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# Characteristics of Guava Probiotic Drink on Various Fruit Type and Fermentation Time

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***ABSTRACT***

*Guava (Psidium guajava) is a fruit that may be used as raw material for probiotic drinks. Guava contains fructose, and glucose which are used by lactic acid bacteria during the fermentation process. Use guava as the basic ingredient of probiotic drinks can increase the selling value of guava. The production of guava probiotics involves the bacterium Lactobacillus casei FNCC 0090 as a probiotic agent. This study aimed to determine the effect of fermentation time and type of Psidium guajava fruit on the characteristics of probiotic drink from guava. This study used a Randomized Complete Block Design (RCBD) with a factorial pattern with two factors, factor I was the time of fermentation (20, 24, 28 hours) and factor II was the type of fruit (red guava, white guava, crystal guava). The data obtained were analyzed using ANOVA and if there was a significant difference between treatments, it was continued by further testing at 5% DMRT level. Crystal guava juice probiotic drink fermented for 28 hours was the best treatment, with the resulting physicochemical characteristics, namely pH 3.3; total BAL 9.09 log CFU/ml, total acid 0.89%, total sugar 5.38%, vitamin C content 152.24 mg. The resulting organoleptic properties include color, aroma, taste, overall 3.4 (slightly like), 3.65 (slightly like), 4.55 (like), 3.60 (slightly like).*

**Keywords***: Fermentation Time; Guava; Probiotic Drink*

## INTRODUCTION

The number of people with degenerative diseases in Indonesia has increased. Based on Basic Health Research (RISKEDAS) data, in 2018 it reached 5.137.637 people suffering from degenerative diseases. The main cause of this increase is meal patterns (Fridalni *et al*., 2019). This causes the importance of public awareness of the benefits of functional food. Functional food is food that contains active components that can provide health benefits, in addition to the benefits provided by the nutrients contained in it (Barrientos *et al*., 2016). One of the functional food products that are starting to be widely consumed today is probiotic drinks.

Probiotic drinks contain bacteria such as lactic acid bacteria, especially the genus *Lactobacillus* and *Bifidobacterium*, which can be beneficial for the alimentary canal because they can improve the balance of intestinal microflora. Fahrozi (2017) stated that guava juice which was fermented for 20 hours with a concentration of 1:3 juice, it was known that the total lactic acid bacteria value was 8.77 log CFU/ml and was chosen as the best treatment. This is in line with the results of Khotimah and Kusnadi (2013) research, the lower the dilution of probiotic fruit juice drinks, the total LAB will increase where lactic acid bacteria use sugar as an energy source and produce metabolites in the form of lactic acid during the fermentation process.

One of the media that can be used for probiotic beverage products is guava juice. Guava is one of the fruits that contain high protein, 4 g/100 g (USDA, 2022). In addition, according to the research results of Sanz et al., 2004 (Nurainy, 2018) that the types of sugar contained in *Psidium guajava* are fructose (2.74±0.26 g/100ml) and glucose (0.95±0.08 g/100ml).

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Carbohydrate contents of crystal guava reaching 24 g/100 g, while red guava 11.8 g and white guava 12.2 g (Ramayulis, 2013). Therefore, the processing of guava fruit is fermented with lactic acid bacteria where these bacteria utilize the amino acids and simple sugars contained in guava to grow and produce lactic acid. Guava has an antioxidant effect which is known to come from the active components contained in guava fruit, especially vitamin C. In connection with this, it is necessary to research on the effect of guava fruit type and duration of fermentation on the characteristics of probiotic drinks with a fruit and water ratio (1:3) good for health so that it is expected to be a fermented beverage product with a better taste and provide benefits which is multifunctional health effects, namely containing probiotics.

## METHODS

### *Material*

The ingredients used in probiotic drink were red guava, white guava, crystal guava, sucrose, FNCC 0090 (UGM). Chemicals used for analysis included MRS Broth (Merck), MRS Agar (Merck), NaOH (Merck), Phenolphtalein (Merck), amylum (Merck), iod (Merck), anthrone (Merck), glucose (Merck).

### *Tool*

The tools used in the processing of probiotic drink were laminar (Thermo Scientific, 1300 series A2), incubator (Memmerth), autoclave (Hirayama), juice extractor (Philips). The tools used for analysis included spectrophotometer (Genesys UV-VIS 10S), centrifuge, colony counter (Stuart Scientific), hot plate, micropipet, buret, pH meter (Mediatech), glassware (Iwaki).

