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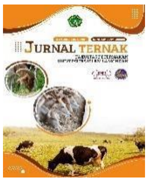

Combined Ultraviolet-C and Pasteurization Methods to Extend the Shelf Life of Cow's Milk

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ABSTRACT

Milk is classified as an animal food that is easily damaged because it contains high nutrients and water, so it is ideal for microbial growth. The common milk preservation technique is pasteurization. However, in pasteurization, heat-resistant bacteria are still able to survive. This study aims to find Total Plate Counternative methods of processing cow's milk, namely by innovating the combination of ultraviolet-C radiation and pasteurization methods to reduce the number of milk microbes while still prioritizing quality and safety. The ultraviolet -C modified pasteurization apparatus was given three treatments, i.e. radiation process then pasteurization process, pasteurization process then radiation process, and simultaneous (combined) process. The results showed that the simultaneous process resulted in a reduction percentage of bacteria of 99.996%, from the number of bacteria in milk which was originally 6.2×106 to 1×103 CFU/mL. The shelf life of milk with the simultaneous method showed the best results with an TOTAL PLATE COUNT test of 10 CFU/ml after 6 months of storage. The results showed that the degree of acidity (pH) before and after treatment of the three sterilization methods did not show a significant change. Simultaneous method or ultraviolet C radiation modified pasteurization process can be an Total Plate Counternative method in milk sterilization and increase shelf life.

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INTRODUCTION

Milk is classified as an animal food that cannot be stored for a long time and is easily damaged [1]. This is because milk contains nutrients and high water content, with a pH between 6.5 - 6.8 which is an ideal condition for the growth and proliferation of various types of microbes [2]. There are two groups of bacteria that commonly contaminate milk, namely spoilage bacteria and pathogenic bacteria. Spoilage bacteria include Pseudomonas sp., Clostridium sp., and Bacillus sp., while pathogenic bacteria in milk include those belonging to the genus Staphylococcus (72.1%), Streptococcus (16.1%), Bacillus (10.3%) , and Escherichia coli (1.5%) [3]. Therefore, milk needs to be handled with proper processing to ensure the quality of milk is maintained [4].

Some common milk preservation techniques include Low Temperature pasteurization, Long Time, High Temperature pasteurization, Short Time, vat pasteurization, ultra-pasteurization and Ultra High Temperature [5]. However, in thermal processing of milk, thermophilic bacteria, thermoduric bacteria, and spore-forming bacteria can still develop so that milk becomes easily damaged [6]. Heat-resistant bacteria such as Micrococcus and spore-forming bacteria such as Bacillus sp. still able to survive in pasteurized conditions [7]. Therefore, another method is needed that can increase the shelf life of milk while still prioritizing quality and safety. One Counternative Total Plate that can be applied to reduce the number of milk microbes with additional ultraviolet C irradiation [8].

Ultraviolet irradiation is an energy source that is capable of penetrating the cell walls of microorganisms and changing the nucleic acid composition of these microorganisms [9]. Ultraviolet irradiation is considered the newest food processing technology with higher commercial applications and is recognized as having 94% quality, 92% safety, and 91% longer shelf life [10]. In addition, the use of ultraviolet light does not cause chemical residues in food [11]. The type of ultraviolet that has strong antimicrobial power is Ultraviolet C because it has antimicrobial properties against bacteria, viruses, protozoa, fungi and algae. The most effective antimicrobial power occurs at a wavelength of 253.7 nm which can kill organisms by affecting cell function and changing cell structure or DNA [12]. Ultraviolet -C ultraviolet irradiation has several advantages compared to other methods in milk processing, such as low maintenance costs, can maintain physicochemical features [11] and nutritional value without causing sensory changes [13], low energy consumption, and suitable for commercial processing.

Based on this background, in this research a cow's milk processing process was carried out with an innovative combination of ultraviolet C irradiation and pasteurization methods to reduce the number of milk microbes. The combined thermal method of modified ultraviolet C pasteurization is expected to be able to overcome heat-resistant bacteria and spore bacteria which so far cannot be removed by conventional pasteurization methods. Therefore, the aim of this research is to combine the pasteurization process with ultraviolet C irradiation which can reduce the number of pathogenic bacteria and have no impact on the environment. This ultraviolet C modified thermal pasteurization device is expected to extend the shelf life of milk which has been a problem so far.

