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Development of Spontaneous Fermented Drink from Red Dragon Fruit Peel (*Hylocereus polyrhizus*): Chemical and Microbiological Perspectives

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ABSTRACT

Red dragon fruit peel (Hylocereus polyrhizus) has a higher antioxidant content than red dragon fruit flesh. However, the peel of red dragon fruit is still not widely utilized in manufacturing functional foods. Fermented drink is one type of product that uses microbes to decompose nutrients contained in raw materials therefore the nutritional value of the resulting product can be increased. This study aimed to make fermented drink from red dragon fruit peel which was done spontaneously with the addition of palm sugar as a source of nutrients for microbes. The method used was a complete randomized design (CRD) with one factor: the concentration of palm sugar (10%, 20%, 30%, and 40%) and the weight of red dragon fruit peel as much as 60%. The results showed that the best dragon fruit peel fermented drink formulation was using 30% palm sugar which produced the highest total titratable acidity of $0.36\pm0.05\%$ with a pH value of 3.39 ± 0.03 . Total lactic acid bacteria also had the highest value of $3.30\times10^9\pm0.06$ CFU/ml while the reducing sugar content was $4.47\pm0.015\%$. The highest reducing sugar ($5.04\pm0.065\%$) was obtained in the formulation that uses 20% of palm sugar as carbon sources.

Keywords: Lactic acid bacteria; Reducing sugar; Red dragon fruit peel; Spontaneous fermentation; Total titratable acidity

INTRODUCTION

One fruit variety that appeals to the general public is dragon fruit. Mexico, Central America, and South America are the origins of dragon fruit. But nowadays it has been widely cultivated in tropical and subtropic regions. In Southeast Asia, such as Thailand, Vietnam, and Indonesia, dragon fruit has become one of the most popular horticultural commodities. Its unique shape, refreshing flavor, and health benefits are the main attractions that have boosted the fruit's popularity in the global market (Shah et al., 2023). Hylocereus undatus, a white flesh dragon fruit, Hylocereus polyrhizus, a red flesh dragon fruit, Hylocereus costaricencis, and a white flesh yellow dragon fruit are the four varieties of dragon fruit (Saha et al., 2024). One variety of dragon fruit that is widely available to the general public is red flesh. Dragon fruit is well-known for its abundant nutrients, including bioactive ingredients, minerals, vitamins, fiber, and carbohydrates, in addition to its distinctive form and color. These components support digestive health, strengthen the immune system, and may even act as a natural antioxidant, among other health advantages (Nishikito et al., 2023). Dragon fruit is used only in processed forms such as juice, chips, crackers, and other preparations. The nutritional content of dragon fruit peel is also quite a lot and good for health, especially its antioxidant activity which is higher than dragon fruit flesh (Suryaningsih et al., 2021). Therefore, dragon fruit peel may also be a viable functional food alternative. About 30-35% of the weight of the dragon fruit is made up of the peel (Nurhafsah et al., 2023). The amount of dragon fruit production in Indonesia in 2023 reached 3,174,06 quintals, resulting in up to 1.5 million quintals of dragon fruit peel waste (BPS, 2024). This peel waste offers a great opportunity to be utilized as a raw material for functional food products and reduce the environmental impact of organic waste.

One of the emerging ways to process dragon fruit peel waste is through fermentation. Fermented drinks have become a trend in modern society because they offer not only a unique

flavor, but also higher health benefits compared to non-fermented products. Fermented drink is a product that results from the process of controlling microbial growth to produce a product with higher functional value (Keşa et al., 2021). Currently, plant-based fermented drink products are a lifestyle choice that is in demand by the public. Lactic acid bacteria (LAB) that play a role in the process of making fermented drinks can break down the substrate into products with better functional value, nutritional content, and sensory characteristics (Cichońska et al., 2024; Wang et al., 2021). The fermentation process can be carried out spontaneously or under control. In spontaneous fermentation, the microbes responsible come from the environment or naturally come from the raw materials without any intervention. In controlled fermentation, the type and number of microbes used are controlled because there is an external intervention to condition the microbes grown (Alagbe et al., 2022; Canonico et al., 2022). Currently, there have been many studies conducted to develop fermented drinks. Research by Lu et al., (2020) showed that making wine from grapes through spontaneous fermentation produced wine with a higher content of bioactive molecules and volatile compounds and was able to improve the flavor of the wine. Furthermore, spontaneous fermentation is known for its ability to produce diverse and complex flavor characteristics due to the presence of a wider range of microbes. Research by Gong et al., (2023) showed that spontaneous fermentation of red dragon fruit produced 69 yeast species divided into 49 genera with a fermentation time of 1 day. While spontaneous fermentation of white dragon fruit produced 37 yeast species in 32 genera with fermentation time of 3 days. This microbial diversity promotes the complexity of fermentation, as different species and strains interact dynamically to produce a broad array of metabolic byproducts, including organic acids, alcohols, and bioactive compounds.

