# Effect of Starter Concentration and Length of Incubation on Protein and Fat Levels of Yogurt with Palm Sugar

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**Abstract.** Palm sugar yogurt is a product of fermented milk with palm sugar in the form of a thick to semi-solid liquid with a specific sour taste. The making of this yogurt utilizes palm sugar to help form a texture and give a sweet taste to yogurt, which is acidic. This research aims to determine the effect of adding palm sugar sweeteners to protein and fat levels in yogurt, to study the activity of starter bacteria Lactobacillus bulgaricus and Streptococcus thermophiles in various concentrations, as well as knowing the functional value of low-fat yogurt with high nutrients that are good for health. This research method consists of 3 stages: preparation of raw materials, making palm sugar yogurt, and incubation (fermentation). The study used a completely randomized design with one factorial, namely with the breakdown of fixed variables with the addition of palm sugar (5% w/v) and lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) and independent variables with the addition of the number of starters (2.5% and 5% v/v) with an incubation time of 8 and 10 h. The results showed that the highest protein content was obtained in the B1D treatment, which was 4.17%. Meanwhile, based on the fat analysis, the best results obtained were the lowest levels in the A2C treatment, having a test content of 1.89%.

**Keywords:** Starter · Incubation · Fat Levels · Yoghurt · Palm

## 1 Introduction

Yogurt is known to increase immunity, lower blood pressure, bad cholesterol, and the risk of a heart attack. People prefer low-fat yogurt because of the nutrients and benefits contained therein. Lower fat levels will keep your body ideal. In addition, yogurt contains various essential nutrients such as protein, calcium, potassium, zinc, phosphorus, and vitamins B2 and B12. Yogurt is also relatively safe for consumption for people who are lactose allergic because bacterial culture during the production of yogurt automatically converts the lactose in milk into lactic acid (Ademosun, 2019).

The manufacture of commercial yogurt is mainly done using two types of lactic acid starter bacteria—namely, Lactobacillus bulgaricus and Streptococcus thermophiles. Fermentation of milk with lactic acid bacteria is carried out to extend its shelf life under acidic conditions. Various starter cultures are selected during yogurt manufacturing


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to achieve the desired product characteristics and provide the consumer with a wide selection of the best possible benefits. Depending on the activity, on an industrial scale, usually, add 2–4% yogurt starter culture.

One strategy to increase dairy products' functional value is adding sugar. Apart from being a source of energy, adding sugar aims to form a texture and give the yogurt a sweet taste. Palm sugar was chosen as an additive for yogurt because it has a minimal fat content and very high sucrose. Palm sugar is the sugar with the lowest fat content compared to other types of sugar. The raw material for the milk used is whole cream milk which contains.

High fat and protein. With the addition of low-fat palm sugar, it is expected to produce low-fat yogurt with good nutritional quality.

The fermentation of Lactobacillus bulgaricus and Streptococcus thermophiles produces excellent protein quality. Lactobacillus can ferment lactose, fructose, and glucose to produce lactic acid, while Streptococcus can ferment lactose, glucose, fructose, and sucrose to produce lactic acid. The combination of the two bacteria can make a higher acidity.

High-protein yogurt increases consumer interest in consumption due to its texture, taste, and health benefits. The protein content in yogurt can be increased by adding milk, membrane filtration, and mechanical separation (Jørgensen et al., 2019). The manufacture of commercial yogurt is mainly done using two types of lactic acid starter bacteria, namely, Lactobacillus bulgaricus and Streptococcus thermophiles. It is known that Lactobacillus plantarum is the highest producer of lactic acid. This is because these bacteria have very high lactase activity. Lactobacillus casei can break the polypeptide bonds into shorter and denatured proteins, thereby increasing viscosity (Jaya et al., 2011).

## 2 Methodology

Producing yogurt: Pasteurized fresh milk at a temperature of 70–80 °C for 15 min, then add palm sugar (5% w/v), stirring until homogeneous. Then it was cooled to a temperature of 40 °C and inoculated with a starter of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* with a ratio of 1:1 (2.5%, 5%, v/v), then stirred until homogeneous. It was subsequently incubated for 8 and 10 h.