METHOD

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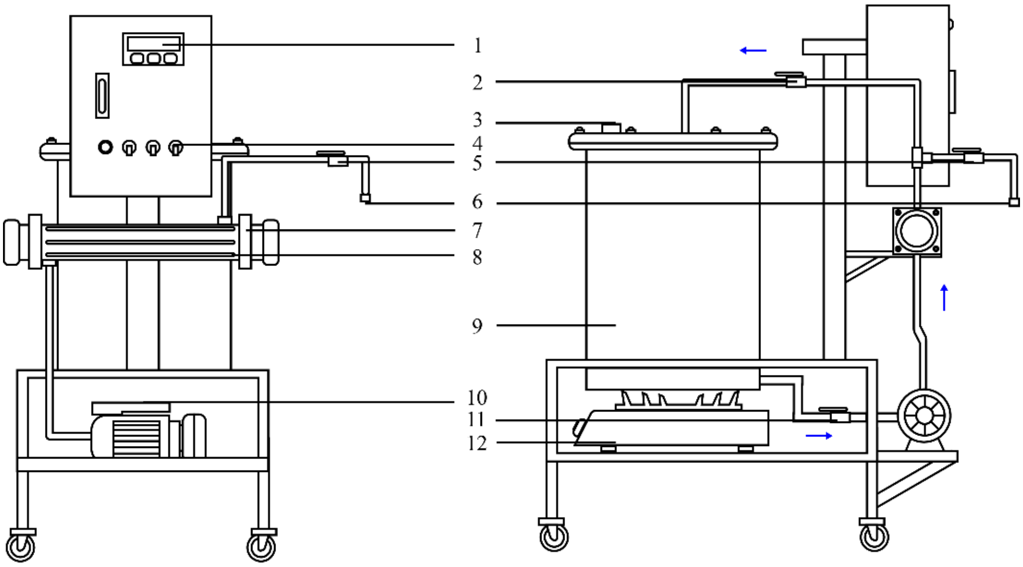
Fresh cow's milk is taken from the Kandri farm in Gunungpati District, Semarang City. The milk standard used is in accordance with SNI 0429 and is stored in bottles at <5°C to prevent bacterial contamination and maintain the condition of the milk so it is not easily damaged. Milk density is measured first using a pycnometer. The density of milk is measured by subtracting the mass of the pycnometer weighing containing the milk sample and the mass of the empty pycnometer then dividing it by the volume of the pycnometer listed. The milk is then put into a series of ultraviolet C modified thermal devices for sterilization.

Ultraviolet C Modified Thermal Apparatus

The series of modified ultraviolet C thermal apparatus is presented in figure 1. The modified ultraviolet C thermal pasteurized apparatus was operated using a temperature of



90˚C, a wavelength of 254 nm, and the number of germedial type ultraviolet C lamps was 7 units with a power of 8 Watt. Ultraviolet C modified pasteurization thermal apparatus is given three which are shown in Table 1.

(A): front view; (B): side view

Description: 1) Temperature Contreller, 2) Tap 1, 3) Milk inlet, 4) On/Off switch, 5) Tap 2, 6) Milk outlet, 7) Irradiation tube, 8) Ultraviolet-C lamp, 9) Pasteurization tank , 10) Pump, 11) Faucet 3, 12) Heating Stove

Figure 1. Desain Alat Pasteurisasi Termodifikasi ultraviolet -C

Table 1. Treatment of Ultraviolet-C Modified Thermal Devices

Sample	Method	Time (minutes)					
		a	b	c	d	e	f
1	Irradiation	0	5	15	30	45	60
2	Followed by pasteurization	0	5	15	30	45	60
3	Pasteurization	0	5	15	30	45	60
4	Followed by irradiation	0	5	15	30	45	60
5	Simultaneous (Pasteurization and Irradiation)	0	5	15	30	45	60

MATERIAL PREPARATION

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Fresh milk is taken from the kandri farm in gunungpati district, Semarang city. the milk standard used is in accordance with SNI 0429 and is stored in bottles at <5°c to prevent bacterial contamination and maintain the condition of the milk so it is not easily damaged.

Setting the Milk Flow Rate in the Sterilization Process

A total of 5 L of milk is put into the pasteurization tank. Next, tap 1 is opened to drain the milk from the tank to tap 2 and collect 600 mL in a 1 L measuring cup. Simultaneously, the stopwatch is turned on from tap 1 to tap 2 to get the flow time. Flow rate can be calculated using the formula volume (L) divided by time (s). Based on calculations, the flow rate value is 4 L/minute.

