



## The Correlation Between pH Values and Acidity Degrees of Dadih from Gayo to Total Bacteria Colonies with Different Storage Time and Temperature

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### Abstract

Dadih is a traditional food product that is produced by storing buffalo milk in bamboo tubes and left for 2-3 days. During the process of making Dadih, milk ferments into Dadih in a bamboo tube. The aim of this research is to determine the quality of buffalo Dadih from pH values, acidity degrees, and total bacterial colonies with different storage durations and temperatures. This research used 12 bamboo tubes with two treatments and three repetitions. This research used a laboratory experimental method with a 2x3 factorial pattern. This research showed that the highest room and refrigerator pH values were found in the first week, with an average of  $7.13 \pm 0.15$  and  $7.9 \pm 0.1$ . The highest acidity degree values from room temperature and refrigerator were in the third week, with  $359.47 \pm 16.95$  and  $254.03 \pm 6.34$ . Another finding was that the highest total bacterial colonies at room temperature and in the refrigerator were in the third week, with  $3.33 \pm 0.89$  and  $2.8 \pm 0.76$ . It can be concluded that the buffalo Dadih from Gayo has a pH value and a total value of bacterial colonies that meet SNI standards. Meanwhile, the total titrated acid in Dadih does not yet have an SNI standard.

**Keywords:** Gayo Dadih, pH, acidity degrees, total bacterial colonies

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### Introduction

Milk is an almost perfect food and contains complete nutrients that are used as basic human needs (Sisriyenni and Zurriyati, 2004). The chemical quality of buffalo milk is higher than cow's milk, but the production is less than cow's milk. This is due to the processing of buffalo farms that are not maximized in terms of quality and quantity (Damayanthi *et al.*, 2014). One of the ways used to extend the shelf life of milk is by preservation. Where this preservation without damaging the characteristics of milk. The preservation of milk can be done by cooling, heating, sterilization, and fermentation of milk into curd.

Curd is a traditional fermented food product produced by storing buffalo milk in bamboo tubes and keeping it for 2-3 days. During storage lactic acid bacteria will help the fermentation process. The lactic acid bacteria can come from bamboo tubes, banana leaves or even the milk itself. During this process, it will change the texture of the milk which initially looks into a solid texture, namely curd (Amri and Putra, 2012).

The quality of curd consists of physical quality and chemical quality. Where physical quality consists of color, aroma, taste, texture and viscosity while chemical quality consists of the presence of

fat, carbohydrate, protein, pH, acidity, vitamins, and total bacterial colonies (Daswati *et al.*, 2009). Microorganisms that play a role in traditionally made curd are lactic acid bacteria (LAB), molds, and yeasts. *Lactobacillus* sp. bacteria dominate in this process. Curd made from buffalo milk generally has protein 5.7-6.6%, fat 7.9-8.2%, total acid content 0.96-1%, and water content around 69-73%, (Wijayati *et al.*, 2016). Meanwhile, curd storage in the room has a pH of 3.91 and a viscosity of 2,866.7 Cp (Fildawati *et al.*, 2017).

The survival of lactic acid bacteria is affected by pH. A decrease in pH value will increase the acidity of the curd. The acidic atmosphere in curd occurs due to the help of homofermentative bacterial enzymes which homofermentative bacteria can produce lactic acid by 85-90% which comes from glucose. The long fermentation time will make the pH value lower while the acid concentration or hydrogen ion concentration produced in the curd will be higher (Meilina *et al.*, 2022). The pH value and lactic acid value do not go together, meaning that the higher the lactic acid value, the lower the pH value and vice versa. the lower the acidity of a material in solution, the less tendency to release protons ( $H^+$  ions) so that the pH rises (Syachroni *et al.*, 2020).

Lactic acid bacteria (LAB) is a type of microbe that has a major role in the process of milk fermentation. Some species of LAB are *Streptococcus* and *Lactobacillus* (Hanum, 2010). Lactic acid bacteria (LAB) in curd are thought to have high activity in the formation of lactic acid due to changes in lactose. due to changes in lactose. The formation of lactic acid is characterized by the higher acidity of the curd. The number of living bacteria and many lactic acid products produced will be high if the curd is fermented for a long time (Chalid *et al.*, 2018).

## Materials and Method

### Research Sample

The samples used were curd from Kutelintang Village, Blangkejeren District. Sampling was done randomly with a total of 12 tubes which were then put into a box and

sent to the Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala.

### Research Methods

This study is a laboratory experiment conducted using a completely randomized design (CRD) with a 2x3 factorial arrangement. The first factor is storage temperature, which includes refrigerator temperature and room temperature, while the second factor is storage duration, categorized as 7 days, 14 days, and 21 days.

