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The Effect of Probiotic Starter Culture Variation on the Quality of Yogurt Based on Indonesian National Standard

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Abstract

Yogurt is usually made by two lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. However, three to seven lactic acid bacteria are also used in several products. Therefore, this study aimed to examine the effect of variations in the number of starter bacteria and compare to Indonesian National Standard (SNI). The experimental method was used with starter variations, including a combination of two (*Lactobacillus bulgaricus* and *Streptococcus thermophilus);* three (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus);* and seven bacteria (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium longum,* and *Bifidobacterium infantis*). The results showed that the yogurt with two and seven bacteria was within the range of SNI value standard. The pH, viscosity, syneresis, lactic acid content, total protein, and total number of bacteria for yogurt with two bacteria were 3.92, 453.76 cP, 88.05%, 1.27%, 3.26%, and 2.47 x 10^{12} Cfu/mL, respectively. The values for yogurt with seven bacteria were 4.11, 478.23 cP, 97,45%, 0,98%, 2.7%, and 1.53 x 10^{10}, respectively, while formulas with three bacteria did not reach the pH standard. However, the atypical taste was given by the seven starter bacteria and the use of various bacteria starters affected the quality of yogurt. Specifically, formulas composed of two bacteria showed the best results according to SNI.

Keywords: Fermentation; Lactic Acid Bacteria; Yogurt Quality.

INTRODUCTION

The concept of "Food as medicine" is being pursued by experts to optimize functional foods in overcoming diseases. An example of functional food is yogurt which is a dairy product that uses fermentation techniques with lactic acid bacteria (Zajác P et al, 2020). The bacteria produce the enzyme lactase to change the remaining sugar in milk and form an acidic environment to inhibit the growth of other harmful bacteria in the body (Hendarto et al, 2019). Yogurt quality is influenced by several factors, such as the type of bacteria culture starter used (Yilmaz-Ersan et al, 2014).

Streptococcus thermophilus and Lactobacillus bulgaricus are common bacteria used to make yogurt because of several health benefits. Yogurt is usually made with different types of bacteria. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* help each other grow by making short peptides and amino acids (Hendarto et al., 2019). The two bacteria produce formic acid but need other probiotics like *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, and Bifidobacterium sp*. These bacteria do not resist bile acids and live only briefly in the digestive system. According to Dabjia A. et al. (2018), these probiotics offer health benefits and stay longer in the digestive tract. To make starter cultures, more than two different strains of probiotic organisms are combined (Putri Y et al., 2020).

Using different bacteria starters when making yogurt leads to helpful interactions. Comparing the initial bacteria cultures is important in the fermentation and acidification processes. This is because health benefits depend on choosing specific microorganisms for therapeutic purposes and consuming enough to have the desired effect (Ningsih E et al., 2019).

Various studies have examined how the number of starter bacteria cultures affects the quality of yogurt. The quality parameters include physical (pH, viscosity, syneresis), chemical (lactic acid content, protein total), and microbiological analysis (total bacteria count) in accordance with Indonesian National Standard (SNI) for yogurt (Yanuarto T et al, 2019).

MATERIALS AND METHODS

Materials

This study used UHT milk (*Diamond*®), starter cultures of two (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), three (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*), and seven bacteria (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium longum,* and *Bifidobacterium infantis*). The materials used include PP indicator (*Merck*®), NaOH 0.1 N (*Merck*®), pH 4 and pH 7 buffer (*Hanna*®), NA media (*Merck*®), H2SO4 concentrated (*Merck*®), and HCl 0.1 N (*Merck*®). The instruments used pot were a stove (*Rinnai*®), thermometer, incubator (*Memmert*®), refrigerator (*LG*®), glass apparatus (*Pyrex*®), burette (*Schott Duren*®), pH meter (*Horiba*®), petri dish (*Anumbra*®), Brookfield viscometer (*Ametek*®), and centrifugation (*Tomy*®).

Methods

Yogurt Production

Milk was pasteurized at 85°C and cooled to 42°C, then the bacteria starter cultures were added, stirred until homogeneous, and incubated for 10-20 hours at 42°C. Yogurt was stored in the refrigerator at 4°C - 5°C (Dabija et al, 2018; BSN, 2009).

Table 1. Yogurt Formulation.

