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# Reduction, and Organoleptic Properties

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# Utilization of Lesser Yam (*Dioscorea esculenta* L.) Flour as Prebiotic in Yogurt to Total Lactic Acid Bacteria (LAB), Sugar Reduction, and Organoleptic Properties

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## Abstract

This study aims to determine the effect of the concentration the addition of lesser yam as prebiotic to total Lactic Acid Bacteria (LAB), reducing sugar content, crude fiber, viscosity, and organoleptic properties of yogurt with a combination of three bacteria (*Streptococcus thermophilus, Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*). The design of this study used a completely randomized design (CRD) with 4 treatments and 5 replications with variations in the addition of lesser yam tuber, namely T1 with a concentration of 0%, T2 with a concentration of 2%, T3 with a concentration of 4% and T4 with a concentration of 6%. The raw materials used are pasteurized fresh cow's milk, lesser yam tuber flour, and yogurt starter. The results showed that the addition of different lesser yam tuber flour had a significant effect ($P < 0.05$) on total LAB, sugar reduction, crude fiber, viscosity, and organoleptic properties of yogurt. The ideal treatment for the addition of lesser yam tuber flour is the concentration of 2% lesser yam tuber, which produces a total LAB is $9.2 \times 10^9$, a sugar reduction is 0.653 mg/mL, crude fiber is 1.3%, 82.25 cPs, and organoleptic properties had sour taste and viscosity is rather thick which the most preferred.

## Keywords

yogurt, prebiotic, lactic acid bacteria, yam tuber flour.

## 1 Introduction

Functional drinks began to develop in various communities in Indonesia, which of one was yogurt. Yogurt is a fermented milk product obtained from the milk or the milk products by the lactic acid fermentation through the action of probiotics bacteria. Yogurt is made using a mixture of two *Streptococcus thermophilus* bacteria and *Lactobacillus bulgaricus* bacteria where both of these bacteria have the ability to convert lactose to lactic acid during the fermentation process [1]. Probiotics are defined as living microorganisms that are consumed by humans or animals in sufficient quantities, are able to live and pass through the digestive tract and improving its intestinal microbial balance [2].

*Lactobacillus acidophilus* is a species of bacteria belonging to probiotics. The ability of *L. acidophilus* to utilize lactose and sucrose supplements contained in milk for its metabolic activity is optimal so that it produces relatively high lactic acid. *L. acidophilus* strains are the source of lactase, the enzyme needed in the digestion of milk products, which lacks in lactose-intolerant people [3]. The combination of probiotics and prebiotics can be called synbiotics. Prebiotics are live microbial feed supplements which beneficially affects the host animal by improving its microbial balance [4].

The lesser yam tuber is known to have a prebiotic content in inulin. Inulin is polysaccharides composed of fructose units with the configuration of the anomeric C2, which makes inulin-type fructans resistant to hydrolysis by human intestinal digestive enzymes that have specificity for glycosidic bonds [5]. Inulin is soluble in water, cannot be digested by digestive enzymes. The large intestine has inulin which is almost all inulin fermented into short-chain fatty acids and lactic acid produced by several microflorae. Inulin is digestible by certain microorganisms living in the gut that have inulinase activity including lactobacilli. This means inulin can pass through the human digestive system in relatively intact condition until it reaches the large intestine, this makes inulin a valuable probiotic dietary fiber [6]. Lesser yam (*Dioscorea esculenta* L.) is largely consumed in the local farmsteads and urban areas as boiled or fried yam. It had relatively short

 https://doi.org/10.29037/digitalpress.22325

(sometimes about 2 months) shelf life of the harvested tubers at shaded tropical ambient conditions [7]. The addition of lesser yam tuber flour to yogurt functional drinks is expected to increase the content of food fibers in the form of inulin in yogurt.

