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Characterization of Probiotic Beverage Based on Tangerine (*Citrus Reticulata*) With Variations in Probiotic Propagation Time and Skim Milk Addition

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Abstract: *Probiotic beverages are nutrient-rich substrates with the help of probiotic microorganisms that are beneficial for digestive health. Tangerine (Citrus reticulata) is a local fruit rich in vitamin C, organic acids, and natural sugars, making it a potential raw material for probiotic beverages. Lactobacillus plantarum PR6105 is one of the lactic acid bacteria (LAB) isolated from bamboo shoot pickles, known for its probiotic properties. This study aims to determine the effect of propagation time and skim milk addition on tangerine-based probiotic beverages' characteristics and identify the best treatment. The experiment was conducted on a laboratory scale, which was designed using a randomized block design (RBD) with two factors, including propagation time (6, 12, 18, and 24 hours) and skim milk addition (3%, 4%, and 5%). Analysis of variance results showed that the treatments of propagation time and skim milk addition significantly affected several parameters, including total LAB, total acidity, total reducing sugar, pH, antioxidant capacity, and organoleptic evaluation. The best treatment was obtained at the combination of 5% skim milk and 24 hours of propagation time, with the following characteristics: total LAB of 1,23 x 10^{10} CFU/mL, total reducing sugar of 0.10%, pH of 4.0, total acidity of 4.43%, antioxidant capacity of 0.90 ppm, and a hedonic taste score of 6.25 (liked category).*

Keywords: Fermentation, LAB, Lactobacillus plantarum, Probiotics, Skim milk, Tangerine

I. INTRODUCTION

Probiotics are live microorganisms which, when consumed in adequate amounts, confer health benefits to the host, particularly by maintaining the balance of intestinal microflora [6]. These microorganisms play a role in improving digestion, enhancing immunity, and preventing disorders such as diarrhea or constipation [20]. One of the most popular probiotic products is fermented beverages, especially those utilizing lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium*, such as *L. plantarum*, which is known to produce bacteriocins and inhibit pathogenic bacteria [24;27].

Probiotic beverages are not limited to dairy-based products but have also been developed from tropical fruits rich in vitamins, antioxidants, and natural sugars that support LAB growth [9]. Several studies have investigated fruits such as guava [15], salak (Utami, 2018), and beetroot (Neha et al., 2018) as fermentation substrates. One local fruit with high potential yet underutilized is the mandarin orange (*Citrus reticulata*), which is abundantly produced but often experiences post-harvest surplus (Badan Pusat Statistik, 2023; Rahman et al., 2007). Its vitamin C, flavonoid, and organic acid contents—such as citric acid and ascorbic acid—make mandarin orange an ideal substrate for probiotic fermentation (Alodokter, 2022; IDNMedis, 2024).

In addition to the substrate, supplementary nutrients such as skim milk significantly affect the fermentation process. Skim milk is rich in protein and lactose, which support LAB growth and viability while enhancing the texture and flavor of the beverage (Michaelsen et al., 2009; Siswanto et al., 2021). Studies have shown that the addition of 5% skim milk can increase LAB counts and improve the stability of fermented products (Sintasari et al., 2014). Propagation time also plays a crucial role, as it influences total LAB, pH, total acidity, reducing sugars, and sensory characteristics. Fermentation for 18–24 hours has been shown to produce an optimal number of LAB and improve the sensory profile of the product (Yunus et al., 2015; Sihombing, 2022; Granato et al., 2010; Rathore et al., 2012).

However, to date, few studies have specifically evaluated the combination of propagation time and skim milk concentration in producing mandarin orange-based probiotic beverages. Therefore, this study aimed to determine the effects of these two factors on the microbiological, chemical, antioxidant, and organoleptic characteristics of the product and to identify the best treatment that could produce a functional probiotic beverage based on a local fruit with optimal quality (Sanders et al., 2010; Suhartini et al., 2009).

II. RESEACRH METHOD

2.1 Place and Time of Research
This research was conducted at the Bioindustry Laboratory and Food Microbiology Laboratory, Agrocomplex Building, 3rd Floor, Faculty of Agricultural Technology, Udayana University. from January to April 2025.

2.2 Experimental Design
This study employed a Randomized Block Design (RBD) with two factors. The first factor was skim milk concentration at three levels: 3% (S1), 4% (S2), and 5% (S3). The second factor was propagation time, consisting of 6 hours (P1), 12 hours (P2), 18 hours (P3), and 24 hours (P4). The experimental design resulted in 12 treatment combinations. Each treatment was grouped into 2 blocks based on the time of execution, resulting in a total of 24 experimental units.

2.3 Variables Observed
The variables observed in this study included total lactic acid, total lactic acid bacteria (LAB), total reducing sugar, pH, antioxidant activity, and organoleptic evaluation. Total lactic acid was measured using the titration method. LAB count was determined using the Total Plate Count (TPC) method. The pH was measured using a pH meter. Reducing sugar content was analyzed using a standard chemical method, while antioxidant capacity was measured using the DPPH method. The organoleptic test was conducted to evaluate the sensory attributes of the final probiotic beverage, including aroma, color, taste (sweetness, sourness, and off-flavor), and overall acceptability. The sensory evaluation was carried out by semi-trained panelists.

