Effect of *Lactococcus lactis* inoculation on characteristics and microstructure of dangke cheese with different ripening temperature and incubation time

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**Abstract.** Physical properties of Dangke cheese can be improved by the ripening process through starter culture inoculation. The purpose of this study was to analyze the characteristics of Dangke cheese with different temperature and incubation time of ripening as well as their interaction by *Lactococcus lactis* inoculation. The research method used was experimental using a completely randomized design (CRD) with 2 (two) factors. The first factor (A) is the ripening temperature (5, 15, and 25°C) and the second factor (B) is the Ripening time (6, 9, and 12 days). Dangke is made by adding a papaya sap solution as coagulation. Microstructure was observed by a scanning electron microscope. The treatments were replicated three times each process. The data were processed statistically with variance analysis, followed by the least significant difference test.

The results indicated that the ripening temperature affected the activity of *L. lactis* in carbohydrate degradation to produce lactic acid so that it affected the hydrogen potential (pH) and the dangke protein content. Long time ripening provides an opportunity for *L. lactis* to continue performing activities resulting in a decrease in carbohydrate and pH levels, but there is an increase in total lactic acid and protein levels. The temperature and duration of ripening in dangke manufacture provide an interaction of carbohydrate and protein levels. The Ripening temperature used in the manufacture of Dangke cheese with the addition of *L. lactis*, preferably 5°C and the duration of 12 days of ripening. The microstructural features show that the duration of ripening causes the Dangke structure to be more compact, as indicated by the proximity of casein molecules with other casein molecules and fat spread evenly in the three-dimensional network of molecules.

1. Introduction

One of the dairy products that are very popular today is cheese, which has a very diverse variety in the market. Cheese is a milk product whose nutritional value is very high and is very important in enhancing its characteristics in overall characterization, for example, texture and flavor, where this quality is crucial in food products [1]. The type of cheese that exists today is also very much determined by the quality of the ripening process. Dangke, which is a traditional local cheese of South Sulawesi, is an example of a cheese that does not have a ripening process [2-8]. Therefore, to improve the quality of Dangke, a ripening process can be carried out [9, 10], both natural ripening process and culture inoculation starter.
Cheese ripening is a complex phenomenon involving a wide range of biochemical reactions [10, 11] and the microbial starter culture plays a significant role in the ripening process.

*Lactococcus lactis* is one of the starter culture bacteria that can be used in the cheese ripening process [12; 13]. *Lactococcus lactis* requires a suitable growth temperature to optimize the cheese ripening process, and prevent the growth of pathogenic microorganisms [14]. The growth temperature of *L. lactis* is 5-40 °C and optimal at 37°C, and the determination of the ripening temperature is also greatly influenced by the ripening time. The process of cheese ripening can be used at a temperature of 10-16°C for 15-30 days to increase cheese quality [15]. That cheese dipping should be at 5°C for 3-4 weeks [16]. The results of [17] can be seen that cheese agglutinated with passion fruit extract and ripened at 10°C for 4 weeks depicts an increase in the physical properties of the cheese. The temperature and time of ripening should be known in order for *L. lactis* to activate optimally and to improve the characteristics of Dangke cheese. *Lactococcus lactis* activity requires a source of energy (carbohydrates) to grow and develop because carbohydrate fermentation by *L. lactis* produces lactic acid causes the cheese to decrease its acidity (pH) during the ripening process. *Lactococcus lactis* has the ability to cut the protein-peptide bonds causing agglutinate milk. The metabolism effects of milk composition affect the microstructure of dangke cheese [7]. For further investigation relating to profiling bacterial growth at the protein-fat interface was suggested by [18]. Rational relationships between microstructure and another quality parameter of physical properties are important to describe how the chemical reaction of cheese products.

The purpose of this study was to analyze the effect of temperature and duration of ripening as well as their interaction with the physicochemical properties and microstructure of dangke cheese inoculated by *L. lactis* and interaction between protein-fat.

2. Methods

This research was conducted experimentally by using a complete randomized design factorial pattern (3 × 3) with 3 replications. The first factor (A) is the ripening temperature (5°C, 15°C, and 25°C) and the second factor (B) is the ripening time (6, 9, and 12 days).

