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THE CHARACTERISTICS OF FERMENTATION DRINK FROM JACKFRUIT SEED JUICE (Artocarpus heterophyllus) IN VARIATION THE GIVING OF SUCROSE AND SKIM MILK WITH THE BACTERIA Lactobacillus casei

KARAKTERISTIK MINUMAN FERMENTASI DARI SARI BIJI NANGKA (*Artocarpus heterophyllus*) PADA VARIASI PEMBERIAN SUKROSA DAN SUSU SKIM DENGAN BAKTERI *Lactobacillus casei*

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ABSTRAK

Tujuan dari penelitian ini adalah untuk menentukan pemberian sukrosa dan susu skim pada kombinasi yang terbaik terhadap karakteristik minuman fermentasi sari biji nangka. Percobaan ini mengimplementasikan rancangan acak lengkap dua faktor. Faktor pertama dan kedua berturut – turut adalah pemberian sukrosa dan susu skim yang terdiri dari lima taraf perlakuan, yaitu: 0; 5; 10; 15; and 20%. Terdapat 25 kombinasi perlakuan dan diulang dua kali. Pemberian sukrosa dan susu skim dengan variasi berbeda menghasilkan interaksi yang signifikan terhadap total bakteri asam laktat (BAL), total asam laktat, pH, dan uji stabilitas. Pemberian sukrosa 0% dengan penambahan susu skim 15% meningkatkan total BAL tertinggi dibandingkan perlakuan lainnya (9,2 x 10^7 CFU/mL). Total BAL tersebut memenuhi standar total BAL pada SNI 01-2981-2009. Pemberian sukrosa 0% dengan penambahan susu skim 20% meningkatkan total asam laktat tertinggi dibandingkan perlakuan lainnya (2,46%) yang memenuhi standar total keasaman sebagai asam laktat SNI 01-2981-2009 yaitu 0,5 – 2%. Nilai pH yaitu kisaran 3,1 – 5,2 dengan waktu fermentasi selama 48 jam. Uji stabilitas dari minuman probiotik telah memenuhi standar SNI 7552:2009 terhadap kestabilan yoghurt yang tinggi (> 85%).

Kata kunci: biji nangka, pangan fungsional, probiotik, sukrosa, susu

ABSTRACT

This study aims to determine the giving's effect of sucrose and skim milk concentrations on the features of jackfruit seed juice probiotic drinks. This research implemented a completely randomized design with two factors. The first and second factor is sucrose and skim milk given, with five treatment levels: 0, 5, 10, 15, and 20%, respectively. There were 25 treatment combinations and they were repeated two times. Sucrose and skim milk in different variations resulted in considerably varied interactions with total lactic acid bacteria, total lactic acid, pH, and stability tests. Giving 0% sucrose with 15% skim milk resulted in the highest overall lactic acid bacteria (LAB) concentration compared to other treatments (9.2×10^7 CFU/ml). The total LAB is calculated using SNI 01-2981-2009 at 107 CFU/mL. Giving 0% sucrose with 20% skim milk elevated total lactic acid the most compared to other treatments (2.46%). After 48 hours of fermentation, the pH ranged between 3.1 and 5.2. The probiotic drink's stability test follows the SNI 7552:2009 standard for yogurt stability, indicating a high stability rating (> 85%).

Keywords: functional foods, jackfruit seed, milk, probiotic, sucrose

INTRODUCTION

Bioactive compound components are generally found in every food consumed by humans as a source of nutrition and are safe. Bioactive compounds in these foods can also be categorized as functional food ingredients (Zhang *et al*., 2019). One category of functional food ingredients is fermented milk with probiotics, which are selected based on their functional capabilities so that they become milk products that can improve the digestive system.

Processing jackfruit seeds into a probiotic drink because they contain high oligosaccharides and nutrients. According to (Tadimalla, 2023) the carbohydrate content of 100 g of jackfruit seeds is quite high, namely around 38 g, 7 g protein, 1.5 g fiber, and < 1 g fat. Carbohydrates in the form of oligosaccharides in jackfruit seeds can be absorbed easily by the body by fermenting them. One way is to process jackfruit seeds into probiotic drink products.

