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Effect of Red Guava (*Psidium guajava L.*) Juice Concentration on Product Quality and Antioxidant Activity of Red Guava Synbiotic Yoghurt

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Abstract: Red guava juice has very strong antioxidant activity and has the potential to be processed into synbiotic functional beverage products that have health benefits. This study examined the effect of red guava juice concentration on product quality and antioxidant activity of red guava synbiotic yoghurt. Product quality tested included microbiological quality (total LAB) and chemical quality (pH value and Total Tritable Acid (TTA)value). The concentrations of red guava juice used were 0%, 10%, 20%, 30%, and 40%. Fermentation was carried out for 6 hours at 37ºC by Lactic Acid Bacteria (LAB) from powdered mixed culture. Then organoleptic testing using the hedonic test, total LAB testing using the Total Plate Count (TPC) method, pH value testing using a pH meter, TTA value testing using the acid-base titration method, and antioxidant activity testing using the DPPH (2,2-diphenyl-1-picrylhydrazil) method. Test data were analyzed using SPSS software to determine the effect of red guava juice concentration on test results. The concentration of red guava juice in red guava synbiotic yoghurt affects the pH value, TTA value, and IC_{50} value as the result of antioxidant activity testing. Red guava juice concentration did not affect total LAB testing. Organoleptic assessment of red guava symbiotic yoghurt is in the range of like to like very much. The best formulation was red guava synbiotic yoghurt with 30% red guava juice concentration, which had strong antioxidant activity with an IC_{50} value of 59.637 ppm, pH value of 3.94, TTA value of 1.98%, and total LAB as much as 1.81×10^8 colonies/g, so it has met the quality requirements of yoghurt according to Indonesian National Standard SNI 2981: 2009.

Keywords: Antioxidant Activity; Red Guava Juice; Synbiotic; Yoghurt; LAB.

Introduction

Red guava (*Psidium guajava L.*) is a fruit commodity often found and cultivated in Indonesia. Red guava contains bioactive compounds that function as food fiber and prebiotics. Red guava contains fructo-oligosaccharides (FOS) that can act as prebiotics, which are food ingredients and can increase the survival and activity of probiotic bacteria. Non-starch polysaccharide fibers such as pectin, hemicellulose, and cellulose are prebiotics [1]. Red guava is known to have high levels of antioxidants, including lycopene, vitamin C, and phenols [2].

Phenol is an antioxidant known for treating diabetes mellitus because it can reduce glucose levels by inhibiting the α-glucosidase enzyme. One of the phenol group antioxidant compounds in red guava is flavonoid [3]. Flavonoids in red guava still have glycoside bonds, so their function as antioxidants is not maximized [4]. One of the easy ways to break glycoside bonds is through fermentation. In fermentation, Lactic Acid Bacteria (LAB) produce enzymes that can degrade glycoside bonds. Free antioxidant compounds will inhibit the activity of the α-glucosidase enzyme and reduce blood sugar in people with diabetes mellitus [5].

A fermented product often consumed by the public is yoghurt. Yoghurt has a higher nutritional value than fresh cow's milk as the solids in yoghurt increase. UHT milk yoghurt has moderate antioxidant activity with an IC_{50} value of 105,708 ppm [6]. Yoghurt is considered practical for consumption as a healthy food with a distinctive taste. The yoghurt flavour comes from organic acids formed during fermentation [7].

Yoghurt-making development has expanded to include functional foods, such as synbiotic yoghurt. Synbiotic yoghurt is a yoghurt product that combines probiotics and prebiotics. Probiotics can benefit their host (digestive tract) in specific amounts. Probiotic growth in the body tends to be slow, so it can reduce the performance of the digestive tract. Probiotic growth can be supported by prebiotics, compounds that cannot be digested by humans but can become food for microorganisms (probiotics) that live in the host so that they can affect the growth of these microorganisms [8]. The prebiotic content in red guava can be utilized to make synbiotic yoghurt to support the activity of probiotic bacteria. The use of red guava in yoghurt products can also increase antioxidant activity. Synbiotic yoghurt, as a functional food, has a good effect on people with certain infectious disorders such as diarrhea and colitis, and the latest case is irritable bowel syndrome [9]. Synbiotic components are more effective than probiotic components in improving intestinal disorders that lead to obesity. This is because synbiotics can support the growth and survival of the positive bacteria in them [10].

