Optimizing the quality and antimicrobial ability of yogurt through a combination of starter and dates puree at different levels

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Abstract. Research on the optimization of the quality and antimicrobial ability of yogurt using a combination of starters (Lactobacillus acidophilus and Streptococcus thermophilus) and dates puree with different levels has been carried out to determine the changes in the quality of yogurt in terms of lactic acid levels, protein content, syneresis and also looking for an antimicrobial ability which was measured based on inhibition zone formed against the tested bacteria (E.coli and S aureus). A completely randomized design (CRD) factorial pattern was applied consisting of 2 factors, those are factor A which is the level of starter, a1 = 2.5 percent, a2 = 5 percent, and factor B which is the level of dates puree namely b0 = 0 percent, b1 = 10 percent, b2 = 20 percent and b3 = 30 percent. The results showed that the level of dates puree had a very significant effect on lactic acid levels but not so with the starter level. The combination of treatments (P <0.01) affects the protein content but there was no interaction between treatments. Furthermore, there is interaction between treatments and it decreases the syneresis value significantly. It can be concluded that the levels of lactic acid, protein and syneresis of yogurt still have the best quality obtained in the treatment of 5 percent starters and the addition of 20 percent of dates puree. On the other hand, it had a weaker antimicrobial ability

1. Introduction
Yogurt is a fermented milk product using lactic acid bacteria that has a distinctive taste and is useful as a source of nutrition. In general, lactic acid bacteria that often used as a starter in milk fermentation are Lactobacillus acidophilus, Lactobacillus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei and Streptococcus thermophilus [1]. In this study the starter used was Lactobacillus thermophilus, and Lactobacillus bulgaricus. The addition of dates puree in yogurt aims to increase the nutritional value and increase the benefits of yogurt in suppressing the growth of pathogens. This is supported by the excellent potential of dates as healthy foods such as vitamin A, vitamin B1, vitamin B2, vitamin B3, tannin, anthocyanin, antioxidant, tannin, beta carotene, lutein, and zeaxanthin [2]. Fiber components in dates are expected to improve the quality of yogurt produced and also increase the benefits of yogurt as a probiotic drink which is reflected in its ability to suppress the growth of pathogens [3]; [4]. Therefore, the potential development of probiotic products from dates can increase the functional effectiveness of the dates themselves.
2. Material and Methods

2.1. Material
The material used are milk powder, dates (Phoenix dactylifera L) and lactic acid bacteria (*Lactobacillus acidophilus* and *Streptococcus thermophilus*).

2.2. Methods

2.2.1 Preparation of Fermented Milk. Powdered milk was reconstituted with sterile water and added with dates puree (dates which have been mashed using water and filtered). The mixture was inoculated with a starter according to the treatments, and then incubated for 8 hours at 37°C.

2.2.2 Lactic Acid Analysis. Fermented milk in amount of 18 mL was put it into Erlenmeyer and added by 3-4 drops of phenolphthalein as an indicator before titration with 0.1 N NaOH to form a stable pink color. The volume of NaOH was noted. Lactic acid concentration was calculated using the following formula of [5].

\[
\text{Lactic Acid concentration} = \frac{\text{ml NaOH} \times N \times 0.009}{\text{Sample weight (g)} \times 100}\]  

(1)

2.2.3 Protein Analysis. As much as 25 mL of sample was put into Erlenmeyer, added with 1 mL of potassium oxalate solution and 0.25 mL of phenolphthalein, and left for 2 minutes. Next, it was titrated with 0.1 N NaOH until forming pink color. Furthermore, 2 ml of formaldehyde was added and shaken until it became homogeneous and the pink color disappeared. After 1 minute incubation, it was titrated with 0.1 N NaOH until a red color was formed. The volume of NaOH (V1) used was recorded [6]. Similar procedure was done for blank (V2), however, the sample was replaced with aquadest in similar amount with the sample.

\[
\text{Protein Level} = \frac{V_1 - V_2 \times 1.83}{100}\]  

(2)

2.2.4 Syneresis Value. The sample was weighed as much as 10 grams, put into the test tube and then stored in the refrigerator at 5°C for 1 h. The sample was centrifuged for 10 minutes at a speed of 3000 rpm. After then the weight of the supernatant (whey liquid) was obtained. [7]

\[
\text{Syneresis} = \frac{\text{Supernatant weight (g)}}{\text{Sample weight (g)} \times 100}\]  

(3)

2.2.5 Antimicrobial Activity Assay. Antimicrobial ability of samples was measured by the extent of the inhibitory zone formed in the testing bacteria as a response of its growth. This test used two types of testing bacteria, *Escherichia coli* and *Staphylococcus aureus*. These bacteria were grown in physiological NaCl for 24 hours at 37°C. After dilution to 10³, 1 mL dilution was put into a sterile petri dish, then the nutrient agar (NA) media was poured into a petri dish, homogenized, let it harden well. Next, place the disc paper that has been soaked with a sample on the surface of the Petri dish that has been planted with the test bacteria, then incubated for 24 hours at 37°C. The diameter of the inhibition zone is measured using a caliper and presented in millimeter [8].

