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Optimum condition of *Streptococcus termophilus*, *Lactobacillus fermentum*, and *Lactobacillus plantarum* producing yoghurt starter

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Abstract. The study aimed to reveal the interaction of temperature and duration of incubation onto total LAB yogurt starter producer. The bacteria inoculated was used are *Streptococcus termophilus*, *Lactobacillus fermentum*, and *Lactobacillus plantarum*. The research method was used are randomized block factorial design was A factor variety of temperature A1 (25 0C), A2 (30 0C), A3 (37 0C), and A4 (42 0C), then B factor range of duration of incubation B1 (12 h), B2 (18 h) and B3 (24 h) within three replications. Best treatment determined by the highest Lactic Acid Bacteria (LAB) level consisted of treatment. Only the best treatment will analyze the proximate. The result showed an interaction between temperature and duration onto total LAB on the yogurt starter producer. It can be concluded that the best results from starter yogurt using three bacterial cultures were obtained at an incubation temperature of 370C for 18 hours with the number of LAB is 5,5 x 1010. Proximate results are the pH value of 4.46, Total Titrations Acid 2.20%, the water content of 82.48%, the protein content of 6.39%, and fat content of 4.36%.

Keywords: *Streptococcus termophilus*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, starter yogurt, optimum conditions

1. Introduction

Yogurt is fermented milk that has a thick texture and a sour taste. Yogurt has recently been in great demand by the public, apart from having an acid taste and having health benefits for those who consume it. Further, consuming yogurt can help the digestive tract maintain the microflora balance in the digestive tract, especially those in the intestine. Yogurt is a source of probiotics because it combines two types of bacteria in the manufacturing process. Those bacteria are *Streptococcus termophilus* and *Lactobacillus bulgaricus*. The combination of the two bacteria produces a thick, sour yogurt texture that is popular lately.



Streptococcus termophilus and *Lactobacillus bulgaricus* are Lactic Acid Bacteria (LAB). Both bacteria will break down milk lactose into lactic acid. [1] stated starters manufactured on yogurt have symbiosis in their growth. *Lactobacillus bulgaricus* releases amino acids and peptides that are useful for *Streptococcus termophilus* to grow faster. On the other hand, *Streptococcus termophilus* will produce formic acid to stimulating the growth of *Lactobacillus bulgaricus*. The interaction of the two bacteria makes yogurt with different characteristics from other fermented milk and helps accelerate the fermentation process in milk.

The substitution of *Lactobacillus bulgaricus* in the manufacture of yogurt has been widely researched. This bacterium could be replaced by 1 or 2 different types of bacteria. The purpose of the switch to produce a thicker yogurt texture with the dense consistency of other previous yogurts. [2] substituted *Lactobacillus bulgaricus* with *Lactobacillus fermentum* L23 in yogurt with dragon fruit extract. The best results were obtained for the total LAB 170×10^8 CFU/ml and pH 4.4 in the addition of 5% starter and 2% dragon fruit extract. [3] substituted *Lactobacillus bulgaricus* with *Lactobacillus fermentum* MGA40-6 in yogurt with the addition of spoon fruit juice (*Melastoma malabathricum*, L.) obtained the best results with a pH value of 4.4 and a titration acid value of 5.18% at the addition of starter 5% and spring fruit juice 4.5%.

In this study, the substitution of *Lactobacillus bulgaricus* with *Lactobacillus fermentum* and *Lactobacillus plantarum* was carried out. To confirmed the best results from using three different types of bacteria in yogurt, the tests were carried out on various incubation times and temperatures to make the starter. [4] states that in making yogurt, 1.5% -3% bacterial culture is usually added and incubated at a temperature of 42-45 °C for 3 hours, and the best results are obtained in the addition of 1.5% mixed cultures of *Streptococcus termophilus* and *Lactobacillus bulgaricus* bacteria to promote lactic acid. [5] on making yogurt is carried out at a temperature of 37 °C for 12 hours. Processing three bacterial cultures in making yogurt, the procedure was carried out regarding the different fermentation times and temperatures on the quality of the yogurt starter. Considering each bacterium have a specific condition to grow optimally.

This study aimed to determine the best incubation time and temperature to achieve the best yogurt starter production to produce yogurt with three different bacteria. Each bacterial culture has a specific time to break down lactose in milk into lactic acid in the fermentation process. In this study, tests were carried out using temperatures of 25°C, 30°C, 37°C, and 42°C and the fermentation time of 12 hours, 18 hours, and 24 hours. It is expected that later it will produce good quality yogurt in terms of texture and total LAB.

