Journal of Biotechnology and Natural Science
Vol. 5, No. 2, December 2025, pp. 75-81
ISSN 2808-2508

The Effect of Fermentation Duration on Alcohol Content of Local Variety of Kelud Pineapple *Tepache*

Shalsa Neha Kharisma Devi[1*], Dwi Ari Budhiretnani[1], Elysabet Herawati[1]

[1]Biology Department, Universitas Nusantara PGRI, Kediri, Indonesia
[1]shalsanehakd17@gmail.com*; [1]dwiari@unpkediri.ac.id; [1]elysabet@unpkdr.ac.id
*corresponding author

ARTICLE INFO

Article history
Received: June 11st 2025
Revised: July 14th 2025
Accepted: July 22nd 2025

Keywords
Alcohol
Kelud Pineapple
Tepache

ABSTRACT

The purpose of this study was to obtain data on the effect of fermentation time optimization on alcohol content in *Tepache* made from local varietity of Kelud pineapple and to evaluate the alcohol content with the consumption safety standards set by BPOM RI to ensure that the product remains safe and suitable for consumption. This study is an experimental study and used a completely randomized design (CRD) method with 4 treatments and 6 replications. The data obtained ware analyzed statistically through normality tests, homogeneity tests, one-way analysis of variance (ANOVA), and continued with Bonferroni's further test to determine significant differences between treatments. Based on the results of the study, it was found that the longer the fermentation duration, the higher the alcohol content produced. In 0-hour and 24-hour fermentation, the alcohol content was recorded at 0%, followed by 48-hour fermentation with an average alcohol content of 1%. Meanwhile, 72-hour fermentation recorded the highest alcohol content with an average of 1,3%. The maximum alcohol content produced, which is 1,3%, is still far below the maximum threshold set by the BPOM RI, which is 5% for category A alcoholic beverages. Thus, this fermented *Tepache* product is still considered safe for consumed as a low alcohol beverage.

How to Cite: Devi, S.N.K., Budhhiretnani, D.A. & Herawati, E. (2025). The Effect of Fermentation Duration on Alcohol Content of Local Variety of Kelud Pineapple *Tepache*. *Journal of Biotechnology and Natural Science*, 5(2):75-81.

1. Introduction

Mount Kelud is a volcano located in Kediri Regency. This mountain was formed as a result of explosive eruptions that occurred from 1000 AD to 2014 (Delfianto *et al*., 2021). The eruption of Mount Kelud had a negative impact such as damage to infrastructure and agricultural land. However, the volcanic material that was spewed out actually made the soil more fertile. This fertility greatly supports agriculture including the cultivation of local pineapples known as Kelud pineapples.
According to Dzulqaidah *et al*. (2021), pineapple is a fruit that is rich in vitamins A and C, important nutrients that help maintain healthy eyesight and strengthen the body's immune system. Furthermore, this fruit also contains various minerals, dextrose, sucrose, and the enzyme bromelain, which have various benefits such as aiding digestion, preventing blood clots, supporting fibrinolytic activity, and potentially inhibiting the growth of cancer cells. Pineapple peel is known to contain various secondary metabolite compounds, including alkaloids, steroids, tannins, saponins, and flavonoids, which play a role in inhibiting inflammation, reducing allergic reactions, acting as antioxidants, as well as acting as antiviral and antibacterial agents (Patimah *et al.*, 2024).
Probiotic drinks come from a fermentation process that has a fresh taste and is safe to consume. One example is *Tepache*, a traditional Mexico beverage made by fermenting pineapple peels in a sugar water solution. As a probiotic drink, the quality of *Tepache* can be assessed from various aspects such as taste, aroma, color, alcohol content, the appearance of bubbles during the fermentation

process, and the level of cleanliness during manufacture. The ideal taste of *Tepache* is a balanced combination of sweet and sour with a prominent pineapple flavor. The aroma should be fresh and distinctive from fermentation, blending with the natural aroma of fruit and not smelling bad. Visually, *Tepache* has a brownish yellow color with a slightly cloudy appearance due to the fermentation process (Sagita *et al.*, 2023).

