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Differences in Thawing Methods in Broiler Chicken Meat on Total Plate Count (TPC) of Bacteria

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Abstract: Total Plate Count (TPC) determines the number of microorganisms, both bacteria and fungi. Thawing is a step in thawing food that was previously stored frozen. This study aims to analyze the differences in the Total Plate Count of bacteria with variations in thawing methods in broiler chicken meat. Thawing methods commonly used include thawing at room temperature, soaking in water, and leaving it in the refrigerator (chiller). The type of research is experimental design with a posttest-only control design. Repetition was carried out five times for each control group KN (negative control) and test groups R0 (fresh chicken), R1 (frozen chicken), R2 (chicken thawed at room temperature), R3 (chicken soaked in water) and R4 (chicken thawed in the chiller) then the number of bacteria that grew was calculated. The average number of R0 colony results ranged from 4.1 x 10^3 CFU/g, R1 8.6 x 10^2 CFU/g, R2 1.6 x 10^4 CFU/g, R3 8.9 x 10^3 CFU/g and R4 1.2 x 10^3 CFU/g. The Kruskall-Wallis test results showed an Asymp sig value of 0.000 (<0.05); it can be concluded that there are differences in TPC in broiler chicken meat with variations in thawing methods that are thawed at room temperature, soaked in water and thawing in the chiller. It is recommended to thaw frozen chicken meat using the method of leaving it in the refrigerator because it has the lowest number of germs and can inhibit the growth of germs.
Keywords: Broiler chicken; thawing; Total Plate Count (TPC).

INTRODUCTION

Biological damage to chicken meat is mainly caused by the growth of microbes from livestock and environmental pollution during slaughtering and marketing. Handling is needed to extend the shelf life, one of which is by freezing method. Freezing aims to suppress the activity of microorganisms, enzymatic reactions, chemicals and physical damage[1]. Frozen meat that will be used is first thawed, which is the process of thawing. Different thawing methods on frozen broiler chicken meat can affect the meat's physicochemical quality and sensory quality[2]. Correct meat thawing practices must also be chosen to ensure the quality of the final product. Ensuring that chicken meat does not experience microbial growth, chemical damage, and excessive water loss during thawing is essential. This may be due to the characteristics of poultry muscle fibres, which are softer and thinner than livestock meat[3,4,5].

Thawing methods have a variety of ways; it is necessary to select the optimal process, and it is essential to understand the advantages of each technique. The thawing

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method can potentially affect the quality of broiler chicken meat by causing changes in the physicochemical and sensory properties of meat that has been stored frozen[6].

There are various methods of thawing meat, such as using a refrigerator, soaking it in cold water, thawing it at room temperature, and thawing it in a microwave oven. Thawing food, such as chicken, in the refrigerator is better for maintaining natural juices, flavours and textures. However, this technique is inefficient, time consuming and takes up more space needed to cool other foods[7].

Most previous studies focused on the effects of different thawing methods on the physicochemical and structural characteristics of chicken/beef meat. Still, there is a lack of information on evaluating different thawing methods on the bacteriological quality of chicken meat[8,9,10].

Studies have shown the effect of different thawing methods (household refrigerator, kitchen counter, and microwave oven) on the bacteriological quality of frozen broiler chicken meat[7]. However, other findings still need to be added to the data, including different variations of thawing methods. This study analyzed the differences in bacterial TPC with variations of thawing methods (thawing at room temperature, soaking in water, thawing in the chiller) in broiler chicken meat.

MATERIALS AND METHODS

The research design used is the Post-test with control design; the researcher conducted a post-test on the control group and the experimental group, but only the experimental group received treatment[11]. Conducting a comparison of the thawing method on broiler chicken meat by means of R0, namely, the sample was examined without any treatment, R1 was immediately examined after being frozen, R2 was left at room temperature, R3 was soaked in water, R4 was thawed in the chiller

The research sample was fresh chicken from Pasar Harapan Baru in Samarinda. The inclusion criteria were fresh chicken that had never been stored in an ice box before and sellers who were willing to be respondents. This study had five treatments for each sample with five repetitions.

The tools used in this study were an autoclave, bunsen, petri dish, knife, analytical balance, erlenmeyer, test tube, tube rack, incubator, micropipette, microtip, cotton, aluminium foil, measuring cup, beaker, oven, and paper. The materials used in this study were sterile aquades, Plate Count Agar (Merck), 0.9% NaCl and broiler chicken meat.

