The Best Pasteurization Method in Reducing the Amount of Bacteria in Cow’s Milk on People Dairy Farm

E N Nawangsih¹,², I I Rahmat³, L S Halimah³, and D N Hidayat⁴

¹,² Department of Microbiology, Faculty of Medicine, Universitas Jenderal Achmad Yani, West Java, Indonesia
³ Department of Bio Chemistry, Faculty of Medicine, Universitas Jenderal Achmad Yani, West Java, Indonesia
⁴ Medical Education Program, Faculty of Medicine, Universitas Jenderal Achmad Yani, West Java, Indonesia

*eka.noneng@lecture.unjani.ac.id

Abstract. One method to reduce the number of bacteria and kill pathogenic bacteria is pasteurization. The purpose of this study is to get the best pasteurization method to reduce the number of bacteria and meet the Indonesian national standard requirements. This research is a comparative study to compare the methods of HTST (High Temperature Short Time) and LTLT (Low Temperature Long Time) pasteurization using samples of cow’s milk from the people’s dairy farms in Sunten Jaya Village, Lembang. The results showed the average total colonies number of cow’s milk before pasteurization was $1.19 \times 10^4$ CFU/m, post pasteurization using LTLT method was $8.8 \times 10^2$ CFU/ml, and HTST method was $3.2 \times 10^2$ CFU/ml. There was a significant decrease in the number of bacteria after pasteurization, with a p-value of 0.027 after being analyzed by the Kruskal Wallis test (p-value < 0.05). In addition, there was a significant difference between the total colonies number of HTST and LTLT post-pasteurization after being analyzed by the Mann Whitney test, with p-values = 0.05 (p-value < 0.05). Thus the HTST method is the best method in reducing the number of bacterial colonies in cow’s milk from Sunten Jaya Village, Lembang.

1. Introduction

Bacteria that pollute cow’s milk can cause diseases, especially diseases in the digestive tract, and some can even cause poisoning. Bacteria that can contaminate cow’s milk can be classified into two, namely pathogenic bacteria and spoilage bacteria. Rotting bacteria that can damage cow’s milk include, among others, Micrococcus sp., Pseudomonas sp., and Bacillus sp. and several types of pathogenic bacteria that often contaminate milk are Staphylococcus aureus, Salmonella sp., and Escherichia coli[1]. There are several factors that can cause milk pollution and affect the quality of cow’s milk, including 1) pre-milking, not cleaning the cage and bathing cattle before milking. 2) At the time of cast, do not wash hands before milking, and 3) after milking, milk is not stored in a sterile and closed container, and milk processing exceeds three hours from the time of collection [2].

One method to reduce the number of bacteria and kill pathogenic bacteria in cow’s milk without damaging the nutritional content is pasteurization. The pasteurization method can prevent the growth and development of bacteria in milk. According to the Food and Drug Association (FDA) based on the statement of Pasteurization Marketing Ordinance (PMO) there are 3 recommended methods, namely...
Low Temperature Long Time (LTLT), High Temperature Short Time (HTST), and Ultra High Temperature (UHT) [3].

In this research pasteurization will be conducted using the LTLT and HTST methods. Both methods are widely used in the community because they are easy and do not require expensive equipment. The purpose of this study is to obtain pasteurized cow milk in accordance with SNI standards so that it is safe for consumption and can be made advanced products.

2. Materials and methods

2.1. Materials

Cow milk samples came from productive cows in Sunten Jaya Village, Lembang. Sampling was carried out by cattle ranchers in Sunten Jaya Village, Lembang. Cow's milk samples were stored in cool boxes with temperatures around 4-8°C. Culture media used were plate counting agar (PCA).

2.2. Methods

This research is a comparative analytic study with cross sectional approach. All samples of cow's milk were mixed until homogeneous and then grouped into 3 groups: Group 1 (G1) was pasteurized using the LTLT method, group 2 (G2) was pasteurized by HTST method, and group 3 (G0) was fresh cow's milk group without pasteurization. Bacterial culture using pour plate method and counting the number of colonies using the TPC formula.

TPC formula [4]:

\[
N = \sum c \left[ \frac{1}{(1 \times n_1) + (0.1 \times n_2)} \right] d
\]

Formula description

\( N \) = total number of bacterial colonies
\( \sum c \) = the number of bacterial colonies in all plates was counted
\( n_1 \) = the number of plates in the first dilution calculated
\( n_2 \) = the number of plates in the first dilution calculated
\( d \) = first calculated dilution.

2.2.1 Sampling cow's milk

Sampling was carried out by cattle ranchers in Sunten Jaya Village, Lembang. Cow milk samples were collected using sterile syringes as much as 25 ml. Milk samples are then collected and put in a cool box at 4-8°C. Pasteurization and total plate count (TPC) examination were conducted at the Faculty of Medicine, Universitas Achmad Yani.