Tepache is a beverage obtained through a fermentation process that produces a small amount of alcohol. The relatively short fermentation time allows for the formation of a distinctive taste. In general, the alcohol content in *Tepache* is 0.5% - 2% (Apriliyanto *et al.*, 2021). As a country with a Muslim majority population, Indonesia refers to Islamic principles in determining consumption rules. MUI (2018) states that fermented drinks with an alcohol content of less than 0.5% can be categorized as halal if the production process is in accordance with Islamic law. Based on BPOM RI regulation No. 14 of (2016), alcoholic beverages are classified into three groups based on their alcohol content, namely group A (maximum 5%), group B (5% - 20%), and group C (20% - 55%). Meanwhile, fermented drinks made from fruit are only allowed to have an alcohol content of up to 24%.

Optimizing fermentation time is crucial in tepache production to achieve the ideal alcohol content without compromising sensory qualities such as flavor, aroma, and texture. Microbiologically, this process relies on yeast activity, which ferments sugars into alcohol. Fermentation that is too short results in incomplete breakdown of sugars, while excessive fermentation produces excess alcohol and organic acids, lowering the pH and compromising the flavor. From a biotechnological perspective, controlling fermentation time is key to optimally directing microbial metabolism (Rifdah *et al.*, 2022). However, to date, no specific studies have examined the effect of fermentation time on *Tepache* made from the local Kelud pineapple variety, even though each variety has different sugar and acidity characteristics that can influence the fermentation process.

This study aims to obtain data on the effect of fermentation time optimization on alcohol content in *Tepache* made from local variety of kelud pineapple. In addition, this study also aims to evaluate the suitability of alcohol content in the *Tepache* product with the consumption safety standards set by the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI) to ensure that the product remains safe and suitable for consumption.

2. Methods

2.1. Tools and Materials

The tools used in the research include glass jars, knives, basins/buckets, stirrers, measuring cups, clean cloths for covering, rubber bands, sieves, digital scales, and alcohol meters.The materials used in this study consisted of 200 grams of pineapple peels, 100 grams of brown sugar, and 600 mL of boiled water.

2.2. Pineapple Peels Sample Collection Procedure

Pineapple skin samples were obtained from fresh pineapples purchased directly from traders to ensure their quality and freshness. The pineapples were then washed using clean water to remove dirt, then the pineapple skin was cut into small pieces to speed up and facilitate the fermentation process. This stage is important to maintain the cleanliness of the ingredients, minimize contamination, and support the smooth fermentation of *Tepache*.

2.3. Procedure for Making *Tepache*

The process of making *Tepache* from the peels of the local Kelud pineapple variety involves several steps. The pineapple peels cleaned and weighed, 200 grams for each treatment. Brown sugar is sliced to speed up dissolution, then mixed with the pineapple peels and water in a fermentation jar until all ingredients are evenly submerged. The mixture is stirred gently, then the jar is covered with a clean cloth tied with a rubber band to ensure a tight seal. Fermentation occurs spontaneously without the addition of a microbial starter, and takes place in a clean environment with normal room lighting or out of direct sunlight. Treatments are carried out based on varying fermentation times, namely 24 hours, 48 hours, and 72 hours. During the fermentation process, gas bubbles and a white film form on the surface of the solution as a result of natural microbial activity. After fermentation is complete, the *Tepache* is filtered to separate the liquid from the pulp and impurities, resulting in a clean and clear final product.

2.4. Alcohol Content Testing Method

Alcohol content measurement is carried out using an alcoholmeter (alcohol hydrometer) whose working principle is based on Archimedes' principle. The higher the alcohol content in the solution, the lower the density of the solution, so that the alcoholmeter will sink deeper. The measurement process is carried out by pouring the sample into a transparent cylindrical tube with a capacity of 100 mL, then cooling it to a temperature of 20°C according to the measurement standard (Dupa *et al.*, 2022). After that, the alcoholmeter is slowly inserted into the solution and allowed to float until it reaches a stable position. The alcohol content recorded by reading the scale on the alcoholmeter scale which is parallel to the surface of the liquid in the tube (Sitorus *et al.*, 2024).

