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Total lactic acid bacteria, total dissolved solids, and organoleptic properties of cocogurt with the addition of stevia leaf extracts

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Abstract

The term "cocogurt," a combination of the words "coconut" and "yogurt," is commonly used to describe yogurt prepared with coconut milk. Cocogurt is a lactic acid bacteria-fermented beverage made from coconut milk that has similar properties to yogurt. Sugar is used as the carbon source in the majority of commercially available fermented beverages. Customers, particularly diabetics, are demanding that food items have lower calorie levels, so sugar, which is known to have a high concentration of calories, is beginning to be avoided as a sweetener in some products. In the case of cocogurt, replacing sugar with stevia leaf extract could be an alternative solution. However, there has yet to be any reporting on the inclusion. This study aimed to determine the effect of adding stevia leaf extract to the total lactic acid bacteria, total dissolved solids, and organoleptic properties of cocogurt. This study involved four treatments and five replications, with additions of stevia leaf extract at varying concentrations (T0 = 0%, T1 = 0.5%, T2 = 2%, and T3 = 3.5%). The materials used in the research were grated coconut, stevia leaf powder, skim milk, and yogurt starter. According to the study's findings, adding stevia leaf extract in various quantities had a significant impact ($p<0.05$) on the amount of total lactic acid bacteria, total dissolved solids, sweetness, and acidity of cocogurt. The T0 treatment had the most lactic acid bacteria, with an average of $4.47\times10^8\pm0.125$ log CFU while the T3 treatment had the least, with $6.61\times10^7\pm0.20$ log CFU. Each treatment had at least 10^7 CFU of total lactic acid bacteria which is the minimum required by the Indonesian National Standard for fermented beverages. The T0 treatment had the lowest total dissolved solid value of 2.92±0.11°Brix and the T3 treatment had the highest total solid value of 3.26±0.17°Brix. Therefore, it can be concluded that cocogurt made with 0.5% stevia leaf extract contained the optimal amount of total lactic acid bacteria, meeting the requirements of the Indonesian National Standard, and had a moderately sweet and sour flavor in terms of sweetness and acidity.

1. Introduction

Many people's eating habits are changing as a result of increased public awareness of the importance of health, with people now preferring natural and nutritious food that attempts to prevent disease. This occurrence has established functional food as a trend in current food products and has driven numerous food industry parties toward the notion of "healthy, functional, and delightful foods" in the manufacturing of their products. Functional food is consumed to supply energy, thereby improving the health of the individual who consumes it (Havrlentová *et al.,* 2011). Functional food differs from food supplements or pharmaceuticals in that it helps to prevent disease, boost immunity, and lower the risk of disease (Adawiah *et al.,* 2015).

Fermented beverages containing LAB (Lactic Acid Bacteria) are common sources of functional food (Bekoglu *et al.,* 2016). The fermentation process of yogurt makes it beneficial for the digestive system and safe for lactose-intolerant individuals to consume. Yogurt is often manufactured from dairy products such as cow's milk, either with or without the inclusion of skim milk and cream (Lee and Lucey, 2010). Yogurt can also be made using several additional components, including oat milk, soybean juice, maize juice, and coconut milk (Lestiyani *et al.,* 2014).

Indonesia contributes approximately 27% of global

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coconut production and has the world's largest coconut area (Wibowo *et al.,* 2016). As a result, coconut can be used as an alternative to processed foods, particularly the flesh of the fruit converted into coconut milk. The addition of coconut milk can enhance the flavor of a culinary product. Although coconut milk possesses properties similar to cow's milk, it has not been widely used in fermented beverages such as yogurt. Furthermore, due to its high viscosity (Yaakob *et al.,* 2012), coconut milk is ideal for fermenting goods with a rich texture. Yogurt made with coconut milk is known as cocogurt, a combination of coconut and yogurt. One of the ingredients used in the production of cocogurt is a sweetener. Sweetener is a food additive used to enhance flavor and aroma, improve physical and chemical properties, as preservative, and as a calorie source (Cheung and Mehta*,* 2015).

