The Effect of Brown Sugar Addition on Total Lactic Acid Bacteria (LAB) and Total Dissolved Solids (TDS) of Young Coconut Water *(Cocos nucifera L.)* Probiotic Drinks

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Abstract. Probiotic drinks are fermented drinks that contain lactic acid bacteria and have various health benefits, including maintaining gastrointestinal health and increasing immunity. In this study, probiotic drinks were developed using young coconut water, which is expected to be an alternative fermented product made from plant-based with affordable prices. The research aimed to determine the total lactic acid bacteria (LAB) and total dissolved solids (TDS) in young coconut water probiotic drinks with different brown sugar additions. The experiment of this study was conducted using a single-factor Completely Randomized Design (CRD) method with brown sugar addition (0%, 5%, 10%, and 15%), skim milk concentration (3%), starter of *Lactobacillus casei* (2%), and 48 h of fermentation. The research was conducted in two treatment replications with two laboratory analyses. Total LAB was examined using CFU/mL calculations, and TDS was examined using a hand-refractometer with 0–32% Brix. The results showed that the brown sugar addition to young coconut water probiotic drinks did not affect the total LAB with $p = 0.134$. However, the total LAB in all treatments fulfilled the minimum standard of SNI 10^6 CFU/mL. Furthermore, brown sugar addition significantly affected the TDS, with $p = 0.000$. This result was subsequently continued using the LSD test $\alpha = 5\%$ and showed a noticeable difference in all treatments. The TDS produced in this study has also fulfilled the minimum standard of SNI, which is 8.2% Brix in brown sugar additions of 5%, 10%, and 15%.

Keywords: Young Coconut Water · Probiotic Drink · Total Lactic Acid Bacteria · Total Dissolved Solids

1 Introduction

Lifestyle and diet can become problems that impact human health. Contemporary diets, such as consuming junk food and high-fat and low-fiber foods, generate various diseases, especially those related to the digestive tract. It prompts people to consider the significance of selecting foods and drinks with high nutritional content and positive health effects [1].

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Functional food is also defined as food or drink that affects one or more beneficial target functions in the body. Furthermore, it has health effects that are relevant in improving health or reducing disease risk. Most functional food products utilize the metabolic activity of bacteria, which can produce bioactive composition and specific flavors in functional food products [2]. One of the functional food products that are beneficial for human health and can balance the microflora in the digestive tract is probiotic food or drink [3].

Probiotics are good bacteria that live and can provide health benefits and maintain gastrointestinal health if provided in sufficient quantities [4]. Probiotic products can benefit from modulating the immune system and supporting gut health by stabilizing good microflora in the gastrointestinal tract [5]. In addition, microbes added to probiotic drinks can also serve to eliminate toxins in the body [6]. The wider community has widely recognized probiotic drinks. They are frequently produced from fermented milk products such as yogurt, Bulgarian milk, kefir, kumiss, piima, skyr, taetee, leben from Egypt, dahi from India, hamao from Central Asia, yakult, etc. [7]. However, fermented products from dairy products are relatively more expensive. Therefore, it is necessary to develop probiotic drink products with non-dairy or plant-based ingredients as an alternative to milk-based probiotic drinks to be more widely accessible to the community.

Coconut water is an endosperm fluid that fills the middle cavity of the coconut fruit. Coconut water is considered one of the most versatile natural products in the world because it has benefits in various fields, especially tissue culture, food, and medicine. Based on the nutritional content of young coconut water, several researchers and health experts have outlined multiple properties that can be obtained when consuming young coconut water, including as a thirst reliever, a healer of several types of diseases, medicine, beauty, and also a growth medium [8].

The main challenges coconut water producers encounter are product shelf life, and the preservation of the product's characteristics, and the distribution of the product to non-coconut-producing areas. Coconut water naturally contains oxidative enzymes that are easily damaged when it is still in the fruit, called sterile coconut water, but when it is extracted and exposed to direct air, it can undergo oxidation or microbial contamination related to improper extraction, which will result in a loss or decrease in the quality of coconut water [9]. Employing adequate processes or innovations to preserve coconut water is necessary to extend its shelf life, maintain its quality and optimize its utility.