2.4 Research Procedur
2.4.1 Revival and Propagation Preparation of Lactobacillus plantarum PR6105
The initial step of reviving Lactobacillus plantarum involved preparing MRS broth medium composed of triammonium citrate, peptone, manganese sulphate $4H_2O$, lab-lemco powder, yeast extract, glucose, dipotassium hydrogen phosphate, magnesium sulphate $7H_2O$, and sorbitan mono-oleate, each weighing 2.6 grams, which were then dissolved in 50 mL of distilled water following the method of De Man et al. (1960). The solution was heated and stirred on a hotplate for 5 minutes until homogeneous, then sealed tightly. Sterilization of the medium was carried out using an autoclave at 121°C under 1 atm pressure for 15 minutes. After sterilization, the medium was aseptically dispensed into five test tubes, each containing 10 mL. Subsequently, inoculation was performed by adding 1 mL of pure L. plantarum culture into the MRS broth medium aseptically. The inoculated tubes were then incubated at 37°C for 24 hours until visible bacterial colony growth was observed [31].

2.4.2 Preparation of Keprok Orange Substrate
The preparation of raw materials began by washing 8 kg of fresh Citrus reticulata under running clean water to remove dirt and contaminants, followed by draining. The fruits were manually peeled, and the pulp was pressed to extract the juice, which was then filtered using sterile cloth or filter paper to obtain a clear orange juice. This process yielded approximately 3000 mL of juice, which was used for a single experimental time group consisting of 12 treatment combinations. The juice was then stored in sterile bottles until the propagation stage.

2.4.3 Preparation of Lactobacillus plantarum PR6105 Starter Culture
Starter culture preparation began by placing 100 mL of fresh Citrus reticulata juice into a sterile container to maintain material hygiene and prevent cross-contamination. Subsequently, 5% (v/v) of sterile skim milk was added to the juice as an additional nutrient source, providing lactose and proteins essential for supporting the growth and metabolic activity of lactic acid bacteria. The homogenized mixture was then pasteurized in a water bath at 80°C for 5 minutes to inactivate contaminating microorganisms and ensure media stability [28]. After pasteurization, the medium was gradually cooled to the optimal incubation temperature of 37°C. A 1% (v/v) inoculum of previously revived and washed Lactobacillus plantarum PR6105 was aseptically introduced into the medium under laminar airflow to ensure culture purity. The inoculated starter was then incubated under optimal conditions for subsequent propagation.

2.4.3 Production of Keprok Orange Probiotic Beverage
The production of the probiotic beverage began by preparing 250 mL of fresh Citrus reticulata juice, which was transferred into sterile jars for each treatment unit to prevent contamination by wild microorganisms. Sterile skim milk was then added according to the treatment levels at 3%, 4%, and 5% (v/v) as a nutritional source of lactose and protein, while also

functioning as an emulsifier to aid in media homogenization. The mixture was pasteurized in a water bath at 80°C for 5 minutes to inactivate pathogenic and contaminating microorganisms. After pasteurization, the mixture was gradually cooled to the optimal incubation temperature of 37°C. Subsequently, 10% (v/v) of previously revived Lactobacillus plantarum PR6105 starter culture was aseptically inoculated into the medium, followed by gentle shaking to ensure even bacterial distribution. The propagation process was carried out at 37°C with varying incubation periods of 6, 12, 18, and 24 hours according to the treatment conditions.

III. Result and Discussion

3.1 Total LAB Count

The analysis of variance showed that the addition of skim milk and the duration of propagation had a significant effect (P<0.05). However, the interaction between skim milk concentration and propagation time was not significant (P>0.05). The total lactic acid bacteria (LAB) count in the Citrus reticulata probiotic beverage is presented in Table 1.

Table 1. Effect of Skim Milk Concentration and Propagation Time on Total LAB Count (log CFU/mL) of Citrus reticulata Probiotic Beverage

Propagation	Skim Milk Concentration (%)			$\bar{X}$ (%)
	3%	4%	5%	
6 Hours	9,54±0,07	9,84±0,01	9,90±0,02	9,76±0,03d
12 Hours	9,90±0,02	9,92±0,02	9,96±0,03	9,93±0,08c
18 Hours	9,97±0,02	10,00±0,01	10,01±0,01	9,99±0,01b
24 Hours	10,01±0,01	10,06±0,01	10,09±0,01	10,05±0,01a
$\bar{X}$ (%)	9,85±0,03c	9,95±0,01b	9,99±0,01a	

Note: Mean values followed by different letters in the same column/row indicate significant differences (p < 0.05).

In Table 1, it can be observed that the average total lactic acid bacteria (LAB) count in the tangerine probiotic drink ranges from 9.54 log CFU/mL to 10.09 log CFU/mL. This indicates that the increase in skim milk concentration and propagation duration contributes to the rise in total LAB count. This condition occurs because a higher concentration of skim milk provides more nutrients available for the bacteria to utilize as an energy source to support their growth. [22] Which states that the addition of skim milk concentration affects the growth of lactic acid bacteria in the production of probiotic drinks made from red rice extract. The graph of total LAB can be seen in Figure 1 below.