Sterilization process: Radiation Process then Pasteurization Process

The pump is turned on by pressing the pump switch button. Furthermore, milk in the tank is flowed by opening faucet 1 to the chamber to be exposed to UVC light for 5 minutes. During this process, the valve connecting the chamber and the pasteurization tank is closed so that milk does not flow into the tank. Next, the heating stove is turned on and the temperature is regulated up to 90C through the temperature controller. The irradiated samples were then pasteurized in a heating tank for 5 minutes. Then tap 3 is opened to flow the sample from the chamber to the tank. During this process, tap 1 which is the valve connecting the chamber and the pasteurization tank is closed so that the milk does not flow into the chamber. The final stream of milk that has been irradiated and pasteurized is taken from the outlet pipe of tap 2 and put into a 250 mL agro bottle. This treatment was carried out in three trials. These stages are in 8. Repeating steps 1 – 3 with time variations for each process for 15, 30, 45, and 60 minutes....A total of 5 L of milk is put into the pasteurization tank. The density of milk was carried out before and after treatment for 60 minutes. An empty pycnometer measuring 10 ml is first weighed using a scale. The milk sample before treatment is inserted into the pycnometer until it reaches the filling limit. The density of milk is measured by subtracting the mass of the pycnometer weighing containing the milk sample and the mass of the empty pycnometer then dividing it by the volume of the pycnometer listed. The procedure was repeated 3 times to calculate density in treated milk samples.

RESULTS AND DISCUSSION

Comparison of Single Process with Simultaneous Process

Milk processing using the pasteurization method can be shown in Figure 2A. The results showed that after 60 minutes of treatment, the sample pasteurization method produced a total plate count value of 1.3×105 CFU/mL or decreased by 99.19% from the original number of bacteria which was 1.6×107 CFU/mL. In the first 5 minutes, there was an increase in the bacterial content in the sample. This is because the optimum temperature and time for pasteurization have not been reached which causes thermophilic bacteria, thermoduric bacteria, and spore-forming bacteria to survive and develop [7]. After 10 minutes, a decrease in the number of bacteria began to occur. The duration of pasteurization causes more and more bacteria to experience cell damage because they cannot withstand the heat of pasteurization. The length of pasteurization time not only damages the bacteria which can shorten the shelf life of milk, but can also destroy some of the nutritious elements found in milk [14]. This is also supported by the pasteurization process with a temperature of 65˚C for 30 minutes resulting in a 90% reduction in the number of bacteria [15].

Furthermore, in processing milk with the irradiation method for 60 minutes can be seen in Figure 2B.

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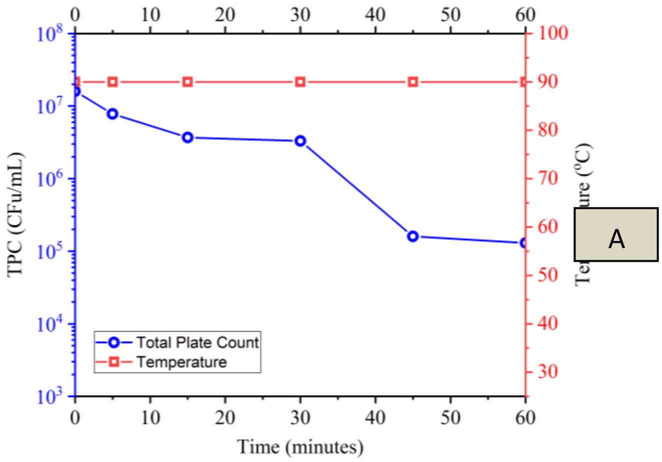
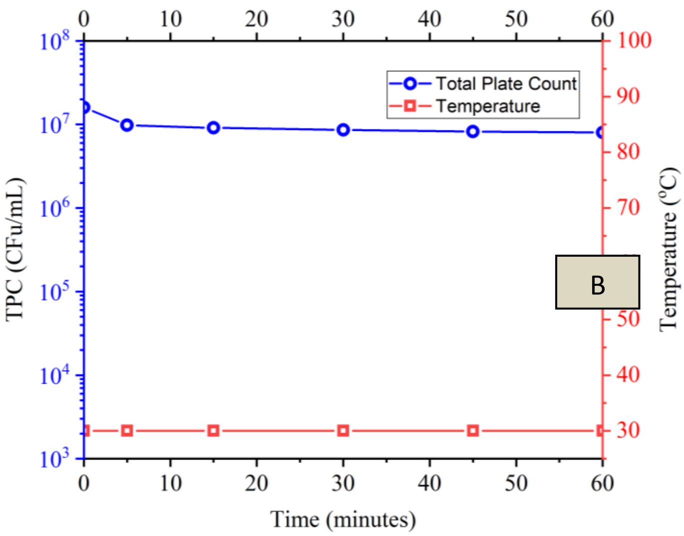
ISSN 2086-5201 (Print)

