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Effect of Sucrose Concentration on Microbiological, Physicochemical, Antioxidant Activity, and Organoleptic Characteristics of Salak Fruit Juice Beverage Fermented with *Lactobacillus Plantarum* InaCC B153

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ABSTRACT

Salak is a tropical fruit that has great potential to be made into a healthy fermented beverage. The carbohydrate content and bioactive compounds such as phenolics in salak provide benefits as a source of antioxidants. The fermentation process will produce a probiotic drink that is good for digestive health. The fermentation process involves Lactobacillus plantarum bacteria as probiotic agents. This study aims to determine the effect of sucrose concentration on the characteristics of salak fruit juice probiotic drinks. This study used a completely randomized design (CRD) factorial pattern with sucrose concentration factor (3%, 6%, 9% b/v). The data obtained were analyzed using ANOVA followed by DMRT further test at the 5% level. The results showed a significant interaction ($p \leq 0.05$). Fermented salak fruit juice using 9% sucrose was the best treatment with characteristics of pH 3.80; total microbes 9.023 log CFU/ml; total acid 1,11%; total sugar 13,88%; viscosity 118,35 mPa.s; DPPH antioxidant activity 89.99%. The resulting organoleptic properties including colour, aroma, and taste, overall were 3.40 (moderately liked), 3.08 (moderately liked), 3.76 (moderately liked), and 3,48 (moderately liked).

1. INTRODUCTION

1.1. Research Background

Salak is a tropical fruit that contains carbohydrates, protein, phosphorus, calcium, vitamins, and fibre as well as bioactive compounds such as polyphenols and flavonoids that have antioxidant activity. The limited knowledge and exploration in processing salak fruit has led to a lack of variety in the utilization of this fruit. In addition, the lack of promotion and research on the various potential uses of salak fruit can also limit the variation in its utilization. The processing technology that can be done to extend the shelf life and maintain the content contained in salak fruit is fermentation because, in the fermentation process, some nutrients of bioactive compounds that produce antioxidant activity are maintained and can even be increased.

From 2019-2024, the global functional beverage market is predicted to grow at an Annual Growth Rate of 8.66% [1]. One of the functional beverages is probiotic fermented beverages, by processing salak fruit into beverages through the fermentation process, it can be an attractive and healthy alternative for those who want to improve gut health and benefit from probiotic microorganisms. Therefore, salak fruit has the potential to be used as a basic ingredient for making fermented beverages.

The utilization of probiotic microorganisms in beverage manufacturing is gaining popularity due to the health benefits associated with probiotic consumption. One type of probiotic microorganism that is often used is *Lactobacillus plantarum*. *Lactobacillus plantarum* can show probiotic characteristics, namely resistance to gastrointestinal pH conditions and bile salts, resistance to antibiotics, and is antagonistic to pathogenic bacteria [2].

In addition to fermentation media, probiotic starter cultures require energy or nutrients to thrive. The addition of sucrose is an important factor that can affect the final result of probiotic drinks, especially the flavour. In addition, sucrose also affects the final result of the drink in terms of physicochemicals.

1.2. Literature Review

Salak Pondoh has a round shape with a relatively small (30-100 grams), blacker skin colour but whiter flesh colour compared to other types of salak, besides that salak Pondoh has a harder texture [3]. Salak Pondoh is a type of salak that has better quality than other types of salak, where salak Pondoh is famous for its sweetness. The sweet flavour is influenced by its chemical composition, namely a relatively small tannin content of 0.08% and a relatively high sugar content of 23.30% [4].

Lactobacillus plantarum is a Gram-positive, nonmotile bacterium, Rods, generally measuring 0.6-0.8 μm x 1.2-6.0 μm, single or many chains and short. These bacteria are catalase-negative, do not spore, do not reduce nitrate to nitrite, do not produce NH3 from arginine, are facultatively anaerobic, and grow optimally at 30-35ºC but cannot grow at 7ºC [5].

