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Development Probiotic Drink of *Siwalan* Sap Ready to Drink Using *Lactobacillus acidophilus* FNCC0051, *Lactobacillus plantarum* FNCC0027, and *Bifidobacterium breve* BRL131

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A B S T R A C T

Siwalan sap is highly perishable due to its elevated sugar content, which promotes spontaneous fermentation shortly after collection. Converting siwalan sap into a probiotic beverage offers a promising alternative for extending shelf life and diversifying its utilization. This study aimed to see how different fermentation times and amounts of starter affect the physical and taste qualities of probiotic siwalan sap and to find the best combination of these factors. A factorial completely randomized design (CRD) was applied, comprising two factors: starter concentration (3%, 6%, and 9%) and fermentation time (16, 20, and 24 hours), with three replications. Data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) for significant results. Both factors significantly influenced total lactic acid bacteria (LAB), total titratable acidity, pH, reducing sugar content, total soluble solids, and organoleptic attributes (taste, aroma, and color). The best formulation was achieved with a 3% starter concentration and 20 hours of fermentation, yielding an LAB count of 8.751 log CFU/mL, pH of 4.07, a titratable acidity of 0.750%, a reducing sugar of 1.39 mg/100 mL, and total soluble solids of 17.167°Brix. Sensory evaluation indicated moderate acceptance with scores of 3.2 (taste), 3.35 (aroma), and 3.6 (color) on a 5-point hedonic scale.

Contribution to Sustainable Development Goals (SDGs):
SDG 3 – Good Health and Well-being
SDG 9 – Industry, Innovation, and Infrastructure
SDG 12 – Responsible Consumption and Production:
SDG 13 – Climate Action:

1. INTRODUCTION

1.1. Research Background

Siwalan sap, which is extracted from the flower bunches in sterile conditions, has a high sugar content of around 10-15 g/100 ml [1] and contains other nutrients such as water, protein, fat, and ash [2]. People usually consume *siwalan* nira as a traditional drink called *legen*. *Legen* drinks have a short shelf life because spontaneous fermentation. Some people utilizing this fermentation process to produce traditional fermented drinks with relatively high alcohol content, commonly called *tuak*. Yeast play a role in the spontaneous fermentation of sap [3].

Lactic acid bacteria is a type of microorganism that is safe when added to food because it is non-toxic (food grade microorganism). Lactic acid bacteria have the ability to break down complex compounds into simple compounds and produce lactic acid. The acid product causes the growth of other unwanted microbes to be inhibited [4]. Probiotic bacteria are generally from the lactic acid bacteria (LAB) group, especially the genus *Lactobacillus* and *Bifidobacterium* which are part of normal flora in the human digestive tract [5].

Fermentation time will affect the characteristics of probiotic drinks because during the fermentation there is a breakdown of nutrients in the raw material where the more fermentation time can increase the total acid and decrease the pH of the product, so that it will affect the organoleptic properties of the resulting probiotic drinks. Starter concentration can also affect the characteristics of probiotic drinks where if an excessive starter concentration is added, it will increase the acid [6]. Research is needed to determine the optimal starter concentration and fermentation time for producing probiotic drinks from *siwalan* sap.

1.2. Literature Review

Siwalan sap is a liquid from the tapis vessels tapped on the flower bunches of the *siwalan* plant [2]. The sugar content in nira is 12.30-17.4% [7], and it has a pH of around 5-6 and alcohol >5% [3]. There is also a vitamin content that can be beneficial to the human body, which is vitamin C. *Siwalan* sap also contains more nutrients, consisting of fat (0.19%), calcium (0.861%), phosphorus (0.052%), iron per 100 g of nira (11.01mg), and copper per 100 g of nira (0.767 mg). The composition of nira is influenced by several factors, among others: plant variety, plant age, plant health, soil condition, climate, stacking and irrigation [8]. The quality of legen can be affected by the length of storage, because the sugar content in legen can be fermented. Damage to legen begins to occur when the legen starts to come out of the panicle and then falls into the storage container. The liquid just out of the panicle is sterile with a neutral pH, but some time later, the pH will decrease [9].

Probiotic drinks are beverage products produced from the fermentation process of a substrate by certain probiotic bacteria that produce organic acids and contain living cells that can survive in active conditions in the digestive tract [10]. The bacteria contained in probiotic drinks are beneficial for the digestive tract. Probiotic drinks can maintain gut microflora balance by suppressing pathogenic bacteria in the intestine [11].