Sucrose is the sweetener and carbon source for most fermented products on the market (Jayabalan *et al.,* 2014). However, because consumers, particularly diabetics, prefer low-calorie foods, sucrose, which contains many calories, is being avoided. Therefore, substituting sucrose with the additional stevia leaf extract can become one of the alternative methods. According to the research by Widodo *et al.* (2015), yogurt with the addition of stevia sweetener can be categorized as low-calorie yogurt because it has a calorific value of ≤ 60 kcal/100 g. Furthermore, stevia leaves contain polyphenols that have antioxidant, anti-inflammatory activity (Myint *et al.,* 2020) and an anti-diabetic effect (Shivanna *et al.,* 2013), which may benefit the body. However, there is no report yet about adding stevia leaf extract into cocogurt. This study aimed to determine the effect of adding stevia leaf extract to total lactic acid bacteria, total dissolved solids, and organoleptic properties of cocogurt.

2. Materials and methods

2.1 Materials

This study was conducted in the Laboratory of Food Engineering and Agricultural Products, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia. The materials used in this study were grated coconut obtained from the Semarang Teak market, stevia leaf powder, skim milk, starter yogurt (yogourmet), distilled water, and MRS agar. The instruments used were thermometers, pans, analytical scales, beaker glasses, test tubes, petri dishes, ovens, hot plate stirrers, autoclaves, micro pipets, *UV* light laminar air flow, hand refractometers and incubators.

2.2 Coconut milk preparation

Coconut milk was made by mixing 600 g of grated coconut with 1200 mL of hot water at 60°C or in a ratio of 1:2. Grated coconut was then squeezed and filtered. Coconut water was then stored in a closed container prior to use.

2.3 Preparation of stevia leaf extract

Stevia leaf powder measuring 80 mesh is dissolved in hot water at a temperature of 100°C with a ratio of 1:30. The solution is then allowed to stand for 30 min to form a precipitate, and then the solution is filtered so that the filtrate will be used to make the cocogurt. The stevia leaf extract is stored in a closed container until used (Wuryantoro and Susanto, 2014).

2.4 Starter preparation

Yogurt starter consisted of a mixture of *Lactobacillus bulgaricus, Streptococcus thermophilous,* and *Lactobacillus acidophilus* bacteria*.* Starter F1 was prepared by preparing 500 mL of skim milk, then pasteurized at 80°C for 10 mins. The skim milk was stored and cooled down until it reached the temperature of 43-45°C. Later, skim milk was inoculated with 1 sachet of yogurt starter package and incubated for 24 hrs at 37°C. After the F1 incubation, the F2 starter was made by mixing 50 parts of skim milk medium and 50 parts of coconut milk. The homogeneous medium was then pasteurized at 80°C for 10 mins using an autoclave. The medium was allowed to stand until it reached a temperature of 43-45°C, then was inoculated with 5% of the F1 starter. The incubation process was carried out at 37°C for 24 hrs. The F2 starter was then ready to use.

2.5 Cocogurt manufacture

Cocogurt was produced based on the method described by Syaputra *et al.* (2015) with some modifications. Coconut milk previously prepared (1200 ml) was divided evenly and put into 4 jar bottles (300 mL each). A total of 15% skim milk was added to each jar bottle. Stevia leaf extract powder was added at 0% for T0, 0.5% for T1, 2% for T2, and 3.5% for T3, and the mixture was stirred until it was homogeneous (Reis *et al.,* 2011). After that, coconut milk was pasteurized for 10 mins at 80°C. The pasteurized coconut milk was quickly cooled to 45°C. Then it was inoculated with a 5% starter. A six-hour incubation was carried out at 37 degrees Celsius. The finished yogurt was kept in a 5°C temperature-controlled environment.