*Lactococcus lactis* subsp. *lactis* FNCC-0086 was obtained from Center for Food and Nutrition Studies of Gadjah Mada University Yogyakarta. Preparation of culture starter using methods from research [17] which were modified. Full cream milk was reconstituted to 10% (w/v) and sterilized at 105°C for 5 minutes, inoculated with 1% *L. lactis* after the milk culture at 40°C, then incubated at 30°C for 12 h.

The papaya sap is tapped from papaya fruit, still attaching to the tree, done from 06:00 to 07:00 am and 17:30-18:30 pm. Tapping is done by incised a knife on the skin of papaya fruit, starting from the base to the tip of the fruit. The papaya sap was collected, frozen and then dried using freeze dryer for 50 hours. The crude papain used was first dissolved with sterile aquades of 1: 100 (w/v) ratio.

Fresh cow's milk is obtained from Sinjai District of South Sulawesi. The milk of 500 ml was heated to 40°C and 0.2% papain solution was added. The temperature of the milk was increased to 95°C maintained for 5 minutes until the milk coagulated. The curd filtration process for 5 minutes was carried out after the temperature reaches 70°C. The molded curd (mold modified) and pressurized for the whey dispensing process. Dangke is left in the mold for 20 minutes to maximize whey expenditure.

The addition of a starter and curing process was modified from research methods before [17]. The starter of 1% (w/v) of dangke weight is added by injecting the 5 sides of dangke, then it is incubated at 5°C, 15°C, and 25°C for 6, 9 and 12 days. Samples for microstructure examination were made according to [16] and observed using scanning electron microscopes (Hitachi S-4 type SEM).

Measured parameters were viscosity (mpa/s), lactic acid (%), and pH. The data were calculated by statistical variant analysis based on the completely randomized design factorial pattern. The treatment of significant effect was further tested by the smallest real difference test.
3. Results and discussions

3.1. Viscosity

The results of the study (table 1) indicated that the temperature and time of ripening and interaction between temperature and time of ripening had a significant effect (p<0.01) on the viscosity of dangke cheese. The least significant difference (LSD) test showed that the viscosity was significantly different (p<0.05) between the temperature and time of the ripening, and there was an interaction between temperature and duration of the ripening of dangke cheese.

**Table 1. Viscosity (mpa/sec) of dangke cheese at different temperature and time of ripening.**

| Temperature | Time of ripening | Average |
|-------------|------------------|---------|
|             | 6 days           |         |
| 5°C         | 205.26±4.30<sup>b</sup> |         |
| 15°C        | 112.53±1.81<sup>c</sup> |         |
| 25°C        | 97.58±1.22<sup>d</sup> |         |
|             | 9 days           |         |
| 5°C         | 223.04±3.03<sup>g</sup> |         |
| 15°C        | 115.16±6.16<sup>e</sup> |         |
| 25°C        | 102.08±8.18<sup>a</sup> |         |
|             | 12 days          |         |
| 5°C         | 258.20±5.30<sup>f</sup> |         |
| 15°C        | 135.08±9.20<sup>b</sup> |         |
| 25°C        | 116.39±8.03<sup>c</sup> |         |
|             | Average          |         |
| 5°C         | 228.83±4.21<sup>c</sup> |         |
| 15°C        | 120.92±5.72<sup>b</sup> |         |
| 25°C        | 105.53±5.80<sup>a</sup> |         |

<sup>ABC</sup>superscripts that follow the mean values on the same rows and columns showed significant difference (p<0.05).
<sup>abcd</sup>e superscripts that follow the mean values in different treatment interactions showed significant difference (p<0.05).

The ripening temperature and time significantly affected on the viscosity of the dangke cheese. The longer the ripening time, which caused an increase in viscosity, the more significant increase in the ripening temperature which caused a decrease in viscosity.

3.2. Lactic acid presentation

The results of the study (table 2) showed that total lactic acid was not significantly different from the variation between 0.49 to 0.54%. The results of the variance analysis showed that the temperature and time of the ripening and interaction of both had no significant effect (p>0.05) on the lactic acid content of ripening dangke cheese produced.