### *Research Design*

The treatment in the study was two factors. First is type of *Psidium guajava* (A) with 3 levels, namely A1 = red guava, A2 = white guava, A3 = crystal guava, and the second factor is the time of fermentation (B) with 3 levels, namely B1 =20 hours, B2 = 24 hours, B3 = 28 hours.

### *Production of Guava Juice*

Guava fruit is washed and cleaned of dirt attached. Then peeled the fruit into cubes measured 2x1 cm and weighed. The guava fruit is mashed using a blender with the ratio of guava fruit: aquadest (1:3) for 3 minutes then filtered with a filter cloth.

### *Production of Probiotic Drink*

Guava juice was put into a sterile glass bottle, then pasteurized at 70 °C for 10 minutes. Guava juice was added with 5% sucrose (w/v), then homogenized and cooled to a temperature of 31 - 32 °C.Guava juice was inoculated with a starter containing *Lactobacillus casei* FNCC 0090 4% (v/v) culture at 37 °C.

### *Test Parameters*

Test was carried out to measure the quality of probiotic drink in this test include pH (Amalia, 2022), total lactic acid bacteria (LAB) (Amalia, 2022), total sugar content (Primurdia and Kusnadi, 2014), total lactic acid (Ulum, 2022), vitamin C (Yenrina, 2015), and organoleptic (Kartikasari and Nisa, 2014).

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### *Data Analysis*

This study used a Randomized Complete Block Design (RCBD). Significant differences between data were analyzed using ANOVA followed by Duncan's multiple distance test (DMRT) at 5%.

## RESULT AND DISCUSSIONS

### *Chemical Characteristics of Probiotic Drink*

The results in the chemical analysis of probiotic drink can be seen in Table 1.

Table 1. Chemical characteristics of probiotic drinks

| Sample | Ph | Sugar Content (%) | LAB (CFU/ml) | Lactic Acid (%) | Vitamin C Content (mg) |
|---|---|---|---|---|---|
| A1B1 | 3.85±0.07$^{ab}$ | 6.15±0.03$^{f}$ | 8.80±0.05$^{ab}$ | 0.55±0.02$^{b}$ | 99.44±1.25$^{b}$ |
| A1B2 | 3.75±0.07$^{b}$ | 5.91±0.03$^{c}$ | 8.96±0.03$^{b}$ | 0.68±0.01$^{de}$ | 109.12±1.49$^{c}$ |
| A1B3 | 3.35±0.07$^{f}$ | 5.63±0.05$^{ab}$ | 9.08±0.01$^{f}$ | 0.73±0.02$^{e}$ | 123.20±1.49$^{e}$ |
| A2B1 | 4.25±0.07$^{a}$ | 6.71±0.03$^{h}$ | 8.75±0.01$^{a}$ | 0.34±0.03$^{a}$ | 84.48±1.49$^{a}$ |
| A2B2 | 3.85±0.07$^{bc}$ | 6.34±0.02$^{cg}$ | 8.97±0.04$^{bc}$ | 0.48±0.06$^{ab}$ | 91.52±1.49$^{ab}$ |
| A2B3 | 3.55±0.07$^{c}$ | 6.08±0.02$^{e}$ | 9.01±0.03$^{c}$ | 0.61±0.06$^{c}$ | 102.08±1.99$^{bc}$ |
| A3B1 | 3.65±0.07$^{d}$ | 6,00±0.08$^{d}$ | 9.02±0.02$^{d}$ | 0.67±0.03$^{d}$ | 110.88±1.49$^{d}$ |
| A3B2 | 3.45±0.07$^{e}$ | 5.78±0.02$^{b}$ | 9.04±0.04$^{e}$ | 0.75±0.02$^{f}$ | 132,00±1.49$^{f}$ |
| A3B3 | 3.25±0.07$^{g}$ | 5.38±0.03$^{a}$ | 9.09±0.02$^{g}$ | 0.89±0.01$^{g}$ | 152.24±1.73$^{g}$ |

### *pH*

Based on Lee *et al.* research (2018) that fermentation of tomato juice fermented by *Lactobacillus casei* bacteria for 24, 32 and 48 hours obtained pH values of 2.27, 3.43, 3.11, respectively. The results in the analysis of variance showed that was interaction between factors. The pH value of guava juice fermented drinks with different time of fermentation and types of *Psidium guajava* showed an average ranged from 4.2 to 3.3. Based on the analysis of variance, it can be seen that there was a significant interaction (p≥0.05). There was a decrease in the amount of pH with increasing fermentation time because during the fermentation process, there is an accumulation of lactic acid as the main product of bacteria. This is supported by Umam *et al.,* (2012) In the fermentation process, lactic acid bacteria will produce some lactic acid and several other acids that can lower the pH value. Crystal guava and fermentation time of 28 hours produced the lowest pH value of 3.3.