The growth of lactic acid bacteria (LAB) is one of the things that must be considered in the fermentation process of jackfruit seed juice drinks. The increase in LAB growth is influenced by carbon and nitrogen as media nutrients (Karim *et al*., 2020). The content of 403.44 mg/g (bk) polysaccharides and 29.35 mg/g oligosaccharides (bk) in jackfruit seeds cannot be digested by digestive enzymes. This can stimulate the growth of *Lactobacillus* bacteria. Apart from that, in in vitro tests using artificial intestines,

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jackfruit seeds were selective in microflora fermentation (Rahayu *et al.*, 2020).

This research focuses on providing sucrose as a source of carbon (C), while skim milk as a source of nitrogen (N) to support the activity of LAB *Lactobacillus casei*. This research aimed to identify the best combination of sucrose and skim milk for the characteristics of fermented jackfruit seed juice drinks. It is hoped that the results of this research can increase literacy regarding the use of jackfruit seeds as a basic ingredient for making fermented drinks as a form of diversification of functional food products.

MATERIALS AND METHODS

Time and Place

The research was conducted at the General Plant Cultivation Laboratory of the Yogyakarta LPP Polytechnic from December 2022 to August 2023 (8 months).

Tools and Materials

This experiment used equipment, including a blender, stove, tight glass jar, pan, thermometer, filter, petri dish, test tube, 1 mL pipette, pH meter, Erlenmeyer, vial, bunsen, spatula, digital scale, measuring cup, and a 100 mL bottle. It also used jackfruit seeds, skim milk, starter/bacterial source *L. casei*, sucrose, water, MRSA, distilled water, 70% alcohol, and 0.1N NaOH.

Method

This experiment implemented a two-factor completely randomized design. The first and second factors respectively are the administration of sucrose and skim milk, which consists of five treatment levels: 0; 5; 10; 15; and 20%. These treatments resulted in 25 treatment combinations. Each treatment combination was repeated twice so that there were fifty experimental units. The experiment began with preparing tools and materials, then extracting jackfruit seed juice using a grinder (blender), next was making a working *L. casei* starter from the pure starter, testing experimental units, data collection, and data processing (Figure 1). Extraction of jackfruit seed juice is done using a grinder (blender). A ratio of jackfruit seeds and water (w/v), namely 1:1, is used in the extraction. The ground jackfruit seeds are then filtered to obtain jackfruit seed juice (Figure 2).

Starter Preparation

Making a starter refers to the method used by (Suharyono *et al.*, 2012). The *deMan Rogosa Sharpe* (MRS) culture medium is used by bacterial cultures (*L. casei*) that have been transferred from the culture stock to a test tube. The culture medium was sterilized and incubated at 37°C for two days. Bacterial cultures of 1-2 cycles were then incubated in skim milk medium (0.5 g of skim milk in 10 mL of sterilized distilled water at a temperature of 121°C for 15 minutes) for two days at a temperature of 37°C. The result of this culture is the parent culture. The mother culture was inoculated into sterile skim milk medium (4% (v/v)) and incubated at 37°C for two days to form an intermediate culture. The intermediate culture was obtained by inoculating 4% (v/v) of the intermediate culture (4 mL added to 96 ml of skim milk) which was added to a sterile skim milk medium with the addition of 3% (w/v) sucrose. The culture composition was then incubated at 37°C for 24 hours.

