Aplication of *Lactobacillus Rhamnosus* in Probiotic Juice as Functional Drink for Type 2 Diabetes Mellitus Patients

Eko Farida[1]*, Yanesti Nur Avianda Lestari[1]

[1] Nutrition Study Program, Faculty of Medicine, Universitas Negeri Semarang, Semarang, 50237, Indonesia
*e_farida@mail.unnes.ac.id

Abstract. The number of people with type 2 diabetes mellitus (T2DM) increases every year so that low-sugar products are needed as an alternative to functional drinks for them. The aim of the study was to obtain a probiotic juice formula mixed with carrots, tomatoes and guava so that it could be used as a functional drink for people with T2DM. The research was conducted in 3 stages, namely: (1) Formulation of probiotic juice, (2) Analysis of microbiological quality of probiotic juice, and (3) Sensory analysis of probiotic juice. The results showed that dilution had a very significant effect on the overall acceptance of probiotic juice, but decreased total lactic acid bacteria (LAB) and total acid. Storage time has a very significant effect on increasing total LAB, increasing lactic acid and overall acceptance of probiotic juice. The interaction between dilution and storage time was found in total LAB and overall acceptance which resulted in the best mixed probiotic juice characteristics, namely P2-H14 treatment with a total LAB of Log 10.98 CFU/mL, total of acidity 0.98%, and overall acceptance score of 3.92 (like).

Keywords: diabetes mellitus, juice, lactic acid bacteria, probiotic, sensory analysis

1 Introduction

The number of people with Diabetes Mellitus (DM) is increasing every year. In 2019 people with DM in the world reached 463 million and is estimated to reach 578 million in 2030 [1]. The World Health Organization (WHO) predicts an increase in the number of people with DM in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030. Based on a report by the International Diabetes Federation (IDF) in 2019, Indonesia was ranked 7th in the world with the number of sufferers reaching 10.7 million and 73.7% of DM sufferers in Indonesia did not realize that they had DM. The results of the Basic Health Research show that the prevalence of DM in the Indonesian population is 10.9 [2]. The largest case of DM in the world is Type 2 Diabetes Mellitus (DMT2), so it is necessary to find alternative ways of prevention and control.

Lactic Acid Bacteria (LAB) is one of Indonesia's biodiversity and is found in various fermented foods. Some LAB have the potential as probiotics that have functional benefits for the body, including lowering blood glucose. LAB isolate has the potential to prevent and control T2DM because it is reported to be able to inhibit the -glucosidase

C. D. M. Putri et al. (eds.), *Proceedings of the International Joint Conference on Science and Engineering 2024 (IJCSE 2024)*, Advances in Engineering Research 250,
https://doi.org/10.2991/978-94-6463-626-0_4

enzyme and has antioxidant activity [3]. Research in experimental animals also showed that LAB were able to control blood glucose, such as *Lactobacillus rhamnosus* [4] and *L. casei* CCFM419 [5]. Fermented products containing LAB is also reported to be able to control blood glucose, for example carrot juice fermented with *L. plantarum* NCU116 [6]. Fruit or vegetable juices fermented with probiotic bacteria also have sensory properties that are acceptable to consumers such as pineapple juice fermented with *L. acidophilus* LA-5 [7], lime juice fermented with *L. plantarum* SI-1 and *L. pentosus* MU-1 [8], orange juice fermented with *P. acidilactiti* CE51 [9] and mixed juice made from oranges, carrots, apples and jujubes fermented with *S. thermophilus* [10]

Lactobacillus rhamnosus R23 has been isolated from breast milk and has the potential as a probiotic [11]. Studies of its functional properties have also been carried out, namely to prevent diarrhea [12], reduce cholesterol [13] and lower blood glucose [4] These potential isolates need to be applied to food or beverage products as functional food. Fermented products that are widely used are usually milk-based, but their consumption is limited because they cannot be consumed by lactose intolerant sufferers and vegetarians. One alternative product that can be developed is probiotic juice. Probiotic juice is juice made from fruits and vegetables (carrots, tomatoes and guava), then fermented with *L. rhamnosus* R23. Carrots, tomatoes and guavas have a low glycemic index of 35.86, 38.38, 32.25 and are rich in antioxidants, respectively [14]. Fruits and vegetables are also rich in vitamins and minerals that can be fermented by *L. rhamnosus* R23 so as to increase their viability.

2 Method

The research was conducted in 3 stages, namely: (1) Formulation of probiotic juice, (2) Analysis of microbiological quality of probiotic juice, and (3) Sensory analysis of probiotic juice.