The independent variable in this study is the thawing method, which is placed at room temperature, soaked in water and thawing in the chiller. The dependent variable in this study is the bacteria's Total Plate Count (TPC).

Chicken meat sampling was done by selecting five fresh chickens and then wrapping them in sterile plastic. The samples were delivered by placing them in an ice box and then taken to the Bacteriology Laboratory at the Poltekkes Kemenkes Kaltim Indonesia.

The chicken samples were stored by first weighing the chicken with a weight of 25 grams aseptically using a knife and cutting board that had been sterilized using an autoclave. The weighed chicken was put into sterile plastic and labelled based on the treatment R0 (fresh chicken), R1 (frozen samples were immediately examined), R2 (samples were placed at room temperature), R3 (samples were soaked in water), and R4

(thawing in the chiller). Samples R1, R2, R3 and R4 were put into the refrigerator for one day until the samples were frozen.

Sample treatment was carried out by directly examining R0, while samples R1, R2, R3 and R4 were put into the refrigerator for one day until the sample was frozen. R1, which had been frozen for one day, was immediately examined; sample R2 was thawed by being placed at room temperature for 5 hours; sample R3 was thawed by being soaked in water for 3 hours, and R4 was thawed by being left in the refrigerator for one night.
The sample is processed by diluting the sample from 10-1 – 10-4 using sterile distilled water. A sample of 1 ml of each dilution in a pipette is inserted into a petri dish, and 15 ml of Plate Count Agar (Merck) media is added at 40-50ºC, then homogenized. Incubate at 37˚C for 24 hours. Colonies that grow between 30-300 are counted using a colony counter. The TPC value (CFU/g) is the number of colonies on the test petri dish minus the number of colonies on the control multiplied by the dilution divided by the number of test petri dishes counted.

RESULTS AND DISCUSSION

In this research, the sample used was fresh chicken, which had previously been asked about by the trader with the condition that it was new chicken that had never been stored in an ice box. Five chicken samples were needed for research, and chicken sampling was carried out in two stages on different days. This was done because the equipment and materials needed were limited. The first collection requires three chickens with a length of time for the chickens on the trader's table for 4 hours, and the second collection requires two chickens for the same time.

Table 1 Total Plate Count of Broiler Chicken with Thawing Method Variations

Sample code	Replication					Average
	1	2	3	4	5	
KN	0	0	0	0	0	0
R0	$2{,}2 \times 10^4$	$1{,}2 \times 10^4$	$2{,}1 \times 10^4$	$8{,}1 \times 10^4$	$7{,}0 \times 10^4$	$4{,}1 \times 10^3$
R1	$6{,}6 \times 10^3$	$8{,}9 \times 10^3$	$5{,}6 \times 10^3$	$1{,}1 \times 10^4$	$1{,}1 \times 10^4$	$8{,}6 \times 10^2$
R2	$1{,}0 \times 10^5$	$3{,}8 \times 10^4$	$1{,}1 \times 10^5$	$2{,}9 \times 10^5$	$2{,}4 \times 10^5$	$1{,}6 \times 10^4$
R3	$2{,}8 \times 10^4$	$2{,}1 \times 10^4$	$3{,}4 \times 10^4$	$1{,}9 \times 10^5$	$1{,}7 \times 10^5$	$8{,}9 \times 10^3$
R4	$1{,}2 \times 10^4$	$1{,}3 \times 10^4$	$1{,}2 \times 10^4$	$1{,}3 \times 10^4$	$1{,}3 \times 10^4$	$1{,}2 \times 10^3$

Table 2 Thawing Method Temperature

Sample code	Replication					Average
	1	2	3	4	5	
R1	-18°C	-18°C	-18°C	-11°C	-11°C	-15°C
R2	24°C	24°C	24°C	26°C	26°C	25°C
R3	26°C	26°C	26°C	28°C	28°C	27°C
R4	4°C	4°C	4°C	4°C	4°C	4°C

KN: Negative Control
R0: Fresh Chicken
R1: Frozen Chicken
R2: Chicken Thawed at Room Temperature
R3: Chicken Soaked in Water
R4: Chicken Thawed in the Chiller

The results of examining the number of broiler chicken germs carried out at the Poltekkes Kemenkes Kaltim for 19 - 23 February 2024 by comparing variations of the thawing method with five repetitions obtained the results as shown in Table 1.