Composition	F1	F2	F3
UHT Milk	1 Liter	1 Liter	1 Liter
Starter cultures	1 gram (*Lactobacillus bulgaricus, Streptococcus thermophilus*)	1 gram (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*)	1 gram (*Lactobacillus bulgaricus, Streptococcus thermophilus,Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium infantis*)

Control: Branded Greek Yogurt

Organoleptic

Organoleptic test was carried out to identify the characteristics of color, consistency, appearance, smell, and taste (Anjarwati S et al, 2022; Fatmawati U et al, 2013).

pH

pH was determined by a digital pH meter and performed by dipping the electrode into the sample until the pH value was shown (Purwatiningsih et al, 2022).

Viscosity

Viscosity was determined with a Brookfield viscometer. Yogurt samples were prepared up to 500 mL using spindle 2 at a speed of 60 rpm then the results were analyzed (Purwatiningsih et al, 2022).

Syneresis

Syneresis was determined through centrifugation. About 15g of yogurt samples were taken, centrifuged, and then the supernatant was measured (Yanuarto T et al, 2019). Syneresis percentage was calculated by the following formula: the solution as an indicator (Yilmaz E et al, 2014; Anjarwati S et al, 2022; Purwatiningsih et al, 2022).

$$\%\,Syneresis = 100\% - (\frac{supernatan\ weight}{sample\ weight}\ x\ 100\%)$$

Protein Total

Protein total was determined by using the *Kjeldahl* method divided into three stages, namely digestion, distillation, and titration (Anjarwati S et al, 2022; Purukan C et al, 2020). Protein levels were calculated by the following formula:

$$\%\,N = \frac{mL\ HCl\ sample - blanco}{sample\ weight\ (g)}\ x\ n\ H\ x\ 14{,}007\ x\ 100\%$$

Total Bacteria Count

Total bacteria count was determined by using TPC (*Total Plate Count*) method (Lestari Y et al, 2020; Utami et al, 2020). The calculation of the total bacteria count was calculated by the formula:

$$\text{Total}\ \frac{cfu}{mL} = \frac{Total\ Colony}{spread\ volume\ x\ dilution\ factor}$$

Titratable acidity

Lactic acid was determined by using the titration method. Yogurt samples were taken up to 10 grams into Erlenmeyer then 10 mL of aquades was added and 2-3 drops of 1% PP. The result of the lactic acid percentage was calculated using the formula:

$$\text{Lactat acid (\%)} : \frac{mL\ NaOH\ x\ N\ NaOH\ x\ 0{,}009}{sample\ weight}\ \text{x } 100\%$$

Stability

The stability of pH, viscosity, and syneresis was determined repeatedly on day 7 and day 14 during storage at 4-5°C. The results obtained were compared to day 1 and then statisticallyanalyzed.

RESULTS AND DISCUSSION

Organoleptic

Organoleptic tests were conducted based on the guidelines of the Indonesian National Standard (SNI) for yogurt with a minimum of three panelists or one expert (BSN, 2009). The result after the fermentation process showed no changes in all formulas for 14 days of storage. Yogurt with two and three bacteria had similar characteristics such as white or yellowish color, distinctive aroma, typical sour taste, thick texture, and homogeneous consistency. Meanwhile, yogurt with a combination of seven bacteria had characteristics such as yellowish-white color, distinctive aroma, atypical taste, liquid texture, and homogeneous consistency. The physical appearance of yogurt is shown in Figure 1.

(a) 2 bacteria (b) 3 bacteria (c) 7 bacteria

Figure 1. Physical appearance.

Based on these results, yogurt with two and three bacteria conformed to SNI, while the seven bacteria did not. This was attributed to the atypical taste (BSN, 2009).

Physical, Chemical, and Microbiological Analysis

Yogurt samples were analyzed after being stored in a refrigerator overnight. The parameters analyzed include physical (organoleptic, pH, viscosity, syneresis), chemical (lactic acid content, total protein), and microbiological analysis (total bacteria count). All parameters were conducted for 14 days. The result of parameters analysis on day 1 is shown in Table 2.

Table 2. Parameters analysis on day 1.