One of the methods employed for the process of preserving food or drinks is fermentation. Fermentation is a process that brings chemical changes in each protein, carbohydrate, or fat component of a product through the action of enzymes released by specific microorganisms. Microorganisms that contribute to the development of fermented products are recognized as starters. The most constant starter employed in the fermentation process is lactic acid bacteria (LAB) [10]. Coconut water can be applied as a fermented drink or probiotic. Coconut water probiotic drink is one of the non-dairy products made from plant-based. Thus, it can be a solution to develop probiotic beverages at affordable prices that the wider community accepts.

Young coconut water is rich in nutrients such as sugar, protein and fat, which is relatively complete. The content of these nutrients contributes vitally to supporting the growth of good bacteria-producing food products in the fermentation process. Young coconut water also has an acidic pH, which is appropriate for bacterial growth. In the fermentation process, one of the nutritional components required is sugar. Young coconut water has sugar content in the form of simple carbohydrates such as glucose and fructose. The existence of this sugar contributes vitally as fermentable sugar to be a source of carbon and nutrients for microorganisms [11].

The use of young coconut water as a fermentation medium in the production of probiotic drinks can be a valuable option for development. Young coconut water contains a type of sugar, monosaccharides with glucose, fructose, and disaccharides in sucrose. The content of monosaccharides such as glucose and fructose in coconut water is easier to utilize by LAB [12]. However, the sugar content in the required young coconut is still relatively low, and sugar addition is necessary as a carbon source to support the growth of microorganisms during the fermentation process and as a sweetener so that the probiotic drink products produced are not too acidic [13]. In previous studies, sugar in sucrose or granulated sugar was added as a carbon source for microorganisms to obtain probiotic drinks from young coconut water. Furthermore, in this study, researchers innovated using another alternative to sucrose from granulated sugar, brown sugar, as an added sugar in producing young coconut water probiotic drinks.

Brown sugar, commonly recognized as Javanese sugar, is a type of sugar made from coconut juice. This sugar is normally a brown cylindrical shape and can also be employed as a sweetener in food or drinks. Brown sugar has a higher content of micronutrients such as calcium, phosphorus and iron than that of granulated sugar [14]. In addition, based on previous research on the effect of brown sugar addition and fermentation time on metabolites and microbial communities of Yibin Yacai, it was found that brown sugar addition can increase the activity of microorganisms such as *Lactobacillus* and *Debaryomyces* [15]. Brown sugar also has a lower glycemic index of 35% compared to cane sugar which is 75%. Regarding the glycemic level limit of sugar that is good for health is 40%, brown sugar can be considered better for health [16].

Brown sugar is frequently applied to enhance sweetness or flavoring spices in cooking on a small and large scale. In addition to solid printed sugar, brown sugar processing is also widely applied in the form of brown sugar syrup or liquid brown sugar. Due to the numerous applications for brown sugar and its source of protein, brown sugar can also be applied as a sweetener and source of carbohydrates in developing drink products such as probiotic drinks.

LAB is a potential microorganism and has been widely applied in food fermentation worldwide due to its recognized safe status. Through the fermentation process, the presence of LAB can improve food taste, improve organoleptic attributes, enrich nutrients, and increase health benefits [17]. Changes in the total amount of LAB occurred during fermentation. In fermentation, lactic acid bacteria convert lactose into lactic acid [18]. Increased activity of microorganisms, which can affect the total LAB. LAB alters substrates' biochemical and organoleptic characteristics to produce various metabolites and enrich foods or drinks with micronutrients such as minerals, vitamins, amino acids, probiotics, prebiotics, and digestive enzymes [19]. Some factors that affect the viability of probiotic bacteria are storage temperature, oxygen availability, pH, and the emergence of microorganism competitors. The suitability of probiotic bacteria to substrates can be evaluated through several parameters such as organoleptic, microbiology and chemistry in probiotic beverage finished products [20].