Therefore, this study aimed to make fermented drinks from red dragon fruit peel waste through the spontaneous fermentation process. The resulting fermented drinks could have a high content of lactic acid bacteria that affect the functional properties of the resulting drink. The carbon source used in this research was palm sugar which plays a role in microbial growth. Suharman et al., (2021) showed that sucrose concentration affected the total lactic acid bacteria produced. Total lactic acid bacteria increased then decreased after adding excessively high sucrose in yogurt. Excessively low sucrose concentrations can inhibit microbial growth, while overly high concentrations can cause an imbalance in microbial metabolism. Furthermore, the study by Sinamo et al., (2022) proved that the concentration of sugar impacted the secang kombucha drink's total acid, pH, total soluble solids, and viscosity. Liu et al., (2023) showed adding sugar to blueberry wine could stop the phenol and anthocyanins from degrading. Therefore, adding sugar to the fermentation process can enhance the final beverage's functional qualities. Thus, the main goal of this study was to investigate how the concentration of palm sugar affects the chemical and microbiological properties of fermented drinks made from dragon fruit peel. This will inform the judicious selection of the lowest palm sugar concentrations that will produce fermented drinks with the best quality based on the parameters of reducing sugar content, pH, total titratable acidity, and the number of lactic acid bacteria. Reducing sugar content indicates the microbial metabolic process during fermentation, while pH and total titratable acidity show the level of acidity produced. The amount of LAB is an indicator of the efficacy of fermentation and its potential as a functional drink. It was expected that the findings of this study would not only offer a way to make use of leftover dragon fruit peels but also produce novel goods that customers would find appealing. Plant-based fermented drinks have become part of the healthy lifestyle of modern society. By utilizing red dragon fruit peels, this product can compete in the global functional beverage

market, while supporting sustainability principles by reducing organic waste.

METHODS

Material

Red dragon fruit (*Hylocereus polyrhizus*) with an average weight of 400-600g, without defects in the skin obtained from traditional markets in Jember Regency. Palm sugar was obtained from a store in Jember Regency.

Chemicals used included ethanol 95%, Luff-Schoorl reagent, potassium iodide 20%, H₂SO₄ 26.5%, sodium thiosulfate, sodium hydroxide, phenolphthalein, and amylum obtained from Sigma Aldrich (St. Louis, MO, USA). Methicillin-resistant Staphylococcus aureus (MRSA) was used as the bacterial strain

Equipment

The equipment used included knives, cutting boards, bowls, spatulas, pans, containers, glassware (Pyrex) such as Erlenmeyer flasks, burettes, beakers, and volumetric flasks, a water bath (Memmert), and analytical balances (Ohaus).

Preparation of red dragon fruit peel fermented drink

The surface of the table was sterilized using 70% ethanol before the making process to prevent contamination. The preparation process of red dragon fruit peel fermented drink refers to Lim et al., (2023) with modifications. Red dragon fruit was washed with tap water to remove dirt and residue. Furthermore, the dragon fruit was peeled to separate the flesh and peel. The peel of the dragon fruit was sliced into tiny, roughly 1 cm pieces. The peel of the dragon fruit was rewashed. The weight of dragon fruit used in the formulation was 60% w/w and the concentration of palm sugar used was 10%, 20%, 30%, and 40%. The container used in the fermentation process was washed and sterilized with hot water. Palm sugar was dissolved using hot water, and the dragon fruit peel was put into the container when the temperature started to warm up. The fermentation process lasted 48 hours at room temperature (37°C). The resulting fermented drink was separated between the liquid and the solid (peel). The separation process was carried out on a flame lighted by Bunsen to prevent contamination. The red dragon fruit peel fermented beverage was stored at 4°C for further analysis. Research conducted by (Anyiam et al., 2023) stated that fermentation time of 48 hours produced Cassava mahewu fermented drinks with low pH and the highest total titratable acidity. These circumstances result in fermented drinks that are more microbially stable and have a longer shelf life. This is because harmful bacteria found in food, including Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli, cannot survive at low pH levels.

Research Design

The research design used was a complete randomized design (CRD) with one factor, namely palm sugar concentration (P1 = 10%, P2 = 20%, P3 = 30%, and P4 = 40%) with a concentration of red dragon fruit peel of 60%. Each treatment was repeated three times. According to research by Dianasari *et al.*, (2020), red dragon fruit peel contains glucose, fructose, and maltose, which serve as carbon sources for the growth of lactic acid bacteria. This explains why red dragon fruit peel is used up to 60% of the time. A 60% concentration of red dragon fruit peel may boost lactic acid bacteria activity during fermentation and result in fermented beverages with the ideal amount of lactic acid bacteria.