Preparation of *Lactobacillus rhamnosus* culture

LAB isolates in the form of dry cultures (lyophiles) were transferred to sterile MRS broth (MRSB) media. The tube containing the LAB culture suspension was incubated at 37°C for 24 hours, then the culture was streaked using a loop on an inclined MRS agar (MRSA) medium. LAB cultures were incubated at 37°C for 48 hours. LAB culture on slanted MRSA was stored for a period of 1 month and stored in a refrigerator at 5°C. Each time the test will be carried out, 1 ose is taken and inoculated in 10 ml of MRSB, then incubated at 37°C for 24 hours. Culture refresher was performed twice by inoculating 0.1 ml of liquid bacterial culture in 9.9 ml of MRSB, and incubated at 37°C for 24 hours. LAB culture is ready for use.

Probiotic Juice Making[10]

The flow chart for making probiotic juice is presented in Figure 1. The probiotic juice produced will be stored at 4^0C for 28 days. Observations will be made on days 0, 7, 14, and 21.

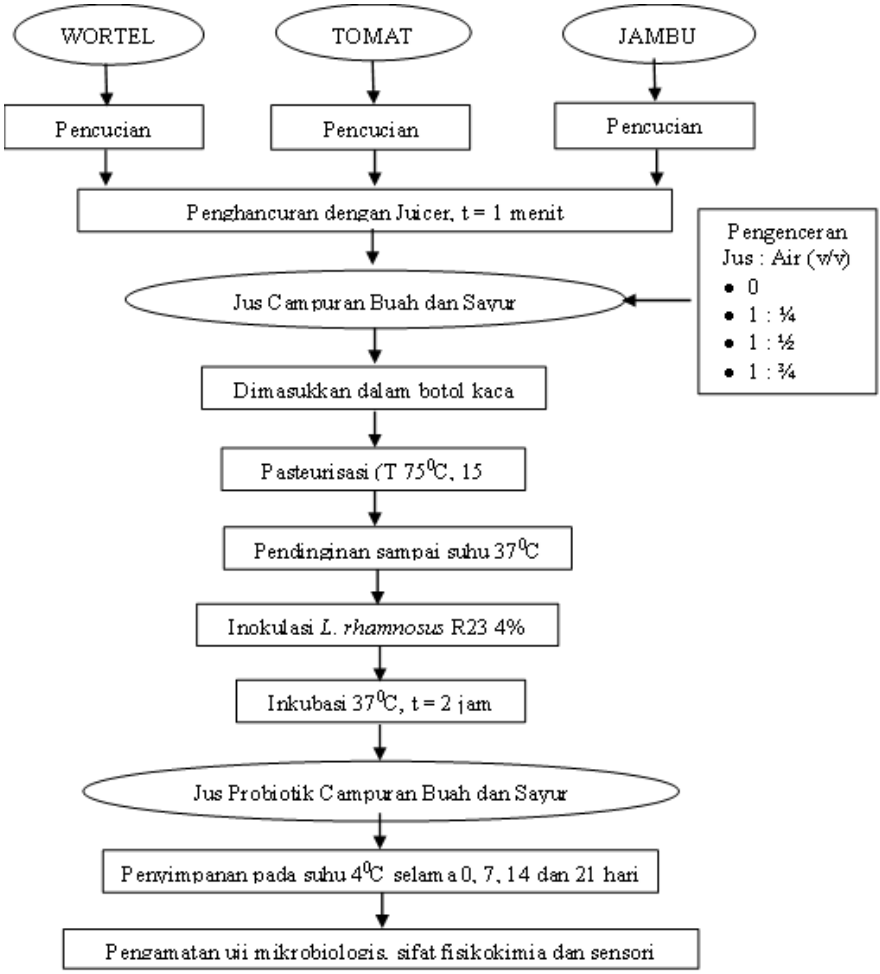

Figure 1. Probiotic Juice Making Flow Chart

Total BAL Test

Total LAB testing was carried out using the Total Plate Count method. A total of 1 mL of probiotic juice was put into 9 mL of sterile physiological saline solution. From the mixture obtained a dilution of 10^{-1}. The mixture was then homogenized and 1 mL of the solution was taken from the first tube and put into the next test tube containing 9 mL of physiological saline solution so that a dilution of 10^{-2} was obtained and so on until the appropriate dilution was obtained (10^{-5} to 10^{-7}). From the desired dilution, 1 mL was taken and put into a sterile petri dish, then about 15 mL of sterile MRSA medium was added. The plates were incubated at 37°C for 48 hours and the colonies

growing were counted using the Colony Counter. The total colonies counted were between 25 and 250 colonies per petri dish.

Total Acid Test

Acidity testing is done by measuring the level of acid equivalent to lactic acid by the titration method. Probiotic juice was centrifuged at 3500 rpm for 15 minutes. A total of 1 ml of the supernatant was diluted 10 times, then titrated with 0.1 N NaOH until a constant pink color (phenolphthalein indicator) was formed.

