

The Potential of Tepache Drinks with Addition of Guava Skin as Probiotic Drink

Potensi Minuman Tepache Dengan Penambahan Kulit Jambu Biji Sebagai Minuman Probiotik.

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Abstract: *The development of probiotic beverage innovation is now growing. Probiotic products are in demand by the public because of their benefits in maintaining digestive track Health. One drink that has potential to be a probiotic drink is tepache, a fermented drink made from pineapple skin dissolved in a sugar solution. The fermentation process that occurs produces lactic acid bacteria that have potential to be probiotics. In this study, an experimental design was used where tepache drinks were made with six different formulas, where the differences are found in the types of sugar, namely palm sugar and granulated sugar, and the amount of sugar added: F1 (16%), F2 (22%) and F3 (27%) is the same for added brown sugar in F4, F5, F6. Identification of bacteria was carried out by growing on PCA (Plate Count Agar) media to determine the Total Colony Count and MRSA (de Mann Rogosa Agar) media to grow lactic acid bacteria in this case selectively in the grow of lactobacillus. From the results of observations for 3 days, showed that bacteria grew on PCA media at a dilution 10^{-4} , 10^{-5} , and 10^{-6} and on MRSA media grew at a dilution of 10^{-4} . From the pH measurements, the results obtained for all formulas were pH 4 and brix degress 60. Organoleptic test showed that this tepache drink was liked by panelists, data taken from 20 panelist because its refreshing, sour taste and fruity aroma. The study concluded that tepache has the potential to be a probiotics beverage, as the bacteria found to grow were lactic acid bacteria with pH that matches the probiotic drink and was preffered by panelists.*

Key word: Tepache, Probiotic, Lactobacillus, Guava Skin

1. INTRODUCTION

Fermented beverages have gained increasing attention in recent years due to their potential health benefits and their role as functional foods. Fermentation is a traditional food processing method that enhances nutritional value, improves flavor, and extends shelf life through the activity of beneficial microorganisms. In addition, fermented products often contain probiotics, organic acids, and bioactive compounds that contribute to improved digestive health and overall well-being. As consumers become more aware of the relationship between diet and health, interest in naturally fermented beverages continues to grow. (1.) One traditional fermented beverage that has attracted scientific interest is tepache, a lightly fermented drink originating from Mexico. Tepache is typically produced by fermenting pineapple peels or pulp with sugar and water, allowing naturally occurring microorganisms to initiate the fermentation process. During fermentation, various lactic acid bacteria and yeasts convert sugars into organic acids, carbon dioxide, and small amounts of alcohol, resulting in a refreshing beverage with a slightly sweet and tangy flavor. Traditionally, tepache is

consumed as a refreshing drink and is considered a low-cost product because it utilizes pineapple by-products that might otherwise be discarded.(2) The fermentation process of tepache not only contributes to its unique sensory characteristics but also enhances its nutritional and functional properties. Previous studies have reported that tepache contains beneficial microorganisms, organic acids, vitamins, and antioxidant compounds derived from pineapple and microbial metabolism.(3) The presence of these compounds suggests that tepache has the potential to function as a probiotic or functional beverage that may support gut health and improve nutrient absorption. Furthermore, the utilization of pineapple peel in tepache production aligns with current efforts to reduce food waste and promote sustainable food systems. Pineapple peel, often considered an agricultural by-product, is actually rich in bioactive compounds that offer significant health benefits for humans. It contains a variety of nutrients such as dietary fiber, vitamins (especially vitamin C), and essential minerals. In addition, pineapple peel is a natural source of phytochemicals, including flavonoids, phenolic compounds, and the enzyme bromelain, which contribute to its functional properties.(4) One of the main health benefits of pineapple peel is its antioxidant activity. The presence of phenolic compounds helps neutralize free radicals in the body, thereby reducing oxidative stress and lowering the risk of chronic diseases such as cardiovascular disorders and certain types of cancer.(5) The high dietary fiber content also supports digestive health by improving bowel movement and promoting the growth of beneficial gut microbiota. Furthermore, bromelain found in pineapple peel is known for its anti-inflammatory and digestive properties. It can aid in protein digestion and may help reduce inflammation in the body. The antimicrobial properties of compounds in pineapple peel also make it beneficial in inhibiting the growth of harmful microorganisms.(6)