Reducing sugar analysis

Reducing sugar analysis refers to the AOAC (1995) method. 5 grams of the sample were weighed and placed in a 100 mL volumetric flask. Distilled water was then added until the desired amount was reached. A 250 mL Erlenmeyer flask was filled with 25 mL of the filtrate. The Erlenmeyer was then filled with 25 mL of Luff-Schoorl solution, and the mixture was heated for 10 minutes in a water bath. After that, the solution was cooled quickly using tap water, and then 10 mL of 15% KI solution was added followed by 25 mL of 25% H₂SO₄ solution. Conversely, a blank was made in an Erlenmeyer by combining 25 mL of Luff-Schoorl solution with 25 mL of distilled water. After that, 0.1 N sodium thiosulfate solution was used to titrate the samples and blanks. Toward the end of the titration, 2-3 mL of amylum indicator was added to make it easier to see color changes. Titration is considered complete when the blue color turns milky white. The difference in the volume of thiosulfate solution used for the sample and blank was calculated and then converted based on the relation table between the volume of thiosulfate solution used and the amount of reducing sugar.

Total Titratable Acidity

A titration method based on AOAC (2000) was used to determine total titratable acidity (TAT). After weighing up to 10 grams, the sample was diluted to 100 ml. Three drops of phenolphthalein indicator were added to 10 ml of sample in an Erlenmeyer. After that, 0.1 N NaOH was added to the sample until it reached the titration's end point, which was indicated by the appearance of a stable pink color.

pН

Measurement of pH value based on AOAC, (1995), was carried out with an OEM pH-meter (EZ-9909), the pH meter was first calibrated using pH 4.01, pH 6.86, and pH 9.18 buffers before being used to measure pH. The pH value was determined by placing the electrode on the dragon fruit peel fermented drink sample and the pH value of the sample was read on the screen.

Total Lactic Acid Bacteria

Total Lactic Acid Bacteria (LAB) analysis was carried out based on (Fardiaz, 1993). The calculation of LAB was done by calculating the total LAB that grew on de Man Rogosa and Sharpe Agar (MRSA) culture media. The calculation of total LAB was done by diluting sample into distilled water in a ratio of 1:9. Dilution was done up to 10⁻⁸ dilution. The first dilution process was carried out by diluting 1 ml of sample into 9 ml of sterile distilled water (10⁻¹). The second dilution was carried out using 1 ml of the first dilution results were put into 9 ml of sterile distilled water (10⁻²), and carried out until a dilution (10⁻⁸) was obtained. Media preparation was made by dissolving 5.2 g MRSA into 100 ml of distilled water and sterilized in an autoclave 121°C for 15 minutes. Sampling was done by taking 1 ml of samples from dilutions 10⁻⁶, 10⁻⁷ and 10⁻⁸ and put into a Petri dish. Then as much as 10 ml of MRSA that had been cooled (temperature 47-50°C) was poured into a Petri dish. Furthermore, the media in the Petri dish was flattened. The dish was incubated for 48 hours at 37°C inverted after solidifying. The following formula can be used to calculate the total lactic acid bacteria using the Total Plate Count method:

$$CFU/ml = \frac{\text{number of colonies} \times \text{dillution factor}}{\text{volume of culture plate}}$$
 (1)

Data Analysis

Data were analyzed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) to determine significant differences (p < 0.05). All statistical analyses were performed

using IBM SPSS Statistics 25.

RESULT AND DISCUSSIONS

A number of biochemical processes called fermentation are carried out by microorganisms and are crucial in converting substrates into a wide range of products that have an impact on the final product's chemical makeup and environment. Therefore, fermentation is not just a simple reaction where sugar is converted into alcohol. Instead, fermentation involves the complex breakdown of sugars into various by-products, such as alcohols, organic acids, and metabolites, each of which contributes to the chemical characteristics of the product (Ngoc et al., 2024). At low sugar concentrations, the conversion of sugar into alcohol will happen faster than at high sugar concentrations. In this case, microorganisms can more easily convert sugar into alcohol making the fermentation process more efficient (Dimero & Tepora, 2018). These differences in fermentation rates are critical in understanding how different substrates influence microbial activity and the resulting products. In the fermentation process, sugar provides a carbon source for the growth of microorganisms. The breakdown of sugar in the fermentation process by microorganisms will produce various types of products including alcohol, acids, and metabolites that can affect the chemical characteristics of the resulting fermentation product (Xie & Gänzle, 2023). Table 1 presents the chemical characteristics of fermented drinks from red dragon fruit peel, while Table 2 displays the total lactic acid bacteria contained in the fermented drinks. Palm sugar is selected as a carbon source in the preparation of fermented drinks because palm sugar contains glucose and fructose as well as sucrose. Thus, palm sugar is more easily broken down by microbes converted into alcohol and organic acids. According to research conducted by Maryani et al., (2021), palm sugar contains 89.94% sucrose, 3.61% glucose, and 3.50% fructose. Meanwhile, sugar cane had a sucrose content of 94.75%. The different sugar composition of palm sugar makes it an excellent choice for fermentation processes, as it promotes microbial activity and the formation of more diverse products.