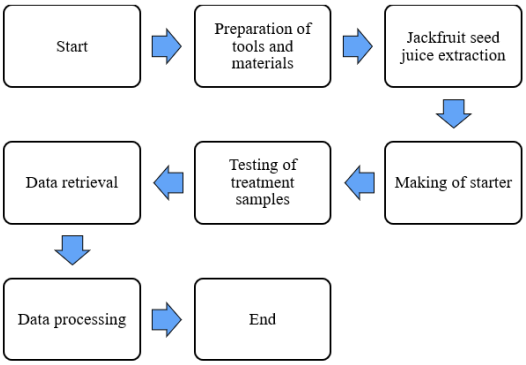

Figure 1. Flow diagram of research implementation

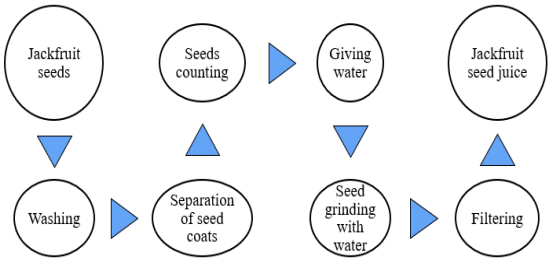

Figure 2. Flow diagram for making jackfruit seed juice

Observation

The variables observed for analyzing the characteristics of fermented drinks from jackfruit seed juice include total lactic acid bacteria (LAB), total lactic acid, pH, and stability tests. The culture sample was 10 mL and put into a plastic centrifuge tube, then stored at low temperature for one day for stability testing. The stability of the culture is determined by measuring the volume of the cloudy part of the sample against the total volume of the sample. Apart from that, organoleptic tests were also carried out using a hedonic approach. The fermented jackfruit seed juice drink samples taken for organoleptic testing have met the requirements of SNI 7552:2009, namely containing total LAB and lactic acid, an acidity level that does not inhibit LAB growth activity (pH 3 - 6) and is very stable (> 85%).

RESULT AND DISCUSSION

A significant interaction was shown by the ANOVA results in the combination of sucrose and skim milk with different variations in total Lactic Acid Bacteria (LAB), total lactic acid, pH, and stability test ($P < 0.05$) (Table 1). These interactions are significantly different, then an interaction test is carried out between the two factors on each observed variable.

Total Lactic Acid Bacteria (LAB)

The interaction response between giving sucrose and skim milk with different variations in the total LAB variable is presented in Table 2 below. The addition of sucrose affected the total LAB combined with skim milk. Giving skim milk with different variations also significantly affected all levels of sucrose addition to total LAB. Giving 0% sucrose with the addition of 15% skim milk increased the highest total LAB compared to the treatment with the addition of sucrose or other skim milk, namely 9.2 x 10^7 CFU/mL, and the lowest in the treatment with the addition of 20% sucrose with 10% skim milk, namely 1.2 x 10^7 CFU/mL. The total LAB in this study met the standard for total lactic acid bacteria from the National Standardization Agency or NSA with Indonesian National Standards number 01-2981-2009, namely 10^7 CFU/mL. Lactic acid bacteria can produce organic acids, such as lactic acid, acetate acid, oxalate acid, and citric acid during the fermentation process. The high increase in total acid produced by LAB is indicated by the high sugar consumption during the fermentation process (Setyaningsih *et al*., 2019).

The combination of sucrose and skim milk in this study shows that the increasing concentration of added skim milk will result in variations in total BAL. The breakdown of lactose into lactic acid is carried out during fermentation, producing flavor and aroma components. Lactic acid is formed from the conversion of lactose by LAB during the process.

Table 1. Anova test results of giving sucrose and skim milk with different variations in fermented jackfruit seed juice drink

Treatments	Total LAB (10^7 CFU/mL)	Total lactic acid (%)	pH	Stability test (%)
Factor I: Addition of sucrose (%)				
0	6.19a	1.33b	3.7c	87.5d
5	3.82b	0.41e	5.0a	93.5b
10	2.05d	1.02c	3.6c	93.0b
15	3.58c	1.52a	4.2b	92.0c
20	2.00e	0.77d	4.2b	95.0a
F test	*	*	*	*
Factor II: Addition of skim milk (%)				
0	2.46e	0.19e	4.5a	84.0e
5	3.43d	0.81d	4.3b	91.0c
10	3.94b	1.04c	4.2c	89.0d
15	4.30a	1.31b	3.9d	97.0b
20	3.50c	1.69a	3.90e	100.0a
F test	*	*	*	*
Interaction Sucrose * Skim milk	*	*	*	*