2.6 Calculation of total lactic acid bacteria

Calculation of total lactic acid bacteria (LAB) was done by the total plate count method. The method was in

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accordance with the experiment of De Man *et al.* (1960) cited in Rahman *et al.* (2015) with modifications. Man Rogosa and Sharpe (MRS) was used as the culture medium for the growth of lactic acid bacteria such as *Lactobacillus* sp.

The dilution of samples of sterile distilled water at a ratio of 1:9 was the first step in calculating the total number of lactic acid bacteria (LAB). Dilutions were performed from 10^{-1} all the way down to 10^{-8}. The first dilution consisted of 1 mL of sample diluted in 9 ml sterile distilled water. The 10^{-1} dilution sample was diluted in 9 mL of sterile 0.85% NaCl physiological solution for the second dilution, and so on. Then, 1 ml of the diluted sample was placed in a sterile petri dish, followed by 10 mL of MRS agar. It was carried out in duplicate, with the final three dilutions being 10^{-6}, 10^{-7}, and 10^{-8}. Then, it was incubated at 43°C for 48 hrs. Total lactic acid bacteria were expressed in CFU/mL and calculated as follows:

$$\text{Total LAB} = \text{The number of colonies} \times \frac{1}{\text{The dilution factor}}$$

2.7 Measurement of total dissolved solids

The total dissolved cocogurt added with stevia leaf extract was measured by a refractometer following the method from the National Standardization Agency of Indonesia (2004). A small amount (1-2 drops) of the sample was put into the refractometer's prism, which was previously calibrated with distilled water. The prism end of the refractometer was pointed toward a light source, and the eyepiece was focused until the scale was visible. Then the scale in °Brix was read as the total amount of dissolved solids. The refractometer was rinsed with distilled water after each sample change.

2.8 Organoleptic analysis

Organoleptic analysis was carried out by twenty semi-trained panellists selected from students of the Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang. The assessment was carried out by providing 20 mL of T0, T1, T2, and T3 treatment samples for each panellist to be sampled and assessed based on the ranking method with sweetness and acidity parameters. The panellists evaluated and ranked the samples from most intense to least intense, with 1 being the most intense and 4 being the least intense in terms of sweetness and acidity.

2.9 Statistical analysis

Total LAB and total dissolved solids were analyzed statistically by ANOVA (Analysis of Variance) test using the SPSS 16.0 application for Windows. DMRT (Duncan's Multiple Range Test) was later used to analyze significant differences among the results. Meanwhile, the organoleptic test results were analyzed by Kruskal-Wallis nonparametric analysis with a significance level of 5%.

3. Results and discussion

Based on the results in Figure 1, there was a significant effect ($p<0.05$) on the total lactic acid bacteria with T0, T1, T2, and T3 treatments. However, there is no significant difference between T1 and T2 treatments. The highest total lactic acid bacteria were found in the T0 treatment, with an average value of 5 replicate samples was 4.47×10^{8} ±0.125 log CFU while the lowest value was in the T3 treatment which was 6.61×10^{7} ±0.20 log CFU. Each sample contained a minimum of 10^{7} CFU of total lactic acid bacteria, as required by the Indonesian National Standard for fermented beverages. It also indicated an increase in the quantity of stevia leaf extract reduced the total number of lactic acid bacteria. Adding stevia leaf extract may inhibit the growth of lactic acid bacteria. Widodo *et al.* (2015) found that while stevia leaf extract does not affect the fermentation process, it can inhibit the proteolysis of lactic acid bacterial cells during the first three hours of fermentation. Stevia leaf extract contains stevioside as well as other antimicrobial active substances such as alkaloids, flavonoids, and tannins. The main content of stevia leaves, stevioside, cannot be hydrolyzed and utilized by bacteria during the fermentation process (Putri *et al.*, 2017). However, it has the potential to kill pathogenic microbes, using stevia leaf extract as a sweetener will not result in the death of all lactic acid bacterial cells in cocogurt. This finding is in accordance with the research carried out by Davoodi *et al.* (2016), who demonstrated that certain strains of *Lactobacillus* possess properties that make them resistant to the bacteriocidal effect of stevia leaf extract. Therefore, even though it is not as good as sucrose sugar, using stevia as a carbon source in fermented beverages is still possible.