3. Results and Discussion

This research is experimental research conducted in the Botany laboratory of Nusantara PGRI Kediri University to determine the alcohol content of *Tepache* of local Kelud pineapple variety. Testing of alcohol content in *Tepache* of local Kelud pineapple variety was carried out by testing using an alcoholmeter. For more details about the results, see Figure 1.

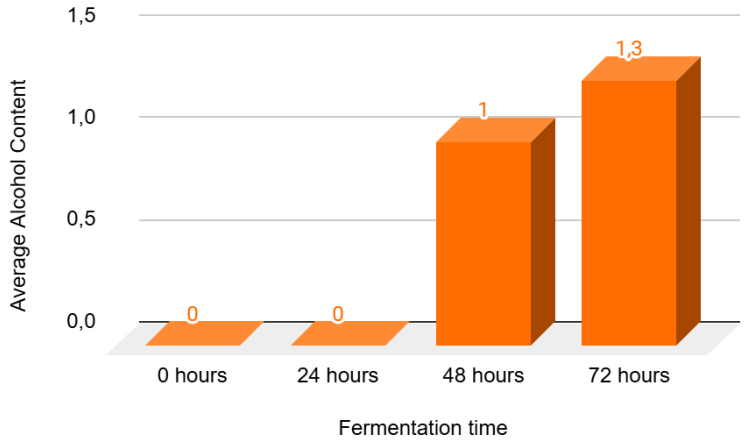

Figure 1. Graph of the mean alcohol content of *Tepache* pineapple Kelud local variety

In Figure 1, it can be seen that the difference in the average alcohol content of the four treatment groups. At fermentation times of 0 hours and 24 hours, the alcohol content was detected at 0%. However, an increase in alcohol content began to occur after fermentation lasted for 48 hours with an alcohol content of 1%, and continued to increase at 72 hours reaching 1.3%. The results of the study identified that the duration of fermentation plays an important role in the formation of alcohol. *Tepache* contains various microorganisms including bacteria and yeast. The fermentation process in *Tepache* is dominated by lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Acetobacter*, and *Lactococcus*, as well as yeast groups such as *Saccharomyces cerevisiae*, *Gibberella*, *Zygosaccharomyces*, *Candida*, *Meyerozyma*, and *Talaromyces* (Nurfauziati, 2021). These microorganisms work synergistically in changing the substrate during fermentation. Sugars such as glucose, fructose, and sucrose are converted into alcohol through microbial activity with the help of the Invertase enzyme which breaks down disaccharides into monosaccharides, which are then fermented by the Zimase enzyme into alcohol (Najini *et al.*, 2024).

Strengthened by Firdaus *et al.* (2020) the longer the fermentation time, the activity of fermentation microbes increases in converting sugar into alcohol. The presence of simple sugars, such as glucose and fructose which are naturally found in local variety of Kelud pineapple is one of the important factors that support fermentation efficiency. Thus, the longer the fermentation lasts, the greater the possibility of increasing alcohol levels, as long as conditions continue to support microbial activity for fermentation.

Temperature is one of the important factors that affect the effectiveness of microbial activity during the fermentation process. Wahyuningtyas *et al.*, (2023) clarified that fermentation activity takes place optimally at temperatures between 25°C and 30°C. In this study, the fermentation process was carried out at room temperature around 27°C - 29°C, which is still within the optimum temperature range.

Thus, temperature stability during the fermentation process plays a key factor in producing alcohol content that meets the expected standards.

Based on the analysis of alcohol content conducted, the average alcohol content in various *Tepache* fermentation treatments of 0 hours and 24 hours had an average alcohol content of 0%, while the 48-hour and 72-hour treatments showed an average alcohol content of between 1% and 1.3%. According to the provisions of the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI, 2016), drinks with an alcohol content of less than 5% are included in the Group A category, which includes types of low-alcohol drinks that are still permitted for consumption.

The alcohol content produced in the 48-hour and 72-hour treatments, which were below the 5% threshold, indicated that the fermentation process was still in accordance with the consumption safety standards. Therefore, the optimization of fermentation time in all treatments can be considered to have taken place optimally because it was able to produce alcohol content that met the set quality standards.