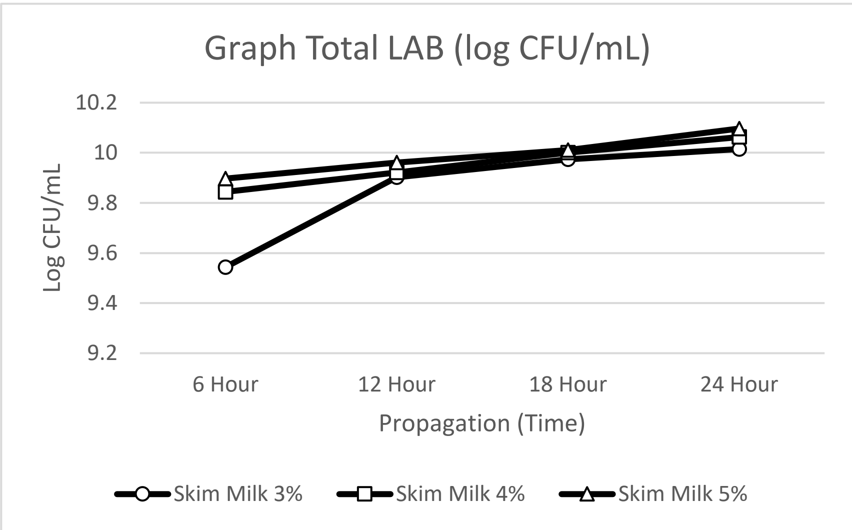

Figure 1. Graph of Total LAB in probiotic beverage based on tangerine with variations in probiotic propagation time and skim milk addition

In Figure 1, it can be observed that during the propagation period of 6 to 24 hours, there was an increase in the number of bacteria. [28] Which demonstrated a logarithmic growth phase of bacteria from 0 to 20 hours, with an increase of 11.57 log CFU/mL. [11] On fermented rice drinks also indicated that the longer the propagation time, the greater the growth of lactic acid bacteria, peaking at 22 hours. After 22 to 24 hours, the bacteria entered the stationary phase, where the rates of growth and death were balanced, while at 26 hours, they began to enter the death phase. The total lactic acid bacteria (LAB) in all treatments met the minimum probiotic standards according to SNI 7552:201 (minimum of 10^6 CFU/mL). The treatment with 5% skim milk and a propagation time of 24 hours resulted in the highest total LAB of 10.09 log or 1,23 x 10^{10} CFU/mL, which meets the eligibility standards for a probiotic drink.

3.2 Total Acidity

The results of the analysis of variance indicate that the treatment of skim milk addition and propagation duration had a significant effect (P<0.05). However, the interaction between skim milk concentration and propagation duration did not have a significant effect (P>0.05). The total acid content of the citrus reticulata probiotic drink is presented in Table 2.

Table 2. Average Total Acid Content of Citrus Reticulata Probiotic Drink

Propagation	Skim Milk Concentration (%)			$\bar{X}$ (%)
	3%	4%	5%	
6 Hours	1,73±0,05	2,50±0,68	3,08±0,12	2,44±0,28d
12 Hours	3,33±0,07	3,87±0,12	4,05,±0,12	3,75±0,10c
18 Hours	4,23±0,12	4,32±0	4,43±0,10	4,33±0,07b
24 Hours	4,59±0,12	4,91±0,02	4,43±0,12	4,64±0,09a
$\bar{X}$ (%)	3,47±0,09c	3,9±0,22b	4,00±0,11a	

Note: Mean values followed by different letters in the same column/row indicate significant differences (p < 0.05).

Table 2 shows that the addition of skim milk and variations in propagation duration significantly affected the total acid content of the tangerine probiotic drink (P<0.05), while the interaction between the two factors did not show a significant effect (P>0.05). The total acid produced ranged from 1.73% to 4.43%. The increase in skim milk concentration indicated a tendency for higher total acid content, with a value of 3.47% at the addition of 3% skim milk, increasing to 3.90% at 4%, and reaching 4.46% at 5%. This is due to the increasing amount of lactose available as a substrate for lactic acid bacteria to produce lactic acid during the fermentation process [6].

Additionally, the duration of propagation was directly proportional to the increase in total acid content produced. The average total acid recorded was 2.44% at 6 hours of propagation, increasing to 3.75% at 12 hours, 4.33% at 18 hours, and reaching a peak value of 4.64% at 24 hours. This increase is closely related to the more active metabolism of lactic acid bacteria in converting lactose into organic acids as fermentation time increases [11]. The optimum treatment for producing the highest total acid was achieved with a combination of 5% skim milk addition and a propagation duration of 24 hours, resulting in 4.43%. A longer fermentation time provides a greater opportunity for bacteria to maximize the utilization of sugar substrates, thereby increasing the production of organic acids such as lactic acid generated during fermentation (Yunus et al., 2015). The changes in total acid values in the tangerine probiotic drink as a result of variations in propagation duration and skim milk concentration can be seen in Figure 2.