Table 1. Chemical Analysis of Red Dragon Fruit Peel Fermented Drinks

No	Sample	Reducing Sugar (%)	Total Titratable Acidity (%)	рН
1	P1	4.14±0.078 ^b	0.27±0.015 ^b	3.54±0.015 ^b
2	P2	5.04±0.065 ^d	0.31±0.004bc	3.53±0.035 ^b
3	P3	4.47±0.015°	$0.36\pm0.05^{\circ}$	3.39±0.03 ^a
4	P4	1.98±0.144a	0.18±0.025a	3.57±0.025 ^b

Note: Different superscripts show significant differences (p < 0.05) based on the DMRT test

Reducing Sugar

Reducing sugar is the product of hydrolysis of sugar, where complex sugars, such as sucrose, are broken down into simpler sugars, mainly glucose and fructose. Reducing sugar acts as a carbon source in the fermentation process that fuels microbial growth and the production of various fermentation products. Microorganisms further break down reducing sugars to produce alcohol and other byproducts such as acids and metabolites, which contribute to the fermented product's characteristics. Higher alcohol content in fermented products indicates lower levels of reducing sugars consumed by microorganisms during the fermentation process (Kim *et al.*, 2018). Table 1 indicates that the reducing sugar of the fermented drink made from red dragon fruit peel is significantly impacted (p<0.05) by the concentration of sugar. The highest reducing sugar value (5.04%) was obtained in fermented drinks with a sugar concentration of 20%. The amount of reduced sugar produced increases

with the concentration of sugar. Microorganisms in the fermentation process grow more quickly at higher sugar concentrations, emphasizing the relation between microbial activity and substrate availability (González-Garcinuño *et al.*, 2017). This leads to more sugar being hydrolyzed into glucose and fructose, which are the reducing sugars (Dali *et al.*, 2024). This research aligns with the findings of Najri *et al.*, (2022), who discovered that the higher the sugar concentration in the preparation of bacterial cellulose, the greater the value of reducing sugar content. The bacterial cellulose made by adding 40g sugar and 7 days fermentation time had the highest value of reducing sugar content (2.612%). These results highlighted the importance of sugar concentration in influencing microbial metabolism and the subsequent formation of fermentation products.

However, when the sugar concentration was increased again, there was a decrease in the value of reducing sugar content in the fermented drink. As seen in Table 1, at 30% sugar concentration, the reducing sugar content was 4.47% and decreased again at 40%, which was 1.98%. The higher sugar concentration causes an increase in osmotic pressure in the microbial growth environment which can interfere with the growth of microorganisms (Huan et al., 2020). This leads to a decrease in the quantity of hydrolyzed sucrose into glucose and fructose, which reduce sugars. This is in line with research conducted by Timmermans et al., (2022) which indicated that the higher concentration of sucrose used in pastry making caused a decrease in glucose and fructose levels. As osmotic pressure increases, it creates an unfavorable environment for microorganisms, reducing their ability to effectively hydrolyze sucrose into glucose and fructose. This phenomenon highlights the delicate balance between providing sufficient sugar for microbial metabolism and avoiding excessive concentrations that might hinder microbial performance. Consequently, the quantity of reduced sugars decreases, resulting in lower alcohol production.

Total Titratable Acidity and pH

Total titratable acidity (TTA) and pH value are some of the parameters that determine the quality of fermented drinks. These parameters serve as indicators of the acid-base balance, which directly influences the flavor, shelf life, and overall acceptability of the product. Based on Table 1, the value of total titratable acidity is inversely proportional to the pH value. The higher the sugar concentration, the lower the total titratable acidity value but higher pH value. The addition of sugar in the preparation of red dragon fruit peel fermented drinks has a significant effect on the value of total titratable acidity and pH value (p<0.05). The pH value was at its lowest of 3.39 at 30% sugar concentration, and the total value of titratable acidity reached the optimal point of 0.36%. The total titratable acidity of fermented drinks is influenced by the concentration of organic acids produced during the fermentation process. Organic acids produced in the anaerobic fermentation process include lactic acid, citric acid, succinic acid, malic acid, tartaric acid, and oxalic acid which is the result of glycolysis process of microorganisms (Bangar et al., 2022). The higher the concentration of sugar used in making fermented drinks, the more sugar is hydrolyzed into reducing sugar which in turn forms alcohol and organic acids by microorganisms causing an increase in the value of total titratable acidity (Sabira & Suryani, 2023). However, as the sugar increases after passing the optimum conditions, the value of total titratable acidity decreases and causes an increase in pH value. These findings are in line with research conducted by Zheng et al., (2024) that the addition of more sugar inhibited the formation of acetic acid, citric acid, and succinic acid. The research conducted by Sinamo et al., (2022) also showed that the total acid in fermented drinks using kombucha increased along with the increase in sugar concentration, but after passing the optimum conditions, the total acid decreased. This is because the increase in sugar will cause an increase in alcohol content which inhibits the growth of lactic acid bacteria causing the total acid to be decreased (Ferreira & Mendes-Faia, 2020). In addition, excess alcohol will react with acids to form esters, therefore the amount of organic acids decreases. This reaction not only lowers the TTA value but also contributes to changes in the aroma and flavor profile of the fermented drink (Kłosowski & Czupryński, 2006). Understanding this interaction emphasizes the important role of sugar concentration in fermentation. Appropriate control of sugar levels can optimize acid production, promote microbial growth, and improve overall beverage quality. It also points to the need for careful monitoring during the fermentation process to avoid conditions that could lead to reduced acidity and compromised flavor profiles.