Table 1. Mean and Standard Deviation of Tepache Alcohol Content

Fermentation Time	Mean (%)	Standard Deviation (%)
0 hours	0	0.00
24 hours	0	0.00
48 hours	1	0.00
72 hours	1.3	0.52

The results of alcohol content measurements at various fermentation times showed a significant increase at 48 and 72 hours of fermentation. At 0 and 24 hours, the average alcohol content was still 0.0% with a standard deviation of 0.00%, indicating no fermentation activity that produces ethanol. Alcohol content began to be detected at 48 hours with an average value of 1.0% and a standard deviation of 0.00%, indicating uniform results between replicates. A further increase occurred at 72 hours with an average alcohol content of 1.3% and a standard deviation of 0.52%, indicating variation between replicates in the advanced fermentation phase. The low to moderate standard deviation values reflect a relatively stable fermentation process, although on the 3rd day results began to show differences between samples. The summary of the completely randomized design calculations can be seen in the table below:

Table 2. Results of normality test

Fermentation Time	Statistik Shapiro-Wilk	Sig. (p-value)	Information
0 hours	0.866	0.212	Normal
24 hours	0.960	0.820	Normal
48 hours	0.958	0.804	Normal
72 hours	0.982	0.960	Normal

Normality test was conducted using Shapiro-Wilk method to determine whether the data of each treatment was normally distributed. In table 1, the analysis results show that the significance value (p-value) for fermentation time 0 hours is 0.212; 24 hours is 0.820; 48 hours is 0.804; and 72 hours is 0.960. All sig values exceed the significance limit of 0.05, so it can be concluded that the data in the four treatment groups are normally distributed.

Next, a homogeneity test was conducted to test the equality of variance between treatment groups to ensure that the homogeneity assumption was met before further statistical analysis was conducted.

Table 3. Results of homogeneity test

Fermentation Time	df1	df2	Sig. (p-value)	Homogeneous (p > 0.05)
0 hours	3	20	0.455	Homogeneous
24 hours	3	20	0.433	Homogeneous
48 hours	3	20	0.436	Homogeneous
72 hours	3	20	0.462	Homogeneous

In table 3, the results of the homogeneity test using Levene's Test show that all p-values are above the significance limit of 0.05, namely 0.455 at 0 hours, 0.433 at 24 hours, 0.436 at 48 hours, and 0.462 at 72 hours. These values identify that the data of all fermentation time groups have uniform or homogeneous variance. Thus, the assumption of homogeneity has been met, so that the data is worthy of further analysis using parametric statistical methods, namely the ANOVA test.

Table 4. ANOVA test results

		Sum of Squares	df	Mean Square	F	Sig.
0 hours	Between Groups	0.061	3	0.020	14.412	0.000
	Within Groups	0.028	20	0.001		
	Total	0.090	23			
24 hours	Between Groups	0.551	3	0.184	66.818	0.000
	Within Groups	0.055	20	0.003		
	Total	0.606	23			
48 hours	Between Groups	4.651	3	1.550	286.231	0.000
	Within Groups	0.108	20	0.005		
	Total	4.760	23			
72 hours	Between Groups	10.125	3	3.375	675.000	0.000
	Within Groups	0.100	20	0.005		
	Total	10.225	23			

Data analysis using one-way ANOVA showed a significance of 0.000 with a significance level of 0.05. because the sig value is less than 0.05, hypothesis 0 (H0) is rejected. This shows that there is a statistically significant difference in alcohol content based on variations in fermentation time (0 hours, 24 hours, 48 hours, and 72 hours). This means that the fermentation time has a real effect on the amount of alcohol formed.

As a step to specifically identify treatment groups that show significant differences, further analysis was carried out using the Bonferroni test. This test was applied to provide clarity regarding the fermentation time that significantly contributed to the increase in alcohol content, thus strengthening the evidence that fermentation time has a real influence on the effectiveness of the alcohol production process.