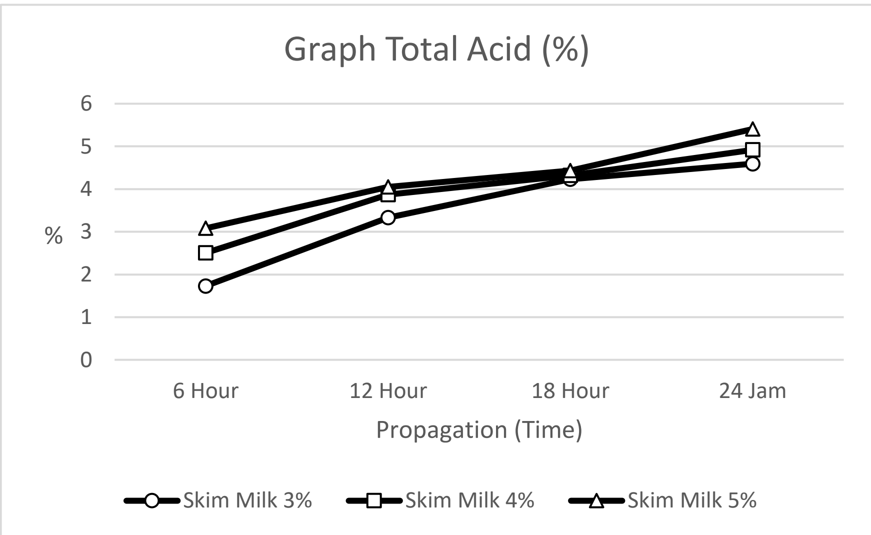

Figure 2. Graph of Total Acid in probiotic beverage based on tangerine with variations in probiotic propagation time and skim milk addition

In Figure 2, it can be observed that the total acid content tends to increase with the duration of propagation at each concentration of skim milk. This indicates that the metabolic activity of Lactobacillus plantarum PR6105 becomes more optimal in converting the substrates of tangerine juice and skim milk into organic acid compounds, such as lactic acid, acetate, and citrate. Higher concentrations of skim milk provide additional nutrients in the form of lactose and protein, which accelerate the growth and activity of bacteria during the propagation process. [21] The availability of sufficient nutrients can enhance acid production by lactic acid bacteria. The accumulation of this acid also contributes to a decrease in pH, thereby improving the microbiological stability of the resulting probiotic drink.

3.3 Total Reducing Sugar

The results of the analysis of variance indicate that the treatment of skim milk addition and its interaction did not have a significant effect (P>0.05), while the duration of propagation had a significant effect (P<0.05) on the total reducing sugar content. However, the interaction between skim milk concentration and propagation duration did not show a significant effect (P>0.05). The total reducing sugar content of the citrus reticulata probiotic drink can be seen in Table 3.

Table 3. Average total reducing sugar (%) of citrus reticulata probiotic drink

Propagation	Skim Milk Concentration (%)			X̄ (%)
	3%	4%	5%	
6 Jam	0,10±0,05	0,10±0,05	0,14±0,11	0,11±0,07b
12 Jam	0,07±0,00	0,08±0,03	0,09±0,05	0,08±0,03ab
18 Jam	0,07±0,03	0,07±0,01	0,08±0,02	0,07±0,02ab
24 Jam	0,06±0,01	0,05±0,00	0,07±0,00	0,06±0,00a
X̄ (%)	0,07±0,02a	0,07±0,02a	0,10±0,05a	

Note: Mean values followed by the same letter in the column/row indicate no significant difference (p > 0.05).

Table 3 shows that the treatments with skim milk concentrations of 3%, 4%, and 5% did not differ significantly. The highest average total reducing sugar content was obtained at a skim milk concentration of 5% (v/v) (0.10%), followed by the treatment with a skim milk concentration of 4% (v/v) (0.07%), while the lowest average total reducing sugar content was produced at a skim milk concentration of 3% (v/v) (0.07%).

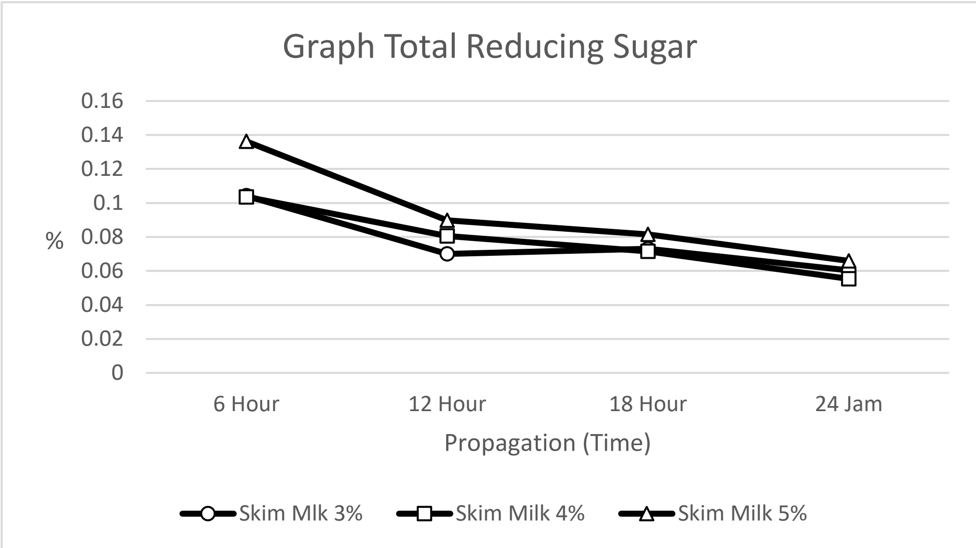

Figure 3. Graph of Total Reducing Sugar in probiotic beverage based on tangerine with variations in probiotic propagation time and skim milk addition