Total Lactic Acid Bacteria (LAB)

Spontaneous fermentation involves microorganisms that are naturally present in the raw materials (Sionek *et al.*, 2023). In addition to relying on microorganisms derived from raw materials, the efficacy of the fermentation process is also influenced by other substrates such as sugar as a source of nutrients for bacteria. The amount of sugar that acts as a carbon source will affect the total lactic acid bacteria that can live in the resulting fermented drink (Cahyanti *et al.*, 2022). Table 2 shows the effect of sugar concentration on total lactic acid bacteria in red dragon fruit peel fermented drinks.

Table 2: Total Lactic Acid Bacteria of Dragon Fruit Peel Fermented Drinks

No	Sample	Total Lactic Acid Bacteria (CFU/ml)	
1	P1	2.65 x 10 ⁹ ± 0.075 ^b	
2	P2	$3.06 \times 10^9 \pm 0.055^{\circ}$	
3	P3	$3.30 \times 10^9 \pm 0.06^d$	
4	P4	$6.94 \times 10^8 \pm 0.017^a$	

Note: Different superscripts show significant differences (p < 0.05) based on DMRT test

Table 2 shows that the total lactic acid bacteria (LAB) in dragon fruit peel fermented drinks is significantly influenced by the sugar concentration (p<0.05). Alongside the rise in the amount of sugar used, there were more lactic acid bacteria (LAB). Dragon fruit peel fermented drink with 30% sugar content had the highest number of lactic acid bacteria at 3.30 x 109 CFU/mL while at 10% sugar content, the number of lactic acid bacteria was 2.65x109 CFU/ml. The increase in sugar concentration which acts as a substrate or carbon source for bacteria causes an increase in bacterial growth. Lactic acid bacteria grow more quickly when there is more substrate available for them to use as a source of nutrients (Fuso et al., 2023). The availability of nutrients will significantly affect the growth of bacteria. This is following research conducted by Suharman et al., (2021) which showed that revealed the number of lactic acid bacteria produced increased with the amount of sucrose used to make bay flower yogurt. At 12% sucrose concentration, the number of lactic acid bacteria in bay flower yogurt was 2.86x107 CFU/ml, while at 4% sucrose concentration, the number of lactic acid bacteria was only 7.60x10⁶ CFU/ml. As demonstrated in Table 2, however, the quantity of lactic acid bacteria produced decreased as the sugar concentration increased. There were smaller lactic acid bacteria at 40% sugar concentration (6.94 x 10⁸ CFU/ml). This was caused by unfavorable environmental conditions for the growth of bacteria. High osmotic pressure causes a decrease in cell viability and cell production levels of the bacteria themselves (Douradinho et al., 2023). This research was under research conducted by (Liu et al., 2017) which showed that the higher the concentration of sugar in making "Shuangyou" red wine, the smaller the number of yeasts will be. This is due to environmental conditions that are less than optimal for the growth of microorganisms and result in many

microorganisms dying. The decline in LAB at higher sugar concentrations can also be explained by the metabolic byproducts produced during fermentation. As LAB metabolizes sugars, they generate organic acids and other compounds that can accumulate and alter the fermentation environment. At elevated sugar levels, the production of these byproducts may reach inhibitory concentrations, further complicating the survival of LAB. Additionally, high sugar concentrations may favor the growth of other microorganisms, potentially leading to competition and resource depletion, which can negatively impact LAB populations. Currently, fermented drinks standards in Indonesia are still limited to fermented drinks with high alcohol content such as wine and beer and fermented milk drinks. The research conducted has not met the standards of fermented drinks with high alcohol because the fermentation time only lasts for 48 hours. Therefore, the standard used refers to the production of fermented milk drinks (SNI 7552-2018) where the fermentation process is also carried out for 24-48 hours with a total bacterial culture of at least 1x10⁶ CFU/mL. Based on the research conducted on all sugar concentrations, the total lactic acid bacteria that meet the standards are produced, but the highest total lactic acid bacteria (LAB) produced, which is 3.30 x 10⁹ CFU/ml, is found in the use of sugar concentration of 30%. In addition, the use of palm sugar at 30% also produces high reducing sugar levels of 4.47±0.015%. Therefore, fermented red dragon fruit peel drinks with the use of 30% palm sugar and 60% red dragon fruit skin can be a good probiotic drink candidate.