Sensory / Hedonic Test

Sensory assessment of probiotic juice was carried out by hedonic test to assess the acceptance of aroma, color, taste and overall acceptance of the product. Before the sample was presented to the panelists, 80 mL of mixed probiotic juice was added with 40 mL of a 65% sucrose solution. Then, 12 cups containing 20 mL of mixed probiotic juice samples were coded with 3 digits and presented randomly to 25 panelists with the criteria of semi-trained panelists. Sample presentation was carried out at 4 test booths with 2 presentations (6 samples each). Each sample cup has 1 main spoon and each panelist is given another 12 spoons to try each sample in turn. Before moving to another sample, the panelists neutralized the senses by drinking mineral water that had been provided.

3 Result and Discussion

The quality of probiotic juice includes microbiological, chemical and consumer assessments of the product sensory. Total LAB of probiotic juice in various treatments and storage are presented in Table 1. The difference in total LAB was caused by differences in the adaptability of each isolate to the growth media used. The amount of LAB required to be consumed and has a positive effect on health ranges from 107 to 109 CFU/ml. The results of this study indicate that the total LAB of probiotic juice meets the requirements for consumption, thus causing a positive effect on health.

Table 1. Total LAB of probiotic juice in various treatment and storage

Treatment	Total LAB (log CFU/ml)			
	Day-0	Day-7	Day-14	Day-21
P0	11.25 ± 0.57	11.32 ± 0.87	11.45 ± 0.57	11.75 ± 0.68
P1	11.02 ± 0.81	11.21 ± 0.83	11.30 ± 0.81	11.45 ± 0.54
P2	10.32 ± 0.37	10.83 ± 0.57	10.98 ± 0.64	11.05 ± 0.96
P3	10.04 ± 0.27	10.35 ± 0.89	10.55 ± 0.34	10.75 ± 0.58

LAB can grow to the maximum amount depending on the nutrient content available in the growth medium. A high amount of LAB will cause metabolic activity to increase so that more metabolites are produced. During the fermentation process, sugar undergoes a metabolic process into organic acids, including lactic acid, so the more LAB, the

higher the amount of lactic acid produced. The total acidity of probiotic juice in various treatments and storage is presented in Table 2. LAB cultures use more glucose than galactose as an energy source in their metabolic processes. Furthermore, the results of the hydrolysis of lactose are converted into lactic acid through the Embden Meyerhof pathway by homofermentative bacteria.

Table 2. Total Acids of Probiotic Juices at Various Treatments and Storage

Treatment	Total acid (%)			
	Day-0	Day-7	Day-14	Day-21
P0	1.15 ± 0.02	1.32 ± 0.05	1.43 ± 0.05	1.55 ± 0.08
P1	1.02 ± 0.03	1.10 ± 0.02	1.15 ± 0.07	1.34 ± 0.04
P2	0.67 ± 0.04	0.77 ± 0.04	0.98 ± 0.02	1.25 ± 0.07
P3	0.54 ± 0.03	0.66 ± 0.06	0.75 ± 0.07	0.99 ± 0.02

Generally, drinks containing probiotics are stored at cold temperatures. Probiotic juice stored at room temperature will be more easily damaged, so the method of storing probiotic juice at cold temperatures is used to help slow down the activity of probiotics. During the cold storage process there will be a process of changing the microbiological and sensory properties of the probiotic juice. The quality criteria that must be met to obtain a good mixed probiotic juice in addition to maintaining the viability of microorganisms is that its sensory characteristics must be accepted by consumers.

The level of acidity in probiotic juice due to LAB metabolism will affect consumer acceptance of the sensory properties of the product, especially the characteristic aroma and taste of probiotic drinks. The panelists' overall acceptance of probiotic juice can be seen in Table 3. Panelists still prefer probiotic juice stored at 4 ^{0}C for 21 days. Overall, the color, aroma and taste of the probiotic juice were acceptable to the panelists. The interaction between dilution and storage time was found in total LAB, Total of acidity and overall acceptance which resulted in the best mixed probiotic juice characteristics, namely P2-H14 treatment with a total LAB of Log 10.98 CFU/mL, total of acidity 0.98%, overall acceptance score of 3.92 (like).

Table 3. Probiotic juice acceptance based on sensory analysis

Treatment	Storage Duration			
	Day-0	Day-7	Day-14	Day-21
P0	3.85	3.83	3.75	3.67
P1	3.95	3.90	3.95	3.75
P2	4.00	3.95	3.92	3.85
P3	4.00	3.87	3.75	3.75

4 Conclusion

Dilution had a very significant effect on increasing LAB viability and overall acceptance of probiotic juice. Storage time has a very significant effect on increasing total LAB and LAB viability, increasing lactic acid, and overall acceptance of probiotic juice. The interaction between dilution and storage time was found in total LAB and overall acceptance which resulted in the best mixed probiotic juice characteristics, namely P2-H14 treatment with a total LAB of Log 10.98 CFU/mL, total acid 0.98% and overall acceptance score of 3.92 (like).

