Effect of the addition of honey on the growth of lactic acid bacteria in functional drinks of fermenting cassava stored at frozen temperatures

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Abstract. Cassava is a food that contains fat, protein, and minerals so that it can be processed by fermentation. The purpose of this study was to find out how to increase the content of honey against the growth of lactic acid bacteria associated with the probiotic tapai based cassava functional drinks stored in frozen storage conditions. Functional drinks based on cassava with additional honey 0%, 2%, 4%, 6%, and 8% which will then be analyzed by the total lactic acid bacteria. The results obtained indicate that the total lactic acid (LAB) bakery in cassava-based functional drinks (Manihot esculenta) in freezing temperature storage decreased in the treatment of honey additions of 2% and 4% but increased in the treatment of adding honey 6% and 8% despite the increase and decrease in total bacteria the results are not significantly different.

1. Introduction

Sinjai is one of the 24 regencies/cities in South Sulawesi Province, which is bordered by three districts, namely Bone Regency in the north, Bulukumba Regency in the south, and Gowa Regency in the west. Administratively, Sinjai has not experienced subdistricts or villages. Sinjai is still divided into 9 districts with 80 villages [1].

Types of food plants cultivated in Sinjai Regency are rice, corn, cassava, sweet potatoes, and peanuts. Cassava is one type of food that has a role as a complementary source of carbohydrates in addition to rice and corn. Cassava production in Sinjai District in 2017 was 481 tons of wet cassava [2]. Cassava contains four groups of nutrients, namely carbohydrates, fats, proteins, and minerals. The advantages of cassava is especially on carbohydrates and fats, which are the main source of fuel for the body's energy generation. One of the cassava products traditionally processed with the fermentation process is cassava tapai. Fermentation in food processing is the conversion of carbohydrates into alcohol and carbon dioxide using yeast, bacteria, fungi or a combination of the three [3]. Apart from the higher nutritional value of the original ingredients, tapai also contains lactic acid bacteria (BAL). Lactic acid bacteria are classified as probiotics which provide several health benefits because they can help maintain a good balance and composition of intestinal flora, and increase resistance to pathogenic infections [4].

We found that a group of people in Sinjai District processed the cassava tapai into a functional beverage known as Minas. At first glance this functional drink is similar to an energy-boosting drink sold on the market, the color is bright yellow with a rather thick texture. The main ingredient is
cassava tapai with several other additional ingredients such as coconut water, milk, eggs, sugar, and honey.

Honey is one of the ingredients added in the manufacture of the tapai based functional drink formula. Honey is a natural sweetener [5]. Research conducted by Olofsson et al. (2014) that around 40 types of lactic acid bacteria (BAL) were identified from honey bees with 13 of them being Lactobacillus (9 spp.) and Bifidobacterium (4 spp.) [6]. Nofrianti et al. [3] applied the addition of honey concentration and the results significantly affected the total lactic acid bacteria of corn yogurt. Honey added is able to meet the nutrition of lactic acid bacteria and improve product quality [7]. According to Herawati and Wibawa [8], sugar (sucrose, lactose, glucose, or fructose) in addition to being a source of sweetness is also a good source of energy for microorganisms. The greater the amount of sugar added, the more available substrate for microbes and more rapid growth.

However, because the main ingredient is fermentation, this drink does not last long. According to the manufacturer, Minas which is stored in a freezer can last for a week but if only stored in an ordinary refrigerator it can last for 3-5 days. If it is not stored at all in the cooler then, of course, the durability is lower. Minas which are packaged in medium-size mineral water bottles can explode on their own because they cannot stand the heat.

This is related to the development of foods with adequate doses of probiotics when consumed because several factors during processing and storage affect the viability of probiotic organisms. Probiotic microorganisms can last longer in product freezing time. However, probiotic cell membranes can be damaged during the freezing process due to mechanical stresses of ice crystals that form on external media or inside cells, thus causing fatal injuries to them [4].

Therefore, it is important to conduct this research to determine the effect of the addition of honey to the growth of lactic acid bacteria contained in a cassava functional probiotic beverage stored in frozen storage conditions. This is based on research that honey can increase total lactic acid bacteria but other opinions indicate that frozen storage conditions can damage the probiotic cells of lactic acid bacteria.

2. Method

2.1. Materials
The materials used are functional cassava tapai, Indonesian organic honey obtained from the Faculty of Forestry, Hasanuddin University, MRS Agar, MRS Broth, alcohol, aquadest. The tools used are HPLC (High-Performance Liquid Chromatography), LAF (Laminar Air Flow), autoclaves, incubators, micropipets, test tubes, Petri dishes, measuring flasks, Erlenmeyer, vortex, ose, bunsen.