### *Total Lactic Acid Bacteria*

The total value of lactic acid bacteria in guava juice fermented drinks with different time of fermentation and types of *Psidium guajava* showed that was a significant interaction (p≥0.05) between factors. Based on the Table 1, the total lactic acid bacteria ranged from 8.75 to 9.09 log CFU/ml. There was an increased in the amount of lactic acid bacteria with increasing fermentation time. Rizal *et al*. (2016) stated that probiotic fruit juice fermented with Lactobacillus casei produced pineapple juice fermented drink with a total LAB of 1.1x1010 log colonies/mL and a total acid of 3.45. Guava fruit is a source of prebiotics which is a carbohydrate in the form of glucose as a carbon source. Lactic acid bacteria utilized fructose and glucose in guava in probiotic drinks as a source of carbon and nitrogen for

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growth. The higher glucose and the duration of fermentation, the higher the total lactic acid bacteria. Crystal guava and fermentation time of 28 hours produced the higher total lactic acid. The nutritional composition, especially in crystal guava fruit which has a higher C source than red and white guava, is used by lactic acid bacteria to grow and reproduce.

***Total Sugar Content***

Based on the analysis of variance (Table 1), there was a significant interaction (p≥0.05). The total gula content of guava juice fermented drinks with different time of fermentation and types of *Psidium guajava* showed an average ranged from 5.38 to 6.71%. There was a decrease in total sugar content with increasing fermentation time. This is because the longer the fermentation time, the number of lactic acid bacteria increase and affects the total sugar that is converted into lactic acid as a metabolite of lactic acid bacteria. The analysis of total sugar content results has a pattern that is inversely proportional to the total acid value test results. The lower the sugar content, the higher the total lactic acid.

***Total Lactic Acid***

Total lactic acid in guava juice fermented drinks with different time of fermentation and different types of *Psidium guajava* showed that was a significant interaction (p≥0.05). White guava and 20 hours of fermentation, produced the lowest total acid which was 0.34%. While crystal guava and 28 hours of fermentation produced the highest total lactic acid which was 0.89%. After the fermentation process, the total value of lactic acid bacteria increased. This high total lactic acid value is due to the longer and optimal process of glucose reshuffling by lactic acid bacteria, so that the lactic acid produced is even higher. Increased activity of lactic acid bacteria in splits glucose will become lactic acid as the end product of metabolism. The pH value has a relationship with the amount of lactic acid. The higher the lactic acid produced,the lower the pH value.

***Vitamin C***

The results in the analysis of vitamin C levels in guava juice fermented drinks with different time of fermentation and different types of *Psidium guajava* showed that was a significant interaction (p≥0.05) between factors. White guava and 20 hours of fermentation, produced the lowest vitamin C level content of 84.48 mg. While crystal guava and 28 hours of fermentation produced the highest vitamin C level contentof 152.24 mg. This indicates an increase in vitamin C with increasing fermentation time. This is because during fermentation produces acidic compounds that can increase the stability of vitamin C. This is supported by the statement of Nurkhasanah (2013) that during the fermentation process, lactic acid bacteria will convert sugar into several important substances, including glucaric acid, acetic acid, lactic acid. Lactic and amino acids that will form acidic conditions, where these conditions will increase the stability of vitamin C.

More lactic acid bacteria will make the product more acidic and increase levels of vitamin C (Jamilah, 2019). Pranayanti (2015) stated that the longer the fermentation, the more organic acids, one of which was ascorbic acid caused by the hydrolysis of accumulated lactic acid. Guava contains ascorbic acid (vitamin C) which can affect antioxidant activity. The vitamin C content in crystal guava is relatively higher, reaching 228.3 mg /100 g (USDA, 2022) while in white guava the vitamin content is only slightly at 87 mg /100 g (Directorate of Nutrition, Ministry of Health RI, 2009). With the activity of lactic acid bacteria will stabilize the vitamin C contained in fruit juice.