Based on table 1, the average KN value is 0 CFU/g, R0 is 4.1 x 10^3 CFU/g, R1 is 8.6 x 10^2 CFU/g, R2 is 1.6 x 10^4 CFU/g, R3 is 8.9 x 10^3 CFU/g and R4 is 1.2 x 10^3 CFU/g. Table 2 shows the average temperature of R1 -15°C, R2 25ºC, R3 27ºC, R4 4ºC.

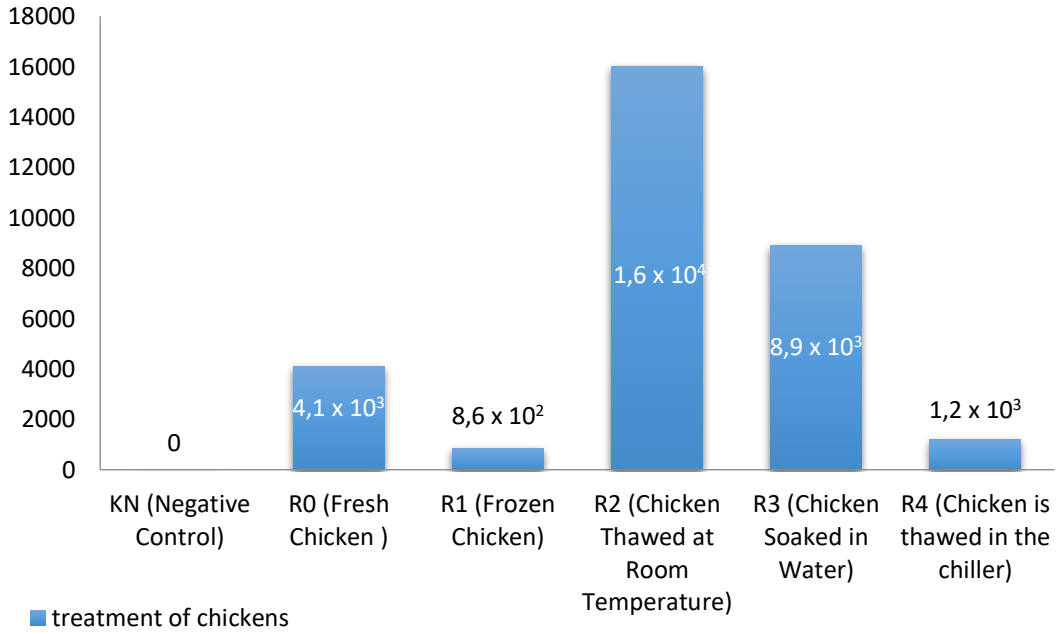

Figure 1 Average Total Plate Count (TPC) of Broiler Chickens

Based on Figure 1, there is a diagram showing that the highest average number of colonies was in sample R2 at 1.6 x 10^4 CFU/g and the lowest colony was R4 at 1.3 x 10^3 CFU/g. More complete germ numbers in broiler chicken samples are presented in Tables 3 and 4.

Table 3. Normality Test Results of Broiler Chicken Meat Germ Count Examination

Chicken Treatment:		
		Shapiro-Wilk
TPC Broiler Chicken Meat	Sig	0,000

The results of the Shapiro-Wilk normality test obtained a sig. value of 0.000 < 0.05, so the data is not normally distributed, then the Kruskal Wallis Test was carried out. In Table 4, the output of test statistics shows the Asymp sig. A 0.000 <0.05 means a significant difference in the average chicken colonies for the five treatments.

Table 4 Kruskal Wallis Test Results of Total Plate Count Examination of Broiler Chicken Meat

Chicken Treatment:			Conclusion
	Kruskal-Wallis H		
TPC Broiler Chicken Meat	Sig	0,000	There is a Meaningful Difference

This study aims to determine the difference in the number of germs in broiler chickens with variations in thawing methods according to the habits carried out in everyday life. The test groups related to variations in thawing methods are KN (negative control), R0 (fresh chicken), R1 (frozen chicken), R2 (chicken thawed at room temperature), R3 (chicken thawed with water), R4 (Chicken Thawed in the Chiller). The negative control plate showed no colony growth, meaning that the examination was carried out aseptically, which was one factor that guaranteed the test's validity.

