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Effect of stevia sugar on innovation of guava, apple, lemon, and melon juice probiotic drink using *Lactobacillus casei* bacteria on amount Lactic acid bacteria

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Abstract

As time goes by, more and more attention is paid to functional drinks. Probiotic cultures provide several health benefits, namely maintaining a healthy gut microbiota, protection against gastrointestinal pathogens, boosting the immune system, reducing cholesterol levels, reducing blood pressure, and anticancer activity. One of the probiotic drinks to be tested in this study was made from guava, apple, lemon, and melon juice This research is a quantitative study with a laboratory experimental research design. In this study, *Lactobacillus casei* was used as a starter. The test material used was juice added with *L. casei* starter and stevia sugar with concentrations of 10%, 20%, and 30%. The average value of the total BAL of the Jamale juice probiotic drink without added sugar was 1,80 x 107 CFU/mL, added sugar 10% was 4,83 x 107 CFU/mL, added sugar was 20 % was 4,91 x 107 CFU/mL and the addition of 30% sugar was 6,05 x 107 CFU/mL. Jamale juice probiotic drink with 30% added sugar resulted in the best condition with a total number of lactic acid bacteria of 6,05 x 107 CFU/mL.

Keywords: *Lactobacillus casei;* pH; temperature; total lactic acid bacteria; zone of inhibition

Introduction

Functional drinks are receiving more and more attention as time goes by, mainly because people are becoming more interested in consuming a drink that has health benefits, including probiotic drinks. The ability to maintain a healthy gut microbiota, protection against gastrointestinal pathogens, immune system enhancement, cholesterol reduction and Blood pressure, and anticancer activity are some of the benefits of the drink probiotics (Dewi *et al*., 2021). Currently, there is much focus on adding probiotics to non-dairy drinks because few people choose to avoid the product milk due to lactose intolerance, milk allergy, or just a fundamental dislike. Required product innovations can open opportunities to combine the benefits of culture probiotics with bioactive molecules.

One of the benefits of probiotic drinks is that they are beneficial for health. Probiotic drinks have a very sour taste and intense aroma, so many people are not interested (Hidayah, 2019). Study this aims to overcome this by using fruit juice as an ingredient, the basis for making probiotic drinks, so that they are tastier to the general public and increase their appeal. Probiotics supplement live microbial brew and produce a variety of beneficial biomolecules, including bacteriocins (Al-Dhab *et al*., 2020). One of the probiotic drinks you want to develop in this research were red guava, melon, apple and fruit juices with *Lactobacillus casei* starter.

According to Winarni *et al*. (2020), giving juice drinks probiotic red guava is more effective than orange juice in increasing haemoglobin levels to prevent anaemia. Yusmarini *et al*. (2021) proposed using melon fruit as an essential ingredient for making drinks. Probiotics are known to be able to meet the nutritional needs of the body. Setyiyaningsih *et al*. (2020) reported that administering a Lemon-based probiotic drink could increase haemoglobin levels by 1.1 g/dl. Hidayah (2019) states that Adding apple juice to probiotic drinks can increase levels of vitamin C. Rosiana and Khoiriyah (2018) prove that making probiotics with apple juice and honey has activity antioxidants of 27.10% to 32.83%. From various previous studies, it is known that red guava, lemon, melon and apple have the potential as ingredients the basis for making juice probiotic drinks with *L.casei* starter as drink health.

Previous research that has been done regarding the feasibility of *L.casei* as the starter in the production of probiotic drinks, namely the research of Boro and Fidyasari (2017), stated that the results of the fermentation of yellow soursop juice were supplemented the *L.casei* line from the Yakult line showed no significant difference ($P<0.05$). Harahap *et al*. (2018) suggested that *L.casei* significantly influenced total lactic acid bacteria, total acidity, sucrose and ash but not on pH when preparing fermented beverages from tomato fruit juice. Nurlilayanti *et al*. (2019) state that *L. casei* bacteria produce cytokines, lymphocytes, antibodies, monocytes, and macrophages that can phagocytose harmful bacteria. *L. casei* can stimulate local and systemic immune responses. Various studies show that *L. casei* can be used as a starter in fermenting juice drinks, such as fruit.