CONCLUSION

Red dragon fruit peel fermented drink made through spontaneous fermentation process demonstrated significant variations in chemical and microbiological properties based on the concentration of palm sugar and dragon fruit peel used. The drink had the highest reducing sugar content of 5.04±0.065% in the use of palm sugar of 20% and dragon fruit peel concentration of 60%. This suggests that the substrate availability was ideal for microbial activity at this level, enabling the effective hydrolysis of sucrose into the reducing sugars fructose and glucose. The highest total titratable acidity content of 0.36±0.05% was obtained with the use of 30% palm sugar with a pH value of 3.39±0.03. These parameters imply that the fermentation process produced organic acids, such as lactic, citric, and malic acids, which are common byproducts of anaerobic microbial metabolism, at this concentration. Because it affects the fermented drink's flavor, preservation, and general acceptability, acidity is important. With 30% palm sugar, the maximum amount of lactic acid bacteria generated during the spontaneous fermentation of red dragon fruit peel was 3.30x10⁹±0.06 CFU/ml. Lactic acid bacteria (LAB) is essential for imparting probiotic benefits, enhancing the flavor, and contributing to the acidic profile of the drink The best treatment obtained in the preparation of dragon fruit skin fermented beverages was seen based on the total lactic acid bacteria contained in it. Thus, the best treatment in the preparation of dragon fruit peel fermented drink was the use of palm sugar concentration of 30% and dragon fruit peel concentration of 60%. This combination not only maximized microbial activity and organic acid production but also ensured a balanced flavor and desirable chemical characteristics, making it an ideal treatment for high-quality fermented drinks. The use of excessively low palm sugar concentration (10%) resulted in a total LAB of 2.65 x 10⁹±0.075 CFU/ml with reducing sugar of 4.14±0.078%, TAT of 0.27±0.015%, and pH of 3.54±0.015%. This shows that with sugar concentration of 10% is still considered insufficient to produce fermented drinks with optimal nutrient content.

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REFERENCES

- Alagbe, E. E., Okoye, G. C., Amoo, T. E., Adekeye, B. T., Taiwo, O. S., Adeyemi, A. O., & Daniel, E. O. (2022). Spontaneous and controlled fermentation to improve nutritional value of Ikpakpa beans, Phaseolus vulgaris. *Cogent Engineering*, *9*(1). https://doi.org/10.1080/23311916.2022.2066823
- Anyiam, P. N., Nwuke, C. P., Uhuo, E. N., Ije, U. E., Salvador, E. M., Mahumbi, B. M., & Boyiako, B. H. (2023). Effect of fermentation time on nutritional, antinutritional factors and in-vitro protein digestibility of macrotermes nigeriensis-cassava mahewu. *Measurement: Food*, *11*, 100096. https://doi.org/10.1016/j.meafoo.2023.100096
- AOAC. (1995). Official Methods of Analysis of Association of Official Analytical Chemist. AOAC International. In AOAC International.
- AOAC. (2000). Official Methods of Analysis of Association of Official Analytical Chemist. In *AOAC International*.
- Bangar, S. P., Suri, S., Trif, M., & Ozogul, F. (2022). Organic acids production from lactic acid bacteria: A preservation approach. *Food Bioscience*, *46*, 101615. https://doi.org/10.1016/j.fbio.2022.101615
- BPS. (2024). Produksi Tanaman Buah-buahan, 2021-2023. Badan Pusat Statistik.
- Cahyanti, A. N., Nofiyanto, E., Sampurno, A., Sudjatinah, M., & Wibowo, C. H. (2022). Viability of Lactic Acid Bacteria and Its Sugar Metabolism Capability in Fermentation Using Local Salak Extract. *The 1st International Conference on Agricultural, Nutraceutical, and Food Science (ICANFS)* 2022.
- Canonico, L., Agarbati, A., Comitini, F., & Ciani, M. (2022). Assessment of Spontaneous Fermentation and Non-Saccharomyces Sequential Fermentation in Verdicchio Wine at Winery Scale. *Beverages*, 8(3), 49. https://doi.org/10.3390/beverages8030049
- Cichońska, P., Kostyra, E., Piotrowska, A., Ścibisz, I., Roszko, M., & Ziarno, M. (2024). Enhancing the sensory and nutritional properties of bean-based and lentil-based beverages through fermentation and germination. *LWT*, 199, 116140. https://doi.org/10.1016/j.lwt.2024.116140
- Dali, N., Chairunnas, A., Amalia, H. A. M., & Puspitasari, S. A. A. (2024). The Effect of Acidity Level (pH) and Palm Sugar Sucrose Levels on the Quality of Brown Sugar. *METANA*, *20*(1), 14–26. https://doi.org/10.14710/metana.v20i1.57772
- Dianasari, U., Malaka, R., & Maruddin, F. (2020). Physicochemical quality of fermented milk with additional red dragon fruit (Hylocereus polyrhizus) skin. *IOP Conference Series: Earth and Environmental Science*, 492(1). https://doi.org/10.1088/1755-1315/492/1/012050
- Dimero, F. N., & Tepora, T. F. (2018). Processing and Development of Dragon Fruit Wine. International Journal of Environment, Agriculture and Biotechnology, 3(5), 1943–1947. https://doi.org/10.22161/ijeab/3.5.49
- Douradinho, R., Sica, P., Tonoli, F., Mattos, E., Oliveira, M., Pinto, A., Mota, L., Faria, T., Costa, V. F., Leite, G., Arthur, V., Coelho, S., & Baptista, A. (2023). Osmotic Stress Alleviation in Saccharomyces cerevisiae for High Ethanol Fermentations with Different Wort Substrates. *Stresses*, *3*(4), 813–826. https://doi.org/10.3390/stresses3040055