The effectiveness of milk processing using the irradiation method in reducing the total plate count was 50% or decreased to 8.0 × 106 CFU/mL at 30 minutes. This happened because the irradiation time is one of the factors that affect the effectiveness of irradiation [11]. Irradiation time is one of the variables in the level of ultraviolet irradiation exposure which indicates the germicidal effectiveness of ultraviolet -C irradiation [13]. A study of ultraviolet -C irradiation of cow's milk was also carried out by [16] which showed that irradiation at doses of 880 and 1,760 J/liter greatly reduced the growth of mesophilic bacteria and spore formation to below 103 CFU/mL. Another study conducted by [17] showed that ultraviolet -C treatment at a dose of 16,822 mJ/cm2 resulted in a 99% reduction in bacteria. Whereas in the study [15] ultraviolet -C irradiation with seven of ultraviolet -C for 8 W lamps resulted in a decrease in the number of bacteria by 99.9%.





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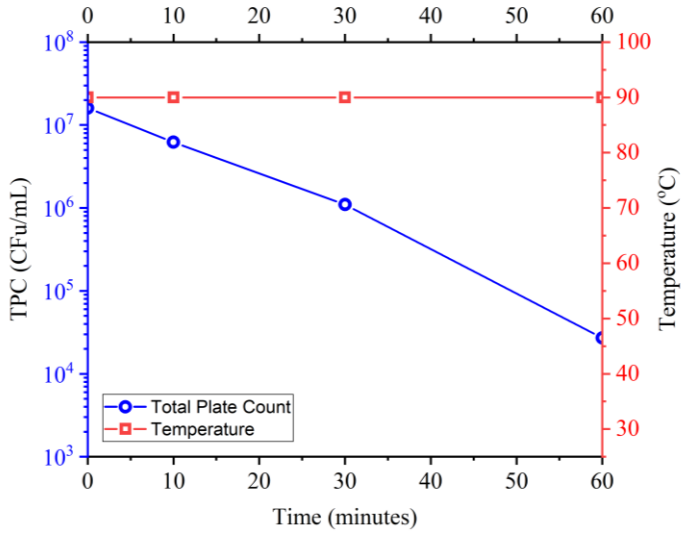

(A): Pasteurization Method; (B): Irradiation Method; (C): Simultaneous Method
Figure 2. Value of total plate count in Milk Processing Process with single and simultaneous methods

Even though the mechanism of nucleic acid from bacteria can be damaged by ultraviolet C light, the damage that occurs still allows cells to re-metabolize. Bacteria contain several enzymes that have the ability to overcome the damage that occurs, thus allowing the microbes to reactivate after exposure to ultraviolet C [18]. This was what happened at 45 and 60 minutes, the total plate count value in the samples that returned increased. The mechanism for microbial repair occurs in two ways, namely photoreactivation and dark repair [13]. Photoreactivation is a mechanism for controlling bacterial damage using photolyase enzymes [19]. On the other hand, dark repair which includes base excision repair and nucleotide excision repair uses an enzyme called N-glycosylase which allows cells to remove damaged DNA bases and does not require energy from the sun in its mechanism. This enzyme is able to increase the chances of survival of microorganisms and cause a reduction in the shelf life of milk [20]. In addition, bacteria can reactivation by other mechanisms such as ultraviolet - damage endonuclease [21].

In the simultaneous method, the percentage reduction in the overall number of bacteria was 99.83% as shown in Figure 2C. The use of pasteurization and irradiation methods simultaneously resulted in the lowest total plate count for 60 minutes, namely 2.7×104 CFU/mL. Research conducted (Bresson et al. 2016) showed that pasteurization and ultraviolet C irradiation could reduce bacteria to <100 CFU/mL based on the results of the total viable count. Another research conducted by [22] using the Ultra High Temperature- ultraviolet C pasteurization method succeeded in reducing bacteria at a temperature of 99.99%. Similar Ultra High Temperature - ultraviolet C pasteurization research was also carried out by [23] which showed a reduction in bacteria of up to 99.999%.