As a food product, yoghurt has a standard from the Indonesian National Standardization Agency, namely in SNI 2981: 2009, with several indicators that must be met to make the product safe for consumption [11]. This study will examine the effect of red guava juice concentration on the leading indicators of SNI yoghurt, namely chemical quality

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(pH and Total Titratable Acid), microbiological quality (total LAB), and organoleptic quality (aroma, taste, and color) according to the standard. This study will also examine the effect of red guava juice concentration on the antioxidant activity of red guava synbiotic yoghurt.

Research Methods

Tools and Materials

The tools to be used are analytical balance sheet (Denver S1-234), laminar airflow (Thermo Scientific 1300-series A2), autoclave (Hirayama HVE-50), incubator (Memmert), shaker (D-Lab SK 220 Pro), water bath (Labtech LWB 122D), UV-Vis spectrophotometer (Shimadzu UV-1700), spray bottle, spirtus burner, lighter, erlenmayer, test tubes, measuring cups, pH meter, sterile glass jars, glass beakers, knives, juicers, cutting boards, burettes, statives, clamps, funnels, volumetric flasks, pipettes, stirring rod, volume pipette, plastic cup, petri dish, and micropipette.

The materials needed are guava, sugar, yoghurt starter powder, liquid skim milk, MRS-A, distilled water/aqua dest, methanol for analysis, DPPH (2,2-diphenyl-1-picrylhydrazyl), alcohol 96%, CaCO3, NaCl 0.85%, NaOH, oxalic acid, and phenolphthalein indicator.

Research Procedure

Red Guava Juice Making

The red guavas were cleaned, peeled, and cut to reduce size. The cut red guava was mashed with a juicer and filtered. Then, it was pasteurized to 70^{o}C for 15 minutes and cooled to 42^{o}C [12].

Yoghurt Making

500 mL liquid skim milk was pasteurized at 63^{o}C for 30 minutes [13]. The milk was cooled to a temperature of 40-45^{o}C. Pasteurized milk was added, and red guava juice was added with variations in the concentration of red guava juice: 0%, 10%, 20%, 30%, and 40% (v/v). Each sample was added with 7 grams of sugar and labelled. Starter powder was added as much as 0,5 grams with a population of more than 10^7 colonies/g on the sample and incubated at 37^{o}C for 6 hours. Samples without adding guava juice were used as a control (0% concentration).

Organoleptic Test

Organoleptic quality testing was carried out using a questionnaire with the hedonic test. The hedonic test determined the panellists' favourite response to more specific properties, such as guava synbiotic yoghurt's colour, aroma, and taste. The test was conducted on 15 untrained panellists.

Total Lactic Acid Bacteria (LAB)

Total LAB was calculated using deMan Rogosa Sharpe (MRS) culture medium. The MRS-agar medium was prepared by dissolving 13,4 grams of MRS-A into 200 mL of NaCl 0,85% and adding 1% $CaCO_3$. Samples were diluted using NaCl 0,85% solution to 10^1-10^7 dilution. Samples at dilution 10^6-10^7 were taken using a micropipette, and as much as 1 mL of dilution resulted in a petri dish (shading process). The media is made triplo. The MRS-agar medium was poured into the cup as much as ±15 mL. Petri dishes are immediately moved following Figure eight so that the inoculated bacteria are evenly distributed and then allowed to solidify. The media in the Petri dish was incubated upside down at 37^{o}C for 48 hours. The number of LAB was counted using the Total Plate Count (TPC) method. All treatments were done aseptically. Colonies that grew were counted between 25-250 colonies. Calculation of the number of bacteria using units of colonies/g with the following formula:

$$\text{Total LAB (colonies/g)} = \text{n} \times \text{F}$$

Description:
n: Number of colonies
F: dilution factor

Acidity (pH)

The pH value is measured using a pH meter calibrated at pH 4 and 7. The electrode on the pH meter is inserted in the sample, and the number on the tool is waited until it does not shift.

Total Titrable Acid (TTA)

The Total Titratable Acid (TAT) test in this study was used to measure the percentage of lactic acid using the titration method. 10 mL of the sample was put in a 100 mL volumetric flask, and distilled water was added until the limit mark was reached for dilution, and then the sample was homogenized. 25 mL of the diluted sample was placed in an Erlenmeyer, and 2-3 drops of 1% phenolphthalein indicator were added and then titrated by a standardized 0,1 N NaOH solution until pink. Titration is done in triplo. The calculation of the amount of titrated acid according to SNI is as follows:

$$\text{TTA (\%)} = \frac{\text{V} \times \text{N} \times 90 \times \text{fp}}{\text{W} \times 1000} \times 100\%$$

Description:
V: volume of NaOH solution for titration (mL)
N: normality of NaOH
90: lactic acid equivalent weight
fp: dilution factor
W: sample weight

Antioxidant Activity Test

Preparation of DPPH solution 40 ppm

DPPH powder weighed as much as 4 mg and was then dissolved in 100 mL of methanol to obtain a 40 ppm DPPH solution.