- Fardiaz, S. (1993). Analisis Mikrobiologi Pangan. PT. Raja Grafindo Persada.
- Ferreira, A. M., & Mendes-Faia, A. (2020). The Role of Yeasts and Lactic Acid Bacteria on the Metabolism of Organic Acids during Winemaking. *Foods*, *9*(9), 1231. https://doi.org/10.3390/foods9091231
- Fuso, A., Bancalari, E., Castellone, V., Caligiani, A., Gatti, M., & Bottari, B. (2023). Feeding Lactic Acid Bacteria with Different Sugars: Effect on Exopolysaccharides (EPS) Production and Their Molecular Characteristics. *Foods*, *12*(1), 215. https://doi.org/10.3390/foods12010215
- Gong, X., Zou, S., Liu, W., Yu, Z., & Liu, X. (2023). Analysis of yeast diversity during spontaneous fermentation of red and white pitaya. *International Food Research Journal*, *30*(3), 796–804. https://doi.org/10.47836/ifrj.30.3.21
- González-Garcinuño, Á., Tabernero, A., Sánchez-Álvarez, J. M., Galán, M. A., & Martin del Valle, E. M. (2017). Effect of bacteria type and sucrose concentration on levan yield and its molecular weight. *Microbial Cell Factories*, *16*(1), 91. https://doi.org/10.1186/s12934-017-0703-z
- Huan, P. T., Hien, N. M., & Anh, N. H. T. (2020). Optimization of alcoholic fermentation of dragon fruit juice using response surface methodology. *Food Research*, *4*(5), 1529–1536. https://doi.org/10.26656/fr.2017.4(5).125
- Keşa, A.-L., Pop, C. R., Mudura, E., Salanţă, L. C., Pasqualone, A., Dărab, C., Burja-Udrea, C., Zhao, H., & Coldea, T. E. (2021). Strategies to Improve the Potential Functionality of Fruit-Based Fermented Beverages. *Plants*, *10*(11), 2263. https://doi.org/10.3390/plants10112263
- Kim, D.-H., Lee, S.-B., Kim, D.-H., Lee, S.-B., & Park, H.-D. (2018). Fermentation characteristics of campbell early grape wine inoculated with indigenous Korean wine yeasts encapsulated in Ca-alginate beads after air-blast drying. *Italian Journal of Food Science*, *30*, 535–552. https://doi.org/10.14674/IJFS-1118
- Kłosowski, G., & Czupryński, B. (2006). Kinetics of acetals and esters formation during alcoholic fermentation of various starchy raw materials with application of yeasts Saccharomyces cerevisiae. *Journal of Food Engineering*, 72(3), 242–246. https://doi.org/10.1016/j.jfoodeng.2004.12.001
- Lim, T. W., Choo, K. Y., Lim, R. L. H., Pui, L. P., Tan, C. P., & Ho, C. W. (2023). The indigenous microbial diversity involved in the spontaneous fermentation of red dragon fruit (Hylocereus polyrhizus) identified by means of molecular tools. *Heliyon*, *9*(11), e21940. https://doi.org/10.1016/j.heliyon.2023.e21940
- Liu, H., Bao, M.-L., Chen, H.-L., & Li, Q. (2017). Impact of Sucrose Addition on the Physiochemical Properties and Volatile Compounds of "Shuangyou" Red Wines. *Journal of Food Quality*, *2017*, 1–11. https://doi.org/10.1155/2017/2926041
- Liu, J., Wang, Q., Weng, L., Zou, L., Jiang, H., Qiu, J., & Fu, J. (2023). Analysis of sucrose addition on the physicochemical properties of blueberry wine in the main fermentation. *Frontiers in nutrition*, *9*, 1092696. https://doi.org/10.3389/fnut.2022.1092696
- Lu, Y., Sun, F., Wang, W., Liu, Y., Wang, J., Sun, J., Mu, J., & Gao, Z. (2020). Effects of spontaneous fermentation on the microorganisms diversity and volatile compounds during 'Marselan' from grape to wine. *LWT*, 134, 110193.