Based on the three treatment methods for the milk, the results of the simultaneous treatment provide a more efficient effect than the pasteurization or irradiation method alone. Bacteria that can be controlled by this method include mesophilic, thermophilic, thermoduric, and spore-forming bacteria [24][7]. However, research conducted by [25] showed that there was no significant difference between the number of microbes obtained in milk that was processed by pasteurization, ultraviolet light, and combinations. In line with this, research [15]

Wara Dyah Pita Rengga, et. al ./ Journal of Animal Husbandry 15 (2) 2024 pp. 11-23
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and [23] states that ultraviolet irradiation is effective against milk pathogenic microorganisms and can be an alternative method of heat treatment.

Comparison Of Double Process With Simultaneous Process.

Milk processing using double and simultaneous processes is compared on the Trent graph. The reduction in the number of bacteria is as shown in Figure 3. The process of irradiation then pasteurization is shown in Figure 3A. During the first 60 minutes of the experiment, a total plate count value of 8.6 × 106 CFU/mL was obtained with a percentage decrease of 50%. Meanwhile, during the second 60 minute experiment, the total plate count value was 1.9×103 CFU/mL with a reduction percentage of 99.99%. Overall, the pasteurization process followed by irradiation can achieve a maximum reduction in the total plate count value of 1.9×103 CFU/mL in an experimental time of 120 minutes. In other words, this process can reduce bacteria by approximately 4-log reduction. These results are in line with research [17] which shows that there is a reduction in bacteria in raw cow milk of more than 4-log reduction.

The results of processing milk using a double process, namely the pasteurization process and then radiation are shown in Figure 3B. During the first 60 minutes of experiment, an total plate count value of 1.3 × 105 CFU/mL was obtained with a reduction percentage of 99.19%. The increase in the ALT value during the 5 minute experiment was caused by the temperature of the pasteurization process being increased towards the optimal operating temperature of 90°C. The optimal operating temperature that has not been reached causes bacterial activity to be still active. Meanwhile, during the second 60 minute experiment, an total plate count value value of 8.2 × 104 CFU/mL was obtained. This value shows an increase from the results obtained in the first 60 minutes. This is because damage to nucleic acids still allows cells to carry out metabolism, because some enzymes are able to repair damage that occurs to nucleic acids. This allows the microbes to be active again after being exposed to UV [18]. Overall, the pasteurization process followed by irradiation was able to achieve a maximum reduction in total plate count value value of 8.2×104 CFU/mL during the 120 minute experiment.

Meanwhile, Figure 3C is a graph of milk processing results using the simultaneous method. The simultaneous process is a modified process that combines irradiation and pasteurization processes in the form of cycles. Experiments carried out for 60 minutes showed a total plate count value of 2,7 × 104 CFU/mL with a reduction percentage of 99.83%. The increase in the total plate count value in the first 5 minutes occurred because the optimal temperature for the pasteurization process in the simultaneous process had not yet been reached. Milk processing experiments using simultaneous processing can achieve a maximum reduction in the total plate count value of 1×103 CFU/mL dalam waktu 120 minutes.

When compared with the three dual processes above, the results of the simultaneous treatment provide a more efficient effect in time. Even though the double process of irradiation then pasteurization gave the smallest Total Plate Count result, namely 1×103 CFU/mL with a reduction percentage of 99.996%, but this reduction only occurred in the 120 minute trial time.

