

Effect of Cooling Time in the Refrigerator Before Frozen Storage on Physical Quality and Total Bacteria of Broiler Chicken Thigh Meat

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ABSTRACT

Broiler chicken meat is a highly nutritious protein source that has a good taste, a delicious smell, a soft texture and is relatively inexpensive. Despite these benefits, it is a perishable food. To address this issue, cold temperature storage can be utilised as a strategy to mitigate the damage. The objective of this study was to determine the physical quality and total microbes of broiler meat stored in a refrigerator before storage in a frozen state. This study was conducted using a Completely Randomized Design (CRD) with four treatments and five replicates. The treatment involved the length of cooling in the refrigerator ($\pm 5^{\circ}\text{C}$) before frozen storage, which was set at 0, 2, 4, and 6 hours. The parameters observed were pH, cooking loss, water binding capacity and total microbial count. The data were analysed using the Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). The results revealed that the duration of refrigerated storage had a significant effect ($P < 0.05$) on the pH value, water binding capacity, cooking loss, and total microbes. The treatment P0 (without refrigeration) resulted in the highest pH value, the lowest water binding capacity, and a low cooking loss and total microbes. The refrigeration of broiler chicken thigh meat was observed to reduce pH value, increase water binding capacity, reduce cooking loss and increase total microbes. Therefore, refrigerating broiler chicken thigh meat for 6 hours before frozen storage can maintain the meat's quality.

Keywords: Cooking loss, cooling, freezing, pH, water binding capacity

INTRODUCTION

Broiler chicken meat is a highly nutritious food ingredient characterised by a favourable taste and aroma, a soft texture, and a relatively cheap price, making it a preferred choice among the public. Broiler chicken meat is perishable, and most of the damage is caused by poor handling, which provides opportunities for the growth of spoilage microbes that subsequently reduce the quality and shelf life of the meat (Soeparno, 2015). One method for mitigating the damage caused to broiler meat is to store it at refrigerated temperatures.

The refrigeration of broiler meat is a crucial aspect of preservation, and it is a relatively straightforward process. There are three methods commonly used for cooling chicken meat: cold air cooling, immersion in ice water, and cold air evaporation (spray) or the integration of both immersion and cold air technology (Sams, 2001). Cooling meat via cold air can be accomplished in a refrigerated room by packing the broiler chicken meat and allowing the cold temperature to be absorbed into the meat over time. The application of cold conditions to chicken meat can inhibit microbial development and enzyme activity,

allowing the meat to maintain its quality for an extended period (Raharjo, 2022). As Ma'rifat and Rahmawan (2017) have observed, the objective of cooling is to keep the quality of meat and to preserve its freshness. Moreover, Jaelani et al. (2014) asserted that broiler chicken meat stored in a refrigerator for 6 days in plastic packaging remains physically suitable for consumption, although the cooking loss value of the meat increases.

The practice of extending the shelf life of meat is commonly achieved through the utilization of freezing temperature storage. Before storing at freezing temperature, the meat is cooled first to ensure the freezing process takes place evenly throughout the chicken and forms uniform and smooth ice crystals. Before storing at freezing temperature, the meat is cooled first to ensure the freezing process takes place evenly throughout the chicken and forms uniform and smooth ice crystals. During the thawing process, the liquid that emerges is relatively small in volume and contains the dissolved food substances within the meat. These substances, including salt, protein, amino acids, and vitamins, are known to dissolve in water and become part of the thawed meat liquid, as observed by Sangaji (2019).



As Jaelani (2014) has observed, the cooling of meat significantly affects the physical quality of beef. The duration of the cooling process for chicken meat has a notable impact on the freezing procedure, allowing for the deepest parts of the meat to be frozen thoroughly. The United States Department of Agriculture (USDA, 2009) suggests that the temperature of chicken carcasses should reach 4.4°C or below within a cooling period of 4 to 8 hours. This is intended to inhibit the growth of microbes in the meat.

Based on the above description, it is necessary to conduct this study to determine the optimal length of time for cooling before frozen storage, thereby maintaining the physical quality and total bacteria of broiler thigh meat before frozen storage.

MATERIALS AND METHODS

Research Materials

The study was conducted using meat from the left thigh and right thigh of 20 broiler chickens with a live weight of 1.7-1.8 kg, obtained from broiler farm cages. The following materials were also utilized: distilled water, NaCl, Nutrient Agar (Merck), Peptone (Merck), and 70% alcohol.

The equipment that were used in the study included a refrigerator, freezer, stopwatch, digital scale, beaker glass, pH meter, blender, knife, Whatman filter paper No. 41, 35 kg iron weight, millimeter block, bimetal thermometer, gas stove, water batch, petri dish, test tube, laminar air flow, autoclave, incubator, vortex, micro pipette, Erlenmeyer flask, mortar, colony counter, spirit lamp, and Ohaus scale.

Research Methods

Before slaughter, broiler chickens were fasted for 8 hours to facilitate the cleansing of the digestive tract. Chickens that have been confirmed dead were then manually plucked and cleaned, and proceeded with the removal of all internal organs until clean. For research purposes, carcass slices were selected from all parts of the left and right thighs, weighed, and then placed into polypropylene plastic containers, divided according to their respective treatments. Furthermore, the samples were cooled in a refrigerator for a period of 0, 2, 4, and 6 hours.

Once the cooling period was complete, the meat specimens were transferred to the freezer for 2 months of storage. Following this, the samples were thawed for 12 hours in a refrigerator. After thawing, the pH, Water Binding

Capacity, Cooking Loss, and Total Bacteria were observed.

