Antioxidant Analysis in Drinks of Guava, Lemon, Melon, and Beet Juice Fermented Using Lactobacillus Plantarum

Afrinia Eka Sari[1], Reza Anindita[2] and Wening Cahyani[1]
[1]*Department of Nutrition STIKes Mitra keluarga, Bekasi, Indonesia*
[2]*Department of Pharmacy STIKes MItra Keluarga, Bekasi, Indonesia*

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Abstract: Antioxidants are active substances that can counteract free radicals, where free radicals can trigger infectious and non-communicable diseases. Antioxidants are found in many vegetables and fruits. Jalembi (Jambu, Lemon, Melon, Bit) drink is an alternative source of antioxidants that can counteract free radicals. Guavas, lemons, melons, beets are fruits that contain vitamin C, which is a substance that acts as an antioxidant. The purpose of this research to determine the antioxidant contain of fermented Jalembi drink using Lactobacillus Plantarum. The reason for using lactobacillus plantarum in this research is because lactic acid bacteria are commonly used in the fermentation process and also have the advantage of being resistant to acidic pH and several studies have stated that these bacteria can survive in the digestive tract. The research design is an experimental study with a completely randomized design. The three formulas differ primarily in sugar content, with formula 1 containing 10% sugar, formula 2 containing 20%, and formula 3 containing 30%. The results of the analysis of antioxidant activity in the three formulas were very weak or there was no antioxidant activity. In terms of toxicity analysis, jalembi probiotic drink is safe for consumption and the average panelist acceptance results of the three formulas are quite favorable. Suggestions for further research should test the antioxidant contain without bacteria fermentation process.

1 INTRODUCTION

Basic Riset Kesehatan Dasar (Riskesdas) 2018 shows 95.5% of Indonesian people consume less vegetables and fruit. The average consumption of the Indonesian people is 81.14 grams/capita/day (BPS, 2021). However, this amount is only 54.09% of the minimum nutritional adequacy rate. Fruits and vegetables not only contain vitamins, minerals and fiber but also contain antioxidants.

Antioxidants have a function to ward off free radicals. Free radicals are defined as molecules containing one or more electrons which are highly reactive and can cause cell damage or death (aging). Free radicals can also trigger non-communicable diseases such as hypertension, stroke, obesity and diabetes mellitus. As a result of continuous exposure to free radicals and low intake of vitamin C and vitamin E can lead to depletion of natural antioxidant production in the body, causing various diseases. Physical activity with low and moderate intensity is needed to increase the antioxidant system because it maximizes free radical removal (Sinaga, 2018).

Antioxidants are compounds that are able to counteract or reduce the negative effects of oxidants in the body, working by donating an electron to compounds that are oxidants so that the activity of these oxidant. This research aimed to determine the antioxidant in Jalembi drinks ousing red guava, lemon, melon, and beet juice and *L. plantarum starter.*

2 MATERIALS AND METHOD

The material ingredients used in the development of Jalembi drinks are guava fruit, lemon, melon, beetroot extract, water, guava flavor, granulated sugar, and *Lactobacillus plantarum* compounds can be inhibited (Wulan, 2019). The fruit used are fruit that have sufficient level of ripeness.

Factors that can affect antioxidant activity in a food include pH, temperature, light, and adding sugar. Factors that can affect antioxidant activity in a food include pH, temperature, light, and adding sugar. Based on research conducted by Fidyasari *et al.*

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(2019), lactic acid bacteria in probiotic drinks can provide antioxidant activity. Fermented drinks can also increase flavonoid compounds as a source of antioxidants.

One of the bacteria used in this study is *Lactobacillus plantarum*. *Lactobacillus plantarum* is a lactic acid bacterium with the potential as a biopreservative because it can inhibit the growth of pathogenic and destructive bacteria. *Lactobacillus plantarum* can inhibit the growth of pathogenic bacteria with the most significant inhibition compared to other lactic acid bacteria (Hariati, 2019).