Based on Food and Agriculture Organization (FAO), probiotics are live microorganisms that benefit the host's health by improving the balance of intestinal microflora [12]. Probiotic bacteria can survive in the digestive tract after consumption and can survive in acidic stomach conditions [13]. Probiotic bacteria that have gone through clinical trials include *Lactobacillus casei* subsp. *casei Shirota* strain found in Yakult, *Bifidobacterium* and *Lactobacillus* acidophilus [14]. Some common probiotics include various species from the genera *Bifidobacterium* and *Lactobacillus* such as *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus* [15].

L. plantarum FNCC0027 has been commonly used as a probiotic strain. *L. plantarum* FNCC0027 is resistant to medium with low pH and media added with bile salts, and is antagonistic to pathogenic *E. coli* [16]. *L. plantarum* FNCC0027 can increase the functional activity of Jamaican cherry juice because, during fermentation, the results can increase the total phenolic content, antioxidant activity, and inhibit diabetes-related enzymes in Jamaican cherry juice [17].

L. acidophilus FNCC0051 is able to survive in acidic conditions (pH < 3) and bile salt conditions [18]. *L. acidophilus* FNCC0051 has the potential to be a probiotic supplement for patients with type 1 DM by reducing the value of insulitis so that it can improve the condition of the pancreas during in vivo test [19]. *L. acidophilus* FNCC0051 in fermented soy milk products has good viability and stability while in the digestive tract in vitro, this indicates that *L. acidophilus* FNCC0051 has potential as a probiotic in functional food products [20]. *L. acidophilus* FNCC0051 has antibacterial activity against *Propionibacterium acnes* [21].

B. breve BRL131 was used as probiotic in the prebiotic activity test of gembili tuber inulin with foaming drying method, which has higher prebiotic activity than *B. bifidum* BRL130 [22]. The use of *B. breve* BRL131 is also used in the manufacture of functional foods, namely synbiotic jelly drinks from soy milk and red dragon fruit extract [23]. *B. breve* ATCC 15700 is resistant to bile salts and can degrade fructooligosaccharides added to its growth medium [24].

2. MATERIALS AND METHODS

2.1. Material and Tools

Materials used in the preparation of legen fermented beverages, which is *siwalan* sap derived from the Lontar, Gresik Regency, pure cultures of *L. acidophilus* FNCC051, *L. plantarum* FNCC027, and *B. breve* BRL131 obtained from the Food Nutrition Culture Collection, Inter-University Center for Food and Nutrition, UGM Yogyakarta, and sugar. Materials used for analysis were distilled water, physiological saline solution, PP indicator, pH 4 and 7 buffer solution, NaOH, and alcohol. The media used were deMan Rogosa Sharp (MRS) broth and agar.

The tools used, which are beaker glass, petridish, burette and stative, pH meter, alcohol meter, pipette, pipette pump, micropipette, test tube and rack, erlenmeyer, analytical balance, vortex, hotplate, refractometer, deconstruction, laminar air flow, autoclave, incubator, colony counter, and spectrophotometer.

2.2. Research Procedure

This research used Completely Randomized Design (CRD) with two factors, the first factor was starter concentration (%v/v) with three levels (3%, 6%, and 9%), and the second factor was fermentation time (hours) with three levels (16 hours, 20 hours, and 24 hours). Each treatment was repeated three times. The data obtained will be analyzed using Analysis of Variance (ANOVA). If there is a difference between treatments, Duncan's Multiple Range Test (DMRT) is continued at the 5% level. From the combination of the two treatment factors, 9 samples were obtained as follows:

A_1B_1= stater concentration 3% with fermentation time 16 jam
A_1B_2= stater concentration 3% with fermentation time 20 jam
A_1B_3= stater concentration 3% with fermentation time 24 jam
A_2B_1= stater concentration 6% with fermentation time 16 jam
A_2B_2= stater concentration 6% with fermentation time 20 jam
A_2B_3= stater concentration 6% with fermentation time 24 jam

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A_3B_1= stater concentration 9% with fermentation time 16 jam
A_3B_2= stater concentration 9% with fermentation time 20 jam
A_3B_3= stater concentration 9% with fermentation time 24 jam

2.3. Starter Preparation

The preparation starter of each bacterial was carried out in the same way, wherein 1 mL of protiotic bacteria was put into 10 mL of MRS Broth medium, then incubated at 37°C for 24 hours.