### Research Procedure Sampling

Buffalo milk (500 ml) was first milked and placed into a bamboo container of the talang type (*Schizostachyum brachyladum*). The milk was then fermented for 48 hours (2 days) to form curd. On the third day, 12 bamboo containers of Gayo curd were prepared and delivered using public transportation. On the fourth day, the samples were placed in a container, stored in a cooling box, and immediately transported to the laboratory for analysis. The Gayo curd was transferred into 12 sterile Erlenmeyer flasks, each containing 100 grams, and covered with aluminum foil. Six flasks were stored at refrigerator temperature, while the remaining six were stored at room temperature. Acidity, pH, and total bacterial colonies were examined with the first, second and third week storage treatments. Where the research method for total bacterial colonies refers to SNI 01-2897-2008 concerning testing methods for microbial contamination in meat, eggs, and milk, and their processed products.

### pH measurement

A total of 10 grams of curd from both room temperature and refrigerator storage was placed into separate test tubes. The samples were homogenized using a stomacher, and the pH was measured with a pH meter calibrated with a standard pH indicator. This procedure was repeated on days 7, 14, and 21 (Sudarwanto, 2012).

### Acid Degree Examination

To prepare the standard color, 0.5 ml of cobalt sulfate solution was added to 25 ml of the sample curd. The standard color remains valid for a maximum of 3 hours, after which it must be replaced with a fresh solution. For analysis, 25 grams of curd samples from both room temperature and refrigerator storage were homogenized using a stomacher and transferred into separate Erlenmeyer flasks. Subsequently, 1 ml of 2% phenolphthalein solution was added to each sample. The samples were then titrated with 0.25 N NaOH solution while continuously shaking until the color matched the standard. The titration process was completed within 30 seconds, and the volume of NaOH used (in ml) was recorded (Sudarwanto, 2012).

### Calculation Method

$$\text{Acid Degree (SH)} = \frac{a \times 100}{b}$$

Description

a = amount NaOH 0,25 N used

b = volume of milk sample used (ml)

### Total Bacteria

A total of 25 grams of curd was put into an Erlenmeyer tube containing 225 ml of sterile Buffered Peptone Water (BPW) solution and then homogenized for 1 to 2 minutes (10-1 dilution solution). Then 1 ml of dilution 10-1 was taken and then diluted using 9 ml of Buffered Peptone Water (BPW) solution as dilution 10-2 and repeated again until dilution 10-6. Furthermore, from dilutions 10-4, 10-5, 10-6, 1 ml each was taken to be put into a sterile petri dish and then poured into 20 ml of Plate Count Agar (PCA) media and homogenized by shifting the cup horizontally or forming a figure eight and allowed to become solid. The next stage is incubated at 37°C with an inverted position for 24 hours. The calculation of the number of colonies formed is carried out after 24 hours and the number of colonies that can be counted in a petri dish is 25-250 colonies (Indonesian National Standard, 2008).

### Data Analysis

The collected data were analyzed using analysis of variance (ANOVA), and if significant differences were found, further analysis was performed using the Duncan test

### Results and Discussion

The measurement results regarding the pH value were measured using a pH meter. Based on the table of research results above, it can be seen that the highest pH value is found in curd stored at refrigerator temperature and stored on day 7, as seen in the picture 1.



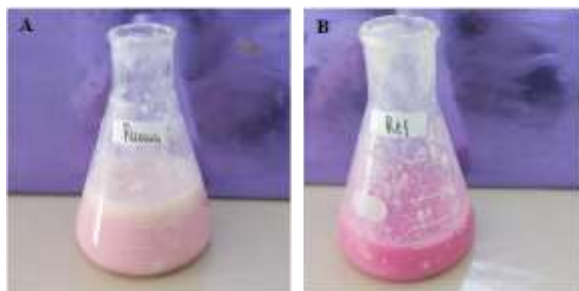
Picture 1. pH values at room temperature and refrigerator day 14.

The picture above shows that in the sample the highest pH value is found in curd stored at refrigerator temperature. The pH value shows the acidity of a solution which is the result of measuring the concentration of dissociated hydrogen ions (Taufik, 2004). Environmental factors such as temperature and poor storage are the causes of pH changes.

Curd at 4°C causes an extension of a product shelf life because, at low-temperature storage, the growth opportunities of pathogenic bacteria will be inhibited or minimized. During the storage period, curd shows a reaction or damage to the constituent components in the curd so that it can reduce the pH value. Changes in pH during storage indicate less stable food during storage (Meilina *et al.*, 2022).

Changes in pH value are strongly influenced by the high average total bacterial colonies in curd derived from this treatment. Meanwhile, if the substrate

needed by lactic acid bacteria is insufficient during the fermentation process, it will cause the pH value to increase which is characterized by reduced lactic acid formation (the minimum pH is 3.4 in yoghurt contained in SNI 2981-2009). Based on Table 1, it can be seen that the highest acidity value is found in curd stored at room temperature and stored on day 21, as seen in picture 2.



Picture 2. Acidity value in curd stored at room temperature (A) and Refrigerator (B).

#### Acidity values at room temperature and refrigerator on day 21

The degree of acidity is the amount of lactic acid formed due to the fermentation process. The total titratable acid (TAT) of buffalo milk curd on storage at room temperature and cold temperature is presented in Figure 2. During fermentation, buffalo milk curd increased every week. This increase is due to carbon and energy sources, namely lactose and casein, which will change into lactic acid which affects the acidity of the product.