Wara Dyah Pita Rengga, et. al ./ Journal of Animal Husbandry 15 (2) 2024 pp. 11-23
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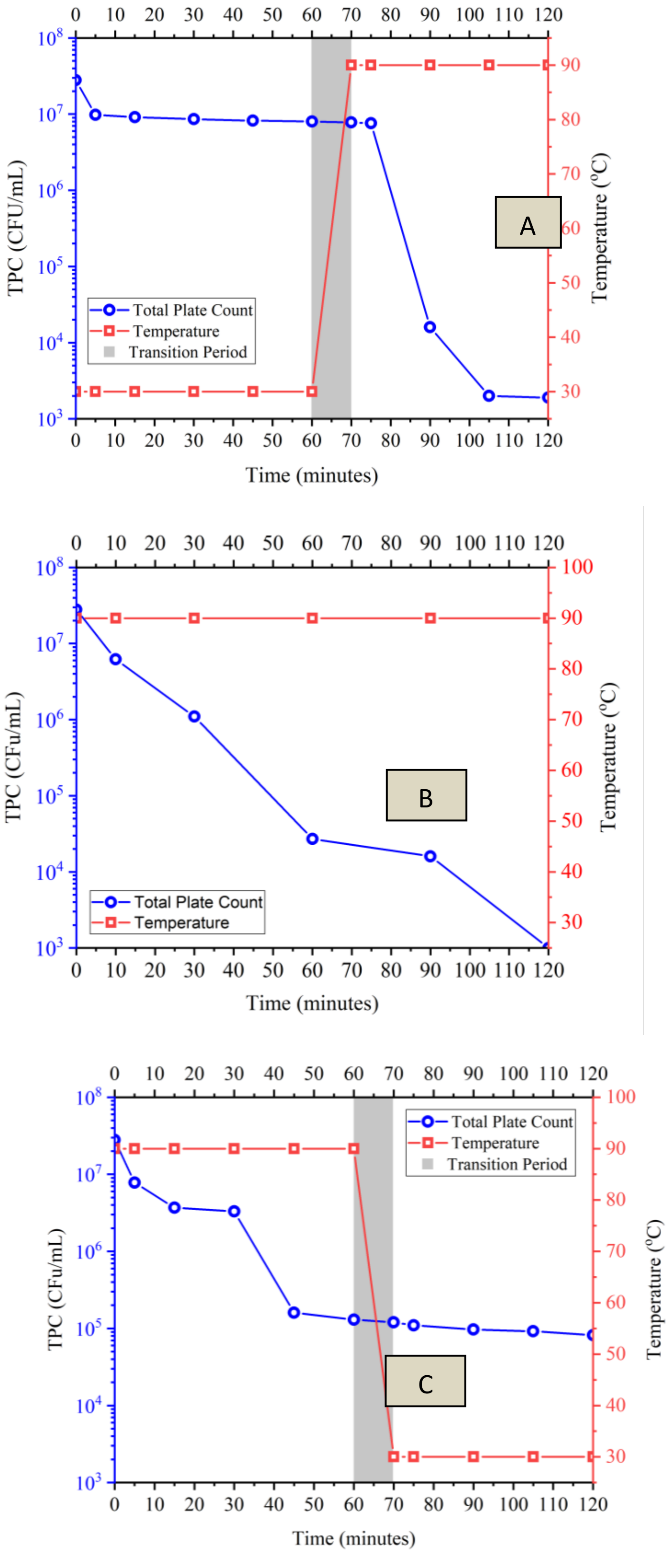

(A) Irradiation-Pasteurization;(B) Radiation Pasteurization; (C): Simultaneous Method
Figure 3. Total Plate Count Value with Double process for 2 hours

Wara Dyah Pita Rengga, et. al./ Journal of Animal Husbandry 15 (2) 2024 pp. 11-23
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ANALYSIS OF ACIDITY DEGREES (PH) of milk products after processing

The degree of acidity before and after treatment did not show a significant change as shown in Table 2, namely it was still in the pH range of 6.5 – 6.8. Ultraviolet-C irradiation treatment had no significant effect on the degree of acidity in milk samples. This is because several water minerals found in cow's milk such as acetate, phosphate and citrate act as pH buffers [26]. If the pH is below 6.5, the milk has formed lactic acid from lactose by bacteria, while a pH greater than 6.8 indicates an abnormality such as mastitis. The degree of acidity at a pH of 6 can be caused by colostrum or the activity of putrefactive bacteria [27]. This is in accordance with the food process caused by several bacteria. Acidity can be indicated by the optimum pH value for the growth of pathogens in food, namely 6-7 (SAKA, 2018). Based on SNI for Fresh Milk (SNI 3141.1:2011), a good pH value for milk and milk products is 6.3 – 6.8 (BSN 2011). Meanwhile, according to International Standards (DKS 2191:2015) states that the pH of milk ranges from 6.5 – 6.8 [28].