In this study, the type of LAB administered for fermentation is *Lactobacillus casei*. Lactic acid bacteria of this species are also admitted as probiotic agents and belong to the Generally Recognized as Safe (GRAS) species in fermented food or beverage products to be consumed by humans [21]. Some general characteristics of an organism as a probiotic agent include non-pathogenic, which is resistant to technological processes such as viability and stability when shipping by vehicle, the resistance to stomach acid, the attachment to the target epithelial tissue, the ability to live in the gastrointestinal tract, the production of antimicrobial substances, the ability to modulate the immune system, positive effects on health, safe to use as a starter, and competitive against pathogenic bacteria [22, 23].

Adding sugar to the fermentation process can also affect the Total Dissolved Solids (TDS) of probiotic beverages [24]. TDS contains elements or materials dissolved in solutions such as glucose, fructose, sucrose and other complex compounds [25]. LAB will apply sugar as an ingredient added to probiotic drinks as a carbon source and energy during the growth phase, so the greater the number of LAB colonies, the more it will affect the total solids to increase [26].

Based on this background, brown sugar can be added to develop young coconut water probiotic drinks. The brown sugar addition with different amounts of variation as an additional source of nutrition for starter bacteria is expected to affect bacterial growth and physicochemical properties such as dissolved solids in probiotic drinks.

2 Research Methods

2.1 Materials

Lactobacillus casei bacteria FNCC 0040 was obtained from the Food and Nutrition Center, Universitas Gadjah Mada. De Mann Rhagosa Agar (MRSA) and de Mann Rhagosa Broth (MRSB) medium were purchased from Chem-Mix Yogyakarta. Young coconut water from green coconuts was purchased from a coconut fruit seller in Pabelan, Sukoharjo. Afterward, the other ingredients were skim milk, brown sugar, and other additives such as NaCl, aquades and alcohol. The utensils utilized for this study include autoclaves, sterilizers, incubators, waterbaths, test tubes, erlenmeyer, electric stoves, thermometers, bunsen, petri dishes and volume pipettes.

2.2 PH and Total Dissolved Solids (TDS) Testing in Young Coconut Water

Prior to fermentation and developing young coconut water probiotic drink products, fresh young coconut water was initially analyzed for pH and TDS levels to determine the increase or decrease in chemical and physical properties after fermentation. pH testing was conducted using a pH meter, while TDS testing used a hand-refractometer with 0–32% Brix.

2.3 Preparation of Bacterial Culture Starter

At the starter for production stage, a culture stock is prepared to obliquely *Lactobacillus casei*, taken as much as one inoculating loop, and grown in 5 mL medium de Mann Rhagosa Broth (MRSB). The culture was incubated at 37 °C for 17 h. The incubated broth culture was taken subsequently 5 mL and added to 50 mL of pasteurized coconut water, eventually incubated at 37 °C for 24 h until it became a liquid starter.

2.4 Development of Young Coconut Water Probiotic Drink

The Erlenmeyer is filled with 800 mL of filtered young coconut water and wrapped in aluminum foil. Young coconut water was subsequently transferred into sterile beaker glasses of 100 mL each, 3% skim milk, and in every two beaker glasses that had been filled with young coconut water, brown sugar with 0%, 5%, 10%, and 15% to be pasteurized for ±10 min at 70 °C. After the temperature decreased to 31–35 °C, pasteurized young coconut water was transferred into sterile vials, inoculated with 2% *Lactobacillus casei* starter, and incubated in an incubator with a temperature of 37 °C for 48 h. This young coconut water probiotic drink was produced at the Microbiology Laboratory, Faculty of Health Sciences, Universitas Muhammadiyah Surakarta.

