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Cookies High in Antioxidants from the Combination of Lactobacillus Reuteri with Papaya Juice as a Functional Food Candidate for Children with Functional Digestive Problems

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ABSTRACT

Lactobacillus reuteri is one of the good microorganisms that play a role in the digestive health of children, such as diarrhea and constipation. Papaya is a on of tropical fruit rich in valuable nutrients and has a good taste. This research aims to process papaya juice with L. reuteri into functional biscuits. There were 3 variations of papaya juice formulation, namely S1, S2, and S3, based on papaya and CO2-free water content (S1 = 1:0.5; S2 = 2:1; and S3 = 3:2). Then, all samples were inoculated for 14 days with Lactobacillus reuteri LRE02-DSM 23878 5% b/v (anaerobic conditions). The part of papayas used was pure ripe flesh. The fermented products were made into flour with a freeze dryer, then the powder was mixed and stirred with the addition of 5% water using a mixer with a power of 102-189 rpm for 30 minutes and then put in the oven for 15 minutes with a temperature of 70 - 90°C so that it became cookies. Sample variation was done to determine the average significance of the antioxidant content in it. In the next step, Vitamin C analysis of all three cookie samples was performed using the Iodometric Titration Method in mg / 100g and antioxidant activity using the 2.2-diphenyl-1- picrylhydrazyl (DPPH) method. The results of vitamin C and antioxidant activity in each sample of cookies; S1 was 98.64mg/100g with 32.91% antioxidant activity, S2 was 91.73mg/100g with 25.70% of antioxidant activity, and S3 was 109.88mg/100g with 38.90% antioxidant activity. The formulation for the number of cookie samples containing the highest vitamin C was S3...

Keywords:
Cookies; antioxidants; L reuteri; papaya; digestive problems

1.Introduction

Papaya is a type of fruit from the papaya carica plant which is native to Central America and South Mexico but now grows in many other parts of the world, including Indonesia.. Papaya is a tropical fruit rich in valuable nutrients and has a good taste (Schweiggert, et al.,2013). Papaya contains an enzyme called papain, which functions to break down the hard protein chains found in muscle or meat. Because of this, people have used papayas to smooth or tenderize meat for thousands of years (LactMed, 2006). Papaya fruit is high in antioxidants that can reduce inflammation, fight disease and help stay youthful (Tomella, et al., 2014). Powerful antioxidants such as lycopene can reduce the risk of many diseases - especially those that tend to appear with age, such as heart disease and cancer, can also protect against visible signs of aging, helping the skin stay smooth and youthful (Somanah, et al., 2012).

Probiotics are living microorganisms that, if administered in sufficient quantities, can provide health benefits to the host by regulating an unbalanced gastroenteric microbiota (Margiotta, et

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al.,2021). One of those probiotics is *Lactobacillus reuteri* LRE02-DSM 23878. *Lactobacillus reuteri (L. reuteri)* are well-studied probiotic bacteria that can colonize a large number of mammals. (Mu, Tavella and Luo, 2018). In humans, L. reuteri is found in various parts of the body, such as the digestive tract, urinary tract, skin, and even breast milk. The abundance of L. reuteri varies from person to person (Mu, Tavella and Luo, 2018). Several beneficial effects of L. reuteri have been noted. First, L. reuteri can produce antimicrobial molecules, such as organic acids, ethanol, and reuterin (Mu, Tavella and Luo, 2018). Due to its antimicrobial activity, *L. reuteri* can inhibit the colonization of pathogenic microbes and overhaul the composition of the commensal microbiota inside the host. Second, *L. reuteri* can be beneficial for the immune system. For example, some strains of *L. reuteri* may reduce the production of pro-inflammatory cytokines while promoting the development and function of regulatory T cells. Third, with the ability to strengthen the intestinal barrier, colonization of *L. reuteri* can decrease the translocation of microbes from the intestinal lumen to the tissues. Microbial translocation across the intestinal epithelium has been hypothesized as an inflammatory trigger (Mu, Tavella and Luo, 2018). Therefore, inflammatory diseases, including those located in the intestines as well as in distant tissues, can be corrected by increasing the colonization of *L. reuteri*. In particular, the decrease in the abundance of *L. reuteri* in humans in recent decades correlated with an increase in the incidence of inflammatory diseases over the same period. Direct supplementation or modulation of *L. reuteri* prebiotics may be an interesting means of prevention and/or therapy against inflammatory diseases (Mu, Tavella and Luo, 2018).
This study aims to process papaya juice fermented by *L. reuteri* into cookies that are high in antioxidants as a functional food for children with functional digestive problems.