Lactobacillus plantarum is salt tolerant, produces acid quickly, and has an optimum pH of 3.0 - 4.6. Lactobacillus plantarum bacteria are more resistant to acidic conditions so they are more present at the last stage of the fermentation process [6]. In addition, *Lactobacillus plantarum* has antagonistic properties against microbes that cause food damage such as *Staphylococcus aureus, Salmonella*, and several other types of Gram-negative bacteria [7].

Probiotic fermented beverages are beverages made by utilizing probiotic bacteria to help the fermentation process of food ingredients [8]. Probiotic drinks are known as functional food products because they can support the function of the human gastrointestinal tract. Probiotic drinks are a type of functional drink that has health effects and contains live microbes commonly called probiotics. Probiotics themselves are live bacteria that can affect health by balancing the microflora in the gut and preventing and selecting dysfunctional microbes [9].

A wide variety of fruit juices have been successfully fermented by L. plantarum strains resulting in final products with potential functional properties. Most of the positive effects were reported and recapitulated into (i) increased antioxidant activity, (ii) increased total phenolic and total anthocyanin contents, (iii) extended shelf life of fruit juices, and (iv) better sensory features. It has been reported, that *L. plantarum* ATCC14917 modified the phenolic composition of apple juice after fermentation and improved the overall antioxidant capacity, as well as the bioavailability of apple polyphenols [10].

To support the growth of the inoculant, the addition of sucrose aims to increase the carbon source for lactic acid bacteria during the fermentation process. During the fermentation process, lactic acid bacteria will metabolize sucrose to fulfill nutrients during fermentation. The result of sugar hydrolysis will result in free phenol compounds that will increase antioxidant activity [11].

1.3. Research Objective

The higher the concentration of sucrose added, the more substrate can be broken down by lactic acid bacteria so that it will cause a distinctive flavor, but if the concentration of sucrose added exceeds the limit, it will cause the environmental conditions to become hypertonic so that plasmolysis occurs [12].

Based on the description above, research was conducted on the development of a salak fruit juice probiotic drink with a sucrose addition concentration factor. The final product will then identify the influence of factors on microbiological, physical, chemical, antioxidant activity, and organoleptic characteristics as a quality evaluation of the resulting fermented beverage.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were salak pondoh fruit, and *Lactobacillus plantarum* InaCC B153 obtained from Indonesia Culture Collection (InaCC) BRIN Cibinong. Materials for analysis include MRS Broth, MRS Agar, distilled water, 2.2-diphenyl-1-picrylhydrazyl (DPPH), PP indicator, 96% ethanol, anthrone solution, and 0.1N NaOH.

2.2 Research procedure

This research was conducted experimentally using a completely randomized design (CRD) factorial pattern with one treatment factor. The treatment factor is sucrose concentration (3%, 6%, 9% b/v) The data obtained were analyzed by ANOVA to determine the effect of treatment with 2 replications. If there were significant differences, further tests were carried out with the DMRT (Duncan Multiple Range Test) method at 5%. Data analysis used the help of the SPSS Statistics 17.0 for the MacOs program.

2.3 Salak Fruit Juice Making

Making Salak Fruit Juice refers to [13] which has been modified and begins with sorting 900 g of fruit with the same maturity level, peeling the skin, removing the seeds, cutting it into small pieces, and washing it thoroughly with water. Salak fruit that has been cut into small pieces is added to water with a ratio of water and salak 4:1. After that, sugar was added according to the research variables: 3%; 6%; 9% (b/v) and pureed using a blender until it became juice. The liquid was filtered using filter paper to obtain salak juice (extract) then poured into a sterile glass jar and closed. The salak juice was pasteurized in a water bath at 70ºC, 1 atm for 10 minutes, and allowed to cool.

