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Characteristics of Buffalo Milk Curd on Variations in Fermentation Time

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ABSTRACT

Curd is a processed product from buffalo milk through a fermentation process. The clumping of fresh buffalo milk into curd occurs due to the presence of Lactic Acid Bacteria (LAB) in buffalo milk. Curd contains lactic acid bacteria (LAB),which are beneficial for health. These bacteria not only extend the shelf life of food by inhibiting the growth of spoilage and pathogenic bacteria but also produce bioactive compounds that are good for the body. LAB contained in curd even has probiotic properties. This research used a starter of 10% buffalo milk curd. In one production, 4500 ml of fresh buffalo milk and 500 ml of starter are used, yielding 3.8 kg of buffalo milk curd. The results of observations during 7 days of fermentation were water content (81.25-72.53%), ash content (0.93-0.89%), protein content (8.97-7.94%), fat content (7 .72-7.10%), carbohydrate content (1.13-11.54%), pH value (5.72-4.27), temperature (26.87-28.43^{o}C), Total Lactic Acid Bacteria ($1.6x10^{9}$- $8.1x10^{9}$ cfu/ml).

1. INTRODUCTION

1.1. Research Background

Curd is a processed product from buffalo milk through fermentation [1]. Equipment generally used in making dadih are bamboo, leaves as a cover, and binding [2]. The clumping of fresh buffalo milk into curds occurs due to the presence of Lactic Acid Bacteria (LAB) in buffalo milk [3]. This research used a starter as 10% buffalo milk curd. This is because, based on Ref. [7] research, regarding the characteristics of curd with variations in the percentage of starter addition using buffalo milk,the best curd characteristic results were obtained, namely with theaddition of 10% starter. This study produced curd on a scale of less than 200 ml. So in this research, curd was produced on a 5000ml scale using a 10% starter. This was done to see the differences in the characteristics of buffalo milk curd produced after increasing production.

During its development, the appearance of curd packaging has also changed. One of the obstacles in its development is that the conventional form of packaging means that the curd is not practical to carry long distances and the shelf life is relatively short, namely around 3 days, so an effort is needed to minimize the existing obstacles in the context of wider marketing development. Therefore, in this research, the curd will be stored and packaged in plastic packaging made from polypropylene. Polypropylene (PP) is a good choice of plastic material for food packaging. Polypropylene or PP plastic is one of the most frequently used plastics because of its characteristics. PP plastic has a smooth surface, can withstand chemicals, has high flexibility and durability, and is easy to recycle [4].

In this study, curd was produced on a 5000 ml scale. This scale is close to the industrial production scale, making it possible to observe the factors that influence the quality of curd in real production. This is due to knowing the comparison of results obtained if produced on a large and small scale between 5000 ml and 200 ml from Ref. [7]. With this large production scale, there are differences in the characteristics of buffalo milk curd if it is produced with a larger fermentation volume. so that it can then be determined what fermentation volume is more optimal.

1.2. Literature Review

Research related to buffalo milk curd in plastic packaging that is stored for several days, the quality of buffalo milk curd in plastic tube packaging is relatively better than in bamboo tube packaging. On the 9th day of storage, the curd in the plastic tube packaging still had the typical milk aroma of curd and was pure white, while the curd packaged in the bamboo tube on the same day of storage had a rancid smell and was yellowish white. The 12th day of storage showed a sharp decrease in protein and fat levels of 5.58% (from 6.81%) and 7.74% (from 8.66) respectively in bamboo tubes, while in plastic tubes a decrease in protein and fat to 4.94% (from 6.34) and 7.09 (from 7.96), respectively [5]. Then, research about the best fermentation time, namely 48 hours, with a protein content of 9%, fat content of 9.89%, water content of 73.02% with a pH of 5.02 and a total bacterial colony of 1.70 x 10^6 CFU/g [6]. Furthermore, research about characteristics and analysis of added value of curd with varying percentages of added starter, with a buffalo milk curd production scale of 200 ml, the results showed that Curd with the addition of 10% starter produced a water content of 73.2%, ash content of 1, 80%, protein content 3.28%, fat content 3.5%, total acid 0.92%, pH value 3.7, total BAL 5.3x109 cfu/ml [7].

1.3. Research Objective

This research aims to determine the characteristics of buffalomilk curd on a 5000 ml production scale using plastic packaging (polypropylene) which was fermented for 7 days regarding chemical, physical, and microbiological.