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### *Organoleptic Characteristic of Probiotic Drink*

The results in the organoleptic analysis of probiotic drink can be seen in Table 2.

Table 2. Organoleptic characteristics of probiotic drinks

| Sampel | Color | Aroma | Taste | Overall |
|---|---|---|---|---|
| A1B1 | 2.70±0.73[a] | 3.10±0.72[a] | 3.60±0.68[a] | 2.80±0.89[a] |
| A1B2 | 2.55±1.09[a] | 3.15±0.93[a] | 4.10±0.72[a] | 2.90±0.85[a] |
| A1B3 | 2.80±1.15[a] | 3.25±1.21[a] | 4.15±0.75[b] | 2.95±0.89[a] |
| A2B1 | 2.85±0.88[a] | 2.90±0.83[a] | 2.95±0.68[b] | 2.50±0.76[a] |
| A2B2 | 3.15±0.88[a] | 3.05±1.04[a] | 3.15±0.72[c] | 3.00±0.97[a] |
| A2B3 | 3.20±0.83[a] | 3.20±0.88[a] | 3.75±0.75[c] | 3.35±0.75[b] |
| A3B1 | 3.20±1.06[a] | 3.45±0.85[a] | 4.35±0.59[d] | 3.40±0.59[c] |
| A3B2 | 3.30±0.92[b] | 3.60±1.15[a] | 4.50±0.61[d] | 3.50±0.76[d] |
| A3B3 | 3.40±0.75[c] | 3.65±1.24[c] | 4.55±0.51[e] | 3.60±0.60[e] |

The results showed that there was a significant interaction (p≥0.05) effect between different time of fermentation and different types of *Psidium guajava* on the color, aroma, taste and overall of guava juice fermented drinks. The panelist preference level obtained an average value of color between 2.55 - 3.4. The lowest preference level value was found in probiotic drink with 24 hours of fermentation time and types of red guava fruit with an average value of 2.55, which mean that they did not like neutral. The color of the probiotic drink with white guava juice and crystals produced a green color. Based on Fahrozi research (2017) that probiotic drink appeared green because guava contains chlorophyll pigment, chlorophyll is a green pigment in fruits and vegetables. The pigment dissolves at the time of extraction or making guava juice. While the color of the guava probiotic drink produces a bright red color. Lycopene is a carotenoid pigment that carries a red color and contains up to 5200 g/100gr of red guava fruit. The results showed that there was a different panelist preference level value of aroma and taste. The lowest preference value was found in probiotic drinks with 20 hours fermentation time and types of white guava fruit with an average value of 2.90 and 2.95 which means neutral.

While the highest preference level value of color, aroma and taste was found in probiotic drinks with 28 hours of fermentation time and types of crystal guava fruit somewhat like. This is supported by Fahrozi (2017) statement which states that the fermentation process occurs when organic acids are fermented, where the sour aroma is caused by the activity of bacteria. On average, the panelists prefer crystal guava juice drinks, where the product has a more sour taste. This is caused by the level of acidity of a product which is influenced by the total amount of acid produced, as well as the lower degree of acidity of lactic acid bacteria metabolism.

The panelists preference level value for the overall probiotic drink with the treatment of fermentation time and type of *Psidium guajava* obtained an average value between 2.80 - 3.60. The lowest preference value was found in probiotic drinks with 20 hours of fermentation time and types of red guava with an average value of 2.80, which means neutral. While the highest preference value is found in probiotic drinks with 28 hours of fermentation time and types of crystal guava with an average value of 3.60 which means

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somewhat like. Treatment with 28 hours of fermentation and types of crystal guava fruit produced the highest score. This is because in terms of taste, aroma and color parameters have a high score, so the overall parameters will be in line with other parameters that have high scores, and become a productwith a high level of preference by the panelists.

## CONCLUSION

The type of fruit and the duration of fermentation of probiotic drink had a significant interaction (p≥0.05) affect the physicochemical and organoleptic characteristic of probiotic drink. The optimal formula for guava probiotic drink treatment A3B3 (crystal guava, fermented 28 hours) became the selected treatment. This treatment had a pH of 3.3; total BAL 9.09 log CFU/ml, total sugar 5.38%, total acid 0.89%, vitamin C content 152.24 mg. The resulting organoleptic include color 3.4 (slightly like), aroma 3.65 (slightly like), taste 4.55 (like), overall 3.60 (slightly like).

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