## 2 Materials and Methods

The materials used in this study were lesser yam tuber, fresh milk, mixed bacterial starter namely *S. thermophilus, L. bulgaricus,* and *L. acidophilus,* physiological saline solution, distilled water, alkaline Cu reagent, arsenomolybdate reagent, $H_2SO_4$, NaOH, ethanol 70%, ethanol, Whatman paper. The tools used are incubators, bacteria-specific incubators, ovens, analytical scales, pans, gas stoves, pH meters, measuring cups, test tubes, Erlenmeyer, Petri dishes, thermometers, Bunsen, refrigerators, micropipettes, spectrophotometers, laminators, cuvettes, buncher funnel, viscometer, filter, porcelain cup, scales, desiccator, measuring flask, magnetic stirrer, Erlenmeyer flask, pipette, cup goblet or cup glass and autoclaves.

Making lesser yam flour is done by preparing the tuber then peeling and washing it. Then the lesser yam tuber is thinly sliced and soaked with salt. The oven is then heated at 60°C for 6-8 hours until it is evenly dried. After drying, it is smoothed using a grinder and sifted using an 80 mesh screen. Making yogurt begins with fresh milk as much as 10 liters divided into 4 treatment units each of 2.5 liters. Addition of lesser yam tuber flour is done by means of flour mixed into the milk according to the treatment, namely T0: without addition of lesser yam tuber flour, T1: addition of 2% lesser yam tuber flour from the amount of milk (b/v), T2: addition of 4% starch tuber from the amount milk (b/v), T3: 6% addition of lesser yam tuber from the amount of milk (b / v). Fresh milk that has been added to the lesser yam flour is pasteurized at 80°C for 15 minutes then cooled to a temperature of 42°C. Pasteurized milk was inoculated with F2 yogurt starter as much as 5% of the milk volume with a density of $\geq 10^6$ CFU/mL [8]. Then incubated at 42°C for 6 hours. The finished yogurt is carried out by a storage process at a temperature of 5°C to inhibit the fermentation process. The yogurt that has been made is tested for total LAB, pH values, sugar reduction, and organoleptic properties were viscosity and sour taste.

### 2.1 Total LAB

Total LAB was calculated by using 1 mL pipette sample into physiological saline 0.85% as much as 9 mL for $10^{-1}$ dilution and carried out until $10^{-8}$. The samples were then taken 1 mL starting from $10^{-6}$ dilutions until $10^{-8}$ dilutions were put in a petri dish in duplicate. The petri dish then poured media de Man, Rogosa, Sharpe (MRS) agar. then incubated for 24 hours at 37ºC in an incubator with the inverted cup position. The amount of LAB is stated in the CFU / mL (Colony Forming Unit) with the formula:

$$T \quad L = n \quad o \quad c \quad \frac{1}{d \quad f}$$

### 2.2 Sugar Reduction

The reduction of sugar content testing was carried out using the Nelson-Somogyi method spectrophotometry which measured visible light absorption at a wavelength of 540 nm.

### 2.3 Total Crude Fiber

Crude fiber analysis was tested by taking 1 mL (x weight) from the sample, then inserted into a 600 mL cup with 50 mL of $H_2SO_4$ 0.3 N and heated for 30 minutes. The sample was added 25 mL of NaOH 1.5 N and reheated for 30 minutes [9]. Whatman paper number 41 is dried in a dryer at a temperature of 105-110 $^0$C for one hour and inserted into a Buchner funnel. During the filtering process, the precipitate is washed repeatedly with 50 mL of hot distilled water $H_2SO_4$, with enough aqua dest, and ultimately with 70% 25 mL ethanol. Filter paper and its contents are inserted into a porcelain dish and dried for 6 hours in an oven at 105$^0$C then cooled in a desiccator and weighed (weight b) porcelain cup and its contents are burned and ignited in an electric furnace at a temperature of 400-600°C for 6 hours then cooled and placed in a desiccator and weighed (weight c).

### 2.4 Organoleptic properties

Tests on the organoleptic properties of thick yogurt texture were carried out with rather trained panelists of 25 people in terms of scale 1 to 5. The hedonic test is done by Mudgil, Barak, Khatkar [10] which has been modified, namely 25 untrained panelists were asked to provide an assessment of preferences for the color, texture, aroma, taste and overall preference of the sample. Hedonic test score using scale range (1) dislike a lot, (2) dislike a little, (3) neither like or dislike, (4) like a little, (5) like a lot.