2.4 Preparation of Lactobacillus plantarum Starter

Preparation of *Lactobacillus plantarum* starter refers to [14] which has been modified starting with inoculating 1000µL of *L. plantarum* isolate into 4 mL of MRS broth. After inoculation, the culture was incubated at 37ºC for 24 hours. Then, the growing culture was centrifuged at 5500 rpm for ± 10 minutes at 4ºC. After centrifugation, the filtrate was decanted and the residue was suspended into 10 mL of sterile NaCl 0.85 solution vortexed with a medium scale for ± 1 minute to be used as a starter culture.

2.5 Fermented Beverage Making

The preparation of fermented beverages refers to [15], starting with pouring 30 ml of salak fruit juice, each of which has been given sugar (3%, 6%, 9%) into sterile glass bottles and pasteurized at 70°C for 10 minutes. After that, the salak juice was cooled to a temperature of 31 - 32°C. After reaching that temperature, the salak juice was inoculated with a starter containing *Lactobacillus Plantarum* culture and incubated at 37°C for 24 hours. After the incubation time, the beverage was

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sampled in a sterile environment, after which it was centrifuged at 4000 rpm for 10 minutes to collect the supernatant. Then the supernatant was stored at 4◦C for further analysis.

2.6 Analysis of Microbiology, Physical, Chemical, and Organoleptic Characteristics Salak Fruit Juice Beverage Fermented

The analysis conducted in this study consisted of physicochemical analysis, antioxidant activity, and organoleptic. The physicochemical analysis includes pH [16], Total LAB [17], Total Acid [18], Total Sugar [19], Viscosity [20], Antioxidant Activity DPPH method [21], Hedonic organoleptic assessment of colour, aroma, taste, and overall Friedman method. Fermented beverages were carried out with 25 semi-trained panelists using 5 rating scales; 1 = very dislike; 2 = dislike; 3 = ordinary; 4 = like; 5 = very like.

3. RESULT AND DISCUSSION

3.1. Microbiological Analysis of Salak Fruit Juice Fermented Beverage

Table 1. Total Microbes Analysis Results of Salak Fruit Juice Fermented Beverage

Sucrose Concentration	Total Microbes (Log CFU/mL)
3%	8.739 ± 0.01^{a}
6%	8.946 ± 0.04^{b}
9%	9.023 ± 0.03^{b}

Description: Numbers accompanied by different letters indicate significant differences ($p \leq 0.05$)

3.1.1 Total Microbes

Table 1. shows an increase in total microbes as the concentration of sugar added increases. This is because the more sugar added, the more microbes produced. This is due to the sugar content in the salak fruit and also the sucrose added, used as a source of carbon and energy for the growth of each inoculant. Following Ref. [22] the speed of growth and viability of microbes in the fermentation process is influenced by the availability of substrate, medium, and fermentation time.

The highest total microbes were produced by 9% sucrose concentration. This is thought to be because sucrose is a source of energy and nutrients needed in the growth of LAB [23], the carbon element in sucrose is utilized by LAB to compose cellular macromolecules in the process of proliferation, and total LAB increases. Total LAB increased as the sucrose concentration increased to the optimal point. In addition, LAB also utilized the fructose in salak in the probiotic drink as a carbon source and protein as a nitrogen source to perform its growth. Carbon source is an important nutrient for LAB growth as its energy source, so the presence of fructose can trigger the growth of LAB colonies [24].