Figure 3 shows a decrease in the total reducing sugar content of the probiotic drink with increasing propagation duration; however, there is an increase in total reducing sugar content with variations in skim milk concentration. During fermentation, disaccharides are broken down into monosaccharides. The lactose present in skim milk is converted into glucose and galactose, while sucrose is hydrolyzed into glucose and fructose. These reducing sugars resulting from the breakdown are then utilized by bacteria to be metabolized into lactic acid [17].

3.4 Total pH

The results of the analysis of variance indicate that the treatment of skim milk addition and its interaction did not have a significant effect (P>0.05), while the duration of propagation had a significant effect (P<0.05) on the degree of pH. The degree of pH of the citrus reticulata probiotic drink can be seen in Table 4.

Table 4. Average total pH of citrus reticulata probiotic drink

Propagation	Skim Milk Concentration (%)			X̄ (%)
	3%	4%	5%	
6 Hours	4,20±0,00	4,15±0,07	4,20±0,00	4,18±0,02a

12 Hours	4,20±0,00	4,20±0,00	4,15±0,07	4,18±0,02a
18 Hours	4,05±0,07	4,05±0,07	4,10±0,00	4,06±0,05ab
24 Hours	4,15±0,07	4,10±0,14	4,10±0,00	4,12±0,07b
$\bar{X}$(%)	4,15±0,03a	4,12±0,07a	4,14±0,01a	

Note: Mean values followed by the same letter in the column/row indicate no significant difference ($p > 0.05$).

Table 4 shows that the duration of propagation significantly affected the pH of the tangerine probiotic drink ($P<0.05$), while the addition of skim milk concentration did not have a significant effect ($P>0.05$). The average pH ranged from 4.06 to 4.18. At 6 hours and 12 hours of propagation, the pH was recorded at 4.18, decreasing to 4.06 at 18 hours, and 4.12 at 24 hours. This decrease in pH reflects the accumulation of organic acids due to the metabolic activity of lactic acid bacteria during fermentation [11].

Although the concentrations of skim milk at 3%, 4%, and 5% did not show significant differences in pH, there was a tendency for pH to decrease with the increase in lactose as a fermentation substrate. In addition to lactic acid and acetic acid, CO_2 is also produced, which contributes to the decrease in pH, while compounds such as butyric acid have a minor contribution [24]. This decrease in pH serves as an indicator of successful fermentation, impacting the microbiological stability and sensory characteristics of the product.

3.5 Total antioxidant capacity

The results of the analysis of variance indicate that the treatment of skim milk addition, duration of propagation, and their interaction did not have a significant effect ($P>0.05$) on the antioxidant capacity. The antioxidant capacity of the citrus reticulata probiotic drink can be seen in Table 5.

Table 5. Average total antioxidant capacity (ppm) of citrus reticulata probiotic drink

Propagation	Skim Milk Concentration (%)			$\bar{X}$(ppm)
	3%	4%	5%	
6 Hours	0,26±0,32	0,29±0,24	0,80±0,18	0,45±0,25a
12 Hours	0,35±0,28	0,59±0,12	0,84±0,26	0,6±0,19a
18 Hours	0,65±0,53	0,70±0,17	0,77±0,37	0,71±0,36a
24 Hours	0,64±0,48	0,82±0,10	0,90±0,39	0,79±0,32a
$\bar{X}$(ppm)	0,48±0,40a	0,6±0,16a	0,83±0,3a	

Note: Mean values followed by the same letter in the column/row indicate no significant difference ($p > 0.05$).

Table 5 shows that the research results indicate the highest average antioxidant capacity was obtained at a skim milk concentration of 5% (v/v) and a propagation duration of 24 hours (0.90 ppm), while the lowest average antioxidant capacity was produced at a skim milk concentration of 3% and a propagation duration of 6 hours (v/v) (0.26 ppm).