Determination of DPPH maximum wavelength

2 mL of methanol was put in a centrifuge tube, and then 1 mL of 40 ppm DPPH solution was added. After that, the mixture was vortexed for 1 minute to mix homogeneously and kept for 30 minutes in a dark place. Then, the absorbance was measured with a UV-Vis spectrophotometer in the wavelength range of 400-700 nm to obtain the maximum wavelength for antioxidant testing.

Preparation of DPPH blank

2 mL of methanol was put in a centrifuge tube, and 1 mL of 40 ppm DPPH solution was added. After that, the mixture was vortexed for 1 minute and kept for 30 minutes in a dark place at 27^{o}C until the color change of DPPH activity occurred. Then, the absorbance was measured triple

with a UV-Vis spectrophotometer at the maximum wavelength that had been obtained.

Measurement of antioxidant potential of samples

10 mg of the sample was dissolved in 10 ml of methanol to obtain 1000 ppm of mother liquor. Then, dilutions of the mother liquor were made with concentration variations of 50, 100, 150, 200, and 250 ppm. 2 mL of sample dilution was put in a centrifuge tube, and then 1 mL of 40 ppm DPPH solution was added. Then, it was vortexed for 1 minute and incubated for 30 minutes in a dark place at 27^{o}C until there was a color change from DPPH activity. Samples incubated are tested for absorbance values using a UV-Vis spectrophotometer at the wavelength obtained. The measurement was done in triplo.

Calculation

$$\%\ \text{Inhibition} = \frac{A_c - A}{A_c} \times 100\%$$

Description:
A_c: Absorbance value of blank
A: Absorbance value of sample

The results of the calculations were made into a linear regression curve. The IC_{50} value can be known by the regression equation obtained. A compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50, said to be strong if the value is 50-100, said to be moderate if the value is 100-150, and said to be weak if the value is 151-200. The smaller the IC_{50} value, the greater the antioxidant activity.

Data Analysis Technique

This research data was processed using the IBM Statistics SPSS 29 program. Product quality testing and antioxidant activity data were processed using the One Way Anova Test, followed by the LSD Post Hoc Test on normally distributed data. Data that was not normally distributed was processed using the Kruskall Wallis Test, followed by the Post Hoc Mann Whitney Test. The data obtained from the organoleptic test was processed using the mode and median test.

Results and Discussion

Effect of Red Guava Juice Concentration on Organoleptic

Organoleptic testing aims to determine the effect of red guava juice concentration on panelists' liking of red guava synbiotic yoghurt products using human senses. Fifteen panelists assessed the aroma, taste, and color of red guava synbiotic yoghurt at each concentration. Panelists were given a questionnaire containing an assessment on a scale of 1 to 4, with a value of 1 for a very dislike assessment, a value of 2 for a dislike assessment, a value of 3 for a like assessment, and a value of 4 for a very like assessment.

Organoleptic test data are ordinal, so conclusions are drawn using the mode method. However, the median method is used if the mode is not found.

Table 1. Effect of Red Guava Juice Concentration on Organoleptic of Red Guava Synbiotic Yoghurt

No	Concentration of The Sample (%)	Aroma	Taste	Color
1	0	3	3	3
2	10	3	3	3
3	20	3	4	4
4	30	4	4	3
5	40	4	4	4

Adding guava juice concentration increases the organoleptic assessment of aroma and taste. The data in Table 1 on the aroma organoleptic results show that adding red guava juice concentration of 0%-20% was rated as liked with a score of 3. The addition of red guava juice concentration of 30%-40% was rated as very liked, with a score of 4. The aroma produced in fermented products involves LAB activity during fermentation, producing organic acids to form a distinctive sour aroma.

In line with this, in the organoleptic results of taste, the addition of red guava juice concentration of 0%-10% received a rating of 3 with the category of liking. In contrast, a concentration of 20%-40% received a rating of 4 with the category of very liking. Adding sugar in the fermentation process can add flavor to the product. In addition, LAB uses sugar for growth. LAB growth is also influenced by prebiotics contained in red guava. LAB will produce lactic acid during fermentation and a sour taste in the product. Apart from lactic acid, the sour taste of the product can also be caused by red guava juice. As the concentration of red guava juice increases, the product has an increasingly sour taste and is preferred by panelists.