Table 2. Interaction response between giving sucrose and skim milk with different variations in fermented jackfruit seed juice drinks on total LAB

Treatments	Total LAB (10^7 CFU/mL)				
	Susu skim (%)				
	0	5	10	15	20
Sucrose (%)					
0	4.30f	6.20c	8.80b	9.20a	2.43k
5	2.19m	3.70j	4.10g	4.30f	4.80d
10	1.82r	2.43k	1.60s	2.21l	2.17n
15	1.88q	3.70j	4.00h	3.90i	4.40e
20	2.10o	1.10u	1.20t	1.90p	3.70j
F test	*	*	*	*	*

The letters following the numbers in the same column indicate that they do not significantly affect the Least Significant Difference follow-up test at a significance level of λ =5%

Milk lactose is broken down by the bacteria *L. casei* into glucose and galactose (monosaccharides), then these bacteria also break down monosaccharides into lactic acid (Zhang *et al*., 2019). In addition, it is suspected that an increase in the total amount of LAB when giving skim milk causes the amount of lactose to increase. This will result in the fermentation process of the substrate used by LAB also being higher. Sintasari *et al*. (2014) stated that the addition of sucrose and skim milk was able to increase the total LAB of probiotic drinks from red rice juice. This is because during the fermentation process protein and lactose are converted by LAB into lactic acid. Increasing honey concentration can increase the number of probiotic lactic acid bacteria (Pangastuti *et al*., 2020). Sucrose acts as a carbon source, while skim milk acts as a nitrogen source which is used for LAB growth (Agustine *et al*., 2018).

Total Lactic Acid

The effect of the interaction between the addition of sucrose and skim milk with different variations on the total lactic acid variable is presented in Table 3 below. The addition of sucrose affects the total lactic acid combined with skim milk. Skim milk given in different variations also had a significant effect on all levels of sucrose addition to total lactic acid. Giving 0% sucrose with the addition of 20% skim milk increased the highest total lactic acid compared to the treatment with the addition of sucrose or other skim milk, namely 2.46%, and the lowest in the treatment with the addition of 5% sucrose with 0% skim milk, namely 0.1%. This is in line with the research results Agustina and Rahman (2010) that the addition of 0% sucrose was able to increase lactic acid levels along with increasing the concentration of skim milk in green bean juice yogurt drinks. This research produces total lactic acid by the total acidity standard as lactic acid from the National Standardization Agency with Indonesian National Standards number 01-2981-2009, namely 0.5 – 2%.

This fermented drink of jackfruit seed juice with added sucrose and skim milk shows that as the percentage of sucrose and skim milk given increases, the total lactic acid production will increase compared to without giving sucrose and skim milk. This is related to LAB activity in the fermentation process. LAB which increases with the addition of sucrose and skim milk will produce metabolite compounds in the form of lactic acid. This increase in lactic acid will also increase total lactic acid. Hendarto *et al*., (2019) stated that the acidity level (pH) of yogurt is influenced by LAB activity. This is because there is a metabolite in the form of lactic acid that is produced. The increased growth and activity of LAB during the fermentation process will increase the results of lactic acid metabolism so it will also increase the total lactic acid (Fadro *et al*., 2015).