The tools used in the development of probiotic drinks are knives, cutting boards, measuring cups, blenders, filters, pans, stoves, spoons, digital scales, basins, glass bottles, pH meters, autoclaves for bottle sterilization, and incubator for incubation. The type of research design used in this research is an experimental method using Completely Randomized Design (CRD).

The analysis included hedonic tests and antioxidant tests. The research was conducted from January to March 2023 in STIKes Mitra Keluarga,using thirty people panelist.

Sample Preparation. First, wash all the fruit, then peel it. Then, weigh all the ingredients according to the formula, crush it with a blender, strain it until there is no pulp, and heat it at 70 degrees Celsius for 10 minutes. The sample that has been heated is cooled and transferred into a sterilized bottle. Then, add 1 ml of Lactobacillus Plantarum bacteria starter. There were three formulas used in this study with a comparison of guava: lemon, melon, beets, flavors, bacterial starter (40:10:27:3:4:1). The variations lie in the amount of added sugar: formula 1 includes 10 grams, formula 2 include 20 grams, and formula 3 includes 30 grams. Before the incubation process is carried out, sterilization of equipment and containers must be ensured, and sterilization is carried out at a temperature of 121 degrees Celsius.

The heated sample is cooled and transferred to a sterilized glass bottle, followed by the addition of 1ml of Lactobacillus Plantarum bacteria starter. Incubate at 37 degrees Celcius for 24 hours..

Organoleptic test results data were analyzed using a non-parametric statistical method, namely the Friedman test. Friedman's test is used to analyze the comparison of three or more averages in dependent samples (paired) where the assumption of normality is not met. In this study, the Friedman test was used to determine the difference between the control group and the experimental group. In this study, the Wilcoxon test was also used to compare the result of organoleptic and hedonic test without having to see whether the data was normally distributed.

3 RESULT AND DISCUSSION

Based on the result of measuring the acidity level, the following result were obtained.

Table 1: pH level of Jalembi drink.

Treatment	pH	Criteria
Formula 1	3,43	Acid
Formula 2	3.39.	Acid
Formula 3	3.32	Acid

Source: Primary date,2023

The three formulas have a fairly high level of acidity which is caused by lactic acid produced from the fermentation process.

The results of the hedonic test for the three formulas did not show significant differences: however, whwn taste preferences were examined. Formula 3 emerged as the most preferred. The following below is a table of hedonic test results.

Table 2: Hedonic test.

	Indicator				Criteria
Treatment	Colour	Odor	Taste	Viscosity	
Formula 1	3.85	3.65	3.30	3.50	Like
Formula 2	3.40	3.53	3.93	3.45	Like
Formula 3	3.95	3.53	4.03	3.58	Like

Source: Primary date,2023

The hedonic test determines panelists' preferences based on indicators of color, aroma, taste, and viscosity (viscosity). The hedonic test scale for probiotic beverage products starts from numbers 1-5. Description of the scale on the level of preference or hedonic.

Data is said to be abnormal if it has $p < 0.05$. If $p < 0.05$, there is a significant difference, and from that, the data analysis is continued with the Wilcoxon test. If the Wilcoxon test is $p < 0.05$, each formula has significant differences. Test, namely value (1) means dislike, value (2) means dislike, value (3) means quite like, value (4) means like, and value (5) means really like. Based on the color criteria, the color contained in the jalembi probiotic drink comes from the betalain compound in beetroot extract, which produces an attractive red or purple color, making it a natural dye. This is by the opinion of Pramesti (2019), which states that determining the quality of food ingredients is generally influenced by several

factors, including taste, texture, and nutritional value Based on the taste criteria, the probiotic drink tastes sour and sweet.