2.5 Testing Method of Total Lactic Acid Bacteria (LAB)

This study conducted the total LAB analysis using the pour plate method in the MRS agar medium. This method is applied by diluting the sample in a solution of physiological salts to a dilution of 10^{-6}. In the last two dilution series, samples were taken 1 ml each and poured subsequently on a sterile petri dish and added MRS agar media. Thus, it was carefully and should not be opened excessively wide to avoid contamination. The MRS agar poured on the petri dish is later displaced on the table by forming the number eight for five times to spread the lactic acid bacteria cells evenly. The petri dish is incubated at 37 °C for 48 h after hardening. The Total Plate Count (TPC) figure was calculated using the colony counter by multiplying the number of colonies by the dilution factor and divided subsequently by the number of samples. Furthermore, the result of this total LAB calculation was stated by colony forming unit/mL (CFU/mL). The calculation of total LAB was conducted at the Microbiology Laboratory of the Faculty of Health Sciences, Universitas Muhammadiyah Surakarta.

2.6 Testing Method of Total Dissolved Solids (TDS)

TDS testing in young coconut water probiotic drink products was conducted using a hand-refractometer with 0–32% Brix. This test begins with the calibration of a hand-refractometer using aquades. Afterward, a sample of 1–2 ml of young coconut water probiotic drink dripped on the refractometer's prism. The results of the percent degree of Brix can be comprehended by reading the scale at the tool's end. This testing was conducted at the Food Science Laboratory of the Nutrition Science Study Program, Faculty of Health Sciences, Universitas Muhammadiyah Surakarta.

2.7 Statistical Analysis

Statistical analysis was implemented through normality and homogeneity test. The data was spread normally but not homogeneously in the total LAB test. Accordingly, the Kruskal Wallis test was administered. The test result showed no significant difference with $P < 0.05$. Furthermore, the TDS testing spread the data normally and homogeneously. It means the One-Way ANOVA test was conducted. After being tested, the sugar addition treatment showed a significant influence. Thus, the evaluation was continued with the LSD test $\alpha = 5\%$ to discover the noticeable differences in each treatment. All data were analyzed using SPSS version 26 for Windows (IBM, Corp., Armonk, NY, USA).

3 Results and Discussion

3.1 Results of Preliminary pH and Total Dissolved Solids (TDS) Test in Young Coconut Water

In the preliminary test, the pH and TDS analysis results were pH levels of 5.8 and TDS of 4.8% Brix. This analysis is implemented to identify the increase or decrease in chemical and physical properties of the product after the fermentation process.

3.2 Effect of Brown Sugar Addition on Total Lactic Acid Bacteria (LAB)

LAB is one of the specific parameters that can be tested on young coconut water probiotic drink products because the total LAB is an indicator of the success of fermented products. Based on Table 1 in the Kruskal Wallis test, it is recognized that the increasing percentage of brown sugar addition shows no significant difference ($p > 0.05$). The absence of this difference can be expected because certain factors affect the growth of LAB during the incubation period, such as less-than-optimal and unstable storage temperatures.

Table 1 shows that the mean of total LAB obtained from the brown sugar addition formula of 0%, 5%, 10% and 15% is 9.9×10^8; 2.7×10^9; 2.0×10^9; and 1.7×10^9, respectively. LAB growth from all brown sugar addition variations treatments has fulfilled the SNI (Indonesian National Standards) 7552: 2009 standard in probiotic drinks, which requires a minimum total LAB of 1×10^6 CFU/mL. Consequently, the starter culture of *Lactobacillus casei* bacteria can survive easily in young coconut water fermented at 37 °C for 48 h. The total amount of LAB that has fulfilled the minimum standards

Table 1. Mean of Total LAB in Young Coconut Water Probiotic Drink with Brown Sugar Addition

Brown Sugar Addition	**Mean of Total LAB ± SD**	***P Value***
0%	9,9 × 108 ± 1,4 × 108	0,134
5%	2,7 × 109 ± 1,6 × 109	
10%	2,0 × 109 ± 4,0 × 108	
15%	$1,7 \times 10^9 \pm 1,5 \times 10^9$	

means that it is safe for consumption and can positively influence the gastrointestinal tract's health.