The high production of lactic acid due to the activity of lactic acid bacteria that are many will make the curd product acidic. The increase in the degree of acidity of buffalo milk curd is strongly influenced by environmental temperature. High ambient temperature supports the metabolic activity of lactic acid bacteria so that bacterial growth will be accelerated or enlarged (Usmiati *et al.*, 2011).

The intensity and amount of lactic acid formation from LAB activity affects the high and low value of curd acidity. Products that are stored for a long time cause further metabolic reactions. This causes a decrease in the TAT value of cow's milk curd on day 21 (Usmiati *et al.*, 2011).



Picture 3. Total bacterial colonies at room temperature and refrigerator day 21

Lactic acid bacteria (LAB) is a factor that determines the success of fermentation. The fermentation activity in buffalo milk is characterized by the increase of LAB population. The total population of LAB in buffalo milk curd is presented in Figure 3. Environmental factors affect the growth of lactic acid bacteria in milk. Environmental factors are supporting aspects in increasing the number of cells and provide an overview of the growth curve (Afzal *et al.*, 2011).

High temperatures cause high metabolism and accelerated growth. Conversely, if the temperature drops, the metabolic rate will also drop and growth will also slow down (Asaminew and Eyassu, 2011). In addition, factors that affect the growth of lactic acid bacteria are incubation time, temperature, humidity, light, pH, and nutrients and if these factors are high, the growth of lactic acid bacteria is more optimal (Mallesha *et al.*, 2010). Milk naturally contains less than  $5 \times 10^3$  microorganisms per ml.

In addition to these factors, milk will also increase due to contamination. Contamination can come from the udder and body of the buffalo, the surrounding environment, water or the highest contamination can occur due to the inappropriateness of the milking process (Nurhayati, 2022). Contamination can be reduced by the use of modern technology during milking. Excessive contamination of pathogenic bacteria in milk causes the shelf life of curd to be low besides that it can also cause food poisoning (foodborne disease) (Susilaningrum *et al.*, 2022).

Based on the table of research results above, the highest pH value was found in curd stored on the 7th day and stored in the refrigerator. Each pH of the highest room temperature and refrigerator temperature has an average of  $7.13 \pm 0.15$  and  $7.9 \pm 0.1$  and the results of the analysis of variance showed that the length of storage ( $P < 0.05$ ) gave a different effect on the pH value and the results of variance showed that the temperature ( $P < 0.05$ ) gave a different effect on the pH value, but the two factors did not interact with each other ( $P > 0.05$ ).

The highest degree of acidity was found in curd stored on day 21 and stored at room temperature. Each of the highest degrees of acidity of room temperature and refrigerator temperature has an average of  $359.47 \pm 16.95$  and  $254.03 \pm 6.34$ . The results of variance on the degree of acidity showed that the length of storage ( $P < 0.05$ ) had a different influence on the value of the degree of acidity, and the results of variance showed that the temperature ( $P < 0.05$ ) gave a different influence on the value of the degree of acidity.

Table 1. Calculation results of pH value, degree of acidity, and total bacterial colonies with different storage and temperature

|                               | pH Value          | Degree of acidity value | Total bacterial colonies |
|-------------------------------|-------------------|-------------------------|--------------------------|
|                               | Log CFU/g         | Log CFU/g               | Log CFU/g                |
| Treatment of Room temperature |                   |                         |                          |
| 1                             | $7.13^a \pm 0.15$ | $175.16^a \pm 6.43$     | $5.03^a \pm 2.61$        |
| 2                             | $6.93^b \pm 0.11$ | $304^b \pm 12.11$       | $4.03^b \pm 2.65$        |
| 3                             | $6.46^c \pm 0.15$ | $359.47^c \pm 16.95$    | $3.33^c \pm 0.89$        |
| Treatment of Refrigerator     |                   |                         |                          |
| 1                             | $7.9^a \pm 0.1$   | $192.93^a \pm 11.12$    | $3.26^a \pm 2.19$        |
| 2                             | $7.36^b \pm 0.15$ | $201.4^b \pm 5.78$      | $2.4^b \pm 0.88$         |
| 3                             | $6.96^c \pm 0.15$ | $254.03^c \pm 6.34$     | $2.8^c \pm 0.76$         |

While the highest total bacterial colonies were found in curd stored at room temperature and storage on day 21, the results of variance analysis on total bacterial colonies showed that the length of storage ( $P < 0.05$ ) gave a different effect on the total bacterial colonies and the results of variance analysis showed that temperature ( $P < 0.05$ ) gave a different effect on the total bacterial colonies, but the two factors were not interrelated. both factors are not interconnected.

## Conclusion

Buffalo milk curd collected from Sangir Village, Dabun Gelang Subdistrict, and produced in Kutelintang Village, Blangkejeren Subdistrict, stored under different conditions of storage time and temperature, demonstrated quality values that meet SNI standards. The pH values ranged from 6.96 to 7.13, and the total bacterial colonies ranged from  $3.26 \times 10^7$  cfu/gram to  $3.33 \times 10^8$  cfu/gram. However, the total titratable acidity ranged from

175.16 to 359.47, and there is no SNI standard available for the degree of acidity.

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