## 3 Result and Discussion

### 3.1 Addition of Palm Sugar

The addition of several types of sugar has a significant effect on the protein content of yogurt. The higher the added sugar will increase the protein content of the yogurt. Adding sugar levels up to 15% will increase energy for metabolic activities or enzymatic reactions from the starter. The percentage of starters does not affect the levels of protein and fat, but there is a tendency for the highest protein and fat content to be found in yogurt with a 5% starter (Zakaria, 2008). This is in line with the results of the palm sugar yogurt protein and fat test that has been carried out using a combination of starter L.

**Table 1.** The results of protein and fat levels of palm sugar yogurt

| Incubation (hour) | Starter (%) | Protein (% weight) | Fat (% weight) |
|---|---|---|---|
| 8 | 2,5 | 2,86 | 1,89 |
| | 5,0 | 3,41 | 2,33 |
| 10 | 2,5 | 3,38 | 2,20 |
| | 5,0 | 3,85 | 2,59 |

bulgaricus and S. thermopiles 5% which tends to have higher protein and fat levels between 3.85–2.59%.

Lactic acid bacteria groups such as Lactobacillus, Bifidobacterium, and Streptococcus can convert carbohydrates such as lactose and glucose through the fermentation process into large amounts of lactic acid so that lactic acid increases with increasing sugar or glucose (Zakaria, 2008). Combined yogurt from Lactobacillus bulgaricus and Streptococcus thermopiles can synthesize B complex vitamins, especially thiamin, and several amino acids that makeup proteins that are useful for health. (Tjatur et al., 2015). Here are the test results for the protein and fat content of yogurt with adding 5% palm sugar and a starter combination of Lactobacillus bulgaricus and Streptococcus thermopiles bacteria (Table 1).

### 3.2 The Protein and Fat Levels

According to Indonesian National Standard, the minimum protein content for low-fat yogurt is 2.7%. The protein content of palm sugar yogurt is between 2.86–3.85%. The results of the palm sugar yogurt test meet the Indonesian National Standard. Yogurt raw materials with high protein will produce yogurt with a higher protein content (Blanchard, 2014). The raw material for palm sugar yogurt uses full cream milk and palm sugar, which are known to increase the activity of lactic acid bacteria at the incubation stage. Yogurt, adding 15% sugar and a starter percentage of 5% Lactobacillus casei produces protein levels between 3.76–3.85% (Zakaria, 2008).

The highest to the lowest protein content is indicated by bacterial activity Lactobacillus bulgaricus, Lactobacillus fermentum, Streptococcus thermophiles, and the combination of Lactobacillus bulgaricus and Streptococcus thermophiles (Cn et al., 2016). The yogurt protein content is comparable to lactic acid (Chairunnissa et al., 2017).

Making yogurt from cow's milk has a protein content between 3.6–4.5% with 5% sucrose. In the manufacturing process, it is necessary to add cow's milk as a source of carbon and bacterial energy during the fermentation process. Besides being a source of energy, cow's milk is also a source of lactose which lactic acid bacteria will break down. Adding 30% cow's milk with a fermentation time of 10 h will increase the consistency of the yogurt. The longer the fermentation time, the higher the thickness of the yogurt. In addition, the high protein content affects the viscosity of the yogurt. The protein coagulated by the acid will form a gel, making the yogurt texture thicker (Perna et al., 2014).