2.4. Preparatin of Siwalan Sap Probiotic Drink

Making probiotic drinks based on [25], which is modified. The sap volume was measured at 100ml for each treatment. Pasteurize at 80°C for 15 minutes. Cooling to 37°C. Addition of starter *L. acidophilus*, *L. plantarum*, and *B. breve* in a ratio of 1:1:1 with three levels of starter concentration treatment (3%, 6%, and 9%). Fermentation of palm sap at 37°C for 16, 20, and 24 hours.

2.5. Research Analysis

2.5.1. Raw Material Analysis

The raw materials researched was *siwalan* sap and starter cultures that will be used. Analysis of *siwalan* sap include pH value, total LAB, total titratable acid, total reduced sugar, and total soluble solids. Starter culture research was analyzed for total LAB

2.5.2. Probiotic Drink Analysis

Probiotic drink products treated with starter concentration and fermentation time were analyzed for pH value, total LAB, total titratable acid, total reduced sugar, total soluble solids, and organoleptic test (taste, aroma, and color).

3. RESULT AND DISCUSSION

3.1. Raw Material Characteristics

Based on the analysis results in Table 1, the pH and total acid values of 5.08 and 0.30% showed a difference from the literature. This can be caused by the presence of lactic acid bacteria in the nira. LAB growing in nira can cause a decrease in pH value due to the breakdown of sugar in nira into lactic acid. In the research of [26], the production of organic acids is in line with the growth of microbial cells that have an impact on decreasing the pH and quality of legen.

Table 1. Raw Material of *Siwalan* Sap Characteristics

Parameters	*Siwalan* Sap	
	Result	Literature
pH Value	5.09±0.100	6.7-6.9[27]
Total Titratable Acid (%)	0.300±0.094	0.11-0.23[28]
Total Reduced Sugar (mg/100ml)	1.909±0.089	0.25-6.37[28]
Total Soluble Solids (ºBrix)	14.167±0.289	11-17[29]
Total LAB (log CFU/ml)	3.342±0.244	

The analysis showed that the total lactic acid bacteria was 3.342 log CFU/ml or 10^3 CFU/ml. Based on [30], microbial growth in nira can come from the air, the tapping barn, or other contaminants that can contaminate the nira during the tapping process. The presence of microbes in nira can also be caused because nira has a relatively high sugar content, so it can be used as a substrate by microbes. Based on [31], the high sugar content along with other micronutrient compositions causes palm sap to become a medium for microbial growth.

The results of the analysis of total reducing sugar of 1,909 mg/100ml and total soluble solids of 14,20ºBrix are consistent with the literature. Based on Ref. [8] stated that the composition of legen depends on plant variety, plant age, plant health, soil condition, climate, stacking and irrigation.

Table 2. Total LAB of Starter Cultures

Parameter	Total Lactic Acid Bacteria	
	Result (log CFU/ml)	Literature (CFU/ml)
L. plantarum FNCC0027	7,307 ± 0,497	
L. acidophilus FNCC0051	7,102 ± 0,358	Min. 10^6[32]
B. breve BRL131	7,479 ± 0,050	

Based on **Table 2.** showed that the total LAB analysis results of *Lactobacillus plantarum* FNCC0027, *Lactobacillus acidophilus* FNCC0051, and *Bifidobacterium Breve* BRL131 were 7,307; 7,102; and 7,479 log CFU/ml or equivalent to 10^7 CFU/ml. The analysis results have met the minimum number of probiotic strains used in food products, which is more than 10^6 CFU/ml.

3.2. Probiotic *Drink Characteristics*

3.2.1. Total Lactid Acid Bakteria

Based on Table 3, it showed that the average total LAB of probiotic drinks of *siwalan* nira ranged from 8.545-9.758 log CFU/ml or equal to 108-109 CFU/ml. The results of the analysis on the probiotic drink of *siwalan* nira have fulfilled the minimum standard of total probiotic bacteria according to SNI 7552:2009, which is 106 CFU/ml. The highest value is treatment of starter concentration 9% with fermentation time 24 hours, which is of 9.758, and the lowest value is treatment of starter concentration 3% with a fermentation time 16 hours, which is 8.545.

This result showed that the higher the starter concentration and longer the fermentation time, the higher the total LAB in the probiotic drink of *siwalan* sap, this is due to the increasing quantity of bacteria and as time passed LAB will continue to grow. LAB will use sugar of sap as a source of carbon and energy for growth. Bacterial growth is one of the characteristics of the fermentation process [6]. Based on [22] the longer the fermentation, the total lactic acid bacteria increases because lactic acid bacteria have a longer time to ferment.