On the other hand, the color organoleptic results showed that the 0%-10% concentration received a like rating and increased to very like at a concentration of 20%. At 30% concentration, the assessment decreased from very like to like, and at 40% concentration, the color organoleptic assessment increased to very like. The resulting pink color comes from the added red guava juice. The greater the concentration of red guava juice, the more intense the color produced.

Effect of Red Guava Juice Concentration on Total LAB

Total LAB testing aims to determine the effect of red guava juice concentration on total lactic acid bacteria in red guava synbiotic yoghurt. LAB growth is characterized by forming a clear zone around the colonies that grow in Petri dishes. The clear zone results from the reaction between lactic acid derived from LAB binding $CaCO_3$, an additional ingredient in making media. The addition of $CaCO_3$ to the media serves as an indicator that a bacterial colony can produce acid [14].

Figure 1. LAB Colonies on Agar Media

Figure 1 shows that LAB colonies can produce a clear zone on agar media. Agar media in this study was made by mixing de Mann Rogose and Sharpe (MRS) agar and $CaCO_3$ with NaCl 0,85%. The reaction between lactic acid and $CaCO_3$ produces calcium lactate with the response:

$$2C_3H_6O_3 + CaCO_3 \rightarrow Ca(C_3H_5O_3)_2 + H_2O + CO_2$$

Table 2. Effect of Red Guava Juice Concentration on Total LAB of Red Guava Synbiotic Yoghurt

No	Concentration of The Sample (%)	Total LAB (colonies/g)
1	0	$0.427x10^8$
2	10	$3.81x10^8$
3	20	$2.49x10^8$
4	30	$1.81x10^8$
5	40	$0.773x10^8$

Description: Values followed by the same letter indicate no significant difference (α > 0.05).

The data obtained was not normally distributed, so the data analysis used the Kruskal Wallis test to see the effect of red guava juice concentration on the total LAB produced. This analysis showed that red guava juice concentration did not affect the total LAB of red guava synbiotic yoghurt, with a significance value of> 0,05. However, from Table 2, it is known that the addition of red guava juice can increase the total LAB by 1 log cycle, which is seen from 0% red guava juice concentration of $4{,}27x10^7$ colonies/g to 10% of $3{,}81x10^8$ colonies/g. The best LAB growth was obtained by adding red guava juice with a concentration of 10%, which produced a total LAB of 3,81x108 colonies/g. The total LAB continued to decrease as the concentration of red guava juice increased.

The results of research on microbiological testing showed that yoghurt with the addition of red guava juice had more LAB than yoghurt without the addition of red guava juice or control. This is because red guava contains FOS, which acts as a prebiotic that can increase the growth of probiotics [15]. LAB is a probiotic whose development can be supported by adding prebiotics [16]. LAB growth can also be influenced by granulated sugar added to yoghurt. Granulated sugar is a carbon source in fermentation media, where carbon is one of the compounds LAB needs to grow.

However, LAB growth decreased in line with the addition of red guava juice, from $3{,}81x10^8$ colonies/g to $7{,}23x10^7$ colonies/g. This can occur due to the composition of yoghurt, where the more the concentration of red guava juice increases, the less skim milk is added. Skim milk, as a source of lactose, has a positive effect on the growth of specific lactic acid bacteria [17]. In addition, the use of mixed cultures can affect the decrease in LAB growth. Research by Kasmiyetti et al. (2022) stated that the reduction in LAB in yoghurt fermented using mixed cultures was due to competition between bacteria and the presence of different compounds produced that could inhibit LAB growth [18].

Effect of Red Guava Juice Concentration on pH Value and TTA

Testing the pH and TTA values aims to determine the effect of red guava juice concentration on the pH (acidity) and TTA values of red guava synbiotic yoghurt. TTA testing uses the acid-base titration method to determine the total amount of acid in the sample.

Table 3 value will decrease, and the TTA value will increase [19]. In addition, LAB will convert lactose in milk to lactic acid at the end of fermentation. The increasing amount of lactic acid will be followed by adding hydrogen ions to decrease the pH value and increase the total acid. The decrease in pH value is based on the activity of LAB and the presence of H^+ ions that indicate acids that have dissociated [20].