**Table 2. Titratable acidity (% lactic acid) of dangke cheese in ripening temperature and time.**

| Ripening temperature | Ripening time | Average |
|----------------------|---------------|---------|
|                      | 6 days        |         |
| 5 °C                 | 0.48±0.05     |         |
| 15 °C                | 0.51±0.10     |         |
| 25 °C                | 0.50±0.08     |         |
|                      | 9 days        |         |
| 5 °C                 | 0.49±0.08     |         |
| 15 °C                | 0.51±0.13     |         |
| 25 °C                | 0.53±0.11     |         |
|                      | 12 days       |         |
| 5 °C                 | 0.51±0.05     |         |
| 15 °C                | 0.53±0.07     |         |
| 25 °C                | 0.58±0.11     |         |
|                      | Average       |         |
| 5 °C                 | 0.49±0.06     |         |
| 15 °C                | 0.51±0.10     |         |
| 25 °C                | 0.53±0.10     |         |

Total lactic acid is the main product of fermentation using Lactic Acid Bacteria (LAB) in dangke products. Lactic acid is the result of the breakdown of milk carbohydrates in dangke during the ripening process by *L. lactis*. The ripening temperature close to the optimum growth temperature of *L. lactis* causes the bacteria’s ability to ferment the increased carbohydrate, resulting in a greater total of lactic acid. At low ripening temperatures, *L. lactis* growth is inhibited so that the production of lactic acid is also less. *Lactococcus lactis* is a type of LAB that can live at 5-50°C and optimum growth at 30°C. The titratable acidity values are similar to reported before [19]. The low acidity of the product can be a reflection of the short ripening period of the product or the inadequate lactic acid fermentation during the ripening process.
3.3. **potential Hydrogen (pH)**

The result of variance analysis (table 3) showed that the temperature and ripening time had a very significant effect \( (p<0.01) \) on the pH value of dangke cheese, but the interaction between temperature and ripening time had no significant effect \( (p>0.05) \) one the pH value of dangke. Further, LSD test results showed that pH values different \( (p<0.05) \) between each ripening temperature and ripening time.

**Table 3.** Characteristics of pH dangke at different temperature and time of ripening.

| Ripening temperature | 6 days   | 9 days   | 12 days  | Average  |
|-----------------------|----------|----------|----------|----------|
| 5°C                   | 4.81±0.03| 4.71±0.07| 4.49±0.11| 4.67±0.16<sup>C</sup> |
| 15°C                  | 4.49±0.11| 4.37±0.00| 4.28±0.17| 4.38±0.14<sup>B</sup> |
| 25°C                  | 4.51±0.02| 4.29±0.03| 4.06±0.05| 4.23±0.20<sup>A</sup> |
| Average               | 4.60±0.17<sup>C</sup>| 4.46±0.20<sup>B</sup>| 4.27±0.21<sup>A</sup> |          |

<sup>ABC</sup>superscript that follows the average values on the same rows and columns showed significant difference \( (p<0.05) \).

The production of lactic acid by *L. lactis* causes a decrease in pH, due to an increase in H<sup>+</sup> ion activity. The incubation at 5°C causes the growth of bacteria to be slow because of metabolic processes inhibited. The pH value is in the range of values reported by [20] which examines Turkish white cheese. Generally, almost all countries, cheese acidity standards, should have 3% maximum titration of acidity in terms of lactic acid, much have a pH value over 4.5.

3.4. **Protein of dangke**

The result of variance analysis (table 4) showed that ripening temperature and time and their interaction had a very significant effect \( (p<0.01) \) on the protein of dangke. The LSD test results showed that protein of dangke at various temperatures and time of ripening and both interaction was significantly different \( (p<0.05) \).