2. Material and method

This study used three bacterial cultures, namely *Streptococcus termophilus*, *Lactobacillus fermentum*, and *Lactobacillus plantarum*. The milk used is goat's milk, goat's milk from Etawa-Simpang-Pelangi-farm, Korong-Gadang, Kuranji District, Padang City. The design used was a factorial randomized block design with 3x4x3 and continued with Duncan's test.

Making starter [5]

Fresh goat milk (300 ml) had been pasteurized at 65 °C for 30 minutes, followed by cooling down the temperature to 40-45 °C. Each isolate (*Streptococcus termophilus*, *Lactobacillus fermentum*, and *Lactobacillus plantarum*) inoculated for 4% on cooled down pasteurize fresh goat milk been enriched on MRS Broth media for 24 hours at 37°C. LAB cultures were centrifuged at a speed of 14,000 rpm for 2 minutes at a temperature of 27°C. Obtained centrifuged pellets and inoculated on pasteurized milk every 100 ml and incubated for 37°C for 12 hours under anaerobic conditions. After incubation, 4% of each starter was taken and inoculated on 50 ml pasteurized milk. Then set according to the arrange treatment being tested. The process conducted triplet.

The total LAB and density observed visually were perceived as an indicator to decide the best treatment based on duration and temperature. The best treatment explored the proximate and chemical content to know the pH, total titration acid, water content, protein content, and fat content of the starter.

3. Results and discussion

3.1. Total colonies of Lactic Acid Bacteria (LAB)

The result of the combination of three isolates (*Streptococcus thermophilus*, *Lactobacillus fermentum*, and *Lactobacillus plantarum*) for total LAB can be seen in table 1.

Table 1. Total colonies of LAB x10⁸.

Faktor A (temperature)	Faktor B (Time)			Average
	12 h	18 h	24 h	
25 °C	134 ^a	129 ^a	140 ^{ab}	134
30 °C	166 ^{ab}	417 ^d	158 ^{ab}	247
37 °C	172 ^{ab}	550 ^e	337 ^{cd}	353
42 °C	204 ^{abc}	264 ^{bc}	223 ^{abc}	230
Average x10⁸	169	340	215	

^{abcde}. Different superscript in the same row and column shows a very significant difference (P <0.05)

As shown in table 1, the highest number of total LAB belong incubated at 37 °C for 18 h (550 x 10⁸). On the other hand, the lowest whole LAB documented starter was set at 25 °C for 18 hours (129 x 10⁸). The various analysis confirms that the period and temperature incubation interact (P<0.05) toward the total LAB of starter. The interaction has indicated joined effect between both treatments on to entire LAB of the starter. The treatment incubation on 37 °C for 18 h was considered the best usage since the superscript is significantly different from others. Indeed, the total number of LAB in a product is influenced by the substrate's temperature, time, humidity, and nutrients. Each type of LAB has an optimum temperature for growth to convert glucose into lactic acid in specific substrates. The optimum temperature of each bacterium will affect the development of these bacteria because LAB is classified into several types based on their optimum temperature. [6] states that bacterial growth is temperature-dependent.

[7] state duration of incubation will affect the viscosity of yogurt. The longer the fermentation time lasts, the microbes will continue to multiply, and the number will increase so that they can break down glucose into lactic acid. The process of bacterial growth has several phases, including the lag phase (adaptation phase), the log phase (growth phase), the stationary phase, and the death phase. According to [8] the bacterial growth rate test aims to determine the nature of the growth of bacteria through a bacterial growth curve.

The interaction between temperature and incubation time significantly affected the total number of LAB (P <0.05). The interaction between incubation temperature 37 °C at 18 hours of incubation time resulted in the best total number of LAB (550x10⁹ CFU / ml) than other treatments. In other words, optimizing utilization of three bacterial cultures at 37 C for 18 h. LAB will produce primary metabolites in the logarithmic phase at the 18th and 24th hours [9]. [10] states that the logarithmic phase in LAB is achieved at 18-24 hours incubation, depending on the medium and type of LAB. [11] stated that the genus of *Lactobacillus sp.* has optimal growth at 37°C in the growth medium. According to [12], *Streptococcus thermophilus* bacteria have the optimum temperature for growth is 37°C. Based on the research that has been done, the total number of LAB is 550x10⁹ CFU/ml with the best incubation at 37°C for 18 hours. The results obtained follow the Indonesian National Standardization Agency (2009), which states that the number of starter bacteria in yogurt is 10⁷ CFU / ml.