Analysis	Formula			
	Control	**F1**	**F2**	**F3**
pH*	4,00± 0,05	3,92± 0,03	3,54± 0,08	4,11± 0,05
Viscosity*	3015,67± 8,08	453,76± 12,40	503,13± 27,46	478,23± 30,65
Syneresis*	99,94%± 0,01	88,05%± 5,56	97,74%± 0,24	97,45%± 1,33
Lactic acid*	1,41%± 0,01	1,27%± 0,01	1,47%± 0,02	0,98%± 0,01
Protein	4,00%	3,26%	2,71%	2,70%
Total bacteria	2,47 x 10^{12}	2,47 x 10^{11}	1,51 x 10^{10}	1,53 x 10^{10}

Note: * = All formulas were tested in 3 replications
Control : Branded Greek yogurt F2 : yogurt with 3 bacteria
F1 : yogurt with 2 bacteria F3 : yogurt with 7 bacteria

The pH value for the yogurt made using two and seven bacteria complied with SNI while yogurt with three bacteria did not, falling less than 3.8. Based on a previous study, the combination of *Lactobacillus acidophilus Streptococcus thermophilus*, and *Lactobacillus bulgaricus* produced yogurt with a lower pH value due to the ability to ferment sugar into lactic acid quickly (Jannah MA et al, 2014). *Bifidobacterium* culture which has an optimal pH of 5.5 – 7 may produce yogurt with a higher pH compared to other starters (Adriani L et al, 2008). This was consistent with the results of yogurt made using seven bacteria which had the highest pH value.

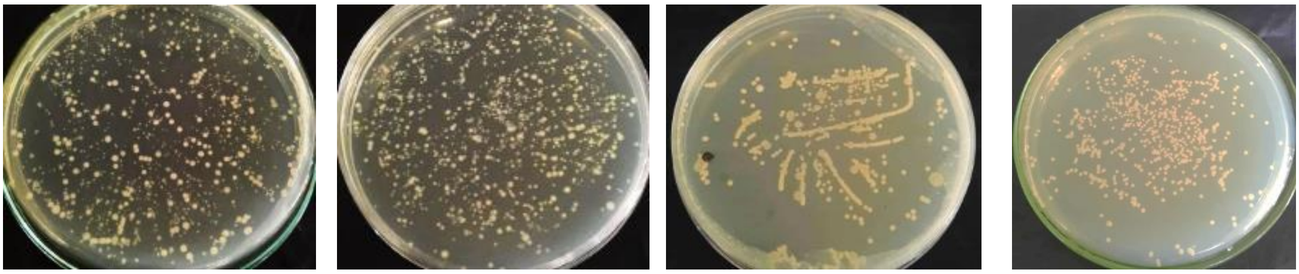
The standard viscosity value for yogurt is yet to be stated in SNI because the measurement is based on the appearance of yogurt to identify thick liquid to solid. However, the viscosity test in this study was carried out to standardize the quality of yogurt and to compare each formula. The result showed a range from 453 cp to 503 cp on day 1. Yogurt viscosity value is inversely comparative to pH, hence, when pH value is low then the viscosity value will increase due to the acidic fermentation process (Rohman E et al, 2020). According to previous studies, viscosity could be influenced by the protein and fat content of raw materials with the ability to bind water and activate the interaction between fat and casein during fermentation (Prastiwi et al, 2019; Setyawardani T et al, 2020). The presence of lactose in raw materials turns into pyruvic and produces lactic acid during the fermentation process to form an acidic atmosphere making casein unstable and coagulate from liquid to gel (Falah et al, 2020).

The lowest syneresis was found in yogurt with three bacteria and the highest syneresis was in yogurt with two. The smaller number of separated liquids indicates better yogurt quality with a higher percentage. In general, yogurt with a high percentage value often directly has a high viscosity value. Each type of lactic acid bacteria has a different ability to metabolize lactose which affects the ability of proteins to coagulate. In addition, the length of storage time affects syneresis, especially in yogurt samples that do not use stabilizers (Falah et al, 2020).

Regarding the lactic acid content value, all formulas conformed to SNI, falling between 0,5% - 2,0%. The highest lactic acid content value was found in yogurt with three bacteria, and the lowest was found in seven bacteria. Lactic acid content is inversely compared to pH value, when pH value decreases, then lactic acid content value increases.

In terms of protein total value, all formulas complied with SNI, reaching 2.7%[8]. The highest protein total value was found in yogurt with two bacteria and the lowest was found in yogurt with seven bacteria. Based on a previous study, yogurt made using two bacteria cultures namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus* had a higher protein level than those with three and four due to competition interactions. Yogurt with a combination of two bacteria has a symbiotic interaction where *Streptococcus thermophilus* produces pyruvic, formic, and folic acid. This expression stimulates *Lactobacillus bulgaricus* to grow, which releases the amino acids valine, glycine, and histidine (Purukan C et al, 2020).