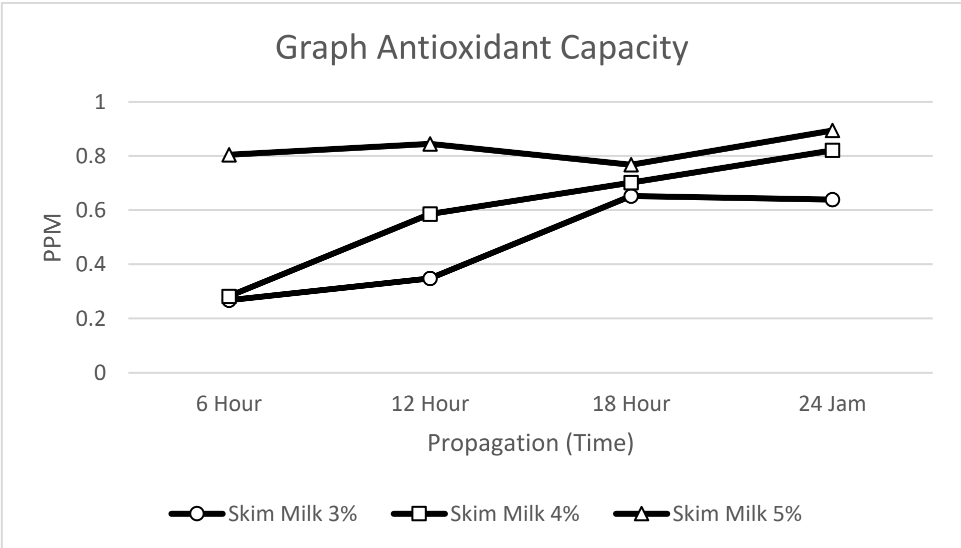

Figure 4. Graph of Total Antioxidant Capacity in probiotic beverage based on tangerine with variations in probiotic propagation time and skim milk addition

Figure 4 shows an increase in the antioxidant capacity of the probiotic drink with the extension of propagation duration and the concentration of skim milk used. A longer fermentation process allows probiotic bacteria to metabolize polysaccharides from the fruit, resulting in metabolites that contribute to enhancing antioxidant activity. One of the main metabolites is lactic acid, which is formed from the breakdown of lactose by lactic acid bacteria and possesses αhydroxyacid properties [1;17]. Additionally, during the stationary phase, bacteria begin to produce secondary metabolites that further strengthen the antioxidant capacity of the fermented product. On the other hand, tangerines, as the raw material, also contain various bioactive compounds, such as alkaloids, flavonoids, terpenoids, phenolics, saponins, tannins, coumarins, and carotenoids, which synergistically play a role in enhancing the antioxidant activity of the probiotic drink [3].

3.6 Sensory Evaluation

3.6.1 Evaluation of Aroma

The results of the analysis of variance indicate that the treatment of skim milk addition and the duration of propagation had a significant effect (P<0.05) on the hedonic aroma of the tangerine probiotic drink. The hedonic aroma values can be seen in Table 6 below.

Table 6. Average hedonic aroma of citrus reticulata probiotic drink

Skim Milk Concentration	Propagation (Hours)	Hedonic Aroma
3%	6	3,05±1,00bc
	12	3,10±0,79bc
	18	2,85±1,18bc
	24	3,20±0,89bc
4%	6	2,65±0,88c
	12	2,85±0,75bc
	18	2,70±1,22c
	24	3,45±0,76b
5%	6	2,80±0,77bc
	12	2,65±0,75c
	18	3,00±1,12bc
	24	4,40±0,68a

Note: Mean values followed by different letters in the same column/row indicate significant differences ($p < 0.05$).

Based on the data in Table 6, the average hedonic rating for the aroma of the tangerine probiotic drink ranged from 2.65 to 4.40, with criteria from "dislike somewhat" to "neutral." The highest aroma preference score was observed for the 5% skim milk treatment with a 24-hour propagation duration, categorized as "neutral," while the lowest preference score was found for the 4% skim milk treatment with a 6-hour propagation duration, categorized as "dislike somewhat." The analysis of variance results indicated that the treatments had a significant effect ($P < 0.05$) on the hedonic aroma. Duncan's test showed that the S3P4 treatment was significantly different from S2P1 and S3P2, but not significantly different from the other treatments.

3.6.2 Evaluation of color

The results of the analysis of variance indicate that the treatments of skim milk concentration and propagation duration had significant effects (P<0.05) on the hedonic color attributes of the citrus reticulata probiotic beverage. The hedonic color evaluation scores are presented in Table 7 below.

Table 7. Average hedonic color of citrus reticulata probiotic drink

Skim Milk Concentration	Propagation (Hours)	Hedonic Color
3%	6	3,35±0,59a
	12	2,45±0,51d
	18	2,85±0,88bcd
	24	2,65±0,93cd
4%	6	3,20±0,52ab
	12	2,90±0,72abcd
	18	2,90±0,72abcd
	24	2,80±0,70bcd
5%	6	2,60±0,60d
	12	2,80±0,89bcd
	18	2,60±0,68d
	24	3,15±0,59abc

Note: Mean values followed by different letters in the same column/row indicate significant differences ($p < 0.05$).

Based on the data in Table 7, the average hedonic rating for the color of the tangerine probiotic beverage ranged from 2.45 to 3.35, with criteria from "dislike" to "dislike somewhat." The highest color preference score was observed for the 3% skim milk treatment with a 6-hour propagation duration, categorized as "dislike somewhat," while the lowest preference score was found for the 3% skim milk treatment with a 12-hour propagation duration, categorized as "dislike." The analysis of variance results indicated that the treatments had a significant effect ($P < 0.05$) on the hedonic color. According to Duncan's test, there were significant differences among several treatments; for example, S3P4 was significantly different from S3P3, but not significantly different from the other treatments.