pH Value

The combination of sucrose and skim milk with different variations produces a significant effect on the degree of acidity (pH) as in Table 4 below. The fermentation time of two days produces a pH of around 3.1 – 5.2. Karim *et al*., (2020) also stated that providing a fermentation temperature of 42°C for 5-6 hours in making yogurt drinks will produce a pH value of around 4.3 – 4.4. Apart from that, the pH will also decrease according to the fermentation time. The pH value of fermented jackfruit seed juice is influenced by the lactic acid content (Rizal *et al.*, 2016) and total lactic acid (Nainggolan *et al*., 2021). LAB will carry out a fermentation process on the carbohydrate content to produce lactic acid. Lactic acid can lower the pH so that acidity increases (Nisa *et al.*, 2022). Increasing the concentration of skim milk causes a decrease in pH. This is because of the influence of the lactose content in milk on LAB activity in producing lactic acid. The sugar content in skim milk will be utilized by LAB which will then turn into lactic acid. The increasing concentration of skim milk will reduce the pH. The addition of sucrose is also utilized by microorganisms that play a role in the yogurt fermentation process (Rahayu *et al*., 2020). The increase in acidity followed by a decrease in pH during the fermentation process is the result of the utilization of carbohydrates into lactic acid by LAB (Hidayati *et al*., 2021).

Table 3. Interaction response between giving sucrose and skim milk with different variations in fermented jackfruit seed juice drinks on total lactic acid

Treatments	Total lactic acid (%)				
	Skim milk (%)				
	0	5	10	15	20
Sucrose (%)					
0	0.35kl	0.69ij	1.28e	1.89c	2.46a
5	0.10n	0.29lm	0.40k	0.59j	0.69ij
10	0.14n	0.66ij	0.91fg	1.28e	2.10b
15	0.17n	1.67d	1.83c	1.90c	2.04b
20	0.20mn	0.73hi	0.80gh	0.91f	1.20e
F test	*	*	*	*	*

The letters following the numbers in the same column indicate that they do not significantly affect the Least Significant Difference follow-up test at a significance level of λ = 15%

Table 4. Response of interaction between the addition of sucrose and skim milk with different variations in fermented jackfruit seed juice drinks on pH values

Treatments	pH value				
	Skim milk (%)				
	0	**5**	**10**	**15**	20
Sucrose (%)					
0	4.0ij	4.1i	3.7k	3.5l	3.1n
5	5.0b	5.2a	5.2a	4.85c	4.8cd
10	5.0b	3.1n	3.3m	3.1n	3.7k
15	3.6k	4.6f	4.7ef	4.3g	4.1hi
20	4.7def	4.8cde	4.2gh	3.9j	3.7k
F test	*	*	*	*	*

The letters following the numbers in the same column indicate that they do not significantly affect the Least Significant Difference follow-up test at a significance level of λ = 15%.

Table 5. Response of interaction between the addition of sucrose and skim milk with different variations of fermented jackfruit seed juice drinks to the stability test

Treatments	Stability test (%)				
	Skim milk (%)				
	0	**5**	**10**	**15**	**20**
Sucrose (%)					
0	85d	80g	82,5f	90d	100a
5	90d	90d	92,5c	95b	100a
10	85e	100a	80g	100a	100a
15	80g	90d	90d	100a	100a
20	80g	95b	100a	100a	100a
F test	*	*	*	*	*

The letters following the numbers in the same column indicate that they do not significantly affect the Least Significant Difference follow-up test at a significance level of λ = 15%.

Stability Test

Stability testing aims to determine the durability of the yogurt preparation during the storage period. The combination treatment of adding sucrose and skim milk had a significant effect on the stability test of fermented jackfruit seed juice. The addition of sucrose and skim milk up to 20% produces the highest level of stability, namely 100%. Characteristics that met the Indonesian National Standards 7552:2009 standard for yogurt stability are that it has a high stability value (> 85%).

Storing yogurt at low temperatures can inhibit the growth of microorganisms so that the stability of jackfruit seed juice is maintained. The lowest level of stability was seen in the treatment of 0% sucrose with 5% skim milk and the treatment of adding 15 – 20% sucrose with 0% skim milk. The decrease in stability was caused by the increasingly low cloudy phase in the fermented jackfruit seed juice. The results of the research Nurainy *et al*. (2018) show that the stability of the red guava juice probiotic drink will decrease with the addition of sucrose and skim milk in a linear manner.