2. Material and Methods

There were 3 variations of papaya juice formulation, namely S1, S2, and S3, based on papaya and CO_2-free water content (S1 = 1:0.5; S2 = 2:1; and S3 = 3:2). Then, all product samples were inoculated with *Lactobacillus reuteri* LRE02-DSM 23878 5% b/v for 14 days under anaerobic conditions. The part of papayas used was pure ripe flesh. The fermented products were made into flour with a freeze dryer, then the powder was mixed and stirred with the addition of 5% water using a mixer with a power of 102-189 rpm for 30 minutes and then put in the oven for 15 minutes with a temperature of 70 - 90°C so that it became cookies. Sample variation was done to determine the average significance of the antioxidant content in it. In the next step, The method for determining vitamin C from 3 samples of cookies was carried out by using the iodometric titration method to determine the amount in mg / 100g and the antioxidant activity was known using the 2.2-diphenyl-1- picrylhydrazyl (DPPH) method.

$$Vitamin\ C\ \left(\frac{mg}{100g}\right) = \frac{V\ I2\ x\ 0{,}88\ x\ Fp\ x\ 100}{Ws\ gram}$$

The determination of water content used was the AOAC drying method (thermogravimetry). The principle of this method is based on the evaporation of water in the material by heating, then weighing it to a constant weight. The weight reduction that occurs is the water content contained in the material. An empty plate is heated in an oven at 105° C for 30 minutes, cooled down in a desiccator for 15 minutes, then weighed (W0). A 2-gram sample was then put in a plate with known weight, weighed (W1), then dried in an oven at 105° C for 3 hours, cooled in a desiccator for 15-30 minutes, then the plates and contents were weighed and dried again for another one hour, cooled in the exicator, and weighed again (W2). The water content was calculated using the following formula:

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$$Water\ Content\ (\%) = \frac{W1 - W2}{W1 - W0} x\ 100$$

The procedure for determining the ash content was carried out using the AOAC 2005 method. Meanwhile, the procedure is as follows: the cup was dried in an oven at 105° C for 1 hour. Then, it was cooled in the cup for 15 minutes in a desiccator and weighed. 2 grams of the sample was put into a furnace where the temperature was 550° C for 3 hours. It was then cooled outside the furnace to a temperature of ± 120° C and put in a desiccator. The plates and ashes were weighed so that a constant weight was obtained. Calculation of the ash content was carried out using the following formula:

$$Ash\ Content\ (\%) = \frac{Weight\ of\ Bowl\ After\ Heated - Constant\ Weight\ of\ Empty\ Bowl}{Sample\ Weight}\ x\ 100$$

3. Results and Discussion

The results of vitamin C and antioxidant activity in each sample of cookies; S1 was 98.54mg/100g with 32.91% antioxidant activity, S2 was 91.73mg/100g with 25.70% of antioxidant activity, and S3 was 109.88mg/100g with 38.90% antioxidant activity. The formula for the number of cookie samples containing the highest vitamin C is S3. There is a significant difference ($P < 0.05$) which determines the level of vitamin C between sample formulations.. The higher the antioxidant activity, the higher the antioxidant levels, and the less food needed to reduce free radicals (Lisdawati and Kardono., 2012). This shows that the formulation of papaya juice which was made into cookies contains vitamin C and has antioxidant activity. This makes formulated cookies a potential healthy snack that is high in vitamin C and antioxidants during the COVID-19 pandemic. The average vitamin C level in the three cookie samples was 100.05mg/100g. S3 showed the best activity, namely antioxidant activity against 2.2-diphenyl-1-picrylhydrazyl (DPPH) of 38.90%.

The average ash content of the three samples was 1.95% and water content was 1.70%, which corresponds to the Indonesian National Standard (SNI) 01-2973-1992. Higher ash content in cookies indicates the higher mineral contents in cookies such as calcium, potassium, and iron (Andarwulan *et al*., 2014). It is clinically known that mineral intake can improve the respiratory system, especially in tuberculosis sufferers (Taslim *et al*., 2020).

Fermented papaya with *L. reuteri* has a great potential to be developed into healthy snack cookies. The vitamin C and antioxidants content in cookies from the fermentation of papaya may be a great substitute for snacks for children with functional digestive problems since antioxidants and vitamin C can improve immunity and anti-inflammatory response. These cookies are also good prebiotics for the gut microbiome which plays a good role in the digestive system and immune system. It needs clinical trials in humans to find out more about its effects on human health and the authors are very open to joint research collaborations.

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Conflict of Interest

The authors authors (J. I. C. Manoppo, F. Nurkolis, M. R. Bahar, S. C. Batubara, N. Mayulu, W. B. Gunawan5, P. S. Augusta, N. A. Taslim, M. F. J. Mantik) declare that there are no conflicts of interest.

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Table 1. Vitamin C Content, Anti-Oxidant Activity, Water and Ash Content in Cookies

Sample	Vitamin C Content (mg/100g)	Anti-Oxidant Activity Towards (DPPH)	Ash Content (%)	Water Content (%)
S1	98.54	32.91%	1.90	1.87
S2	91.73	25.70%	1.96	1.75
S3	109.88	38.90%	1.99	1.49
Mean	100.05 ± 9.16	32.50 % ± 6.60	1.95 ± 0.04	1.70 ± 0.19