3.2.2. Total Titratable Acid

Based on Table 3, it showed that the average total titratable acid of the probiotic drink of *siwalan* sap ranged from 0.62-1.31%. The highest value is treatment of starter concentration 9% with fermentation time 24 hours, which is 1.31%, while the treatment of adding 3% starter concentration with a fermentation time of 16 hours produced the lowest total LAB of 0.62%. Based on SNI 7552:2009 that the quality requirements of good fermented beverages have total titratable acid ranging from 0.2-0.9%. This shows that the total titratable acid in the probiotic drink of *siwalan* sap in some treatments does not meet these requirements. The results of the analysis have a wider range of total titritable acid, which can be caused by the use of a combination of types

and increasing the concentration of starter used. Based on Ref. [33] stated that the high level of lactic acid is influenced by the ability of LAB which is determined by the amount and type of inoculum used, as well as the fermentation environment during incubation.

3.2.3. pH Value

The results showed that the average pH value of the probiotic *siwalan* sap drink ranged from 3.37 to 4.34. The highest value is the treatment of starter concentration 3% starter concentration and fermenting for 16 hours, which is 4.34. The lowest value is treatment starter concentration 9% starter concentration with a fermentation time 24 hours, which is 3.37. According to SNI 7552:2009, good fermented beverages have a pH value between 3.80 and 4.50. Therefore, some of the treatments for making probiotic *siwalan* sap drinks do not meet these requirements. Some samples had a pH value lower than 3.80. This correlates with the results of the total titratable acidity of the probiotic *siwalan* sap drink: the higher the total titratable acidity, the lower the product's pH value. According to [34], lactic acid bacteria utilize carbohydrates in the medium to produce lactic acid, which reduces the pH value during fermentation.

Table l. Characteristics of *Siwalan* Sap Probiotic Drink

Sample	Total LAB (log CFU/ml)	Total Titritable Acid (%)	pH Value	Total Reduced Sugar (g/100ml)	Total Soluble Solids (ºBrix)
A1B1	8.545 ± 0.128	0.62 ± 0.026	4.34 ± 0.079	1.44 ± 0.096	17.58 ± 0.382
A1B2	8.751 ± 0.043	0.75 ± 0.026	4.07 ± 0.048	1.39 ± 0.107	17.17 ± 0.289
A1B3	8.875 ± 0.059	0.92 ± 0.026	3.79 ± 0.050	1.30 ± 0.071	16.33 ± 0.289
A2B1	8.740 ± 0.045	0.72 ± 0.045	4.20 ± 0.051	1.38 ± 0.039	17.00 ± 0.250
A2B2	9.328 ± 0.085	0.96 ± 0.026	3.80 ± 0.040	1.26 ± 0.007	16.42 ± 0.144
A2B3	9.512 ± 0.073	1.07 ± 0.026	3.58 ± 0.054	1.18 ± 0.089	15.92 ± 0.144
A3B1	9.272 ± 0.092	1.02 ± 0.026	3.79 ± 0.020	1.22 ± 0.141	16.42 ± 0.144
A3B2	9.615 ± 0.133	1.10 ± 0.026	3.59 ± 0.032	0.93 ± 0.045	15.08 ± 0.144
A3B3	9.758 ± 0.093	1.31 ± 0.045	3.37 ± 0.064	0.76 ± 0.065	14.75 ± 0.250

3.2.4. Total Reduced Sugar

The results showed that the average sugar reduction content of the probiotic *siwalan* sap beverage ranged from 0.76 to 1.44 mg/100 mL. The highest value is treatment of starter concentration 3% starter concentration and fermenting for 16 hours, which is 1.44 mg/100 ml, and the lowest value is treatment of starter concentration 9% starter concentration with a fermentation time 24 hours, which is 0.76 mg/100 ml. Higher starter concentrations and longer fermentation times resulted in lower sugar reduction content in the probiotic *siwalan* sap beverage. This occurs because LAB uses more reducing sugars as substrates for growth during fermentation. Sugar is broken down by LAB into organic acids, especially lactic acid, which decreases the amount of reducing sugar and increases the acidity of the probiotic drink. Based on [35], microbes use simple sugars from the breakdown of carbohydrates as carbon and energy sources for growth during fermentation. [36] states that reducing sugar content is an indicator of fermentation. Microbes ferment sugar to produce metabolites, most of which are acids. If fermentation occurs, the reducing sugar content decreases, the total acid content increases, and the pH becomes acidic.

3.2.5. Total Soluble Solid

The result showed that the average total soluble solids of probiotic drink of *siwalan* sap ranged from 17.58-14.75ºBrix. The treatment of adding 3% starter concentration with fermentation time of 16 hours produced the highest total soluble solids of 17.58ºBrix, while the treatment of adding 9% starter concentration with fermentation time of 24 hours produced the lowest total soluble solids of 14.75ºBrix.

