) were in different

subsets from the longer fermentation groups, indicating a real difference in the early stage of fermentation.
In addition, day 4, day 6, day 8, and day 10 fermentations were in overlapping subsets. This pattern shows that the decline in TSS occurred gradually and did not always produce significant differences at adjacent fermentation times. These results indicate that the middle fermentation phase was a period of continuous change in soluble components, so TSS continued to decrease gradually until the end of observation.

## 4. Conclusion

Overall, fermentation time had a significant effect on changes in pH and Total Soluble Solids (TSS) in Kelud Simplex pineapple peel kombucha. During 14 days of fermentation, pH decreasd gradually from 3.68 to 2.74, whereas TSS declined from 11.0 °Brix to 8.0°Brix. Statistical analysis showed that these changes were significant, indicating that fermentation time can serve as a determining factor in the physicochemical characteristics of kombucha. All pH values obtained were remained within the safe consumption range for kombucha, indicating that the resulting product met food safety requirements based on acidity parameter.

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