2. MATERIALS AND METHODS

2.1. Materials

The research was carried out at the Integrated Microbiology Laboratory, Agricultural Biochemistry and Food Nutrition Chemistry Laboratory, Faculty of Agricultural Technology, Andalas University, Padang. The ingredients used are buffalo milk and starter (buffalo milk curd) obtained from Alahan Panjang City, West Sumatra. The chemicals used are distilled water, NaOH, K_2SO_4, H_2SO_4, H_3BO_3, HCl, n-hexane, alcohol, phenolphthalein indicator, physiological salt, and MRS Agar media. The tools used are an autoclave, colony counter, pH meter, oven, furnace, thermometer, Kjedahl flask, extraction flask, set of Soxhlet tools, digestion flask, desiccator, fermentation packaging (plastic jar).

2.2. Making Buffalo Milk curd

The curd in this study used fresh buffalo milk as raw material. 4500 ml of fresh buffalo milk was put into a polypropylene container in the form of a jar. Then, 10% (500 ml) of starter from buffalo milk curd is mixed into fresh buffalo milk and stirred until well mixed, so the total mixture is 5000 ml. After that, the container was closed and stored at room temperature for seven days of fermentation.

2.3. Observation of Buffalo Milk Curd

Observations of buffalo milk curd, namely water content, ash content, protein content, fat content, and carbohydrate content carried out following SNI 2981:2009 (yogurt). Then pH value (AOAC, 1995), product temperature, and total Lactic Acid Bacteria (LAB). The average yield is used to analyze the added value of buffalo milk curd production.

3. RESULT AND DISCUSSION

3.1. Raw Materials

The results of mixing 4500 ml of buffalo milk and 500 ml of starter (buffalo milk curd) produced a yield of 76%. So, in one production scale of 5000 ml, 3.8 kg of buffalo milk curd is produced. The results of observations of raw materials can be seen in Table 1.

Table 1. The raw material analysis result

Analysis	Buffalo Milk	Buffalo Milk Curd
Water (%)	82.65 ± 0.96	72.47 ± 1.17
Ash (%)	0.93 ± 0.06	0.92 ± 0.06
Fat (%)	6.17 ± 0.27	7.34 ± 0.11
Protein (%)	6.53 ± 0.18	8.16 ± 0.16
Carbohydrate (%)	30.97 ± 1.02	43.95 ± 2.09
LAB (cfu/ml)	0.9 x 10^9 ± 0.52	8.2 x 10^9 ± 0.64
pH	6.29 ± 0.24	5.13 ± 0.45
Temperature (^oC)	29.93 ± 1.03	27.43 ± 0.63

The water content of buffalo milk curd is influenced by bacterial activity in the process of breaking down carbohydrates, proteins, and fats in the material [6]. The difference of ash content occurs because during the fermentation process, there is decay of dissolved minerals from sucrose which are most likely used by bacteria for metabolic activities [8]. Indonesian National Standard (SNI) No. 01-3141-1998 concerning fresh milk, namelythat the minimum protein content in fresh milk is 2.8%. This shows that the protein content of fresh buffalo milk used meets the protein content according to SNI for fresh milk. Protein levelsin curd are influenced by the growth and development of lactic acid bacteria found in curd which help the fermentation process [6].

The fat content in fresh milk can be influenced by the crude fiber content in the ration [9]. The fat content in curd is influenced by the fermentation of lactose into lactic acid which causes the clumping of casein followed by the clumping of fat. The high fat content in curd results in the texture of the curd being softer [6]. The main carbohydrate in milk is lactose. Lactose is a milk sugar synthesized in the cow's mammary glands and gives the milk a sweet taste [10]. Meanwhile, the carbohydrate content of the starter (buffalo milk curd) is higher because the water content in the curd is lower compared to milk. The total lactic acid bacteria in both materials showed more than 10^7 cfu/mL. Based on the SNI 2981:2009 reference regarding yogurt, fermented beverage products must have a total of lactic acid bacteria of at least 10^7 CFU/ml.

The difference of temperature is because the fermentation conditions that have occurred in the curd require conditions that are warm enough for the maximum growth of lactic acid bacteria. Meanwhile, the pH value produced in fresh buffalo milk was 6.29 and in buffalo milk curd it was 5.13. Based on SNI Number 01-3141-1998 regarding the quality requirements for fresh dairy, namely having a milk pH ranging between 6-7. Milk's high and low pH is caused by the conversion of lactose into lactic acid by microorganisms and enzymatic activity.

3.2. Characteristics of Buffalo Milk Curd

3.2.1. Water Content

Changes in water content of curd fermented using plastic containers for 7 days can be seen in Figure 1.