### 2.5 Data analysis

The results of the total LAB and viscosity value test were carried out by the normality test with the Kolmogorov-Smirnov to find out the data were normally distributed, then analyzed the effect test using Analysis of Variance (ANOVA) and its followed by Duncan's Multiple Range Test (DMRT) to determine the effect between treatments and levels 5% significance. Sugar reduction and crude fiber test were analyzed by descriptive. Organoleptic and hedonic testing data were analyzed by the Kruskal-Wallis test and if there was an effect carried out further testing using the Mann-Whitney U Test at a significance level of 5%. These data are analyzed by SPSS 16.0.

## 3 Result

The total LAB of yogurt with variations in the addition of lesser yam can be seen in **Fig 1**.

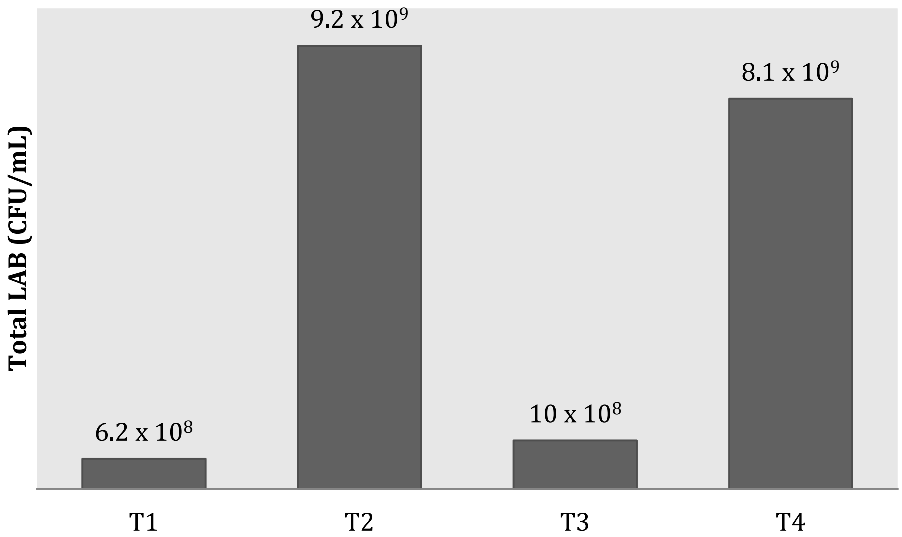


**Fig 1.** Total LAB Test Results of Yogurt

Information:
* Data is displayed as a mean value of 4 replications
*T1, T2, T3 and T4 = The lesser yam tuber flour concentration: 0%, 2%, 4% and 6%

Based on the results of the total analysis of LAB, yogurt with different treatments for the addition of Gembili tuber showed a significant effect ($P < 0.05$). The total LAB results are greater than $10^7$ CFU/mL so that it meets the minimum standards in yogurt as regulated by the Indonesian National Standardization Agency (SNI) 2981: 2009 that the minimum total amount of LAB in yogurt without heat treatment after fermentation is $10^7$ CFU/mL. Yogurt with the treatment of 2% addition of lesser yam tuber shows the highest results of LAB. This is due to the inulin content in lesser yam flour that can support the symbiosis of the three culture bacteria so that BAL can grow optimally. In addition, LAB also requires carbon source as a nutrient to grow. Then the total amount of LAB in T3 treatment higher than treatment T1. The addition of 4% of lesser yam tuber flour can increase the growth of LAB in yogurt. However, it was not significantly different from yogurt with the addition of 6% yam tuber flour. The addition of 6% tuber

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flour showed a lower total LAB for yogurt because the addition of too much sugar can inhibit the growth of LAB. This is consistent with the opinion by Endo, Yonemori, Hibi, Ren, Hayashi, Tsugawa, and Sode [11] that high sugar concentrations are known can inhibit bacterial growth.

### 3.1 Sugar Reduction

Based on the research that has been done, the reduction of sugar content in yogurt is presented descriptively in **Fig 2**.