2.2.6 Experimental Design and Statistical Analysis. The experiment was assigned in a completely randomized design with factorial pattern consisting of 2 factors namely A which is the starter level with 2 levels (a1 = 2.5% and a2 = 5%) and B which is the puree dates with 4 levels (b0 = 0%; b1 = 10%; b2 = 20% and b3 = 30%). All treatments were repeated 4 times. Statistically the data obtained was analyzed by analysis of variance (ANOVA), and differences between treatments were tested by Duncan's Multiple Range Test [9].
3. Results and Discussion

3.1 Lactic Acid

It showed in the table 1 that the starter and dates puree level had significant effect on the lactic acid level. The treatment without dates puree was significantly different from other treatments. The higher the level of the starter and dates puree, the higher was the lactic acid level. It can be explained that the more starter used, the higher was the amount of lactose transformed into lactic acid. Lactic acid comes from not only from milk but also from dates puree. High number of starters will affect the degree of acidity because carbohydrates are converted into lactic acid during the fermentation. Dates also have phenolic compounds that are also acidic [10]. According to [11] and [12], the amount of lactic acid will increase along with the increasing number of bacteria.

| Table 1. Quality of fermented milk with the addition of dates puree. |
|---------------------------------------------------------------|
| **Parameters** | **Starter Level** | **Dates puree (%)** | **0** | **10** | **20** | **30** |
| Lactic acid (%) | 2.5 % | 1.09b | 1.21c | 1.21c | 1.21c |
| | 5% | 1.05b | 1.24b | 1.25c | 1.24c |
| Protein (%) | 2.5 % | 0.97 | 1.09 | 1.22 | 0.67 |
| | 5% | 2.75 | 3.46 | 3.47 | 2.68 |
| Syneresis | 2.5 % | 9.45b | 9.03b | 8.99b | 7.32b |
| | 5% | 7.33b | 6.90b | 4.84c | 4.93a |
| Diameter of Inhibition Zone | E. coli | 2.5 % | 1.2 | 1.85 | 2.5 | 2.4 |
| | 5% | 1.0 | 1.8 | 2.1 | 2.3 |
| Zone S. aureus | 2.5 % | 0.6 | 0.7 | 7 | 0.7 |
| (mm) | 5% | 0.3 | 0.65 | 0.75 | 1.4 |

Means in the same row with different superscript differ significantly at (P<0.01)

3.2 Protein Analysis

Table 2 shows that the level of starter and dates puree have a significant effect on the level of protein. The higher the amount of the starter and dates puree, the higher is the protein concentration in yogurt. This might be the result of the activity of lactic acid bacteria which is able to produce proteolytic enzymes and digest proteins in the media [13]. Protein content of yogurt with 2.5% starter and without addition of dates puree is significantly lower. Application of 5% of starter with high level of dates puree leads to the increase of the protein content. On the contrary, the level of protein increased in combination of 5% starter at 10 and 20% level of puree dates, but there is no significant difference between treatments.

3.3 Syneresis

The texture of fermented milk that is unstable during the fermentation process. The whey is separated from the solids. This indicates a sign of syneresis. The level of starter and dates puree significantly affects the syneresis (table 1). At 2.5% starter and every level of dates puree, the syneresis increases significantly. This has relation with low total solid content in milk which is affected by low levels of puree dates. As found by [14], that syneresis can be led by the low concentration of total solids in yogurt.

At 5% starter in combination with 10 and 20% of dates puree, the syneresis value is low. Low syneresis is a positive indication for yogurt produced at a high level of puree dates. Puree dates contribute to increase total solid the yogurt. Low syneresis indicates that the texture of yogurt is good, because the presence of dates puree supports the stability of the yogurt [15].

Decreasing ability of protein networks to bind water will also lead to syneresis. Syneresis is likely occurred in relation with shrinkage of casein particles [16]. According to [17] syneresis
can be improved by adding stabilizers such as gelatin even though the pH of yogurt approaches the casein isoelectric point.

3.4 Antimicrobial Activity Assay

The antimicrobial ability of yogurt was determined by measurement of the diameter of inhibitory zone. The diameter of inhibition zone was wider for E. coli than S. aureus at all level of starter and dates puree. These results indicated that E. coli is more sensitive to the metabolite compounds formed during fermentation than S. aureus. According to [17], the diameter of inhibition zone formed depend on the concentration of metabolite compounds. The higher the concentration was stronger the ability to suppress the growth of pathogenic bacteria.

4. Conclusion

The best quality of yogurt is produced from the combination of 5 percent starter and 20 percent dates puree. The yogurt contained lactic acid and protein and low syneresis value, although had weak antimicrobial ability.

5. References

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