Despite its traditional use and growing popularity, scientific studies examining the microbiological, nutritional, and functional characteristics of tepache remain relatively limited. Therefore, further research is needed to explore its fermentation dynamics, microbial composition, and potential health benefits. Understanding these aspects can contribute to the development of tepache as a functional fermented beverage with wider acceptance in the food and nutrition field. In this study, we tried to combine tepache drink with pineapple skin and guava skin with the aim of getting a better aroma. Current developments in the food industry demonstrate increasing public interest in functional beverage products that not only provide a refreshing effect but also offer health benefits. One type of functional beverage, which are beverages containing live microorganisms that are beneficial for digestive health, particularly lactic acid bacteria (LAB) such as *Lactobacillus* and *Bifidobacterium*.(7) Guava peel (*Psidium guajava* L) is a fruit waste product with potential for utilization. Guava peel contains various bioactive compounds such as flavonoids, tannins, and vitamin C, as well as dietary fiber that can act as a prebiotic bacteria. The addition of guava peel to the tepache fermentation process is expected to enhance the growth of probiotic microbes, increase antioxidant activity, and enrich the flavor and aroma of the beverage. Furthermore, the use of guava peels also contributes to reducing food waste and supports the concept of sustainable food. Therefore, research into the probiotic potential of tepache drinks with the addition of guava peels is important to determine the extent to which this combination of ingredients can produce a natural functional beverage that offers health benefits and high economic value. The objective of this study is to explore the utilization of fruit peel waste, particularly pineapple (*Ananas comosus*) and guava peel, as raw materials in the production of tepache, a fermented probiotic beverage. This research aims to evaluate the potential of these substrates in enhancing the nutritional value, antioxidant activity, and sensory characteristics of the

final product. Additionally, the study seeks to investigate the role of fermentation in improving functional properties and promoting the growth of beneficial microorganisms. Furthermore, this research intends to contribute to sustainable food processing by reducing organic waste through value-added product development, while also addressing the increasing demand for natural and functional beverages

2. METHODS

This study used an experimental design with completely randomized design. The study was conducted at STIKes Mitra Keluarga in Februari 2026. Here is how to make tepache using pineapple peel. Ingredient needed : Pineapple peel, coconut sugar, water, guava peel, Plate Count Agar (PCA), De mann Rogosa Agar (MRSA). Equipment needed: Glass jar, petridish, test tube, incubator, pH meter. Product preparation procedure: Wash the pineapple peel and guava peel and weigh it, weigh the granulated sugar and palm sugar, place them in separate glass jars, add 600 ml of water, close the jar tightly, fermented in 3 days. The following is a draft tepache formulation:

Table 1. Formulation of tepache

Ingredients	F1	F2	F3	F4	F5	F6
Guava Peel	100 g	100 g	100 g	100 g	100 g	100 g
Pineapple peel	200 g	200 g	200 g	200 g	200 g	200 g
Sugar	150 g	200 g	250 g	-	-	-
Palm Sugar	-	-	-	150 g	200 g	250 g
Water	600 ml	600 ml	600 ml	600 ml	600 ml	600 ml



F1 Sugar (150 g)



F2 Sugar (200 g)



F3 Sugar (250 g)



F4 Brown Sugar (150 g)



F5 Brown Sugar (200g)



F6 Brown Sugar (250 g)

In this study, TPC (Total Plate Count) analysis was also conducted to determine the total bacteria growing in tepache drinks. The tepache analysis procedure is to scratch the sample that has been fermented for 3 days into agar media, then put it in an incubator at 37 degrees Celcius for 24 hours, after which it is observed and counted the number of bacteria growing on the media. Next is the MRSA test which is a selective medium used to grow lactobacillus bacteria, the method is the same as the TPC sample planted into MRSA media in a drugal way, then put in an incubator at 37 degrees celcius for 24 hours , then observed and counted

3. RESULTS

After three days of fermentation, acidity was first tested with pH meter. The following are the results of the measurement using pH meter:

Table 2. Tepache Acidity Levels in Various formula.

Formula	F1	F2	F3	F4	F5
pH (fermentation 3 days)	4	4	4	4	4
pH (fermentation 7 days)	2	2	2	2	2

The acidity level can indicate the success of the fermentation process. The ideal pH Orange for fermented beverages is 3 – 4,5. Beside acidity, another indicator analyzed is the brix level. Measuring the brix level in fermented beverages is done to determine the dissolved sugar content in the product. This is crucial in the fermentation process because directly relates to microbial activity. The following are the results of measuring the brix degree using a refractrometer.