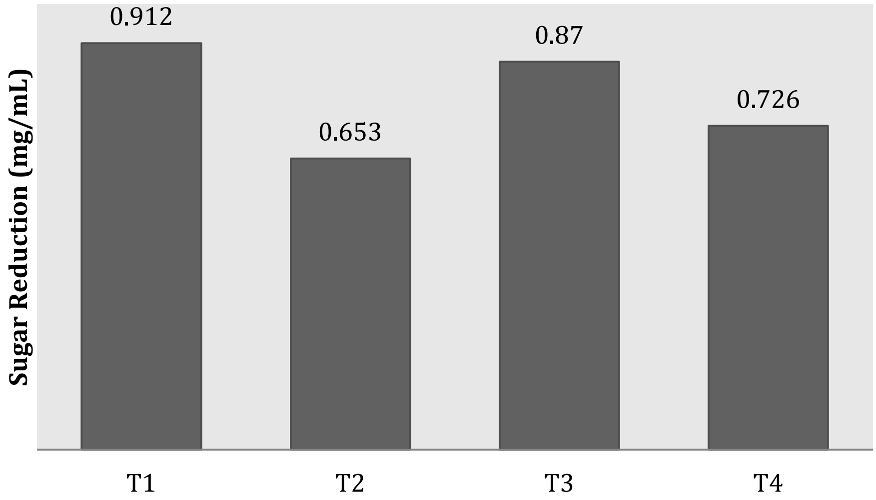


**Fig 2.** Sugar Reduction Test Result of Yogurt

Based on the results obtained, it is known that the reducing sugar level of treatment T1 shows the highest yield of 0.912 mg/mL. The reduced sugar content of treatment T1 shows a large amount caused by the decomposition of milk lactose into lactic acid, but the yield of reducing sugars is not maximally utilized. While the treatment of T2 showed the lowest reducing sugar level of 0.653 mg/mL. Then in the T3 treatment obtained a reduced sugar content of 0.87 mg / mL. T3 treatment showed a fairly high reduction in sugar content, meaning the addition of lesser yam flour as an inulin producer became a provider of growing nutrients for LAB. The amount of reducing sugar increased with heating the yogurt. This is in accordance with the opinion of Glibowski and Bukowska [12] that a higher temperature is necessary to release more than 60% reducing sugar when the acidity decreased. The reduction of sugar level in T4 treatment was 0.726 mg /mL. This is because BAL can utilize inulin to grow nutrients optimally, but LAB, especially Lactobacillus acidophilus takes a long time to convert sugar into lactic acid.

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### 3.2 Crude Fiber

The results of the analysis of crude fiber in yogurt added with yam tuber can be seen in **Fig 3**.

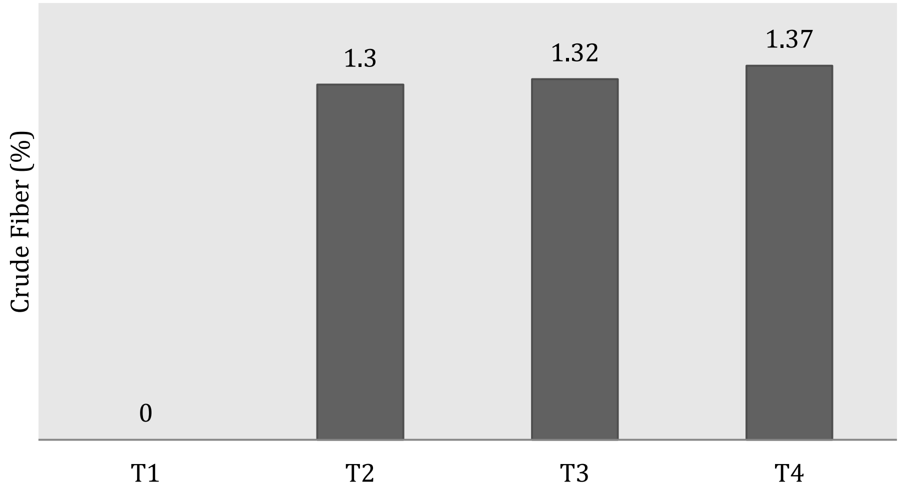


**Fig 3** Crude Fiber Test Results Yogurt

Description: At T1, it was not analyzed because crude fiber at T1 was considered = 0. Data is displayed as mean values: T2, T3 and T4 = Addition of yam tuber flour: 0%, 2%, 4% and 6%.