3.6.3 Evaluation of Taste

The results of variance analysis (ANOVA) demonstrated that both skim milk addition and propagation duration significantly affected ($P<0.05$) the hedonic taste evaluation of the citrus reticulata probiotic beverage. The taste preference scores are presented in Table 8 below.

Table 8. Average hedonic taste of citrus reticulata probiotic drink

Skim Milk Concentration	Propagation (Hours)	Hedonic Taste
3%	6	3,75±1,55bcd
	12	4,20±1,11bc
	18	3,10±1,45d
	24	4,25±1,29bc
4%	6	3,95±1,57bcd
	12	3,80±1,15bcd
	18	3,35±1,39cd
	24	4,50±1,00b
5%	6	3,95±1,36bcd
	12	4,25±1,02bc
	18	3,90±1,29bcd
	24	6,25±0,72a

Note: Mean values followed by different letters in the same column/row indicate significant differences ($p < 0.05$).

Based on the data presented in Table 8, the average hedonic rating for the taste of the tangerine probiotic beverage ranged from 3.10 to 6.25, with criteria from "dislike somewhat" to "like." The highest taste preference score was observed for the 5% skim milk treatment with a 24-hour propagation duration, categorized as "like," while the lowest preference score was found for the 3% skim milk treatment with an 18-hour propagation duration, categorized as "dislike somewhat." The analysis of variance indicated that the S3P4 treatment (5%, 24 hours) was significantly different from S1P3 (3%, 18 hours), while not significantly different from S1P1, S1P2, S1P4, S2P1, S2P2, S2P3, S2P4, S3P1, S3P2, and S3P3.

As the concentration of skim milk increased, the preference scores from the panelists also increased, attributed to the sweetness imparted by the skim milk, which masked the sour taste resulting from the fermentation process. [29] Which stated that the addition of skim milk contributes to an increase in hedonic taste values. Skim milk contains lactose, which can be fermented into lactic acid and flavor compounds by lactic acid bacteria, providing the characteristic taste of fermented milk that is favored by panelists. Additionally, the proteins in skim milk can enhance the viscosity and improve the mouthfeel of the probiotic beverage, resulting in a smoother and more balanced taste sensation.

3.6.4 Evaluation of Sour Taste

The results of the analysis of variance indicated that the treatments of skim milk addition and propagation duration had no significant effect ($P>0.05$) on the sour taste scoring of the citrus reticulata probiotic beverage. The sour taste scoring values are presented in Table 9 below.

Table 9. Average scoring sour taste of citrus reticulata probiotic drink

Skim Milk Concentration	Propagation (Hours)	Scoring taste
3%	6	2,65±1,04c
	12	3,15±1,04bc
	18	2,70±1,17bc
	24	3,25±1,21bc
4%	6	2,65±1,04c
	12	3,20±0,83bc
	18	2,55±1,10c
	24	3,45±1,28b
5%	6	3,10±1,21bc

	12	3,15±0,90bc
	18	3,00±1,00b
	24	4,25±0,44a

Note: Mean values followed by different letters in the same column/row indicate significant differences (p < 0.05).

Table 9 presents the average sourness scores of the tangerine probiotic beverage, which ranged from 2.55 to 4.25, categorized as "slightly not sour" to "sour." The highest sourness score was observed for the 5% skim milk concentration with 24-hour propagation duration, while the lowest sourness score was found for the 4% skim milk concentration with 18-hour propagation duration.

3.6.5 Evaluation of Overall Acceptance

The results of the analysis of variance indicated that the treatments of skim milk addition and propagation duration had a significant effect (P<0.05) on the overall hedonic acceptance of the citrus reticulata probiotic beverage. The overall hedonic acceptance values are presented in Table 10 below.

Table 10. Average hedonic overall acceptance of citrus reticulata probiotic drink

Skim Milk Concentration	Propagation (Hours)	Hedonic Overall Acceptance
3%	6	3,60±1,43cde
	12	4,15±1,31bcd
	18	3,20±1,44e
	24	4,25±1,16bc
4%	6	4,10±1,48bcd
	12	3,85±1,18bcde
	18	3,30±1,38de
	24	4,55±1,05b
5%	6	4,15±1,42bcd
	12	4,20±1,15bcd
	18	3,90±1,25bcde
	24	6,25±0,71a

Note: Mean values followed by different letters in the same column/row indicate significant differences (p > 0,05)

Table 10 presents the average hedonic ratings for the overall acceptance of the tangerine probiotic beverage, which ranged from 3.20 to 6.25, categorized from "dislike somewhat" to "like." The highest overall acceptance score was observed for the 5% skim milk concentration with a 24-hour propagation duration, while the lowest overall acceptance score was found for the 3% skim milk concentration with an 18-hour propagation duration.

Based on the results of the organoleptic tests, which included parameters of taste, aroma, color, and overall acceptance, the treatment with a 5% skim milk concentration and a 24-hour propagation duration (S3P4) demonstrated the best results. This treatment consistently achieved the highest scores across all tested organoleptic parameters. The hedonic taste score reached 6.25, aroma 4.40, color 3.15, and overall acceptance 6.25 (categorized as "like"), while the sourness score reached 4.25. This indicates that the combination of a 5% skim milk concentration and a 24-hour propagation duration produced a product with the most favorable flavor, aroma, color appearance, and overall sensory quality as preferred by the panelists. The addition of skim milk in optimal amounts contributed to an enhanced creamy taste, balanced acidity, and color stability, while the 24-hour propagation duration provided sufficient time for lactic acid bacteria to produce characteristic flavor compounds, thereby improving the overall sensory characteristics of the product.

