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

Muhammad Rahimi Bahar[1], Fahrul Nurkolis[2], Jeanette Irene Christiene Manoppo[3], Nelly Mayulu[4], William Ben Gunawan[5], Siti Chairiyah Batubara[6], Piko Satria Augusta[7], Nurpudji Astuti Taslim[8], Max F. J. Mantik[9]

[1,3,9]Pediatrics, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia.
[2]Biological Sciences, Faculty of Sciences and Technology, State Islamic University of Sunan Kalijaga, Yogyakarta, Indonesia.
[4]Nutrition, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia.
[5]Nutrition, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
[6]Food Technology, Faculty of Food Technology and Health, Sahid University of Jakarta, South Jakarta, Indonesia.
[7]Medical Sciences, Faculty of Medicine, Brawijaya University, Malang, Indonesia.
[8]Professor Clinical Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
[1]Corresponding Author: rahimibahar17@gmail.com

ABSTRACT

Lactobacillus reuteri is one of the good microorganisms that play a role in the digestive health of children, such as diarrhea and constipation. Mango fruit (Mangifera indica) has an abundant content of antioxidants, dietary fiber, and beta-carotene. This research aims to process mango juice with L. reuteri into functional biscuits. There were 3 variations of mango juice formulation, namely S1, S2, and S3, based on mango and CO2-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 mangoes 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 from 3 samples of cookies was done using the Titration Iodometric Method to determine the amount of Vitamin C (mg/100g) and also the antioxidant activity with 2.2-diphenyl-1- picrylhydrazyl (DPPH). The results of vitamin C and antioxidant activity in each sample of cookies; S1 was 89.64mg/100g with 25.78% antioxidant activity, S2 was 81.55mg/100g with 23.77% of antioxidant activity, and S3 was 96.92mg/100g with 28.67% antioxidant activity. The formulation for the number of cookie samples containing the highest vitamin C was S3. There was a significant difference ($P < 0.05$) which determined the vitamin C level between the sample formulations..

Keywords:
Cookies; antioxidants; L reuteri; functional food; digestive problems.

1.Introduction

Mango (*Mangifera indica*) is a tropical fruit plant that contains high levels of nutrients, fiber, macronutrients, micronutrients, and minerals as well as abundant bioactive compounds (Maldonado-Celis *et al*., 2019). The well-known high content in mangoes, including vitamin C, beta-carotene, polyphenol types of quercetin, and kaempferol (Nurkolis *et al.*, 2020; Mantik *et al*., 2021). Recent research has shown that increasing the intake of foods high in antioxidants and polyphenols such as Vitamin C, beta-carotene, quercetin and kaempferol can increase the body immunity against viral infection (Levy *et al*., 2020; Pitsillou *et al*., 2020; Suhail *et al*., 2020). Therefore, the consumption of food sources of antioxidants can be one of the efforts to minimize inflammation, one of which is inflammation caused by functional digestive diseases. Epidemiological studies have shown that approximately 14-25% of infants, young children, and

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adolescents suffer from at least one gastrointestinal function disorder (Robin, et al.,2018).
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 al.,2021). One of those probiotics is *Lactobacillus reuteri* LRE02-DSM 23878. *Lactobacillus reuteri (L. reuteri)* is a well-studied probiotic bacteria that can colonize a large number of mammals (Mu, Tavella and Luo, 2018). In humans, L. reuteri is found in different body sites, including the gastrointestinal tract, urinary tract, skin, and breast milk. The abundance of *L. reuteri* varies among different individuals (Mu, Tavella and Luo, 2018). Some 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 mango 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 mango juice formulation, namely S1, S2, and S3, based on mango 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 mangoes 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 from 3 samples of cookies was done using the Titration Iodometric Method to determine the amount of Vitamin C (mg/100g) and also the antioxidant activity with 2.2-diphenyl-1-picrylhydrazyl (DPPH).

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

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

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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:

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

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 89.64mg/100g with 25.78% antioxidant activity, S2 was 81.55mg/100g with 23.77% of antioxidant activity, and S3 was 96.92mg/100g with 28.67% antioxidant activity. The formulation for the number of cookie samples containing the highest vitamin C was S3. There was a significant difference ($P < 0.05$) which determined the vitamin C level between the 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 mango 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 89.37mg/100g. S3 showed the best activity, namely antioxidant activity against 2.2-diphenyl-1-picrylhydrazyl (DPPH) of 28.67%.

The average ash content of the three samples was 1.85% and water content was 1.87%, 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 mango 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 mango 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 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	89.64	25.78%	1.80	1.97
S2	81.55	23.77%	1.86	1.85
S3	96.92	28.67%	1.89	1.79
Mean	89.37 ± 7.68	26.07 %± 2,46	1.85 ± 0.04	1.87 ± 0.09