Based on Fig. 1, there is an increase from T1 to T4. This happens because the higher concentration of lesser yam tuber flour will increase the crude fiber content in yogurt. As proposed by Amal et al. [13] that the crude fiber content in yogurt will be higher if added with a material containing high crude fiber.

### 3.3 Viscosity

The results of testing the viscosity of yogurt added with lesser yam tuber flour can be seen in **Fig 4**.

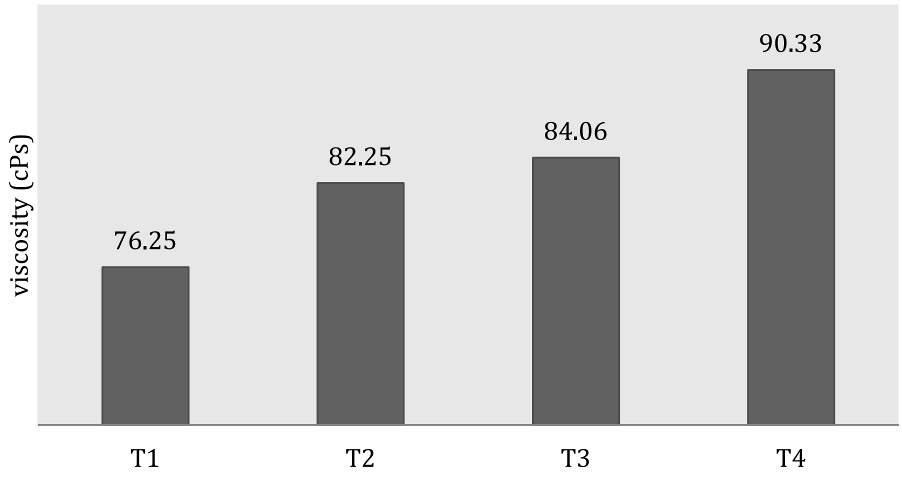


**Fig 4** Viscosity Test Results in Yogurt

Description:
Data is displayed as a mean value of 5 replications. Different superscript notations showed significant differences ($P < 0.05$). T0, T1, T2, and T3 = Concentration of lesser yam tuber flour 0%, 2%, 4%, 6%.

The results of the analysis presented in Table 1 show that treatments T1, T2, T3, and T4 have significant differences ($P < 0.05$). The viscosity continues to increase if the concentration of the lesser yam tuber flour is added more. Viscosity in yogurt is influenced by the coagulation process of clumped milk protein due to the formation of acid by lactic acid bacteria.

## 3.4 Organoleptic Properties

### *3.4.1 Organoleptic*

Organoleptic properties of viscosity and sour taste of yogurt with variations in addition of lesser yam tuber flour can be seen in **Fig 5**.