Sterilization Method pH Before Treatment pH After Treatment
Exposure-Pasteurization 6.74 ± 0.00 6.76 ± 0.00
Pasteurization-Paste 6.77 ± 0.01
Simultaneous 6.74 ± 0.00

Table 2. Milk Acidity Values Before and After Treatment

Sterilization Method	pH treatment	
	Before	After
Irradiation-Pasteurization	6.74 ± 0.00	6.76 ± 0.00
Pasterurization-irradiation		6.77 ± 0.01
Simultaneous		6.74 ± 0.00

Pasteurization treatment can cause an increase in the pH of milk due to the activity of microorganisms that are resistant to certain temperatures [29]. This can happen because the activity of lactic acid bacteria converts lactose into lactic acid and causes the pH of milk to decrease. The process of milk acidification can also be caused by various acidic compounds such as complex phosphate compounds, citric acid, amino acids and carbon dioxide which are dissolved in milk [27].

Increasing the pH value of milk also increases the viscosity of milk as a result of the breakdown of casein granules due to casein aggregation [30]. Milk viscosity is also influenced by the homogenization process [31] when the pH decreases in the range of 6.4 – 5.4. This is because the casein granules are nearly uniform in size and distribution. At pH conditions of 5.4 – 5.3, viscosity increases maximally and casein is in the initial stage of aggregation [26].

MILK STORAGE PERIOD

Analysis of the shelf life of milk was carried out by testing the total plate count value after storage. The results of testing the total plate count value of the milk sample after going through a storage period of 8 months in a showcase at 4°C can be seen in Figure 5. The results of the test of the shelf life of the three sterilization treatments showed that the highest total plate count value after 8 months of storage was the irradiation method. with a total plate count value of 1.3 x 104 CFU/mL, the pasteurization method was 3x105 cfu/mL and the simultaneous method was 1.5 x 103 CFU/mL. Based on the graph, the lowest total plate count value is obtained in samples with simultaneous processing. This indicates that the less the number of bacteria in milk, the shelf life will increase [32]. Ultraviolet C irradiation treatment in pasteurized milk can extend the shelf life from 12 days to 21 days and can increase the

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concentration of vitamin D3 [33]. Similar results were also stated in a study [16] that post-pasteurization ultraviolet-C treatment increased the shelf life of liquid milk from 7 to 35 days. Ultraviolet treatment for use together with pasteurization of milk has also been shown to increase the shelf life of milk by up to 30% [32]. This proves that ultraviolet technology can be used as a Total Plate Counter for non-thermal methods to extend the shelf life of pasteurized milk and whole milk [34].

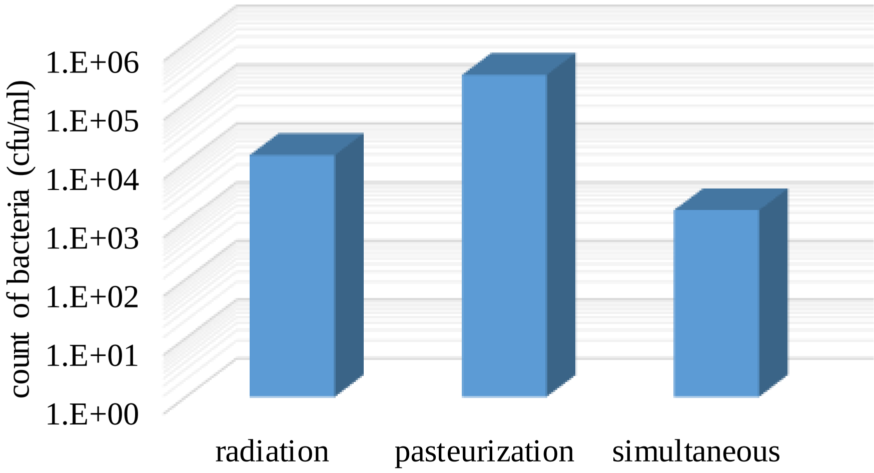

Figure 4. Count of bacteria for a shelf life of 8 months

CONCLUSION

The milk processing process using the method and treatment time using the pasteurization method and the irradiation method with 7 8 Watt ultraviolet-C lamps carried out simultaneously for 120 minutes showed a significant reduction in bacteria. The decrease in bacteria in the simultaneous process, namely the pasteurization process together with ultraviolet C irradiation was 99.996%. The degree of pH acidity before and after treatment from the three sterilization methods did not show a significant change, namely still in the pH range of 6.7 to 6.8. The shelf life of milk using the simultaneous method showed the best results with a total plate count test of 1.3 x 103 CFU/ml after 8 months of storage. The simultaneous method, namely a combination of pasteurization and ultraviolet C irradiation carried out simultaneously, is better than the irradiation and pasteurization methods in sterilizing milk and increasing shelf life.

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