The shelf life of yogurt starter and its derivatives based on the microbiological, physical and sensory aspects

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Abstract. Yogurt quality is affected by the yogurt starter. The aim of this study was to determine the shelf life of yogurt starter and its derivatives based on microbiological and physical and sensory aspects. Yogurt starter is made from commercial dry culture, then lowered to 4th derivative, and stored in the refrigerator for up to 25 days. The result showed that there was stability in the viability of total microorganisms and mold in yogurt starter and its derivatives. The viability of lactic acid bacteria (LAB) decreased from F1 to F4. Decreasing pH and little whey formation occurred during the storage. The yogurt quality made from the starter and its derivatives was acceptable. The shelf life of F1, F2, and F3 starter is 25 days, while F4 is 20 days.

1. Introduction
Yogurt is a fermented drink from milk. It contains significant amounts of nutrients, and as an energy source with its fat and protein content. Other contents are calcium, potassium, vitamins, especially vitamins B1, B2, B6, nicotinic and pantothenic acid [1]. Drinking yogurt can improve the overall quality of the diet and increasing the chances of achieving nutritional recommendations [2]. Due to health benefits, yogurt can be recommended to the people with lactose intolerance, weight control, diarrhea, irritable and inflammatory bowel disease [2,3]. It has an immunologic and therapeutic effect. To have therapeutic benefit, the minimum level of probiotics in yogurt is 5-6 log viable cell per ml or g [4,5]. Yogurt quality is affected by milk quality, storage, incubation temperature and type of starter [6]. The variety of starter will affect the yogurt properties.

Starter culture or starter is defined as a living microorganism that is used to help initial fermentation. It produces a specific change in chemical composition and sensory properties of the substrate to obtain a more homogeneous product [7]. It is an essential component in producing fermented foods and harmless food-grade microorganisms. In the dairy industry, starter cultures widely used in the production of fermented drinks and cheese. The main functions of starter are protecting the product due to a fermentation that results in extending shelf life and enhancing safety, enhancing sensory properties, improving the rheological properties and making functional properties to food [8]. Starter quality is assessed by the ability in adaptation to various manufacturing conditions, fast acid production in vats, minimal acid production during distribution and storage, maintenance of viability during the shelf life of fermented milk, and typical taste, body, and texture formation [9]. Yogurt starters generally contain a combination of Lactobacillus bulgaricus and Streptococcus thermophilus which play a role in flavor and taste formation. Now, it is also added by probiotic such as Lactobacillus casei, Lactobacillus acidophilus, dan Bifidobacterium longum to increase the functional properties.
Shelf life is defined as storage time in which food remains acceptable for human consumption in terms of safety, nutritional attributes, and sensory characteristics [10]. During storage, the product will decrease in quality and acceptability. Oxygen, water, light, and harmful microorganisms can start damage the food. It can cause off-flavor, off-odor and produce toxin. Changing colors and odors is an easy way to assess shelf life food. Starter shelf life is determined by the number of microorganisms and acid resulting from its fermentation.

Making yogurt needs enough knowledge. Qualified ingredients, sterile equipment, and good handling will produce good yogurt. Starter as the heart of fermentation is very important to see the quality. There is a lack of information stating how long the starter can be cultivated to get acceptable yogurt. The purpose of this research was to study the quality of starters and their derivatives during storage at refrigerator temperatures based on the number of lactic acid bacteria, total microorganism counts, total mold, pH value, and sensory test of the yogurt products. The results are expected to be a reference for the dairy industry in choosing the right starter.

2. Materials and methods

2.1. Materials
The materials were fresh milk (Research Center for Biotechnology Dairy Farm), commercial yogurt starter (Yogourmet), microorganism growth media and analysis grade chemical reagents. The equipment used was disposable petri dish, Memmert water bath WNB 14, Eutech Instruments pH 700, Laminar air flow Telstar BH-100, Colony counter digital S and other glassware.

2.2. Methods

2.2.1. Making yogurt starter and its derivatives
Milk is heated using 2 methods, pasteurization (by heated 80 °C, 10 minutes) and pressurization (heated by presto pan). One litter cooled milk (30-35 °C) added 5 g of dry culture and incubating on room temperature for 24 hours (F1). For making the derivatives, 1-liter cooled milk (pasteurized and pressurized) added 5% F1 and incubated like F1 steps (F2). The next derivative was made by adding 10% F2 to 1 L milk (F3). Fifteen percent of F3 was added to 1 L milk to make the last derivative (F4). All yogurt starter and its derivatives stored in the refrigerator (7-10°C) and observed on 0, 5th, 10th, 15th, 20th and 25th day.