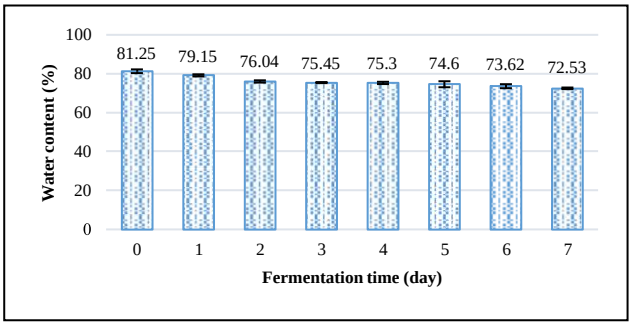

Figure 1. Graph of Changes in Water Content of Buffalo Milk Curd

This water content is close to the results of buffalo milk curd production in general, as in research by Ref. [6] which obtained a whey water content from day 0 to day 2 of around 77.76%-73.02%. The difference in results obtained in this study could be caused by the fermentation container used. Meanwhile, the decrease in whey water content can be caused by LAB activity which produces lactic acid, causing a decrease in the pH of the media. This decrease in pH will cause the milk protein (casein) to clump to form a gel. This protein clumping will trap some of the water previously dispersedin the milk, thereby reducing the water content in the curd [11].

When compared with previous research by Ref. [7] producing 200 ml of buffalo milk curd produced a water content of 73.2%. This is close to the results of this study. However, in this study, along with the length of fermentation, the water content can decrease even with larger production quantities, it is suspected that larger production can produce more LAB so that it uses more water to maintain LAB activity during fermentation.

3.2.2. Ash Content

Changes in the ash content of curd fermented using plastic containersfor 7 days can be seen in Figure 2.

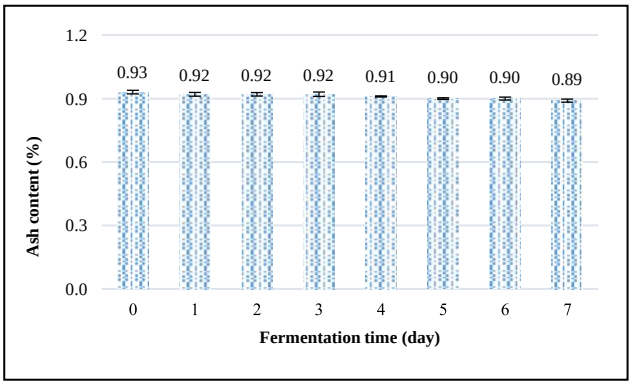

Figure 2. Graph of Changes in Ash Content of Buffalo Milk Curd

This decrease is thought to be related to LAB activity, where LAB can bind or change the shape of minerals, thereby reducing the amount of minerals that can be detected as ash. Lactic acid bacteria produce lactic acid as the main product of their metabolism. The acidic environment that forms can affect the solubility and availability of some minerals. Some minerals are thought to become less soluble in acidic conditions, so they are not fully detected during the ash content determination process. When compared with research by Ref. [7] which obtained the ash content of buffalo milk curd on a 200 ml scale, namely1.80%. This shows a difference because it is thought that themore milk is fermented, the more minerals in the milk will decay during fermentation.

3.2.3. Protein Content

Changes in the protein content of curd fermented using plastic containers for 7 days can be seen in Figure 3.

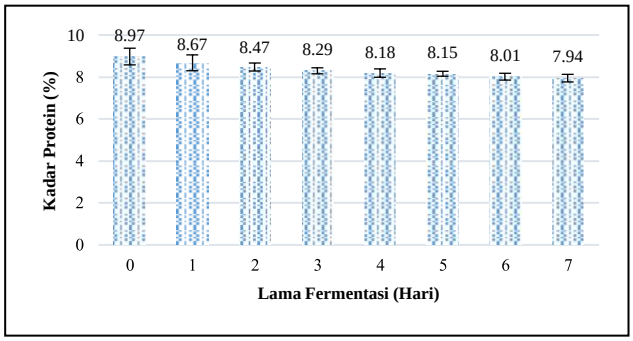

Figure 3. Graph of Changes in Protein Content of Buffalo Milk Curd

The decrease in protein levels in buffalo milk curd fermented for 7 days is related to protein degradation by enzymes. Proteolytic enzymes produced by lactic acid bacteria (for example, Streptococcus thermophilus, Lactobacillus bulgaricus) or those already present in milk can naturally break down milk protein (casein) into smaller peptides or amino acids. In the fermentation process, syneresis also occurs, where syneresis is the process of releasing whey liquid from the curd. When this liquid comes out or is released from the curd, the protein contentin the curd will decrease because some of it is released with the whey [12].