This result showed that the higher starter concentration and the longer fermentation time, the lower total soluble solids of probiotic drinks. This can occur due to the inversion of sugar in the sap due to low pH. The inversion process involves sucrose hydrolyzing into glucose and fructose. LAB will utilize glucose and fructose as substrates for growth, which causes a decrease in sugar and an impact on the reduction of total soluble solids in the probiotic drink of *siwalan* nira. This result agrees with [37] that low pH values can cause the inversion of sucrose into its constituent monosaccharides, which are glucose and fructose. According to [38], lactic acid bacteria will metabolize sugars such as glucose into lactic acid, which directly reduces the concentration of soluble solids in the fermentation medium.

3.2.6. Sensory Test

Table 4. Sensory Characteristics of Probiotic Drink of *Siwalan* Sap

Sample	Taste	Aroma	Colour
A1B1	3.15	3.3	3.65
A1B2	3.2	3.35	3.6
A1B3	3.25	3.25	3.75
A2B1	3.25	3.25	3.6
A2B2	3.2	3.25	3.35
A2B3	3.2	3.15	3.35
A3B1	3.15	3.2	3.2
A3B2	2.8	3.2	3.25
A3B3	2.45	2.85	3.3

Taste

The treatment of starter concentration and fermentation time had a significant effect ($p \leq 0.05$) on the taste of probiotic drinks. The level of preference of panelists for the taste of probiotic drinks obtained the results of the average value between 2,45-3,25. The treatment of 3% starter concentration and fermentation time for 24 hours produced the highest average velue, which is 3,25 (with the highest ranking 113) This treatment classified in the “slightly like” assessment to the panelists. The taste of the probiotic drink formed, namely a sour taste with a distinctive taste of *siwalan* sap. This is in accordance with SNI 7552: 2009 which states the taste of good fermented beverages, which is a typical acid. The more starter concentration and the longer the fermentation time, the more LAB will use the sugar in the nira

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and be broken down into organic acids, creating a sour taste in the product. This is supported by Ref. [39] that the sour taste in fermented beverages is caused by chemical compounds produced during fermentation, such as lactic acid, acetic acid and other organic acids.

Aroma

The treatment of starter concentration and fermentation time had no significant effect ($p \geq 0.05$) on the aroma of probiotic drinks. The panelists' preference for the aroma of probiotic drinks obtained the average value between 2,85 and 3,35. The treatment of 3% starter concentration and fermentation time for 20 hours produced the highest average value according to panelists of 3.35 (with the highest rangking of 110.5). This treatment is classified as "slightly like" according to the panelists. The aroma formed in the product is a sour aroma formed from the fermentation process, the more starter concentration and the longer fermentation time causes higher bacterial activity so that the fermentation process increases which has an impact on increasing metabolite yields.

Colour

The treatment of starter concentration and fermentation time had no significant effect ($p \geq 0.05$) on the color of probiotic drinks. the level of panelist preference for the color of probiotic drinks obtained the results of the number of rankings between 88-120.5. The treatment of 3% starter concentration and fermentation time for 24 hours produced the highest average value according to panelists of 3.75 (with highest ranking of 109). According to the panellists, this treatment is classified in the "somewhat like" assessment category. The color of the *siwalan* nira probiotic drink product is cloudy white, this is following the color of the nira raw material used. The fermentation process can cause turbidity in the product. Following Ref. [10] that in probiotic drinks the turbidity of the product is formed from metabolites resulting from LAB fermentation during fermentation and the color of the product is influenced by the components in the ingredients for making the product.

4. CONCLUSION

The results of the analysis of probiotic drinks of *siwalan* sap can be shown that there is a significant interaction ($p \leq 0.05$) in the treatment of the addition of starter concentration and fermentation time on the parameters of total LAB, total titratable acid, pH value, reduced sugar content, total solids, and organoleptic test (taste). The results of this study obtained the best treatment, that is A1B2 sample, which is probiotic drink of *siwalan* sap with the treatment of adding 3% starter concentration and fermentation time for 20 hours, has the characteristics of total LAB 8.751 log CFU/ml, pH value 4.07, total titrated acid 0.750%, reducing sugar content 1.39 mg/100ml, total soluble solids 17.167ºBrix, and has organoleptic characteristics including taste 3.2 (rather like), aroma 3.35 (rather like), and color 3.6 (rather like).

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