2.2.2. Microbiological quality
The selected microbiological quality was the viability of total microorganism (TPC), lactic acid bacteria (LAB) and mold. The growth media used is Plate Count Agar (TPC), de Mann Rogosa Sugar (LAB) and acidified Potatoes Dextrose Agar (mold). The inoculation method was carried out is a pour plate. The sample was incubated at 30°C for 48 hours.

2.2.3. Physical quality
Acidity (pH) and whey formation were the physical properties tested. Acidity was analyzed using a pH meter. Whey formation was calculated by measuring the height of fluid formed in yogurt starter and its derivatives. Two hundred ml of yogurt starter and its derivatives on a 300 ml bottle and observed until the 25th day.

2.2.4. Preparation of yogurt for sensory tests
Milk is pasteurized at 85°C, for 10 minutes, then put in a sterile sealed container. After the milk was cold, then the yogurt starter was inoculated aseptically. The amount of starter added to make the yogurt product is 5% F1 (for F1 product), 10% F2 (F2 product), 15% (F3 product) and 20% (F4 product).
2.2.5. Sensory test
The method used is the multiple comparison test. The control is yogurt which was produced using an F1 starter. The test scale used is 5 (same as control), 4 (slightly different from control), 3 (different from control but still edible), 2 (worse than control and not edible), and 1 (very bad from control and not eligible for eaten).

2.2.6. Data analysis
Every treatment was done twice. Data were analyzed by analysis of variance (ANOVA) at a 95% significance level.

3. Results and discussions

3.1. Microbiological properties
The main function of the yogurt starter is to produce lactic acid from lactose in milk. Other functions, the starter can play a role in regulating taste, aroma, level of alcohol production, proteolytic and lipolytic activity, and inhibitors for undesirable pathogenic microbes [11]. During the incubation, symbiosis occurs between the two bacteria in the starter yogurt. S. thermophilus develops faster to initiate lactic acid formation (breaking down lactose), this growth continues until it reaches pH 5.5. In addition to the release of oxygen, this condition provides a very good environment for the growth of L. bulgaricus [12]. The activity of proteolytic enzymes from L. bulgaricus causes the degradation of milk protein. It produces amino acids and peptides which will stimulate the growth of Streptococcus. Lactobacillus will also decompose fat to produce fatty acids which give a distinctive flavor to the yogurt's final product. L. bulgaricus and S. thermophilus break down lactose (milk sugar) into lactic acid and various components of aroma and flavor. L. bulgaricus plays a role in aroma formation, while S. thermophilus in the formation of taste [11].

![Figure 1. Total microorganism on yogurt starter (F1 to F4) with pasteurized milk ▲ and pressurized milk ● as material](image)

High viability is one of the criteria for choosing a starter. At the beginning of the fermentation, a large amount of energy is needed. It can be done by microorganisms that can adapt quickly to the growth environment. The total viability of microorganisms and LAB can be seen in figure 1 and 2. All starters have viability above 7 logs CFU/ml. According to SNI and CODEX standards, the minimum
number of starter microbes in yogurt is 7 logs CFU/ml. Based on this microbiological property, the starter can still be used as a yogurt ingredient until 4th derivative and can be stored for up to 25 days.

LAB viability in F1 yogurt starter ranges from 7.99 - 9.32 log CFU/ml, the starter of F2 yogurt ranged from 7.68 - 8.65 log CFU/ml, the starter of F3 yogurt ranged from 7.31 - 8.66 log CFU/ml and the starter of F4 yogurt ranged from 7.37 - 8.17 log CFU/ml. The treatment of milk preparation in making yogurt starters has a significant effect (p < 0.05) on the viability of LAB starter first derivative (F1) and fourth derivative (F4). From the results of variance also known that the storage time does not affect the viability of LAB in yogurt starters, both for F1, F2, F3, and F4 yogurt starters. Compared to the number of LAB in F1 yogurt starters, the viability of LAB in the F4 starter has decreased by about 18%. Many factors affect the microorganism viability during storage i.e. acidity, pH and hydrogen peroxide [13], storage temperature, and oxygen content. Improper environmental conditions and bad nutrition will reduce microorganism viability.