The sour taste is obtained from the fermentation of LAB Lactobacillus plantarum, while the sweet taste is obtained from adding sugar in each sample. The probiotic drink sample that the panelists liked the most in formula 3 was due to the addition of 30% sugar, resulting in a sweet taste that was more attractive to consumers, while the product that was less liked by the panelists was in the control treatment, namely products without the addition of sugar so that the resulting taste was sour. The results of the panelist's preference analysis on the viscosity parameter (viscosity) in formula 1 have a value of 3.50; formula 2 has a value of 3.45; formula 3 has a value of 3.58 and the control treatment has a value of 3.15, where the four formulas fall into the like category. The aroma of this probiotic drink is obtained from the aroma of guava fruit, which is more dominant because it adds guava flavor so that the resulting aroma is sweet and fragrant. Aroma is a determinant of the quality of a food or beverage product. The sense of smell can feel a distinctive aroma depending on the ingredients added to the drink (Feriady, 2018)

Antioxidant activity test using the spectrophotometric DPPH method. The results of the analysis of the antioxidant activity test in each sample can be seen in the table below.

Table 3: Antioxidant.

Sample	Antioxidant activity	Criteria
Formula 1	3586.23 ppm	No antioxidant activity
Formula 2	2178.48 ppm	No antioxidant activity
Formula 3	3785.20 ppm	No antioxidant activity

The results of the analysis on the antioxidant activity of the probiotic drink (guava, lemon, melon, beet) showed that the highest level of antioxidant activity was in the treatment of formula 2 (20 grams of added sugar) with an antioxidant activity level of 2178.48 ppm. The lowest result was found in formula 3 (30 grams of added sugar) with an antioxidant activity level of 3785.29 ppm, which, according to theory, means that the smaller the IC50 value, the higher the antioxidant activity. (Wulansari, 2018).

The principle of the antioxidant activity test method is to measure the scavenging of DPPH radicals by a compound that has antioxidant activity using UV-Vis spectrophotometry so that the value of free radical scavenging activity will be known, which is expressed by the IC50 value. IC50, namely the amount of inhibitory concentration of the test solution on its ability to reduce free radical activity by 50% (Wulansari, 2018).

The study's results on jalembi probiotic drinks found that the antioxidants in the three formulas showed weak activity or did not have antioxidant activity. As for what inhibits the activity of antioxidants in the product, include adding sugar. This by glycosylation reactions, where sugar interacts with antioxidant compounds. This reaction produces glycosylation products that reduce the availability of hydrogen atoms (H) and methyl groups in antioxidant compounds. H atoms and methyl groups play an important role in antioxidant activity, especially as hydrogen donors to unstable free radicals. This loss of H atoms reduces the ability of antioxidant compounds to act as hydrogen donors against free radicals.

As a consequence, antioxidant activity decreases due to the lack of ability of the compounds to capture and neutralize free radicals. (Pelealu, 2019). Another factor that affects antioxidant activity is the length of testing after incubation. After 2-4 days of incubation, the antioxidant activity increases, then on the fifth day, there will be a drastic decrease (Putro H, 2020).

Apart from the influence of the glycosylation reaction, the decrease in levels of antioxidant activity can be caused by the heating process. The ingredients used in the manufacture of this probiotic drink product are ingredients that contain vitamin C. It is known that vitamin C is a substance that can act as an antioxidant. Vitamin C in the probiotic drink tends to be more susceptible to damage at high temperatures.Vitamin C can experience degradation and lose its antioxidant activity when exposed to temperatures of 70-90 ºC or during a long heating process

Based on data analysis using the Friedman test, the results for the aroma and viscosity indicators showed no significant difference because the p-value was > 0.05. The aroma indicator has a p-value of 0.362 and the viscosity indicator has a p-value of 0.378. As for the color and taste indicators, there is a significant difference because they have a p-value <0.05. Where the color and taste indicators have a p-value of 0.0001, this shows that adding sugar affects the color and taste of the probiotic drink.

4 CONCLUSION

Organoleptically, based on taste, color, aroma, and viscosity, the probiotic drink is quite preferred and has the potential as a health drink as a source of probiotics. Further research is needed to determine the factors that cause a decrease in antioxidant activity in probiotic drinks.

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