Table 3. Brix Degree Tepache Formula

Formula	F1	F2	F3	F4	F5	F6
Brix⁰	60	60	60	60	60	60

Brix levels are used to check the sugar content in tepache drinks. The higher brix degree, the higher sugar content. Next, hedonic and organoleptic test were carried out, including taste, colour and aroma. Organoleptic and hedonic test were conducted by 20 panelist, where the hedonic scale used a 5 point scale (1= dislike very much, 2= dislike, 3 = kind a like, 4 = like, 5 = like very much). The following are the result:

Table 4. Hedonic and Organoleptic result

Formula	Average Hedonic result	Organoleptic
F1 (Sugar)	4	Fresh sour taste, pineapple aroma, light yellow colour
F2 (Sugar)	4	Sweet and sour taste, pineapple aroma, light yellow colour
F3 (Sugar)	5	Fresh sweet and sour taste, tropical aroma, light yellow colour
F4 (Brown Sugar)	3	Sweet and sour taste, ripe pineapple aroma, brown colour
F5 (Brown Sugar)	2	Strong sour taste, ripe pineapple aroma, brown colour
F6 (Brown Sugar)	2	Very strong sour taste, aroma of ripe pineapple, brown colour.

Hedonic and organoleptic testing was carried out after 3 days of tepache beverage fermentation, when the tepache was testes it was served at room temperature. The panelist conducting the tests were untrained panelist. The hedonic and organoleptic tests are essential methods used to evaluate the sensory quality and consumer acceptance of fermented fruit beverages. The primary purpose of the hedonic test is to measure the level of preference or liking of consumers toward the product. Meanwhile, the organoleptic test aims to provide a more detailed sensory evaluation of the product's characteristics using human senses. Together, these tests are conducted to ensure that the fermented fruit beverage not only meets quality standards but also achieves a desirable balance of sensory attributes.(Caca Pratiwi1*, 2024)

For the TPC microbiological test by using PCA media, the result obtained were:

Table 5. Result of TPC (Total Plate Count)

Formula	Dilution		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
F1 (Sugar)	TBUD	TBUD	TBUD
F1 (Sugar)	TBUD	TBUD	TBUD
F2 (Sugar)	TBUD	TBUD	TBUD
F2 (Sugar)	TBUD	TBUD	70
F3 (Sugar)	TBUD	TBUD	73
F3 (Sugar)	TBUD	TBUD	70
F4 (Brown Sugar)	TBUD	TBUD	TBUD
F4 (Brown Sugar)	TBUD	TBUD	TBUD
F5 (Brown Sugar)	TBUD	38	TBUD
F5 (Brown Sugar)	TBUD	TBUD	TBUD
F6 (Brown Sugar)	TBUD	TBUD	69
F6 (Brown Sugar)	TBUD	18	29

For the results of microbiological tests with selective MRSA media, the results obtained were:

Table 6. Result Bacteria Growth On MRSA

Formula	Dilution		
	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
F1 (Sugar)	-	-	-
F1 (Sugar)	-	-	-
F2 (Sugar)	-	-	-
F2 (Sugar)	-	-	+
F3 (Sugar)	+	+	+
F3 (Sugar)	+	+	+
F4 (Brown Sugar)	-	-	-
F4 (Brown Sugar)	-	-	-
F5 (Brown Sugar)	+	-	-
F5 (Brown Sugar)	+	-	-
F6 (Brown Sugar)	+	+	+
F6 (Brown Sugar)	+	+	+

4. DISCUSSION

Based on the data, all formulations (F1–F6) showed identical pH values during fermentation. After 3 days of fermentation, the pH of all samples was stable at 4, indicating a moderately acidic environment. By day 7, the pH decreased uniformly to 2 across all formulations, reflecting a significant increase in acidity. This consistent trend suggests that the fermentation process progressed similarly in all formulations, with no observable differences in acid production. The decrease in pH over time indicates active microbial fermentation, leading to the accumulation of organic acids. Therefore, it can be concluded that variations in formulation (F1–F6) did not significantly influence the acidity profile during the fermentation period. The pH level plays a crucial role in determining the quality, safety, and stability of fermented beverages. In general, the optimal pH for the production of fermented drinks ranges between 3.0 and 4.5. This acidic environment is essential because it supports the growth of beneficial microorganisms, such as lactic acid bacteria and yeasts, while simultaneously inhibiting the growth of harmful or spoilage microbes. (8)