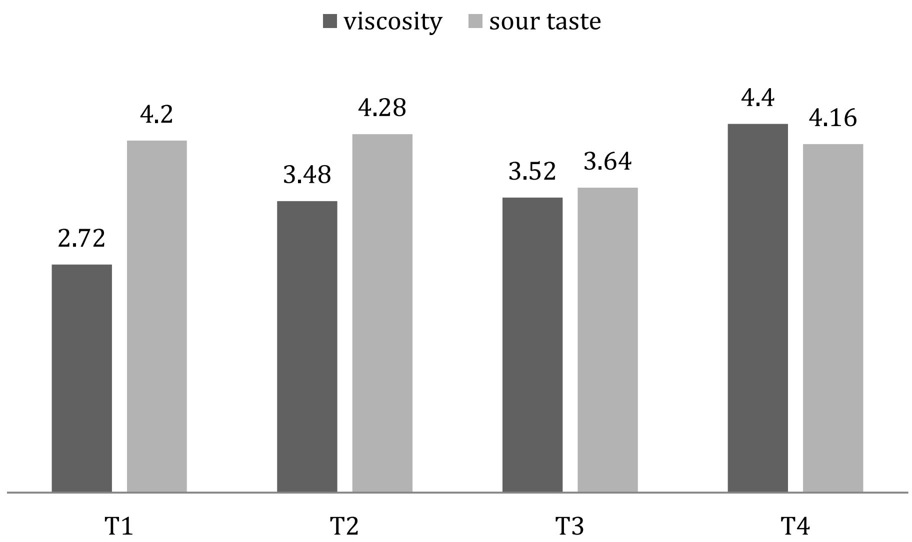


**Fig 5** Organoleptic Test Results of Yogurt

Information:
Data is displayed as a mean value ± standard deviation
Superscripts with different lowercase letters on the same line show significant differences (P <0.05)
T1, T2, T3 and T4 = The lesser yam tuber flour concentration: 0%, %, 4%, and 6%
The organoleptic scale of acidity with a score of 1-5 in a row states that it is not very acidic, not acidic, rather acidic, acidic and very acidic.
The organoleptic scale of viscosity with a score of 1-5 in a row states that it is not very thick, not thick, thick, thick and very thick

Based on the organoleptic test the viscosity of synbiotic yogurt with the addition of lesser yam tuber flour was significant (P <0.05). The average viscosity score in the yogurt of lesser yam flour ranged from 2.72 to 4.40 (not thick). The T1 treatment showed that the mean organoleptic score was not thick. This is due to the formation of yogurt texture due to the coagulation of casein in milk that has not run optimally. Yogurt thickness is formed due to a decrease in pH resulting in coagulation of casein which transforms the texture of the yogurt to be semi-solid. As stated by Meng et al. [14] that the formation of lactic acid by LAB causes pH to decrease so that casein undergoes coagulation, as a result, the thickness increases and the texture becomes semi-solid. While the treatment T2 and T3 showed a rather thick organoleptic score. This is caused by the addition of lesser yam tuber flour to provide additional nutrients in the form of inulin fibers which can be broken down into simple sugars by LAB. Zubaidah and Akhadiana [15] established that the addition of simple sugar from inulin the stimulates the growth and activity of probiotic bacteria. The T1 and T2 yogurt treatments showed an acid rating score, meaning that the addition of 2% of lesser yam tuber flour was not significantly different (P <0.05) with the treatment without the addition of lesser yam tuber flour. While in the T4 treatment, the addition of 6% of lesser yam tuber flour showed an acid rating but the pH of yogurt was higher than that of T2 and T3.

### *3.4.2 Hedonic Test*

The results of the hedonic test of taste, color, aroma, texture, and overall analysis of yogurt, coupled with lesser yam tuber flour can be seen in **Fig 6**.