The highest total LAB obtained in young coconut water probiotic drinks was in brown sugar addition of 5%. In comparison, the lowest total LAB was in brown sugar addition of 0% or control variables. The greater the addition of brown sugar to 5%, the more microbial growth has increased rapidly. In addition, when it is increased to 10%, the number of microbes begins to decrease. However, it presents that the amount of brown sugar added to the fermentation process will be optimal at 5%. This research corresponds to the previous study regarding the total LAB in green coconut water kefir. The study states that in different treatments, the average amount of microbes presenting in fermented drinks increased but did not provide a noticeable difference, and it occurred because the microbes undergo a growth phase, the exponential phase in which the microbes present in the fermented product have adjusted to the substrate conditions to maximize the process of cell growth [27]. In addition, according to the results of other studies, in the exponential phase, microbes can undergo rapid growth along with the increasing number of cells since there are sufficient nutrients and the proper environmental conditions to multiply cells [28].

3.3 Effect of Brown Sugar Addition on Total Dissolved Solids (TDS)

Table 2 presents that the mean of TDS ranges from 6.6 to 19.1 and increases as the percentage of brown sugar increases. The One-Way ANOVA test showed a significant difference ($p < 0.05$) in each percentage of brown sugar addition to the total dissolved solids of young coconut water probiotic drinks. After continuing with the LSD test, $\alpha = 5\%$, a noticeable difference was obtained in each percentage of brown sugar addition (0%, 5%, 10% and 15%).

Based on the requirements of SNI (Indonesian National Standard) 7552: 2009, the minimum amount of TDS contained in probiotic drinks was 8.2% Brix. Regarding young coconut water probiotic drinks with variations of brown sugar addition created for the 0% treatment, the value of TDS is only 6.6% Brix. Furthermore, it had not fulfilled the standards for probiotic drinks that have been set, while the 5%, 10% and 15% treatments fulfilled SNI standards in probiotic drinks.

Table 2. Mean of TDS in Young Coconut Water Probiotic Drink with Brown Sugar Addition

Brown Sugar Addition	**Mean of TDS ± SD**	***P Value***
0%	6,6 ± 0,18a	0,000
5%	10,8 ± 0,21b	
10%	14,5 ± 0,41c	
15%	19,1 ± 0,43^{d}	

Description: Different letters indicate that there are noticeable differences between the treatments.

The test results showed that the TDS value had increased significantly. The TDS value probably increased along with the percentage of brown sugar addition. It can occur since LAB and lactic acid secreted from the cells will accumulate in probiotic drink products and be counted as total dissolved solids [29]. The results of this study correspond to the previous study regarding the total dissolved solids of green coconut water kefir with the addition of High Fructose Syrup (HFS). The study showed that the higher concentration of HFS added, the higher the total dissolved solids contained in a product in which sugar is the main component. The increasing total value of dissolved solids occurs since the substrate required by the microbes in the fermentation product has been reasonably sufficient. The higher the addition of sugar concentrations, the more it can inhibit its activity [30].

The results contained in the total dissolved solids of young coconut water probiotic drinks are simple compounds derived from the breakdown of various nutrients such as carbohydrates, fats, and proteins. In addition, there is also an organic acid content, a type of total dissolved solids, in addition to sugars, pigments, and vitamins [13]. The higher the addition of brown sugar, the higher the total value of dissolved solids. It means that the simple molecules contained in probiotic drinks also increase. These simple molecules will facilitate the work of the digestive tract and can increase the absorption of subsequent nutrients.

4 *C*onclusion

This study concludes that brown sugar addition can increase the total LAB in young coconut water probiotic drinks. However, it did not affect the total LAB ($p = 0{,}134$). In addition, the higher the percentage of brown sugar addition, the higher the TDS contained in the fermented product, showing a significant effect ($p = 0{,}000$). Young coconut water probiotic drinks produced in this study broadly have fulfilled the requirements of SNI 7552: 2009 on total LAB with a minimum standard of 1×10^6 CFU/mL, and TDS also comply with the SNI standards on variations in the brown sugar addition (5%, 10% and 15%) with a minimum standard of 8.2% Brix.

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