Table 5. Bonferroni test results

Time Comparison	Sig (p-value)	Information
0 hours vs 24 hours	0.000	Significantly different
0 hours vs 48 hours	0.000	Significantly different
0 hours vs 72 hours	0.000	Significantly different
24 hours vs 0 hours	0.000	Significantly different
24 hours vs 48 hours	0.000	Significantly different
24 hours vs 72 hours	0.000	Significantly different
48 hours vs 0 hours	0.000	Significantly different
48 hours vs 24 hours	0.000	Significantly different
48 hours vs 72 hours	0.000	Significantly different
72 hours vs 0 hours	0.000	Significantly different
72 hours vs 24 hours	0.000	Significantly different
72 hours vs 48 hours	0.000	Significantly different

Based on the results of the significance calculation with a significance limit value of the corrected results of 0.0041 (obtained from dividing $\alpha = 0.05$ by 12 comparison combinations), all pairs of fermentation times showed a sig of 0.000. This value is far below the specified limit, so it can be concluded that each combination of fermentation times is significantly different. This finding indicates that the changes that occur during the fermentation process take place significantly at each

observation time interval. Thus, the duration of fermentation is proven to have a significant effect on increasing the alcohol content of *Tepache* drinks made from local variety Kelud pineapple.

A study conducted by Virgiana *et al.* (2024) reported that fermentation of pineapple peel for 72 hours produced the highest alcohol content of 1.8%. These results indicate that fermentation duration plays a significant role in increasing ethanol production. These findings align with this study, where significant differences between fermentation times indicate continued microbial activity, thus affecting the accumulation of alcohol content at each observation interval. This is supported by the results of a study by Sukriadi *et al.* (2022) which stated that the duration of the fermentation process significantly affected the alcohol content and final properties of tepache beverages fermented from pineapple peels. The longer the fermentation process lasted until the optimum point, the greater the alcohol produced.

The optimal consumption limit for tepache is based on the alcohol content produced during the short fermentation period. A study by Gutiérrez Sarmiento *et al.* (2022) showed that fermentation needs to be controlled for a specific time, generally around 2 to 3 days, depending on temperature, sugar content, and microbial activity. Therefore, tepache should be consumed in the early stages of fermentation, when the alcohol content is still low, typically between 1% and 3%. Furthermore, these findings also open up opportunities for the commercialization of tepache as a functional beverage made from local pineapple peel. In addition to increasing economic value, this product has broad market potential as a natural, healthy beverage that aligns with current consumer trends.

4. Conclusion

The results showed that the fermentation time had a significant effect on increasing the alcohol content in Tepache drinks. The longer the fermentation time, the higher the alcohol content produced. In 72-hour fermentation, the highest average alcohol content was recorded at 1.3%, followed by 48-hour fermentation with an average alcohol content of 1%. Meanwhile, 24-hour and 0-hour fermentation recorded an average alcohol content of 0%. Based on the results of statistical tests using one-way ANOVA, a significant value of 0.000 ($p < 0.05$) was obtained, indicating that there was a significant difference between fermentation time treatments on the alcohol content of *Tepache*. These results were reinforced by Bonferroni's further test which showed that each pair of fermentation times (0, 24, 48, and 72 hours) were significantly different. Thus, the fermentation time had a statistically significant effect on the alcohol content produced.

Although there was an increase in alcohol content as the fermentation time increased, all results remained below the 5% threshold. Therefore, the fermented *Tepache* drink in this study was still classified as low and safe for consumption according to the provisions set by BPOM RI 2016. This finding indicates that the longer fermentation process provides a greater opportunity for microbes to convert sugar into alcohol, resulting in a higher alcohol content.

REFERENCES

Apriliyanto, U. (2021). The Effect of Differences in Sugar Concentration and Length of Fermentation Process on the Characteristics of Soy Milk Kefir. *Journal of Food Technology and Agricultural Sciences (Jipang)*, 2(1):1–9. https://doi.org/10.36526/jipang.v2i1.1209.

BPOM. (2016). *BPOM Regulation No. 21 of 2016*. Indonesian Food Category, 1–28.

Delfianto, R., Rayes, M. L. & Agustina, C. (2021). Morphology and Soil Classification on The Toposequence of the Western Slope of Mount Kelud, Kediri, East Java. *Journal of Soil and Land Resources*, 8(2):539-552. https://doi.org/10.21776/ub.jtsl.2021.008.2.24.