Disclosure of Interests. There is no conflict of interest in this research.

References

1. [IDF] International Diabetes Federation. 2019. IDF Diabetes Atlas. 9th ed. http://www.diabetesatlas.org.
2. Riset Kesehatan Dasar (Riskesdas). 2019. Badan Penelitian dan Pengembangan Kesehatan. Kementerian Kesehatan RI.
3. Farida E, Jenie BSL, Nuraida L, Giriwono PE. 2019. Aktivitas antioksidan dan penghambatan α-glukosidase oleh ekstrak etanol bakteri asam laktat indigenos. *Jurnal Teknologi dan Industri Pangan.* 30 (1): 56-63. https://doi.org/10.6066/jtip.2019.30.1.56.
4. Farida E, Nuraida L, Giriwono PE, Jenie BSL. 2020. *Lactobacillus rhamnosus* reduces blood glucose level through down-regulation of gluconeogenesis gene expression in streptozotocin-induced diabetic rats. *International Journal of Food Science*. Volume 2020. Article ID 6108575, 12 pages. https://doi.org/10.1155/2020/6108575.
5. Wang G, Li X, Zhao J, Zhang H, Chen W. 2017. *Lactobacillus casei* CCFM419 attenuates type 2 diabetes via a gut microbiota dependent mechanism. *Food Function*. 8(9):3155-3164. https://doi.org/10.1039/c7fo00593h.
6. Li C, Ding Q, Nie SP, Zhang YS, Xiong T, Xie MY. 2014. Carrot juice fermented with *Lactobacillus plantarum* NCU116 ameliorates type 2 diabetes in rats. *Journal Agriculture and Food Chemistry*. https://doi.org/10.1021/jf503681r.
7. Islam MZ, S. Tabassum, M. Harun-ur-Rashid, G.E. Vegarud, M.S. Alam, A. Islam. 2021. Development of probiotic beverage using whey and pineapple (*Ananas comosus*) juice: sensory and physico-chemical properties and probiotic survivability during in-vitro gastrointestinal gestion, *Journal of Agriculture and Food Research*, https://doi.org/10.1016/j.jafr.2021.100144.
8. Yuasa M, Shimada A, Matsuzaki A, Eguchi A. Tominaga M. 20020. Chemical composition and sensory properties of fermented citrus juice using probiotic lactic acid bacteria. *Food Bioscience*. https://doi.org/10.1016/j.fbio.2020.100810.
9. Vieira KCOV, Camila SF, Emili BTB, Yuri ADM, Ana CCGT, Wilson RN, Valeria CT, Lizziane KW. 2020. Development and viability of probiotic orange juice supplemented by *Pediococcus acidilactiti* CE551. *LWT-Food Science and Technology*. 130 (1096637). https://doi.org/10.100016/j.let.2020.1096637.

10. Xu X, Yejun B, Bingbing W, Fei L, Xiaosong H. 2019. Chemical analysis and flavor properties of blended orange, carrot, apple and Chinese jujube juice fermented by selenium-enriched probiotics. *Food Chemistry*. 289: 2550-258. https://doi.org/10.1016/j.foodchem.2019.03.068.
11. Nuraida L, Susanti, AW Hartanti. 2007. Lactic acid bacteria and bifidobacteria profile of breast milk and their potency as probiotics. 10th ASEAN Food Conference. Kuala Lumpur- Malaysia.
12. Nuraida L, Hana, AW Hartanti, E Prangdimurti. 2012. Evaluasi potensi *Lactobacillus* yang diisolasi dari air susu ibu untuk mencegah diare. *Jurnal Teknologi Industri Pangan.* 13(2): 158-165. https://doi.org/10.6066/jtip.2012.23.2.158.
13. Maryati Y, Nuraida L, Dewanti-Hariyadi R. 2016. Kajian bakteri asam laktat dalam menurunkan kolesterol secara *in vitro* dengan keberadaan oligosakarida. *Agritech.* 36(2): 196-205.
14. Oboh G, Ayokunle OA, Margareth A, Olasunkanmi SO, Aline AB, Margareth LA. 2015. Starch composition, glycemic indices, phenolic constituents, and antioxidative and antidiabetic properties of some common tropical fruits. *Journal of Ethnic Food*. 2: 664-73. http://dx.doi.org/10.10016/j.jef.2015.05.003.