Based on the background above and several previous studies that have been carried out using only one to two types of fruit, researchers are interested in making a probiotic drink with more types of fruit, namely from juice (Red guava, Melon, Apple and Lemon) with *L.casei* starter as a drink health. The results of this research are expected to

provide information regarding total Lactic Acid Bacteria from the probiotic drink Jamale juice with *L.casei* starter as a health drink.

Methods

This research is quantitative experimental research. This research was conducted at the STIKes Mitra Keluarga Pharmacology Laboratory, Bekasi, West Java, from January to March 2023. The materials used in this research are Red Guava fruit, Melon, Apple, Lemon and purchased pure culture of *Lactobacillus. casei* bacteria at PT. Agritama Sinergi Inovasi (AGAVI) and bacterial cultures of *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* taken from the Microbiology Laboratory Family Partner STIKes.

This research begins with sterilizing tools and materials, which are then made by DeMan Rogosa Sharp Broth (MRSB) and DeMan Rogosa Sharp Agar media (MRSA). Making MRSB is used to multiply bacteria by means weighing 19.6 g of MRSB, which was then dissolved in 700 ml of distilled water in an Erlenmeyer, covered with cotton and gauze, heated on a hotplate, and then sterilized by autoclave. MRSA preparations were used to count total colonies BAL by weighing 37,512 g MRSA 4 g back to agar, which was then dissolved with 720 ml of distilled water in an Erlenmeyer, covered with cotton and gauze, heated above a hotplate then sterilized by autoclave.

The next stage is the multiplication of L.casei bacteria by taking the pure culture of the bacteria as much as one aseptic ossicle needle, then inserting it into a test tube containing 5 ml MRSB, incubating at 37°C for 18 hours. The next stage is making a starter by inoculating 100 ml MRSB media with 2% L. casei culture incubation for 24 hours at 37°C. The next stage is making the Jamale juice probiotic drink with 75 ml of MRSB, and 25 ml of Jamale juice added with 2% L.casei substrate from a starter solution volume of 100 ml, incubating for 24 hours at 37°C. Results This is considered incubation 1. In the next stage, 50 ml of MRSB is made, and 50 ml of Jamale juice in the sample bottle included 2% L.casei taken sequentially from incubation process 1 sequentially. These results are considered as incubation 2. Do the same for incubation 3 (75 ml MRSB and 25 ml juice) and 4 (100 ml juice). The following is the juice formulation that you can see in Table 1.

Table 1. Juice Probiotic Drink Formula

Material	Formula			
	F1	F2	F3	F4
Red guava	40 g	40 g	40 g	40 g
Apple	20 g	20 g	20 g	20 g
Melon	20 g	20 g	20 g	20 g
Lemon juice	20 g	20 g	20 g	20 g
Stevia sugar	0 g	10 g	20 g	30 g
Water	100 ml	100 ml	100 ml	100 ml

Results and Discussions

This test aims to determine the amount of LAB contained in juice probiotic drink, which is a sign of the success or failure of the process ongoing fermentation. The method used in the total BAL test with using the Total Plate Count (TPC) method. Table 2. the results of this research shows the average value of total lactic acid bacteria.

Table 2. Average Value of the Number of LAB in Jamale Juice Probiotic Drinks

Treatment	CFU/ml ($\bar{x}$) ± SD
No sugar	1,80 x 10^{7a} ± 0,72
Addition of 10% stevia sugar	4,83 x 10^{7a} ± 0,38
Addition of 20% stevia sugar	4,91 x 10^{7a} ± 2,47
Addition of 30% stevia sugar	6,05 x 10^{7a} ± 3,34

Note: The data presented is the average value ($\bar{x}$) ± standard deviation (SD). The mean followed by superscript (a) in the same column shows that the difference is not significant using the One Way ANNOVA test.