2.2.2 Pasteurization and Calculation of the number of bacterial colonies

2.2.2.1 Low Temperature Long Time (LTLT) Method

Heat a water bath to a temperature of 63°C. After the temperature is stable, enter the cow's milk sample. Heat for 30 minutes at this temperature, then immediately cool to a temperature of 10°C, then stored at a maximum temperature of 4.4°C [3,5].

2.2.2.2 High Temperature Short Time (HTST) Method

Heat a water bath to a temperature of 72°C. After the temperature is stable, heat the cow's milk for 15 seconds then immediately cool it to 10°C. The sample was then stored at a maximum temperature of 4.4°C [3,5].

2.2.2.3 Calculation of the number of bacterial colonies

Calculation of the number of bacterial colonies in cow's milk before and after pasteurization using Plate Counting Agar (PCA) media. Dilution starts by making a 10% solution, ie every 1 ml of milk is added to 9 ml of physiological NaCl solution (10-1 dilution). Then put 1 ml of the diluted sample into a tube containing 9 ml of physiological NaCl solution (10-2 dilution). Do the same with dilutions 10-3-10-7. The results of the dilution are then cultured into PCA media and incubated for 24-48 hours. The number of colonies is then calculated using a colony counter. The number of colonies was...
counted three times (triplo). The number of colonies can be counted if the petri dish contains 25-250 colonies and is free of contaminants. Colony calculation uses the TPC method formula [4].

3. Results
The average TPC of fresh milk (before pasteurization) produced by farmers in Sunten Jaya Village, Lembang was 1.19x10^4 cfu /ml. These results have met the SNI standard maximum number of bacterial colonies in fresh cow's milk, which is <1x10^6cfu/ml. The average yield of post-pasteurized milk TPC by the LTLT method was 8.8x10^2cfu/ml and the average yield of TPC by HTST method was 3.2x10^2cfu/ml. Based on these results, the number of bacterial colonies in pasteurized cow's milk by the two methods had met the Indonesian National Standardie <3x10^4cfu/ml.

Table 1. Table of the number of bacterial colonies in pre-pasteurized and post-pasteurized cow's milk using HTST and LTLT methods based on the TPC method

| Variable | Average number of bacterial colonies in milk (CFU / ml) | SNI standard |
|----------|--------------------------------------------------------|--------------|
| G0       | 1.19x10^4                                              | In accordance with the SNI standard |
| G1       | 8.8x10^2                                               |              |
| G2       | 3.2x10^2                                               |              |

Table description:
G0 : Pre-pasteurization group
G1 : Group 1 (pasteurized by the LTLT method)
G2 : Group 1 (pasteurized by the HTST method)
SNI : Standar Nasional Indonesia

Table 2. Comparison of the number of bacterial colonies in post pasteurization cow milk using the Kruskal-Wallis test

| Variable | n | Mean | Std. Deviation | P-Value |
|----------|---|------|----------------|---------|
| G0       | 3 | 119.67 | 13.42          |         |
| G1       | 3 | 1.25  | 0.72           | 0.027*  |
| G2       | 3 | 0.36  | 0.12           |         |

Table description:
G0 : Pre-pasteurization group
G1 : Group 1 (pasteurized by the LTLT method)
G2 : Group 1 (pasteurized by the HTST method)
* p-value ≤0.05 indicates a significant difference

Based on table 2, the statistical test with the Kruskal-Wallis test shows a p-value of 0.027 (p≤0.05), meaning that there are significant differences in the treatment group. Data processing was continued using the Mann-Whitney statistical test.

Table 3. Comparison of the number of bacterial colonies in post-pasteurized cow milk using the Mann-Whitney test

| Variabel | G0 | G1   | G2   |
|----------|----|------|------|
| G0       | -  | 0.05*| 0.05*|
| G1       | 0.05*| -    | 0.05*|
| G2       | 0.05*| 0.05*| -    |

Table description:
G0 : Pre-pasteurization group
G1 : Group 1 (pasteurized by the LTLT method)
G2 : Group 1 (pasteurized by the HTST method)
Based on table 3, the Mann-Whitney test shows that between groups tested are G0 with G1, G0 with G2 groups have a p-value of 0.05 (p≤0.05), meaning that there are significant differences in the number of colonies between the two groups. Thus the HTST and LTLT pasteurization methods can significantly reduce the number of bacterial colonies. In addition, there were also significant differences in the G1 and G2 groups. If we observe table 1, the G2 group has fewer bacterial colonies compared to the G1 group. This shows that the HTST method is better in reducing the number of bacteria when compared to the LTLT method.