The study results showed that the number of frozen chicken meat bacteria was the lowest (Table 1). The total plate count of bacteria in chicken meat at room temperature can grow well compared to when placed in the refrigerator. Bacterial growth at refrigerator temperatures is prolonged compared to bacteria that grow at room temperature, which is very fast. This is because chicken meat is freezing at refrigerator temperatures, which can inhibit bacterial growth[12]. Bacteria that can adapt well to low temperatures are psychrophilic bacteria, namely psychromonas bacteria, colwellia spp, shewanella spp[13]. Temperature causes the growth rate of microorganisms to be inhibited, thereby inhibiting chemical reactions in food ingredients and maintaining the quality of the ingredients to be frozen[14].

Maximum contamination limit according to Indonesian National Standard 7388:2009 (TPC: 1 x 10^6 colonies/gram). The results of the study showed that the average number of fresh chicken colonies was 4,1 x 10^3 CFU/g (Table 1), while other studies showed that broiler chicken meat exceeded the Indonesian National Standard limit with the highest results of 3,7 × 10^7 CFU/g and 6,0 x10^7 CFU/g. This is suspected to be due to contamination during the slaughtering and cleaning process of the broiler chickens and the lack of cleanliness in the traditional marketplace. The high TPC in the chicken can be caused by differences in the number of colonies in each sample and can be influenced by several factors, including environmental conditions, where the hygiene and sanitation factors of the workers are also the main factors. Differences in the number of microbial colonies in each sample can also be influenced by temperature during storage time and in the distribution process[15,16].

The lowest average colony was found in chicken meat samples in the refrigerator, 1.3 x 10^3 CFU/g (Table 1). Our research results are supported by other studies that show similar results that thawing with a refrigerator is the best method for thawing frozen minced red meat compared to thawing under tap water or at room temperature, which can reduce the number of bacteria (3.55Log10 CFU/g) compared to tap water (4.10Log10 CFU/g) and room temperature (4.62Log10 CFU/g)[17]. In addition, overnight thawing frozen minced red meat in a refrigerator produces meat with high and stable microbiological quality[18]. Thawing chicken in the refrigerator takes about 1-2 days, depending on its weight (about 5 hours/half kg). So many consumers prefer to thaw meat at room

temperature because it is easier. However, this technique can pose serious health risks and damage product quality[19].

The highest average colony was found in chicken meat samples thawed at room temperature, 1.6 x 104 CFU/g (Table 1), at an average temperature of 25 oC (Table 2). In Table 3, the Shapiro-Wilk normality test obtained a sig value of 0.000 (<0.05), which means that the data is not normally distributed so that it can be tested for comparison using Kruskal-Wallis with an Asymp sig value of 0.000 (<0.05) meaning that there is a significant difference in the number of germs from various variations of the thawing method (Table 4). These results are supported by other studies showing the highest number of bacteria in frozen chicken meat thawed on the kitchen table at room temperature (27-29ºC) for 5-6 hours[7]. Thawing meat at relatively high temperatures (20-30°C), such as at room temperature, can cause microbial growth and cause a decrease in meat quality[20]. Thawing meat at room temperature will expose it to high-temperature conditions, slower and less uniform thawing with increased water and nutrient content, thus providing an excellent medium for microbial growth[3,21].

The variation in the thawing method affects the number of colonies that grow. The difference in the number of microbial colonies in the sample is influenced by several factors, such as temperature, humidity, and oxygen availability. Temperature is one of the critical factors in the development of microbes; normal temperature or room temperature is the best temperature for the development of microorganisms. The humidity level of an environment is directly proportional to the growth rate of microorganisms[22]. Poultry meat consumed without proper processing can cause disease for those who consume it. The correct processing process can inhibit microbial growth so consumers can avoid disease[23].

The limitation of this study is the non-uniformity of the freezing temperature of chicken meat in repetitions 1-5, so there are variations in the results of the number of bacteria in the treatment with the thawing method, but these results can be averaged out as results that can represent the actual situation.

CONCLUSION

The average result of calculating the Total Plate Number for chicken meat thawed at room temperature is 1.6 x 10^4 CFU/g; soaked with air is 8.9 x 10^3 CFU/g; thawed in the chiller 1.2 x 10^3 CFU/g. There is a difference in the total plate number in broiler chicken meat with various thawing methods and the Asymp sig value. 0.000 (0.05). It is recommended to thaw frozen chicken meat by leaving it in the chiller because it has the lowest germ count and can inhibit the growth of germs.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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