Organoleptic Test

Panelists filled out questionnaires according to the instructions for probiotic drinks from jackfruit seed juice on several samples recommended by researchers. The questionnaire has a scale indicator from 1 to 6 which shows the level of liking of each panelist, as described below: 1 (Strongly disliked), 2 (Disliked), 3 (Somewhat disliked), 4 (Somewhat liked), 5 (Liked), and 6 (Very liked). Data was obtained by collecting questionnaires from 15 untrained panelists regarding the level of consumer preference for probiotic jackfruit seed juice drinks on 3 recommended samples that had been tested. The test parameters are flavor, aroma, and texture. Halik (2024) states that flavor combines smell, taste, and more perception including sight and perception. On the other hand, when the smell enters the nose and is processed through the sense of smell, the aroma of a product can be smelled and identified. The taste of food or drink can be inferred from the aroma, also probiotic jackfruit seed juice drink in this research. Figure 3 below is data on the results of the organoleptic tests that have been carried out.

The results of the organoleptic test of the jackfruit seed extract probiotic drink on the quality parameters like aroma and texture obtained the highest average value in the treatment sample with the addition of 20% sucrose without the addition of skim milk (0%). Based on this, the recommendation for making probiotic drinks is without the use of skim milk in the samples. The use of skim milk will result in an increase in the acidity level and a thicker texture, making it less popular with panelists.

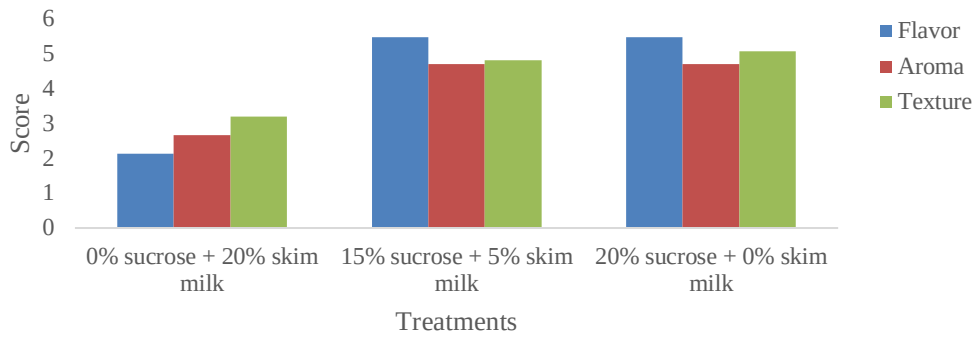

Figure 3. Organoleptic test results of probiotic jackfruit seed juice drinks on several recommended samples
Source: processed by researchers (2023)

Taufik and Maruddin (2020) stated in their research results that adding sucrose with a percentage of 6 – 12% can reduce the acidity level of fermented whey products because sucrose can reduce the activity of the bacteria Lactobacillus acidophilus FNCC 0051 so it will also reduce the formation of lactic acid.

CONCLUSIONS AND RECOMMENDATION

Conclusions

The combination of adding sucrose and skim milk with different variations resulted in significantly different interactions with total LAB, total lactic acid, pH, and stability tests. Giving 0% sucrose with the addition of 15% skim milk increased the highest total LAB compared to other treatments (9.2×10^7 CFU/ml). The total LAB follows the standards in the Indonesian National Standards 01-2981-2009. Giving 0% sucrose with the addition of 20% skim milk increased total lactic acid the highest compared to other treatments (2.46%) which accordance with the total acidity standard as Indonesian National Standards 01-2981-2009 lactic acid, namely 0.5 – 2%. The pH value is in the range of 3.1 – 5.2 with a fermentation time of 48 hours. The stability test of the probiotic drink has met the Indonesian National Standards 7552:2009 standard for yogurt stability, namely having a high stability value (> 85%). The addition of 20% sucrose without the addition of skim milk (0%) was preferred by the panelists.

Recommendation

Testing for total lactic acid bacteria, total lactic acid, pH, and stability tests need to be carried out continuously and are interrelated to maintain the stability of the quality of probiotic drinks from jackfruit seed juice.

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