During the fermentation process, microorganisms convert sugars into organic acids, primarily lactic acid or acetic acid, which gradually lowers the pH. A pH that is too high (above 4.5) may indicate insufficient fermentation and can increase the risk of contamination. On the other hand, a pH that is too low (below 3.0) may result in an overly sour taste and could negatively affect the sensory acceptance of the beverage. Maintaining an optimal pH is therefore important not only for ensuring microbiological safety but also for achieving a balanced flavor profile. (9) Proper monitoring of pH throughout fermentation helps producers control the process, optimize product quality, and ensure consistency in the final fermented beverage. From the result of refractometer for brix degree The initial total soluble solids, expressed as degrees Brix ($^{\circ}\text{Bx}$), were measured for all formulations (F1–F6) at the beginning of the process (Brix_0). The results showed that all samples had an identical Brix value of 60°Bx . This uniformity indicates that each formulation started with the same concentration of dissolved sugars, ensuring consistency in the initial substrate available for fermentation. A Brix value of 60°Bx reflects a high sugar content, which is favorable for microbial activity, as it provides sufficient fermentable substrates for the production of organic acids, alcohol, or other metabolites during fermentation. (10) The consistency across all formulations suggests that any differences observed in subsequent stages of fermentation would not be due to variations in initial sugar concentration, but rather to other factors such as microbial activity, formulation composition, or fermentation conditions. (11) From the results of the hedonic test, the formula most preferred by the panelists was formula 3 using 250 grams of granulated sugar.

The bacterial growth on Plate Count Agar (PCA) was evaluated across six formulations (F1–F6) at serial dilutions of 10^{-4} , 10^{-5} , and 10^{-6} . The results showed that most samples exhibited TBUD (Too Numerous To Be Counted) colonies, particularly at lower dilutions (10^{-4} and 10^{-5}), indicating a very high microbial load in the samples. For formulations F1 and F2 (sugar-based), all replicates at dilutions 10^{-4} and 10^{-5} were consistently TBUD. At the highest dilution (10^{-6}), F2 showed one countable plate with 70 colonies, while the others remained TBUD. Similarly, F3 (sugar-based) demonstrated TBUD results at lower dilutions, but at 10^{-6} , colony counts of 73 and 70 were observed, indicating that this dilution was appropriate for enumeration. In the brown sugar formulations (F4–F6), F4 showed TBUD results across all dilutions, suggesting extremely high bacterial growth. F5 exhibited some countable colonies at 10^{-5} (38 colonies), although most plates were still TBUD. Meanwhile, F6 showed more variation, with countable colonies at 10^{-6} (69 colonies) and both 10^{-5} (18 colonies) and 10^{-6} (29 colonies) in the second replicate. Overall, the data indicate that bacterial populations were generally very high in all formulations, as evidenced by the predominance of TBUD results. Countable colonies were mostly observed at the highest dilution (10^{-6}), suggesting that this dilution level is the most suitable for estimating microbial load in these samples. The variation in colony counts between sugar and brown sugar formulations may reflect differences in nutrient composition influencing microbial growth.

From the results of the MRSA test, it was found that on average lactobacillus appeared in the formula with the addition of 250 gr of sugar/palm sugar. Sugar plays a crucial role in influencing the growth of *Lactobacillus*, a group of lactic acid bacteria widely used in fermentation processes. As a primary carbon and energy source, sugar is metabolized by *Lactobacillus* through fermentation pathways, mainly producing lactic acid. This metabolic activity not only supports bacterial growth but also lowers the pH

of the environment, creating favorable conditions for *Lactobacillus* while inhibiting the growth of spoilage and pathogenic microorganisms. The concentration of sugar significantly affects the growth rate of *Lactobacillus*. (12) At optimal levels, sugar enhances bacterial proliferation by providing sufficient substrates for energy production and cell division. (13) However, excessively high sugar concentrations can create osmotic pressure that inhibits bacterial activity, slowing down growth or even causing cell stress. Conversely, low sugar availability may limit bacterial metabolism, resulting in reduced growth and lower acid production. (14) Different types of sugars, such as glucose, fructose, and sucrose, may also influence *Lactobacillus* growth differently, depending on the strain's enzymatic capability to utilize these sugars. Overall, maintaining an appropriate type and concentration of sugar is essential to optimize the growth and metabolic activity of *Lactobacillus* in fermentation systems. (15) Based on the SNI (Standar Nasional Indonesia) for probiotic drinks, the standard for probiotic fruit drinks has not been set, but we can use the SNI for probiotic drinks as a reference. Based on SNI 2981:2009, tepache drinks have the potential to be probiotic drinks because they contain *Lactobacillus* based on analysis with MRSA and the number of colonies exceeds 10^6 CFU (Colony Form Unit).

5. CONCLUSION

The research found that the optimal fermentation period for tepache production was 3 days. This Fermentation time results in pH that meets the Indonesian National Standard (SNI) probiotic beverage standards and has acceptable hedonic and organoleptic characteristic. Furthermore, differences in sugar use in tepache production did not significantly impact bacterial growth, but did affect organoleptic results.

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