3.2. Physicochemical Characteristics of Salak Fruit Juice Fermented Beverage

Table 2. Physicochemical Analysis Results of Salak Fruit Juice Fermented Beverage

Sucrose Concentration	pH Value	Acid Total (%)	Sugar Total (%)	Viscosity (mPa.s)
3%	3.93 ± 0.05^{a}	0.84 ± 0.04^{a}	12.76 ± 0.26^{b}	117.90 ± 0.55^{a}
6%	3.89 ± 0.04^{a}	1.02 ± 0.11^{ab}	11.01 ± 0.27^{a}	118.15 ± 0.07^{a}
9%	3.80 ± 0.07^{a}	1.11 ± 0.04^{b}	13.88 ± 0.17^{c}	118.35 ± 0.07^{a}

Description: Numbers accompanied by different letters indicate significant differences ($p \leq 0.05$)

3.2.1 pH Value

Table 2 shows that there is a decrease in pH as the concentration of sugar added increases. In the fermentation process, bacteria will break down sugar (carbohydrates) into lactic acid, alcohol, and energy during the fermentation process. This is because the more sugar available, the more lactic acid is produced. This lactic acid is acidic, causing the pH of the beverage to decrease.

The sucrose concentration that produces fermented beverages with the lowest pH is 9%, which is 3.8. This is because, during the fermentation process, there is a buildup of lactic acid as the main product of bacteria. This is supported by the statement of Ref. [25] that in the fermentation process, lactic acid bacteria will produce a certain amount of lactic acid and several other acids that can reduce the pH value. The more acid content, the pH value of the product will decrease and can affect the growth of probiotic bacteria.

3.2.2 Acid Total

Total lactic acid increased with higher sucrose addition. This is due to the higher sucrose added, it will cause the breakdown of glucose and fructose and produce lactic acid by microorganisms through the process of glycolysis. Following Ref. [26], glucose will be broken down into two molecules of pyruvic acid in the glycolysis process accompanied by the formation of two NADH + H^{+}. Pyruvic acid formed in the glycolysis pathway acts as a hydrogen receiver, where the reduction of pyruvic acid by $NADH_2$ will produce lactic acid [27]. Thus, when more sugar is available, more lactic acid will be produced. The lactic acid will then be excreted out of the cell and accumulate in the substrate so that it can increase acidity [28].

3.2.3 Sugar Total

The more sucrose added, the more total sugar produced. This is because the sucrose added to the product is counted as total sugar so it follows Ref. [29], that the higher the concentration of sucrose added, the more the total sugar will increase. However, in the treatment of the type of inoculant, it does not apply or show any difference. Meanwhile, at 6% sucrose concentration, there was a significant decrease in total sugar.

The increase in total sugar along with the addition of sucrose is thought to be because the simple sugars contained in the fruit juice are still sufficient so that the sucrose (disaccharide) added is not all broken down into simple sugars and converted into lactic acid during the fermentation process [30].

3.2.4 Viscosity

Table 2. shows that the higher the sucrose concentration, the higher the viscosity value. This is because viscosity is affected by the addition of sugar and the concentration of sugar added. High sugar contains a high degree of brix so that more double-helix bonds are formed and bind water to form a gel [31].

The highest viscosity value was produced by 9% sucrose concentration which was 118.35 mPa.s. [32] explained that the viscosity of probiotic drinks is influenced by the isolate or bacteria used. LAB itself produces EPS (extracellular polysaccharide) during fermentation, which increases the viscosity of the beverage.

3.3. Antioxidant Activity of Salak Fruit Juice Fermented Beverage

Table 3. Antioxidant Activity Analysis Results of Salak Fruit Juice Fermented Beverage

Sucrose Concentration	Antioxidant Activity DPPH (%)
3%	86.70 ± 0.06^a
6%	88.76 ± 0.04^a
9%	89.99 ± 0.12^a

Description: Numbers accompanied by different letters indicate significant differences ($p \leq 0.05$)

Antioxidant activity increased with higher sucrose addition. This is due to the metabolic products of microorganisms affecting antioxidant activity. Antioxidant activity is related to the increase in total lactic acid bacteria because, during the fermentation process, it is suspected that LAB liberates phenolic compounds that can increase the antioxidant activity of salak fruit juice fermented beverages.

The metabolic products of probiotic bacteria in the form of lactic acid are also one of the triggers for increased antioxidant activity, this is thought to be due to lactic acid in fermentation products in the form of *α-hydroxy acid* (AHA) [33].