2.2. Analysis of Total Lactic Acid Bacteria (LAB)
Functional drinks of cassava are treated with the addition of honey by 0% (control), 2%, 4%, 6%, and 8%. From each variation then a stratified dilution up to 10^{-7} was then inoculated on a petri dish containing MRSA by pouring and incubation at 37^\circ C for 24 hours. Then calculate the Total Lactic Acid Bacteria Total Plate Count (TPC) Method.

3. Results and discussion
The calculation of the number of lactic acid bacteria based on tapai functional cassava (Manihot esculenta) with a variation of the addition of honey using the total plate count method and three times the dilution of 10^{5}, 10^{6}, and 10^{7} in a triple so that each dilution is obtained on an average number of bacterial colonies. The average number of bacterial colonies that met the requirements (25-250 CFU/ml) at three times dilution was then calculated to get the population of lactic acid bacteria in each treatment.
Figure 1. (a) functional drinks with the addition of honey M1 = 0% (control); M2 = 2%; M3 = 4%; M4 = 6% and; M5 = 8% (b) incubation at 37°C for 24 hours.

|                | Honey 0% | Honey 2% | Honey 4% | Honey 6% | Honey 8% |
|----------------|----------|----------|----------|----------|----------|
| Total LAB Room temperature | 1.1      | 1.7      | 2.9      | 3.7      |
| Total LAB Freezing temperature | 1.5      | 1.4      | 0.8      | 1.1      | 1.2      |

Figure 2. Comparison diagram of the growth of lactic acid bacteria in two beverage products stored at room temperature and freezing temperature.

The diagram above shows a comparison between the total lactic acid bacteria (LAB) corn yogurt drinks that have been investigated by Nofrianti et al (2013) [3] with the total lactic acid bacteria found in a functional-based drink with the addition of honey treatment respectively 2%, 4%, 6%, and 8% but by storing freezing temperatures.

A comparison of total lactic acid bacteria with the addition of the same honey variation was done in two different beverage products, namely corn yogurt with cassava-based functional drinks. Total lactic acid bacteria treated with the addition of honey 2%, 4%, 6%, and 8% on corn yogurt products tended to increase. While the total bacteria observed in cassava-based functional beverage products that were stored at freezing temperatures decreased in the treatment of honey additions of 2% and 4%, despite...
an increase in the treatment of adding honey 6% and 8% but were relatively lower than the total bacteria lactic acid in corn yogurt products that are not stored at freezing temperatures. The results of the study of tapai-based functional drinks experienced a decrease in total lactic acid bacteria during the storage of freezing temperatures even though honey has been added as a source of nutrition for the growth of lactic acid bacteria. Although probiotic microorganisms can last longer in product freezing time. However, probiotic cell membranes can be damaged during the freezing process due to the mechanical stresses of ice crystals that form on external media or inside cells [4].

4. Conclusion
The total lactic acid (LAB) bakery in cassava-based functional drinks (Manihot esculenta) in freezing temperature storage decreased in the treatment of honey additions of 2% and 4% but increased in the treatment of adding honey 6% and 8% despite the increase and decrease in total bacteria the results are not significantly different. This means that consideration needs to be given to storing functional cassava-based beverages at freezing temperatures.

References
[1] Sinjai B P S K 2018 Statistik Daerah Kabupaten Sinjai 2018
[2] Sinjai B P S K 2017 Pertanian Tanaman Pangan Kabupaten Sinjai Tahun 2017
[3] Nofrianti R, Azima F and Eliyasmi R 2013 Pengaruh penambahan madu terhadap mutu yoghurt jagung (Zea mays Indurata) J. Apl. Teknol. pangan 2
[4] Tripathi M K and Giri S K 2014 Probiotic functional foods: Survival of probiotics during processing and storage J. Funct. Foods 9 225–41
[5] Bidin N, Zainuddin N, Abdullah M, Marsin F and Yasin M 2015 Sugar Detection in Adulterated Honey via Fiber Optic Displacement Sensor for Food Industrial Applications IEEE Sens. J. 16
[6] Olofsson T C, Butler È, Markowicz P, Lindholm C, Larsson L and Vásquez A 2016 Lactic acid bacterial symbionts in honeybees—an unknown key to honey’s antimicrobial and therapeutic activities Int. Wound J. 13 668–79
[7] Hakim D, Tjahjaningsih W and Sudarno 2019 Antibacterial activity of honey in preserving high-pressure cooked milkfish stored at room temperature IOP Conf. Ser. Earth Environ. Sci. 236 12079
[8] Herawati D A and Wibawa A A 2011 Pengaruh konsentrasi susu skim dan waktu fermentasi terhadap hasil pembuatan soyghurt J. Ilm. Tek. Lingkung. 1 329–452