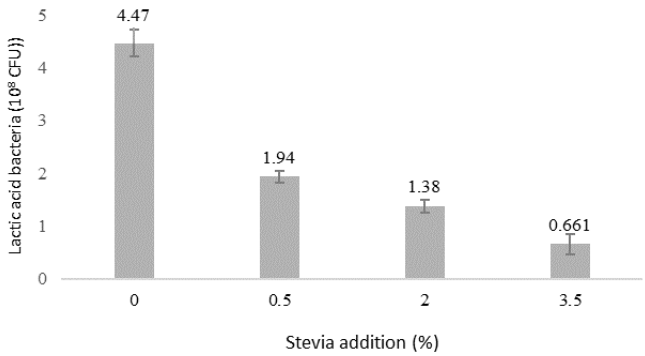

Figure 1. Total lactic acid bacteria of cocogurt.

Figure 2 shows significant differences ($p<0.05$) between the treatments. The lowest total dissolved solids

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were found in the T0 treatment at 2.92±0.11°Brix, while the highest total dissolved solids value was found in the T3 treatment at 3.26±0.17°Brix. Because of its ability to bind water, stevia leaf extract can raise the total value of dissolved solids. Sugar is hygroscopic, which means it dissolves more easily in water; the more bound water, the higher the total dissolved solids (Meikapasa and Seventilofa, 2016). The extraction process influenced the total value of dissolved solids in the stevia leaf extract. According to López Carbón *et al*. (2016), the total dissolved solids of stevia leaf extract ranged from 2.5 to 4.5°Brix, with the value influenced by the type of solvent used, extraction time, and temperature.

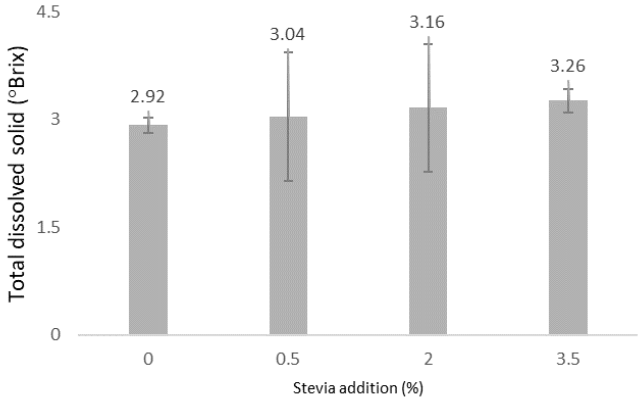

Figure 2. Total dissolved solid of cocogurt.

Total dissolved solids are also frequently used to determine a product's sugar amount. It is possible to calculate the product's total dissolved solids to analyze the total amount of sugar still present after lactic acid bacteria fermentation (Sintasari *et al.*, 2014). This analysis is necessary to determine how much sugar is still present. Sugar in the form of simple carbohydrates will be converted into lactic acid during the fermentation process by the metabolism of lactic acid bacteria. The total dissolved solids will decrease as the amount of sugar used to grow lactic acid bacteria decreases during fermentation (Bayu *et al.,* 2017). However, because stevia leaf extract cannot be used in the fermentation process by lactic acid bacteria, the total dissolved solids increase as the concentration of stevia leaf extract increases.