When compared with the results of research by Ref. [7], regarding the production of buffalo milk curd on a 200 mlscale, a protein content of 3.28% was obtained. This shows that the curd produced on a 5000 ml scale has a higher protein content, presumably because the greater the amount of fermented milk, the greater the total number of lactic acid bacteria, resulting in the formation of many protein molecules.

3.2.4. Fat Content

The decrease in the fat content of the curd is related to the occurrence of syneresis during the fermentation process, where the whey liquid separates from the curd, causing the fat content to decrease because some of it is carried away with the whey liquid. As in research by Naibaho, Simanjuntak, & Silalahi (2023), regarding the effect of temperature and fermentation time on chemical properties, total bacterial colonies and organoleptic curds, fermentation up to the 15th day saw a decrease in the fat content of buffalo milk curd. Changes in fat content of curd fermented using plastic containers for 7 days can be seen in Figure 4.

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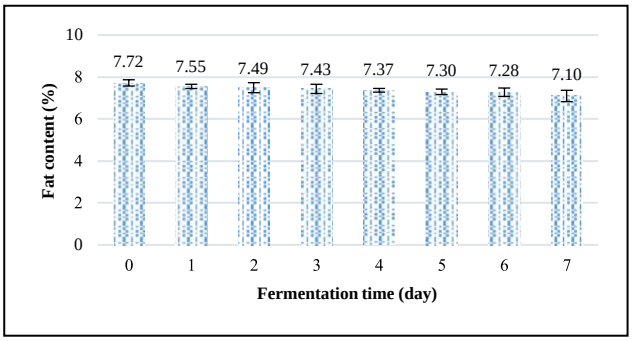

Figure 4. Graph of Changes in Fat Content of Buffalo Milk Curd

When compared with the results of research by Ref. [7], regarding the characteristics of buffalo milk curd produced on a 200 ml scale, a fat content of 3.5% was obtained. This is related to the total number of lactic acid bacteria that grow in the product,where the greater the number of bacteria that can grow and develop, the more casein and milk fat will clot.

3.2.5. Carbohydrate Content

Changes in the carbohydrate content of curd fermented using plasticcontainers for 7 days can be seen in Figure 5.

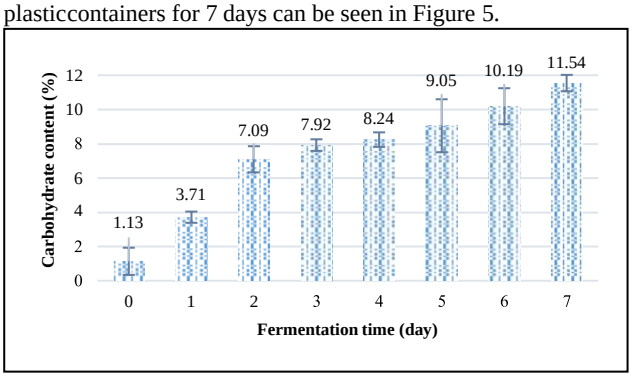

Figure 5. Graph of Changes in Carbohydrate Content of Buffalo Milk Curd

The fermentation process of buffalo milk curd involves the conversion of lactose (milk sugar) into lactic acid by lactic acid bacteria. The conditions in curd fermentation are anaerobic (without oxygen), so pyruvic acid is converted into lactic acid. This reaction is the final step in producing energy under anaerobic conditions and is a characteristic of lactic acid fermentation [13].

3.2.6. pH

These changes in pH are associated with the metabolic activity of the lactic microbiota, characterized by their ability to hydrolyze fermentable milk sugars and proteins [14]. The longer the fermentation time, the more microbes will produce acid so that the pH will decrease and the curd will experience more lumps [15]. The increase in accumulated organic acids causes a decrease in the pH value [16]. Changes in the pH of curd fermented using plastic containers for 7 days can be seen in Figure 6.