All formulas complied with SNI as indicated by a total number of bacteria above 10^7. The desired therapeutic effect in yogurt should be provided in sufficient quantities to compensate for the possibility of probiotic reduction by the stomach and intestines.



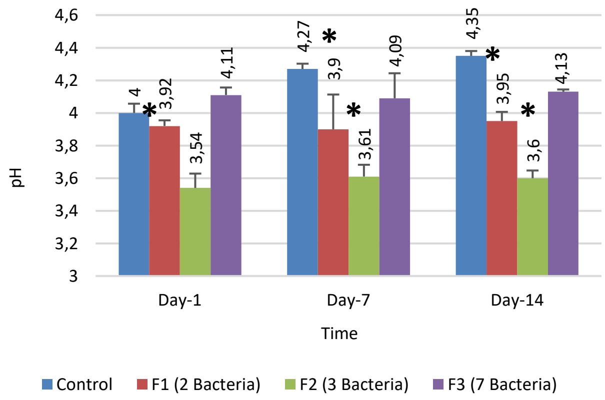
(a) Control (b) F1 (2 Bacteria) (c) F2 (3 Bacteria) (d) F3 (7 Bacteria)

Figure 2. Bacteria on Agar.

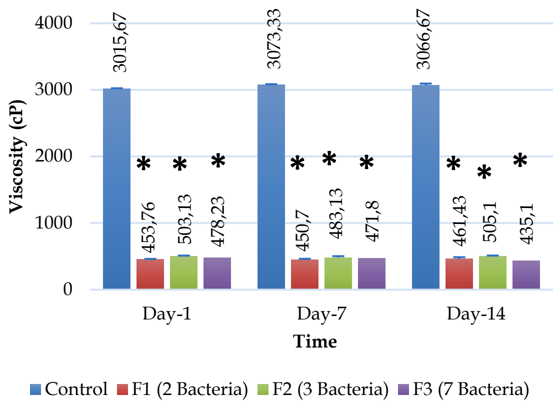
The highest value was yogurt composed of two bacteria and the lowest value was in the sample with three bacteria. The higher number of lactic acid bacteria is directly compared to a higher protein (Ruslian RD et al, 2021). According to a previous study, yogurt with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were synergized in cell multiplication (Jannah MA et al, 2014).

Stability

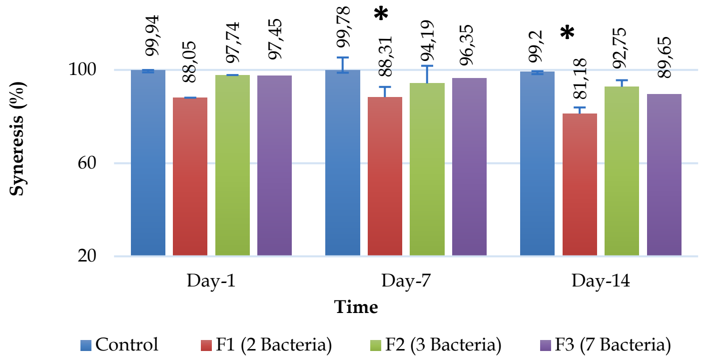
The stability test was conducted three times for 14 days (day 1, day 7, and day 14) consisting of pH, viscosity, and syneresis. For all formulas, the pH and viscosity values showed stable with no significant change ($p \geq 0.05$). The value of syneresis showed no significant change ($p \geq 0.05$) in yogurt with two bacteria, but was unstable with significant change ($p<0.05$) from day 1 to day 14 in yogurt with three and seven bacteria. Changes in stability during storage are shown in Figure 3.



(a) pH



(b) Viscosity



(c) Syneresis

Figure 3. pH (a), viscosity (b), and syneresis (c) percentage determination for 14 days storageNote: * = Significantly different with control (p<0.05)

CONCLUSIONS

In conclusion, the use of variations in the number of different bacteria starters affected the physical, chemical, and microbiological parameters of yogurt in accordance with the Indonesian National Standard (SNI). These parameters include pH, viscosity, syneresis, lactic acid content, protein total, and total bacteria count. The formula with two bacteria and seven bacteria results were within the range of SNI value. However, the formula with three starters did not reach the pH value, and the formula with two bacteria is shown the best according to SNI.

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Competing Interests: The author declares there is no conflict of interest.

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