IV. Interpretation of Analysis Result

The results of the study indicated that the combination of 5% skim milk concentration and 24-hour probiotic propagation time (S3P4) yielded the most optimal characteristics in the probiotic mandarin orange beverage. This treatment produced a total lactic acid bacteria (LAB) count of 10.09 log or 1,23 x 10^{10} CFU/mL, total acidity of 4.43%, antioxidant capacity of 0.90 ppm, and a hedonic taste score of 6.25, categorized as “liked” by the panelists. These findings suggest that increasing skim milk concentration and extending propagation time significantly enhance fermentation activity and the overall quality of the final product.

The increase in LAB count and acidity can be attributed to the availability of lactose and proteins from the added skim milk, which serve as essential nutrients for the growth and metabolism of Lactobacillus plantarum PR6105. The presence of these nutrients allows the bacteria to produce organic acids, such as lactic and acetic acid, more efficiently, resulting in a lower pH and higher total acidity [22].

Moreover, the elevated antioxidant capacity observed in the S3P4 treatment reflects the metabolic activity of LAB during fermentation, which produces bioactive compounds. Extended fermentation periods allow the production of secondary metabolites, such as bioactive peptides, organic acids, and phenolic compounds, that contribute to antioxidant activity. Additionally, mandarin orange juice naturally contains flavonoids, vitamin C, and other phytochemicals, which synergistically enhance the antioxidant properties of the final product. [1;4] Fermentation can improve antioxidant potential through the bioconversion of natural fruit compounds.

[11;28] Longer fermentation times significantly increased LAB counts and acidity levels in fruit-based probiotic drinks. In terms of sensory evaluation, [29] the addition of skim milk improves flavor and mouthfeel, leading to higher consumer acceptance.

From a practical standpoint, the combination of 5% skim milk and 24-hour propagation is recommended for both home-scale and industrial production of probiotic mandarin orange beverages. This formulation not only improves microbiological and functional quality but also provides favorable sensory attributes. Additionally, utilizing surplus or lower-grade mandarin oranges as raw materials adds economic value and supports food waste reduction. Nevertheless, this study has several limitations. It did not assess product stability during storage or evaluate the long-term viability of probiotic bacteria. Therefore, future research should focus on shelf-life studies and explore the incorporation of prebiotics to enhance the functional and synbiotic potential of the product.

V. Conclusion

The addition of skim milk and propagation time interact synergistically to enhance the physicochemical and sensory quality of probiotic beverages. Skim milk, which is rich in protein and lactose, serves as a primary nutrient source for the growth and metabolism of L. plantarum. Lactose is utilized as a fermentation substrate and converted into organic acids, while proteins support bacterial viability and secondary metabolite synthesis. Meanwhile, propagation time determines the duration of bacterial metabolic activity, thereby influencing the accumulation of fermentation products such as lactic acid, the increase in total LAB, and the formation of bioactive compounds. The combination of a sufficiently high skim milk concentration with optimal propagation time creates a fermentation environment that supports maximum LAB growth as well as stable chemical and sensory quality.

The best treatment combination was obtained at a skim milk concentration of 5% with a propagation time of 24 hours (S3P4). Under this treatment, bacterial viability reached its peak (10.09 log or 1,23 x 10^{10} CFU/mL) due to the abundant nutrient availability from skim milk being optimally utilized over a sufficiently long propagation period. The total acidity reached 4.43%, indicating optimal fermentative metabolic activity. The pH decreased to 4.0, reflecting acid accumulation in line with the fermentation level. The reduction of total reducing sugars to 0.10% confirmed the efficient utilization of carbohydrates by the bacteria. In addition, the antioxidant capacity increased to 0.90 ppm, presumably derived from bioactive compounds produced during fermentation. Sensory attributes such as taste, aroma, and overall acceptability also achieved the highest scores, demonstrating that this combination excelled not only in microbiological and chemical aspects but was also most preferred organoleptically.

The relationship between skim milk concentration and propagation time plays a crucial role in producing high-quality probiotic beverages. A higher skim milk concentration provides a stable and continuous substrate supply throughout the propagation phase. A sufficiently long propagation period (24 hours) allows the bacteria to complete the logarithmic phase and enter the stationary phase, during which the production of secondary metabolites such as acids, aroma compounds, and antioxidants reaches its maximum level. These two variables complement each other: without sufficient substrate, a long fermentation time would not be optimal, whereas too short a duration would result in suboptimal bacterial growth and chemical changes.

It is recommended to use a 5% skim milk concentration and a 24-hour propagation duration as the baseline formulation in the development of tangerine probiotic beverages for both industrial and household applications. Further research could be conducted to evaluate the product's stability during storage, as well as the potential addition of prebiotics to enhance functional value and probiotic synergy.

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