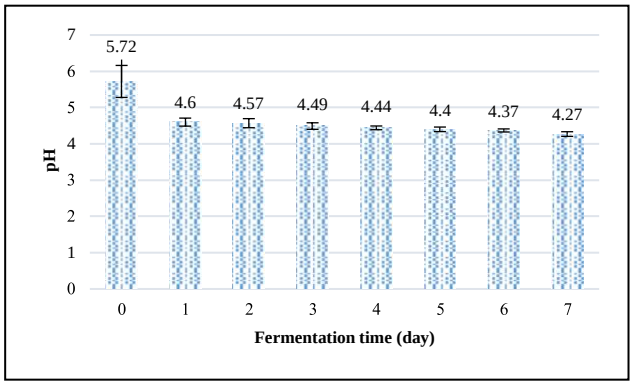

Figure 6. Graph of Changes in pH of Buffalo Milk Curd

When compared with research by Ref. [7], regarding buffalo milk curd produced on a 200 ml scale, the curd pH valuewas 3.7. Even though the numbers are different, the results of thisstudy are close to the results of Rahman's research [7].. This shows that fermented curd will produce a low pH as a result of lactic acid metabolism, where lactose is converted into lactic acidso that the curd becomes more acidic.

3.2.7. Temperature

Increasing the temperature of the curd during fermentation is associated with a decreasing pH value and an increasing total of lactic acid bacteria. The temperature produced by buffalo milk curd in this study is optimal because the enzymes produced by LAB work most efficiently at this temperature (temperature 26-28^{o}C). At optimal temperatures, these enzymes can interact with substrates (such as lactose) at maximum speed, so that the fermentation process takes place more quickly and efficiently. At optimal temperatures, bacteria can divide at a good speed, so that the bacterial population can increase. This is in line with the results of this study which showed an increase in total lactic acid bacteria.

Changes in the temperature of curd fermented using plastic containers for 7 days can be seen in Figure 7.

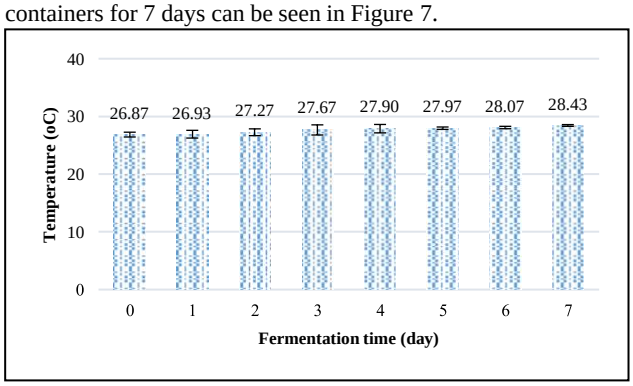

Figure 7. Graph of Changes in Temperature of Buffalo Milk Curd

3.2.8. Lactic Acid Bacteria Total

Changes in Lactic Acid Bacteria (LAB) total of curd fermented using plastic containers for 7 days can be seen in Figure 8.

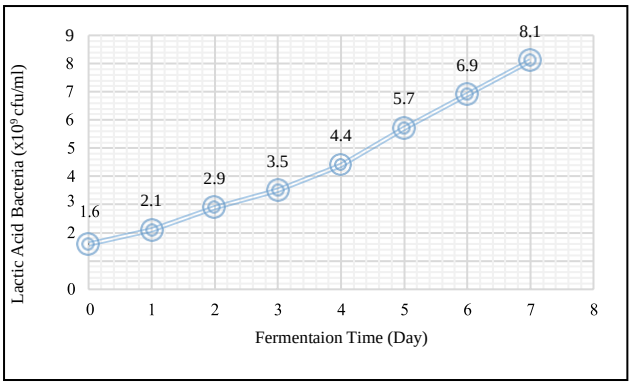

Figure 8. Graph of Changes in Lactic Acid Bacteria of Buffalo Milk Curd

This study, curd showed appropriate results according to SNI 2981:2009 regarding yoghurt, where fermented drinks must have a total of lactic acid bacteria of at least 107 cfu/mL. The total amount of LAB meets the requirements as a probiotic fermented milk drink, because the probiotic effect can be maintained if the carrier food contains a minimum number of probiotic microbes of 10^8–10^{10} cfu/mL. The longer the buffalo milk curd fermentation takes, the greater the number of LAB. This is also related to the decreasing pH, thus triggering the growth of certain microflora, namely Lactobacillus spp. and Pediococcus spp. both are acid-tolerant [17]. Compared with research by Rahman [7], regarding the characteristics of buffalo milk curd at 200 ml production, it produces a total BAL of 5.3x109. These results show that 5000ml of curd produced produces more total BAL. Inthis case, it is thought that the more milk is fermented, the more bacteria will grow.

4. CONCLUSION

This research concludes that the fermentation time influences the characteristics of buffalo milk curd. It was found that there were changes in water content, ash content, protein content, fat content, carbohydrate content, pH and temperature of the product, and total lactic acid bacteria from buffalo milk curd fermented for 7 days at room temperature. This shows that even though it was fermented using plastic packaging, the fermentation still went very well.

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