**Discussion**

Pasteurization of milk is the process of heating milk at a certain temperature and time which aims to kill pathogenic bacteria and reduce the number of spoilage bacteria so that it is safe for consumption and increases the shelf life of milk [25,26]. According to the Indonesian National Standard number 01-6366-2000 maximum limit of microbial contamination based on the Total Plate Count (TPC) method in fresh milk $1 \times 10^6$ cfu/ml and in pasteurized milk $<3 \times 10^4$ cfu/ml [6].

The average number of bacterial colonies in fresh milk from cattle ranchers in Sunten Jaya village, Lembang is $1.19 \times 10^4$ cfu/ml. The average number of bacterial colonies in fresh milk is in accordance with SNI standards. Based on the results of the Mann-Whitney test, there was a significant decrease in the number of bacterial colonies in cow's milk after pasteurization using the LTLT method and the HTST method. The results of this study also showed that the HTST method was better in reducing the number of bacteria than the LTLT method.

This significant decrease in the number of bacterial colonies occurs because bacteria that grow in milk can multiply and live at temperatures between 2.5-57°C, for example: E.coli which can breed at 2.5-45°C, Salmonella at 6, 5-57°C, Staphylococcus 7-48°C, pseudomonadaceae 37°C-42°C and bacillaceae at 6-37°C. The pasteurization method used in this study used a temperature of 63°C for 30 minutes and 72°C for 15 seconds. This can cause bacterial death so that the number can decrease dramatically [7-9]. In addition, research conducted by Russell and Hastings (1983) showed that mycobacterium tuberculosis was found dead in milk that had been heated at 60°C for 10 minutes. From the research data it is recommended to warm up milk with a minimum temperature of 60°C for 20 minutes to ensure that pathogenic bacteria die completely [7].

Low Temperature Long Time (LTLT) method is a pasteurization method by heating milk at 63°C (145°F) for a minimum of 30 minutes. The durability of pasteurized cow's milk from this method is 7-10 days. Pasteurized cow's milk using this method is the best nutritional content, especially protein, therefore this pasteurization method is usually used for making cheese, yogurt, etc [10-11].

High Temperature Short Time (HTST) method is a pasteurization method by heating milk at 72°C (161°F) for 15 seconds. The advantage of this method is the effectiveness of the time used because it is shorter, the nutritional content is slightly damaged, can reduce more pathogenic microorganisms and can deactivate a number of enzymes that can damage cow's milk ie, phosphatase and catalase enzymes. The storability of post milk pasteurization HTST depends on the quality of the cow's milk used, which can last about 2-16 days at room temperature [10-11].

**Conclusion**

The average number of bacterial colonies using the TPC method after pasteurization using the HTST method is $3.2 \times 10^2$ cfu/ml and the LTLT method is $8.8 \times 10^2$ cfu/ml. HTST or LTLT methods can significantly reduce the number of bacterial colonies. There is a significant difference between the number of colonies in the two methods, the HTST method is better than the LTLT method in reducing the number of bacterial colonies. HTST and LTLT methods, can produce cow's milk with a number of colonies in accordance with Indonesian National Standard so that it meets microbiological requirements to make further products.
References

[1] Widodo S 2010 Bacteria that Frequently Contaminate Milk: Detection, Pathogenesis, Epidemiology, and How to Control it. *J R & D Agricul*. 293 96100.

[2] Food and Agriculture Organization of the United Nations 2004 Code of Hygienic Practice for Milk and Milk Products CAC/RCP 57 133.

[3] Britten J et al 2008 Grade A Pasteurized Milk Ordinance 18 8189.

[4] James G and Cappuccino NS 2016 *Manual Laboratory Micobiology* 11th ed.

[5] Indonesian National Standard 1995 Milk Pasteurization.

[6] Indonesian National Standard 2002 Maximum Limits of Microbial Contamination and Maximum Residue Limits in Foodstuffs of Animal Origin.

[7] Holsinger VH, Rajkowski KT, Stabel JR 1997 Milk pasteurisation and safety: a brief history and update. *Rev Sci Tech l’OIE* 162 441451. doi:10.20506/rst.16.2.1037.

[8] Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA 2013 *Jawetz, Melnick & Adelberg’s Medical Microbiology, 26th Edition*.

[9] Willey J, Sherwood L, Woolverton C 2007 Prescott, Harley, and Klein’s Microbiology, Seventh Edition 1088.

[10] Malik AOB, Marouf AAS 2018 Comparison of the Effects of Laser Pasteurization and Heat Pasteurization on The Cow’s Milk. *Haya Saudi J Life Sci.* 31 4650. http://scholarsmepub.com/.

[11] Hutagaol FVA 2013 microbiological quality of milk befor and after pasteurization 1-110 https://repository.ipb.ac.id/jspui/bitstream/123456789/61248/2/B13fva.pdf

Acknowledgements

Thank you to the research institute and community service Jenderal Achmad Yani University for the support of research funding provided.