The best results from the treatment of incubation time and temperature of the starter yogurt were tested on the nutrition value of the best products, namely the incubation time of 18 hours and the incubation temperature of 37°C. Based on the table above, the proximate analysis results of the yogurt starter were obtained at an incubation temperature of 37°C for 18 hours.

Table 2. The average score of proximate tests of yogurt starter.

Proximate content	
Water content (%)	82,48
Protein content (%)	6,39
Fat level (%)	4,36
pH	4,46
TTA (%)	2,20

3.2. pH

Based on the laboratory analysis of the pH value of the starter yogurt, the results were 4.46. The decrease in pH is due to a metabolic process that occurs by bacteria during the fermentation process. Lactic acid bacteria produce lactic acid affects the pH of the milk before fermentation which causes the pH to decrease after the fermentation process is carried out. [2] stated that the more organic acids produced during fermentation, the more H⁺ ions are formed, which causes the pH to decrease.

3.3. Titrated acid

Based on the laboratory analysis of the pH value of the starter yogurt, the results were 2.20%. The titrated acid value shows a large number of organic acids present in yogurt. The value of the titrated acid will be inversely proportional to the pH value. The lower the pH value, the more the titrated acid value will increase. The results of the titrated acid value obtained in this study follow SNI (2009), which states that the amount of acid in yogurt ranges from 0.5-2%.

3.4. Water content

Based on the laboratory analysis of the moisture content in the starter yogurt, the results were 82.48%. During the incubation process, coagulation happened in the process led to a decrease in water content. In the research of [3], with Senduduk powder to yogurt, a reduction in water content and pH value was obtained. [3] stated that a decrease in the pH value of yogurt would coagulate the protein. [13] state viscosity of yogurt is significantly affected by total solids, and the yogurt's pH will be more acidic. [14] state that commercial yogurt produces lower water content, namely 75-80%, due to additional emulsifiers, flavorings, and preservatives, making commercial yogurt content decrease. The best results will be followed by proximate testing to see the chemical content of the yogurt starter. The proximate results of starter yogurt show a pH value of 4.46, Total Titrated Acid 2.20%, water content of 82.48%, protein content of 6.39%, and fat content of 4.36%.

3.5. Protein levels

Based on the laboratory analysis of the protein starter value of yogurt, the results were 6.39%. According to SNI (2009), the protein value in yogurt is at least 3.5%. In the research of [3], making yogurt with dragon fruit extract using two types of bacteria, namely *Streptococcus thermophilus* and *Lactobacillus fermentum* L23, produced a protein value of 2.75% also of 5% starter and 0% dragon fruit extract. [3] stated that a decrease in the pH value of yogurt would affect protein hydrolysis. [15] says the acidity value produced by microbial activity in milk will increase the total solid and create a thicker texture. The acidic pH of the yogurt protein will thicken, which forms a lot of clumps.

3.6. Fat content

Based on the laboratory analysis of the fat value of the starter yogurt, the results were 4.36%. The results obtained in this study were lower than those obtained from [16] on goat milk yogurt with the addition of grapefruit; the results were 5.40%. According to [17] bacteria will experience three main metabolic milk components during fermentation. Changing lactose into lactic acid (*fermentation*), hydrolyzing casein into peptides and free amino acids (*proteolysis*), and breaking down milk fat into free fatty acids (*lipolysis*).

4. Conclusion

Based on the study results, it can be concluded that the different temperatures and incubation times in starter yogurt affected the total colony value of LAB. The interaction between incubation temperature 37°C at 18 hours incubation time resulted in the best total number of LAB (550×10^9 CFU/ml). From the research results, it can be concluded that the best results from starter yogurt using three bacterial cultures were obtained at an incubation temperature of 37°C for 18 hours. The best results will be followed by proximate testing to see the chemical content of the yogurt starter. The proximate results of starter yogurt show a pH value of 4.46, Total Titrated Acid 2.20%, water content of 82.48%, protein content of 6.39%, and fat content of 4.36%.

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