![Figure 2](image-url)  
**Figure 2.** Total lactic acid bacteria (LAB) on yogurt starter (F1 to F4) with pasteurized milk [▲] and pressurized milk [●] as material

In yogurt, the total mold is not required [1]. In this study, mold did not grow. It shows good sanitation in materials, good handling, and good processing.

### 3.2. Physical properties

Lactic acid produced by LAB can reduce pH from its growing environment and cause a sour taste [15]. Other organic acids produced are acetate, butyrate, pyruvate, and formic. Naturally, milk contains citric acid, hippuric, and orotic. The acids can also be found in yogurt [16]. High organic acid production decreases the pH of yogurt. Organic acids have benefits as natural preservatives and determine the sensory properties of yogurt.

Changes in pH during storage in yogurt starter and its derivatives can be seen in Figure 3. In addition to accumulating organic acids, a low pH is also influenced by the pH of the origin of milk. The results showed a trend toward increasing pH values. Accumulation of organic acid increase during storage. Production of organic acid is inversely proportional to pH value. All derivatives have decreased pH during storage, but only F1 and F4 are significant. In the heating type, pasteurized has a lower pH than pressurized in the F1, F2, and F3. Not all microorganism has died during pasteurization. The presence of other bacteria besides the starter is possible to participate in reducing the pH value.
Whey formation or better known as syneresis in yogurt is the process of releasing water from yogurt gel, due to decreased ability of proteins to bind water. The occurrence of syneresis has a negative impact on consumer perception because consumers think there is something wrong with the microbiological properties of the product. Heating, high stirring, and acidity affected syneresis. Heating in milk can produce micelle protein which has a low binding capacity to water compared to the initial micelle. It can cause water released. Hydrogen bonds between water molecules and proteins can be weakened by high stirring and acidic conditions, there is looseness between the pores of casein and can be traversed by water which was previously bound by protein [8]. In addition to causing syneresis, heating can also reduce oxygen which helps the growth of the starter [17].
Figure 4. Whey formation on yogurt starter (F1 to F4) with pasteurized milk [■] and pressurized milk [□] as material

There is no specific pattern of syneresis in the study. In the first derivative, it is more common than other derivatives. It is possible because of differences in the method of stirring and the distribution of samples.

3.3. Sensory properties
Multiple comparison tests are used to determine the difference between samples and controls. The more acidic taste causes the product not to be accepted and not eligible for eaten. Sensory test results (Figure 5) showed that yogurt from the F1, F2, F3, and F4 starter has a value slightly different from control (4) to (different from control but still edible (3), except yogurt from 4th derivative which is stored until the 25th day. The sensory test is very decisive in acceptable food products. Unpleasant tastes and aromas are formed due to changes in the chemical compound of the product during storage. This causes a decrease in consumer acceptance. Spoilage bacteria that are still present in pasteurized milk cause a shorter shelf life. This is the reason why yogurt from the starter yogurt which made from pasteurized milk is faster for panelists not to accept.

The storage time of the starter has a significant effect on sensory properties. The panelists still stated the product was slightly different from the control (4) until the yogurt made from the starter is store 5 days. After the starter has been stored for more than 10 days, the yogurt made has sensory value different from control but still edible until not edible. The acceptance value is also influenced by the accumulation of organic acids that are formed and the decrease in pH. In general, panelists don't like sour taste (low pH products). The type of heating did not affect the consumer acceptance of yogurt.

![Figure 5. Sensory evaluation on yogurt made from F1, F2 F3 and F4 with pasteurized milk [▲] and pressurized milk [●] as material](image)

4. Conclusion
The total viability of microorganisms and coliform in the starter is not different between F1, F2, F3, and F4. The derivative process reduces the viability of the LAB. There is no specific pattern on the whey formation in the starter. A decrease in pH occurs during the storage process. When the starters are used to make products, yogurt from F1, F2 and F3 starters stored for up to 25 days still have
sensory properties that can be accepted by the panellists, but F4 only last 20 days. Storage time affected the viability of starter, pH and sensory properties.

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