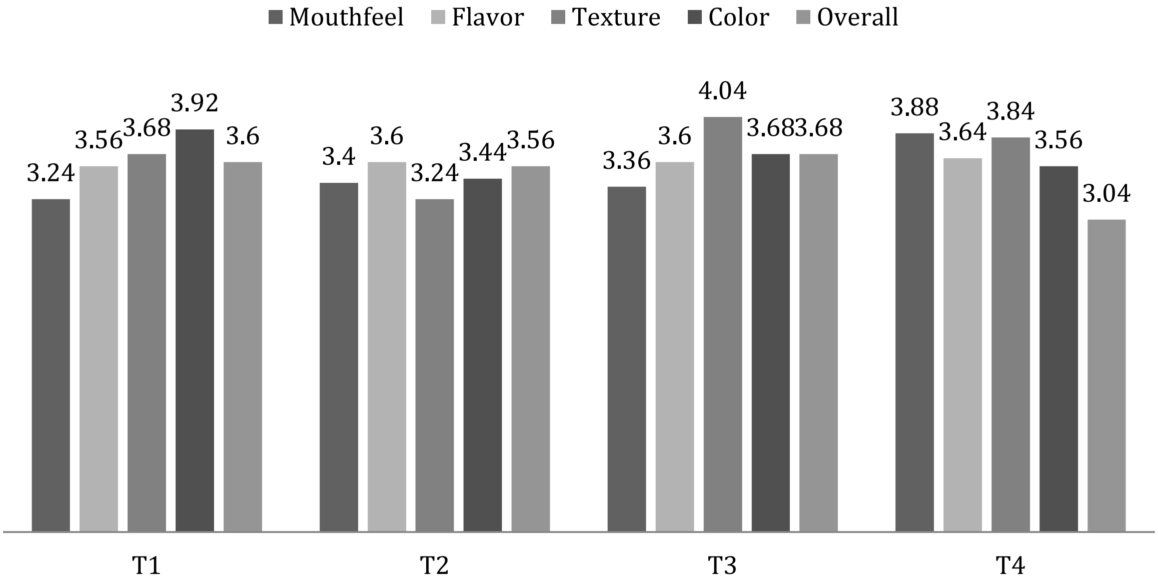


**Fig 6** Hedonic Test Results in Yogurt

Description:
Data is displayed as a mean value of 5 replications. Different superscript notations showed significant differences (P <0.05).
T0, T1, T2, and T3 = Concentration of Lesser yam tuber Flour 0%, 2%, 4%, 6%.

Based on Table 3 the addition of Gembili tuber with different concentrations gave a significant effect (P <0.05) on the texture of yogurt. Whereas the aroma, color, taste and overall did not have a significant effect (P> 0.05). Yogurt in overall preference level did not give a significant effect. Although the sample on T1 has the highest level of preference in terms of color but does not provide a high reception.

## 4 Discussion

The results of the total analysis of symbiotic LAB yogurt showed that the higher the percentage of the addition of lesser yam tuber, the more LAB grows. This is due to the presence of additional nutrients from lesser yam tubers which are used by bacteria to grow. Lesser yam tuber flour contains inulin as a carbon source for lactic acid bacteria. According to Sevim et al. [16], inulin is a water-soluble storage polysaccharide, commonly used as prebiotics, that the consumption of inulin leads to an increase Lactobacillus species in the human fecal microbiota of the colon. The addition of lesser yam tuber flour provides additional nutrients for BAL metabolism. However, a number of reducing sugars formed from the results of hydrolysis inulin have been used to metabolize LAB so that the remaining reducing sugars are relatively small. As reported by Choi et al. [17] that certain species from *Lactobacilli* can ferment fructo-oligosaccharides and degrade types of long-chain fructans such as inulin to produce lactic acid.

There are two types of fiber content in lesser yam tuber, which is water-soluble fiber and crude fiber or water-insoluble fiber. With the addition of lesser yam tuber flour, the total fiber present in yogurt also increases, it can be seen from the total crude fiber value that increases. But the value of water-soluble fibers such as inulin can be reduced because inulin can be used by lactic acid bacteria as an energy source for fermentation. Similar to what Oliveira et al. reported [18] that inulin can be used by lactic acid bacteria as a carbon source to carry out the fermentation process. Ghadge et al. [19] state that milk proteins can clot due to a decrease in pH by lactic acid bacteria and make the texture semi-dense because of viscosity increases. The process of clotting casein occurs when casein at pH approaches the isoelectric point. The largest protein content in milk is b-lactoglobulin and a-lactalbumin [20]. b-Lactoglobulin has a point around pH 5.3 and a-lactalbumin has an isoelectric point of around 4.8 [21]. Another factor that influences the viscosity is because of the presence of solutes and also the carbohydrate content that is in the lesser yam tubers such as glucomannan which can make a gel. Han et al. [22] showed that carbohydrates and total solids can affect viscosity because it affects the stability of the gel. Inulin in lesser yam tuber flour can also affect the texture of yogurt because it can maintain gel stability. In addition, Paz et al. [23] suggested that inulin can affect viscosity because inulin can create a high water retention capacity through hydrogen bonds with proteins in yogurt.

The acidity of yogurt is obtained from the formation of lactic acid by LAB in the presence of lactose in milk. The study by Settachaimongkon et al. [24] corroborated that LAB will ferment carbohydrates to form lactic acid so that acidity increases and pH values decrease. The typical sour taste in yogurt is formed by lactic acid bacteria as a result of milk sugar (lactose) metabolism. To elaborate further, Iyer et al. [25] which states that the typical yogurt flavor is obtained from the formation of lactic acid, acetaldehyde, acetic acid and diacetylated by LAB. For Ward et al., [26] taste will give a higher attraction to try food but the taste is the most dominant factor in giving the highest acceptance effect. The texture also does not give the highest preference for aroma. A study conducted by Kalviainen et al. [27] shows that aroma and taste have the most influence while the texture only gives a slight preference for influence.

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