**Table 2.** Test results of protein and fat levels of palm sugar yogurt

| Treatment | Organoleptic Assessment | | | | | |
|---|---|---|---|---|---|---|
| | Hedonic Score | | | | | |
| | Colour* | Flavor* | Taste* | Overall Acceptance * | Texture** | Consistency *** |
| $A_1C$ | 3,9 | 3,8 | 3.9 | 3.8 | 2.8 | 2.8 |
| $B_1C$ | 3.8 | 3.7 | 3.9 | 4.0 | 3.2 | 2.6 |
| $A_1D$ | 4,0 | 3.9 | 4.0 | 3.9 | 2.9 | 2.7 |
| $B_1D$ | 3,8 | 4.0 | 4.1 | 3.9 | 3.0 | 2.8 |
| $A_2C$ | 4,0 | 4.0 | 4.2 | 4.0 | 3.1 | 2.7 |
| $B_2C$ | 3.8 | 4.0 | 3.9 | 3.9 | 3.0 | 2.9 |
| $A_2D$ | 3.9 | 3.8 | 3.8 | 3.8 | 2.9 | 2,7 |
| $B_2D$ | 3.8 | 3.9 | 4.2 | 4.0 | 3.0 | 2.8 |

Notes:
*: 1 = very dislike, 2 = dislike, 3 = ordinary, 4 = like, 5 = really like
**: 1 = watery, 2 = slightly thick, 3 = thick, 4 = very thick
***: 1 = inhomogeneous, 2 = slightly homogeneous, 3 = homogeny

Increased milk fat levels can affect starter cultures' growth and activity (Aswal et al., 2012). The milk used to manufacture palm sugar yogurt is whole cream milk with higher fat content. The resulting yogurt fat content has decreased due to the heating process and hydrolyzed during fermentation so that it will turn into fatty acids, glycerol, aldehyde, and ketones (Blanchard, 2014). The fat content of palm sugar yogurt is between 1.89–2.59%, which is by the SNI for low-fat yogurt. Yogurt, adding 15% sugar and a starter percentage of 10% Lactobacillus casei resulted in a fat content of 3.37% (Zakaria, 2008) (Table 2).

### 3.3 Organoleptic Test

### 3.4 The Colour

The organoleptic analysis results showed that adding palm sugar had no effect on the color of the yogurt. Panelists stated that the colors of all treatments were the same, namely yellowish white with values obtained. Between 3.8–4.0. The white color of milk comes from the color of casein. Pure casein color is white like snow. In milk, this casein is a colloid dispersion, so it is not translucent, which makes the milk-white (Buda, et al. 1980).

### 3.5 The Flavor

The organoleptic analysis results showed that adding palm sugar had no effect on the aroma of yogurt. This is because the smell formed in each treatment is estimated to be

the same by the panelists. The panelists liked the scent of the resulting product as a whole, with values ranging from 3.7–4.0. Speer (1998) stated that yogurt's distinctive aroma is obtained from lactic acid, acetaldehyde, acetoin, and diacetyl, which are produced during the fermentation process. The unique smell of yogurt is present at each treatment level, so the panelists gave the same assessment. Yogurt has a characteristic aroma, such as a sour aroma. This aroma arises because, during the fermentation process, there is a change in milk lactose into lactic acid by lactic acid bacteria. Lactic acid is what causes yogurt to have a distinctive sour aroma. The aroma of yogurt products is caused by the volatile compounds formed to give a unique sour aroma. Besides playing a role in gel formation, lactic acid also provides sharpness of taste and determines the distinctive smell of yogurt (Anindita, 2002). According to Kusmawati (2008), aroma parameters are closely related to taste parameters. The product's unique taste is because LAB produces chemical compounds from lactic acid, acetaldehyde, acetic acid, diacetyl or 2,3-pentanadione, and other volatile substances.

### 3.6 The Taste

The results of the analysis of variety show that the addition of palm sugar affects the taste of yogurt. Panelists gave values ranging from 3.8 to 4.2, which indicated that they were relatively fond of yogurt at this level. The palm sugar concentration at this level can slightly mask the sour taste of yogurt. According to the panelists, the overall flavor of palm sugar yogurt is somewhat sour. Yusmarini et al. (1998) stated that an organic acid would be formed during fermentation, which causes a distinctive sour taste in yogurt. The use of full cream milk and lactic acid bacteria resulted in a distinctive taste of yogurt products that panelists liked. This is due to the addition of sugar in the manufacturing process, which in its main function is as a sweetener, can also provide nutrition to lactic acid bacteria optimally so that the bacteria are able to produce the right taste and not too sour/distinctive due to the formation of lactic acid and acids. Another organic matter is a result of its metabolites (Sintasari et al., 2014). This led to the construction of the right combination for the panelists when testing yogurt products. Riyanto (2015) adds that during the fermentation process, lactic acid bacteria produce a distinctive taste caused by forming organic acids during fermentation. This is supported by Schornburn (2002), that the sour taste in yogurt is caused by the cellular metabolic activity of lactic acid bacteria in yogurt fermentation. Yogurt has a distinctive flavor and taste because of the chemical compounds produced from lactic acid, acetaldehyde, acetic acid, diacetyl or 2,3-pentanadione, and other volatile ingredients. This sour taste will then mask the distinctive taste of the ingredients, thereby increasing the panelists' preference.