From these results, it can be seen that the total LAB results in probiotic juice drinks Jamale meets the criteria for probiotic drinks according to National Standards Indonesia (SNI) with a minimum total BAL criteria of (6 log CFU/mL) (Tampinongkol *et al*., 2016). The results in the table above show that the total average value of The LAB in

the Jamale juice probiotic drink ranges from 1.80 x – 6.05 x CFU/mL, so it can be seen that the average value already meets the criteria according to SNI 7552:2009. The lowest average total LAB was found in Jamale Juice with a 10% stevia sugar concentration of 1.80 x CFU/mL, and the highest average total LAB was found in Jamale Juice with a 30% stevia sugar concentration of 6.05 x CFU/mL.

Probiotics juice, with a stevia sugar concentration of 30%, has an average total LAB value that is high due to adding more stevia sugar than the other concentrations. The more sugar added, the higher the total LAB value produced, where sucrose is a carbon source of LAB which will degrade various types of sugar into lactic acid so that LAB production will increase (Cinderela *et al*., 2022). Based on these results, it can be concluded that probiotic juice drinks Jamale that have been made meet the total BAL value standards. The highest total BAL value found in Jamale Juice with the addition of 30% stevia sugar concentration proves that the growth of LAB colonies is influenced by the sugar content contained in the sample. This is supported by research conducted by Cinderela *et al*. (2022), which states that glucose is an important nutrient for growth LAB is a source of energy and can trigger rapid growth of LAB colonies in bulk. The high CFU value in the probiotic drink sample juice with the highest addition of sugar is 30% due to the large amount of glucose in the sample. The more lactic acid produced by BAL by means

Glycolysis is breaking down glucose into lactic acid. Fruits are also used can affect total LAB production because the carbohydrates contained in The fruit used is supported by research by Azizah *et al*. (2019), who stated that Lactic Acid Bacteria (LAB) can hydrolyze carbohydrates into lactic acid and can increase the ability of bacteria to break down lactose.

Based on the number of replications, three replications were carried out. It can be seen that in the last replication, there was a decrease in the total value of BAL because The length of storage time causes the stevia sugar content in the sample It has been broken down into lactic acid by LAB, so probiotic drinks are not It is recommended to be stored for a long time and must be consumed immediately. As for the results, the one-way ANOVA test in this study was the total Lactic Acid Bacteria (LAB) test of 0.407 (P>0.05). Results indicate that increasing sugar concentration does not significantly affect the total BAL produced (P>0.05). According to Apriliyanto *et al*. (2020), the concentration of sugar added to the product does not affect the amount of lactic acid. This is caused by the ability of LAB to utilize the glucose to become lactic acid.

The results of the research that has been done are supported by the research that has been carried out by Rizal *et al*. (2016) regarding the study of pineapple juice lactic fermented drinks with a fruit and water composition ratio of 4:1 using a starter *Lactobacillus casei*. The best treatment from research is to produce a total value of BAL 1.1 x CFU/mL, presumably because at the time of incubation, there was a process of glucose breakdown by LAB, which takes longer and is optimal so that the lactic acid produced increases tall. Research conducted by Diza *et al*. (2016) made the drink probiotics with yam-based ingredients using a combination of *Lactobacillus* starter *acidophillus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Producing a total BAL value of 2.38 x in the 0th week, but in the fourth week, there was a decrease of 2.6 log cycles, presumably due to the use of mixed cultures causing there is competition between bacteria in fulfilling nutrition so that it can be concluded that the storage of fermented beverages for quite a long time will reduce the total LAB value contained therein due to the addition of sugar as LAB nutrition decreases because the LAB consumes it used.

Conclusions

Test the total plate number on the probiotic drink juice using a starter *L.casei* with a sugar concentration of 0%, 10%, 20% and 30% is declared to meet the criteria probiotic drinks according to SNI 7552:2009 with a minimum BAL of (6 log CFU/mL) namely probiotics juice without added sugar of 1.80 x CFU/mL, probiotics juice with the addition of 10% sugar of 4.83 x CFU/mL, probiotics juice with the addition of 20% of 4.91 x CFU/mL and probiotics juice with the addition of 30% sugar of 6.05 x CFU/mL.

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