**Table 4.** Protein percentage of dangke at different temperatures and time of ripening.

| Ripening temperature | 6 days   | 9 days   | 12 days  | Average  |
|-----------------------|----------|----------|----------|----------|
| 5°C                   | 24.54±0.08<sup>d</sup>| 22.44±0.25<sup>f</sup>| 24.97±0.07<sup>h</sup>| 23.99±1.18<sup>d</sup> |
| 15°C                  | 21.89±0.03<sup>e</sup>| 22.60±0.10<sup>f</sup>| 19.70±0.03<sup>d</sup>| 21.39±1.31<sup>B</sup> |
| 25°C                  | 18.94±0.10<sup>c</sup>| 15.37±0.02<sup>b</sup>| 14.30±0.01<sup>a</sup>| 16.20±2.10<sup>a</sup> |
| Average               | 21.79±2.34<sup>C</sup>| 20.14±3.58<sup>B</sup>| 19.66±4.62<sup>A</sup> |          |

<sup>ABC</sup>superscript that follows the average value on the same row and column showed significant difference \( (p<0.05) \).

<sup>abcdefgh</sup>superscripts that follow the mean values in different treatment interactions showed significant difference \( (p<0.05) \).

The protein of ripening dangke cheese is strongly influenced by the nutritional content and activity of *L. lactis* in the dangke cheese. The ripening provides an opportunity for *L. lactis* to decompose the dangke protein by proteolysis reaction. Proteolysis is the main biochemical process during cheese ripening which is one of the most important factors for the development of the distinctive taste and texture of cheese [20]. The deposition of milk proteins can occur due to acidification, which causes the solution to reach the isoelectric point [21]. The absolute value for milk casein precipitation is pH 4.7. The results (table 3) show that the 5°C ripening temperature has a pH dangke (4.67), which is equal to the absolute value for casein clotting. Enzymatic hydrolysis that occurs during cheese ripening from the casein matrix is a major biochemical event that is very important in the determination of the final product. Peptides from proteins that are decomposed further by proteinases in the cell wall of starter
LAB (*Lactococcus lactis*) will degrade to produce shorter peptides, which are then further degraded by various peptidases to form free amino acids. The formation of hundreds of peptides and their complement in the form of free amino acids can be converted into volatile compounds through amino acid catabolism [22].

### 3.5. Microstructure

The description of the microstructure of dangke cheese is presented in figure 1. It is seen that increasing the ripening time at 5°C the dangke cheese more compact; otherwise, the increasing the ripening temperature causes the dangke structure softer. Microstructure plays a dominant role in the feature shown by the final product, which is a closely related picture as perceived by consumers [23]. Rational relationships between microstructure and another quality parameter of physical properties are important to describe how the chemical reaction of cheese products. The process of curd formation of clots of milk proteins due to the pull force between proteins whose different molecular or ion charge. The process of molecular imbalance causes the protein denaturation process, followed by aggregation that starting from whey protein.

**Figure 1.** The microstructures of dangke cheese using an electron microscope. A: Ripening at 5°C for 6 days; B: Ripening at 5°C for 9 days; C: Ripening at 5°C for 12 days; D: Ripening at 15°C for 6 days; E: Ripening at 15°C for 9 days; F: Ripening at 15°C for 12 days; G: Ripening at 25°C for 6 days; H: Ripening at 25°C for 9 days; I: Ripening at 25°C for 12 days.
Ripening process influence in water binding capacity of milk protein, coupled with retarded proteolysis, influences the texture of cheese, which becomes progressively firmer, more cohesive, whereas the structure of the protein matrix becomes coarser and more compact, thus resulting in slower softening during ripening [24]. Dangke by inoculated of *Lactococcus lactis* enhance proteolytic and lipolytic activities that promote the acceleration of cheese ripening. During coagulation, papain (enzyme from papaya latex) causes the breakdown of k-casein, which is present on the surface of casein, physical agglomeration, give rise to more uniform protein mass. On the other hand, fat globules normally retain their membranes and are thus observed as single entities, which may form a cluster that is entrapped within the protein micelles called three-dimensional matrix [23].

4. Conclusion
The best ripening temperature for dangke cheese was 5°C and at this temperature, the longer the ripening time will improve the physical properties of dangke cheese.

Acknowledgment
Authors would like to thank the Ministry of Higher Education Research and Technology the Republic of Indonesia, which has funded this research (STRANAS) and the Faculty of Animal Science Hasanuddin University which has provided laboratory facilities for the implementation of this research.

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