https://doi.org/10.1016/j.lwt.2020.110193

- Maryani, Y., Rochmat, A., Khastini, R. O., Kurniawan, T., & Saraswati, I. (2021). Identification of Macro Elements (Sucrose, Glucose and Fructose) and Micro Elements (Metal Minerals) in the Products of Palm Sugar, Coconut Sugar and Sugar Cane. https://doi.org/10.2991/absr.k.210304.051
- Najri, M., Antara, N. S., & Wijaya, I. M. M. (2022). Pengaruh Penambahan Gula dan Lama Fermentasi terhadap Karakteristik Selulosa Bakterial dari Kulit Pisang Kepok (Musa paradisiaca L.). *Jurnal Rekayasa Dan Manajemen Agroindustri*, 10(2), 211–220. https://doi.org/10.24843/JRMA.2022.v10.i02.p09
- Ngoc, T., Thinh, P., Mui, D., Uyen, L., Ngan, N., Tran, N., Khang, P., Huy, L., Minh, T., & Trung, N. (2024). Influences of Fermentation Conditions on the Chemical Composition of Red Dragon Fruit (Hylocereus polyrhizus) Wine. *Beverages*, 10(3), 61. https://doi.org/10.3390/beverages10030061
- Nishikito, D. F., Borges, A. C. A., Laurindo, L. F., Otoboni, A. M. M. B., Direito, R., Goulart, R. de A., Nicolau, C. C. T., Fiorini, A. M. R., Sinatora, R. V., & Barbalho, S. M. (2023). Anti-Inflammatory, Antioxidant, and Other Health Effects of Dragon Fruit and Potential Delivery Systems for Its Bioactive Compounds. *Pharmaceutics*, 15(1), 159. https://doi.org/10.3390/pharmaceutics15010159
- Nurhafsah, N., Laboko, A. I., Gobel, D. G., Fitriawaty, Novitasari, E., Muazam, A., Andriani, I., & Rahmi, R. (2023). Effect of red dragon fruit (Hylocereus polyrhizus) peel and pulp on jelly candy chemical composition and acceptance. *IOP Conference Series: Earth and Environmental Science*, 1183(1), 012059. https://doi.org/10.1088/1755-1315/1183/1/012059
- Sabira, Q. A. N., & Suryani, T. (2023). Glucose Levels and Organoleptic Quality Probiotic Tepache of Pineapple Peel on Variation of Sugar and Fermentation Duration. *Proceeding of International Conference on Biology Education, Natural Science, and Technology, 1*(1).
- Saha, G., Mukhim, C., Chandni, S., Singh, P., & Chanu, N. B. (2024). Dragon Fruit Cultivation. *Vigyan Varta*, *5*(4). https://www.researchgate.net/publication/380214567
- Shah, K., Chen, J., & Qin, Y. (2023). Pitaya Nutrition, Biology, and Biotechnology: A Review. *International Journal of Molecular Sciences*, *24*(18), 13986. https://doi.org/10.3390/ijms241813986
- Sinamo, K. N., Ginting, S., & Pratama, S. (2022). Effect of sugar concentration and fermentation time on secang kombucha drink. *IOP Conference Series: Earth and Environmental Science*, 977(1), 012080. https://doi.org/10.1088/1755-1315/977/1/012080
- Sionek, B., Szydłowska, A., Küçükgöz, K., & Kołożyn-Krajewska, D. (2023). Traditional and New Microorganisms in Lactic Acid Fermentation of Food. *Fermentation*, *9*(12), 1019. https://doi.org/10.3390/fermentation9121019
- Suharman, Sutakwa, A., & Nadia, L. S. (2021). Effects Of Sucrose Addition To Lactic Acid Concentrations And Lactic Acid Bacteria Population Of Butterfly Pea (Clitoria Ternatea L.) Yogurt. *Journal of Physics: Conference Series*, 1823(1), 012038. https://doi.org/10.1088/1742-6596/1823/1/012038
- Suryaningsih, S., Muslim, B., & Djali, M. (2021). The antioxidant activity of roselle and dragon fruit peel functional drink in free radical inhibition. *Journal of Physics: Conference Series*,

1836(1), 012069. https://doi.org/10.1088/1742-6596/1836/1/012069

- Timmermans, E., Bautil, A., Brijs, K., Scheirlinck, I., Van der Meulen, R., & Courtin, C. M. (2022). Sugar Levels Determine Fermentation Dynamics during Yeast Pastry Making and Its Impact on Dough and Product Characteristics. *Foods*, *11*(10), 1388. https://doi.org/10.3390/foods11101388
- Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.612285
- Xie, J., & Gänzle, M. (2023). Microbiology of fermented soy foods in Asia: Can we learn lessons for production of plant cheese analogues? *International Journal of Food Microbiology*, *407*, 110399. https://doi.org/10.1016/j.ijfoodmicro.2023.110399
- Zheng, H., Jiang, J., Huang, C., Wang, X., & Hu, P. (2024). Effect of sugar content on characteristic flavour formation of tomato sour soup fermented by Lacticaseibacillus casei H1 based on non-targeted metabolomics analysis. *Food Chemistry: X, 21,* 101116. https://doi.org/10.1016/j.fochx.2024.101116