3.4. Organoleptic Characteristics of Salak Fruit Juice Fermented Beverage

Table 4. Mean Organoleptic Value of Salak Fruit Juice Fermented Beverage with Sucrose Concentration Treatment

Sucrose Concentration	Average Ranking			
	Colour	Aroma	Taste	Overall
3%	6.54	4.28	3.86	4.82
6%	5.48	4.10	6.30	5.68
9%	4.76	4.20	6.86	5.72

3.4.1 Colour

From Table 4, it can be seen that the level of panelists' liking for the colour of salak fruit juice fermented beverages resulted in an average ranking ranging from 4.76-6.54. The highest number of ranks is the most preferred fermented beverage by panelists. The highest value was found in salak fruit juice with 3% sucrose concentration while the lowest ranking value was produced by salak fruit juice with 9% concentration. From the panelists' notes, they tended to like the light yellow coloured drink rather than the dark yellow (almost brownish) coloured drink. This is because salak juice undergoes colour changes such as caramelization due to the heating process. The heating causes the product to become brownish.

Ref. [34] states that the browning process occurs by the phenolase enzyme that reacts with sugar. Browning is also caused by direct contact with outside air in an oxidative or metal manner. Colour is influenced by the constituent components in the ingredients and the process of making the product. Differences in the addition of sugar and inoculants affect the colour after fermentation through the formation of metabolites, resulting in different final product colours [35].

3.4.2 Aroma

From Table 4. it can be seen that the level of panelists' liking for the aroma of salak juice fermented beverages produces an average ranking that is not much different. This is because panelists tend to like drinks that smell of fresh salak in general, while in the treatment of drinks fermented with *Lactobacillus plantarum*, the aroma produced is sour. The distinctive aroma of probiotic products is obtained from lactic acid as a result of bacterial metabolism that can provide a sharpness of flavor. According to [36], in addition to its role in gel formation, lactic acid can provide sharpness of flavour and determine the distinctive sour aroma of probiotic drinks.

3.4.3 Taste

Table 4. shows that the level of panelists' liking for the taste of salak fruit juice fermented beverage resulted in an average

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ranking ranging from 3.86 to 6.86. The highest number of ranks is the most preferred fermented beverage by panelists. The highest value is found in salak fruit juice with 9% sucrose concentration while the lowest ranking value is produced by salak fruit juice with 3% concentration. This is because the higher the sucrose concentration, the sweeter the flavour and the more desirable it is to the panelists while the low sucrose concentration produces a bland fermented beverage.

3.4.4 Overall

Table 4. shows that the level of panelists' liking for the overall fermented salak fruit juice drink resulted in an average ranking ranging from 4.82-5.72. The highest number of ranks is the most preferred fermented beverage by the panelists. The highest score was found in salak fruit juice with 9% sucrose concentration while the lowest ranking value was produced by salak fruit juice with 3% concentration. The treatment of adding 9% concentration resulted in the highest score due to the highest colour, taste, and aroma parameters. The overall parameter will be directly proportional to other parameters that have a high score, so this product will be favoured by panelists. Product acceptance is determined by a combined assessment of colour, texture, taste, and aroma. A high level of liking indicates that the product is accepted by panelists [37].

4. CONCLUSION

Sucrose concentration significantly interacted with microbiological, physicochemical, antioxidant activity, and organoleptic characteristics of salak fruit juice fermented beverage. The best treatment was obtained by fermented salak fruit juice with 9% sucrose addition which resulted in pH 3.80; total microbes 9.023 log CFU/ml; total acid 1.11%; total sugar 13.88%; viscosity 118.35 mPa.s; DPPH antioxidant activity 89.99%. The resulting organoleptic properties including colour, aroma, and taste, overall were respectively 3.40 (slightly like), 3.08 (slightly like), 3.76 (slightly like), and 3.48 (slightly like).

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