Based on Figure 3, the T3 treatment has the highest sweetness level, whereas the T0 treatment has the lowest sweetness level. The addition of a higher concentration of stevia leaf extract has led to an increase in the sweetness level of the cocogurt. The greater the amount of stevia leaf extract used, the sweeter the cocogurt. Stevia leaf extract is a sweetener that, when added to a product like cocogurt, is expected to improve the product's taste. The addition of a higher concentration of stevia leaf extract has led to an increase in the sweetness level of the cocogurt. Stevia leaf extract is a sweetener that, when added to a product like cocogurt, is expected to improve the product's taste. Furthermore, stevia has fewer calories than table sugar and contains a bioactive compound that may promote positive health effects. Stevia is an artificial sweetener that is effective at increasing sweetness while having a lower calorific value than sucrose sugar (Yaqub *et al.* 2018). Moreover, stevia leaf extract has a much higher sweetness level than sucrose. Stevia has a sweetness level that is 250–300 times sweeter than the sweetness level of sucrose and also possesses stable chemical and thermal conditions, thus having sensory characteristics that are quite similar to those of sucrose (Sukhmani *et al.*, 2018).

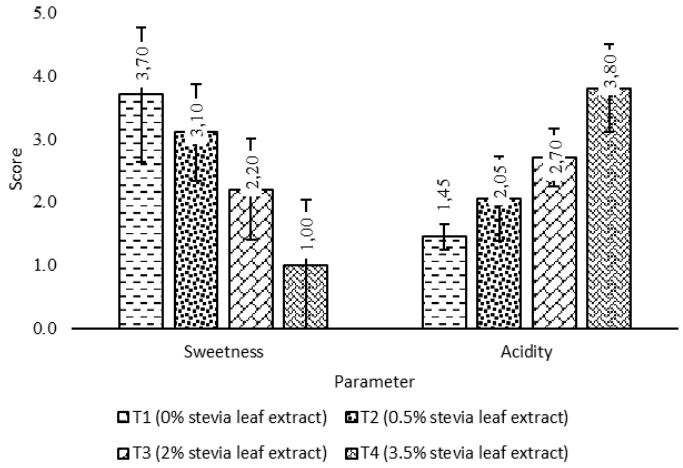

Figure 3. Sensory ranking test of cocogurt.

Despite its sweet flavor, stevia leaf extract has a slightly bitter aftertaste. According to Pinasti *et al.* (2018), even the addition of 1% stevia results in a bitter aftertaste. The bitter taste is caused by the active compounds found in stevia leaf extract. The presence of stevioside and tannin content may cause a bitter taste in the product (Chranioti *et al.,* 2016).

Figure 3 also indicates that the T0 treatment had the highest acidity, while the T3 treatment had the lowest. The addition of stevia leaf extract has a significant effect on the acidity of cocogurt at the 0.05 level of significance. The sour taste of cocogurt is caused by organic acids formed during fermentation. This follows the provisions of the National Standardization Agency of Indonesia (2009) that yogurt should have a distinct sour flavour. The sour taste of cocogurt is caused by organic acids formed during the fermentation process. As metabolites, Setiarto and Widhyastuti (2017) demonstrated that lactic acid bacteria produce organic acid components such as lactic acid, acetaldehyde, propionic acid, butyric acid, and volatile compounds. These ingredients give the product a distinct acid flavor. Because stevia acts as a sweetener, adding stevia leaf extract can help to reduce acidity. Adding sweeteners to yogurt can reduce the intensity of the sour taste

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(Kumalasari *et al.*, 2013).

4. Conclusion

According to the findings of the study, the T0 treatment had the highest levels of lactic acid bacteria, total dissolved solids, and acidity, while the T3 treatment had the lowest levels of lactic acid bacteria, total dissolved solids and acidity. Each treatment had at least 10^7 CFU of total lactic acid bacteria, the minimum required by the Indonesian National Standard for fermented beverages. Therefore, it can be concluded that cocogurt produced using 0.5% stevia leaf extract resulted in cocogurt that contained the optimal amount of total lactic acid bacteria and thus fulfilled the requirements of the Indonesian National Standard. It also had a moderately sweet and sour flavor in terms of sweetness and acidity.

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