Table 3. Comparison of Quality Test Result of Red Guava Sinbiotic Yoghurt with Yoghurt Quality Requirements Based on SNI 2981:2009

Test Criteria	Unit	SNI 2981:2009	Result
Display	-	Thick-Solid Liquid	Thick-Solid Liquid
Aroma	-	Normal/typical	Normal
Taste	-	Sour/typical	Sour
TTA	%	0.5-2.0	1.617-2.103
pH	-	3.8-4.5	3.91-4.07
Bacteria	colonies/g	Minimal 10^7	10^7-10^8

Based on Table 4, the results of total LAB testing and pH value at 0%-40% red guava juice concentration have met the requirements of SNI 2981: 2009, but the results of TAT testing at 40% concentration do not meet the quality requirements of yoghurt.

Effect of Red Guava Juice Concentration on IC_{50} Value

Antioxidant activity testing aims to determine the effect of red guava juice concentration on the antioxidant activity of red guava synbiotic yoghurt. Antioxidant activity can be known from the ability of antioxidant compounds in the product to capture DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals measured using a UV-Vis spectrophotometer instrument. The absorbance value produced in the test will be processed into a practical concentration value or IC_{50} value.

Table 4. Effect of Red Guava Juice Concentration on IC50 Value of Red Guava Synbiotic Yoghurt

No	Concentration of The Sample (%)	IC_{50} Value (ppm)
1	0	106.935^a
2	10	93.784^b
3	20	78.454^c
4	30	59.627^d
5	40	42.139^e

Description: Values followed by the same letter indicate no significant difference (α > 0.05)

The data obtained showed data that were not normally distributed, so data analysis was carried out with the Kruskal Wallis test to determine the effect of red guava juice concentration on the antioxidant activity of red guava synbiotic yoghurt. The data analysis showed that the red guava juice concentration influenced the antioxidant activity of red guava synbiotic yoghurt with a significance value <

0.05. Furthermore, the data were tested using the post hoc Mann-Whitney Test to see the real difference in each sample. The data in Table 4 show significant differences in each concentration from 0% to 40%. The addition of red guava juice concentration affects the decrease in IC_{50} value in red guava synbiotic yoghurt from 106.935 ppm to 42.139 ppm.

The decrease in IC_{50} value defines that antioxidant activity has increased, where the smaller the IC_{50} value, the greater the antioxidant activity. Antioxidant activity is said to be very strong if the IC_{50} value is less than 50, strong (50-100), moderate (100-150), and weak (151-200). The smaller the IC_{50} value, the higher the antioxidant activity [21]. The increase in antioxidant activity in yoghurt is influenced by adding red guava juice, which has high antioxidant activity. Red guava juice has a very high category of antioxidant activity with an IC_{50} value of 11.96 ppm [22]. Antioxidant compounds in guava fruit include vitamin C, lycopene, and phenols. Apart from red guava, the increase in antioxidant activity is also influenced by the fermentation process. Enzymes produced by LAB in the fermentation process will degrade glycoside bonds and leave antioxidant compounds [23].

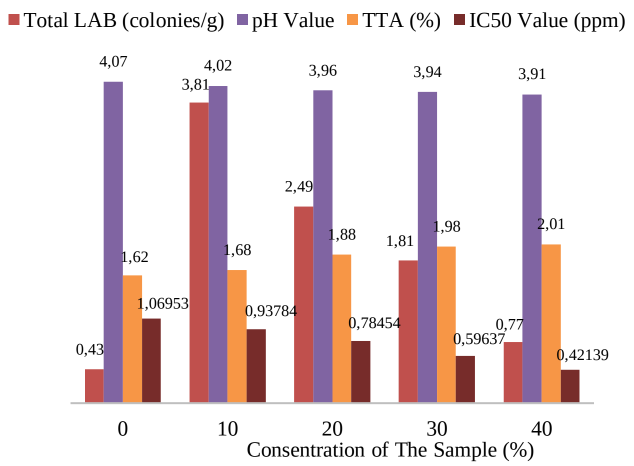

Figure 2. Graph of the Relationship between Red Guava Juice Concentration and Total LAB, pH value, TAT value, and IC_{50} value

Conclusion

Based on the results of research and discussion, it can be concluded that The addition of red guava juice concentration did not affect the total LAB but the pH value, TAT value, and IC_{50} value. The yoghurt produced has met the product quality in total LAB and pH values of>10^7 colonies/g and 3.91-4.07, respectively. The TAT value met the quality requirements at sample concentrations of 0%-30% but did not meet the 40% concentration because it had a TAT value > 2.0%. Meanwhile, antioxidant activity increased with the addition of red guava juice concentration. The red guava synbiotic yoghurt with the best formulation is the yoghurt added with 30% red guava juice, which has strong antioxidant activity, meets the quality requirements of yoghurt, and has quite favorable organoleptic values.

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