### 3.7 Texture

The results of the analysis of variety show that the addition of palm sugar has a significant effect on the texture of yogurt. The longer the incubation time, the thicker the texture of the resulting yogurt. Panelists gave a value between 2.8–3.2, which indicates that the yogurt is thick. The thickness of the product can be caused by the addition of palm sugar in sufficient quantities and the clumping of protein in milk as a result of the formation of lactic acid. Nofrianti et al. (2013) stated that during the yogurt fermentation process,

protein clumping occurred due to lactic acid produced by Streptococcus thermophilus and Lactobacillus bulgaricus. The clumping of protein makes the yogurt texture thick. The consistency of the coagulum determines the texture of the yogurt. This consistency is influenced by pH when the pH is outside the normal pH range of milk because, in this range, casein can form a network with denatured whey protein during pasteurization as an integral part of the yogurt-making process (Bylund, 1995). Buckle et al. (1987) stated that clumping is the most characteristic of milk. Clumping can be caused by enzyme activity or adding acid. Proteolytic enzymes produced by bacteria can cause milk clumping. The action of this enzyme usually occurs in three stages, namely absorption of the enzyme into the casein particles, followed by changes in the state of the casein particles as a result of the enzyme action, and finally, depositing the changed casein as calcium salt or complex salt. The presence of calcium ions in milk is necessary for the precipitation process.

### 3.8 The Consistency

The results of the analysis of variance showed that the addition of palm sugar did not affect the consistency of the yogurt. Based on the score test, the panelists gave scores ranging from 2.6 to 2.9. The consistency of yogurt in question is the formation of a thick liquid or a homogeneous solid (Anon., 2009b). In this case, the determination of whether the yogurt is homogeneous or not is judged by the ability of the solid and liquid components in the yogurt to combine after stirring. Panelists mentioned that the consistency of the resulting palm sugar yogurt as a whole is somewhat homogeneous to homogeneous. The general consistency can be affected by the amount of palm sugar added. Palm sugar, as a type of sugar, is known to have the ability to bind water and form a strong gel structure in yogurt (Winarno, 1992). The more sugar is added, the more water the sugar components can bind with, causing the texture of the resulting yogurt product to become thicker and more homogeneous.

### 3.9 Overall Acceptance

The analysis of variety shows that adding palm sugar has a very significant effect on the overall acceptance of yogurt. Panelists, on average, gave a relative rating of liking palm sugar yogurt. This is likely because adding palm sugar to the palm sugar concentration provides a slightly sour taste and thick texture that the panelists like.

## 4 Conclusion

Based on the research and analysis that has been done, it can be concluded that:

1. From the results of the protein content test, it was found that the highest protein content was in the treatment of incubation time for 8 h, and the starter concentration was 5% v/v.
2. From the results of the fat content test, the highest fat content was obtained in the 10-h incubation time treatment, and the starter concentration was 2.5% v/v.

3. The level of protein test in this palm sugar yogurt research has met the Indonesian National Standard of at least 2.7%, namely between 2.86%-3.85%.
4. The level of fat test in this palm sugar yogurt research has met the Indonesian National Standard for low-fat yogurt between 0.6%-2.9%, namely 1.86%-2.59%.
5. The addition of lactic acid bacteria significantly affected the organoleptic characteristics of aroma and taste but had no effect on color and texture characteristics.

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