Dupa, E. C., Tuju, T. J. & Langi, T. M. (2022). The Effect of Mixing Black Glutinous Rice and White Glutinous Rice *(Oryza glutinosa)* on the Physicochemical Properties of Alcoholic Beverages from Tape. *Journal of Applied Agroecotechnology*, 3(2):279–286. https://doi.org/10.35791/jat.v3i2.44332.

Dzulqaidah, I., Zanuba, R. B., Alwi, A. S. F., Salsabila, A. R. P., Mursidi, S. & Muliasari, H. (2021). Extraction and Activity Test of Crude Bromelain Enzyme from Pineapple Fruit. *Journal of Agritechnology and Food Processing*, 1(2):80. https://doi.org/10.31764/jafp.v1i2.6974.

Firdaus, S., Isnaini, L., & Aminah, S. (2020). "Review" Kombucha Tea as a Functional Drink with Various Tea Ingredients. *In Proceedings of the Unimus National Seminar* (Vol. 3).

Gutiérrez-Sarmiento, W., Peña-Ocaña, B. A., Lam-Gutiérrez, A., Guzmán-Albores, J. M., Jasso-Chávez, R., & Ruíz-Valdiviezo, V. M. (2022). *Microbial Community Structure, Physicochemical Characteristics and Predictive Functionalities of the Mexican Tepache Fermented Beverage*. Microbiological Research.127045.

MUI, M. U. I. (2018). *Food and Beverage Products Containing Alcohol/Ethanol*. Mui, 1–11.

Najini, R., Purwanti, N. U., Mufida, A. R., Kurniawan, A., Alghifary, M. H. H., Syalsabila, R. R., Nuraini, R., & Safitri, W. (2024). Tepache Probiotic Drink Fermented Pineapple Peel *(Ananas comosus* L.*)* Using Various Types and Concentrations of Sugar. *Journal Pharmacy of Tanjungpura*, 1(2):74–79.

Nurfauzianti, R. (2021). Pengaruh Proses Fermentasi Terhadap Kandungan Asam Laktat Pada Makanan Fermentasi. Parapemikir: *Jurnal Ilmiah Farmasi*, 10(2):71–76. https://doi.org/10.30591/pjif.v10i2.2098.

Patimah, R., Susena, B., Febriyanti, A. D. & Effendi, C. P. (2024). The Secret of Pineapple Peel as a Source to Increase Body Resistance: The Secret of Pineapple Skin as a Source to Increase Body Resistance. *Jurnal Pengabdian Indonesia*, 4(01).

Rifdah, R., Kalsum, U. & Anugrah, I. S. (2022). The Effect of *Saccharomyces Cerevisiae* on Ethanol Levels from Pineapple Peels by Fermentation. *Jurnal Teknik Patra Akademika*, 13(02):115–126. https://doi.org/10.52506/jtpa.v13i02.176

Sagita, C. (2023). Making Probiotic Drinks from Pineapple Peel Waste *(Tepache).* Tarbiatuna: *Journal of Islamic Education Studies*, 3(2):205–210. https://doi.org/10.47467/tarbiatuna.v3i2.3017

Sitorus, W.A., Rasyidah. & Mayasari, U. (2022). The Influence of Production Factors on Pineapple Farming Production and Income *(Ananas comosus).* Methodagro: *Journal of Agricultural Science Research*, 8(2):1–9. https://doi.org/10.46880/mtg.v8i2.1677.

Sukriadi, H, E., Rustomo, W.T. & Astiana, R. (2022). Pineapple Peel Tepache. *Indonesian Tourism Journal*, 18(1):28–37. https://doi.org/10.53691/jpi.v18i1.267.

Virgiana, P. A. (2024). The Effect of Fermentation Time and Yeast Type on Bioethanol Production Using Pineapple Peel Waste as Raw Material by Enzymatic Hydrolysis. Repository.Malahayati.ac.id

Wahyuningtias, D. S., Fitriana, A. S. & Nawangsari, D. (2023). The Effect of Temperature and Fermentation Time on the Organoleptic Properties and Antioxidant Activity of Butterfly Pea Flower Kombucha Tea *(Clitoria ternatea* L.*). Pharmacy Genius*, 2(3):198-207.