Research Design

This study was conducted using a Completely Randomized Design (CRD), comprising four treatments with five replications. One experimental unit comprised two pieces, taken from the left and right thighs. The treatment used was the length of cooling in a refrigerator before frozen storage for 2 months:

P0 = without cooling, directly frozen

P1 = 2 hours cooling

P2 = 4 hours cooling

P3 = 6 hours cooling

pH (AOAC, 2005)

The pH of the sample was measured with a pH meter that had been calibrated at pH 7 and 4. A total of ±5 g of the meat sample was then added to 50 ml of sterile aquadets, and later the mixture was crushed with a blender. After blending, the sample was transferred into a glass baker. The pH meter was then inserted into the sample, and the scale indicated on the tool was used to determine the pH.

Water Binding Capacity

The water binding capacity is measured according to the Hamm method (Swatland, 1984, as cited in Soeparno, 2015) by the pressing process. Meat samples are placed between two glasses and then pressed with an iron load weighing 35 kg for 5 minutes. The wet area can be determined by calculating the difference between the outer circumference and the inner circumference, then dividing the result by 100. The wet area is calculated using the following formula:

$$\text{mgH}_2\text{O} = \frac{\text{Wet Area (cm}^2 \text{ mgH)} \cdot 20}{0,0948} - 8,0$$

The percentage of free water can be calculated using the following formula:

$$\text{Free Water Percentage (\%)} = \frac{\text{mgH}_2\text{O}}{300 \text{ mg}} \times 100 \%$$

Cooking Loss

The cooking loss of chicken thigh meat was determined by weighing a sample measuring approximately 3 cm in thickness and inserting a bimetal thermometer into the side. The sample was then heated until the internal temperature reached 85°C (Soeparno, 2009). The cooking loss was calculated using the following formula:

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooking} - \text{Weight after cooking} \times 100}{\text{Weight before cooking}}$$

Total Bacteria (APHA, 1992)

Total bacterial testing on chicken meat was conducted using the pour method. A 5-gram sample of chicken meat was ground, then 1 gram was taken and placed in a 9-ml dilution solution (0.1% peptone solution). Next, 1 ml of the initial dilution solution was taken and put in a second dilution solution containing 9 ml of diluent (10^{-2}), then homogenized.

The process of dilution was repeated with the same amount and method until the 6th dilution (10^{-6}). The subsequent step involved taking 1ml from each of the 10^{-4} , 10^{-5} and 10^{-6} dilutions and transferring them into Petri dishes, followed by the addition of 15-20 ml of Nutrient Agar (NA) media. In the final step, the sample was shaken in a circle to form a figure-8 shape, allowing the

bacteria to spread and form colonies. The sample was then incubated in an incubator at 37°C for 24 hours.

Data Analysis

The data were subjected to analysis using Analysis of Variance (ANOVA), and subsequently, any influential results were evaluated with the Duncan Multiple Range Test (DMRT) (Steel and Torrie, 1995)

RESULTS AND DISCUSSION

The mean values of pH, Water Binding Capacity (WBC), cooking loss, and total bacteria of broiler chicken thigh meat, as determined by the analysis of variance, are presented in Table 1.

Table 1. The mean pH, Water Binding Capacity (WBC), cooking loss, and total bacteria of the broiler chicken thigh meat in each treatment.

Variables	Treatment			
	P0	P1	P2	P3
pH Values	6.40 ± 0.05 ^a	6.07 ± 0.12 ^b	6.04 ± 0.06 ^b	6.03 ± 0.08 ^b
WBC (%)	26.78 ± 0.82 ^c	27.31 ± 0.65 ^b	27.84 ± 0.50 ^b	28.1 ± 0.54 ^a
Cooking Loss (%)	21.56 ± 3.56 ^a	16.86 ± 0.79 ^b	16.04 ± 4.31 ^b	15.36 ± 2.78 ^b
Total Bacteria (cfu/gr)	6.5x10 ⁴ ± 0.36	6.1x10 ⁴ ± 0.07	7.4x10 ⁵ ± 2.28	7.6x10 ⁵ ± 0.27

Notes: Different superscripts in the same row indicate significant differences (P<0.05)

pH of Meat

The pH value of broiler chicken thigh meat, as determined by the analysis of variance, indicated that the duration of cooling before frozen storage had a significant effect (P<0.05). The pH value was observed to range from 6.03 to 6.40. The findings of this study exhibited higher pH values when compared to the results of the research conducted by Jaelani et al. (2014), which demonstrated a pH range of 6.23 to 5.64 in broiler meat stored in a refrigerator. The findings of Risnajat's (2010) study indicated that the pH of chicken carcasses stored in refrigerated conditions ranged from 6.32 to 5.72. Afriani et al. (2024) reported that the pH value of broiler chicken breast meat chilled in a refrigerator ranged from 6.09 to 6.51.

The cooling time of 0 hours (P0) resulted in a pH of 6.40. After 2 hours of cooling (P1), the pH decreased to 6.07. At the 4-hour cooling interval (P2), the pH value differed from that of P0 (6.04), but was not significantly different from P1 and P2. At the 6-hour cooling period (P3), the pH value (6.03) was also different from P0, but not significantly different from P1 and P2. The P0 treatment involved the immediate freezing of

meat that had not undergone a cooling process. In contrast, the P1, P2, and P3 treatments each involved a distinct cooling period, with P1 lasting for 2 hours, P2 for 4 hours, and P3 for 6 hours, after which the meat was frozen. It is suspected that during the cooling process before freezing, microbial activity continued to reform glycogen, resulting in the production of lactic acid and a consequential decrease in pH. As stated by Sangaji (2019), the cooling process results in a reduction of pH, leading to the formation of lactic acid and the breakdown of glycogen by microbes. This phenomenon has also been observed by Ernawati et al. (2018), who noted that freezing and thawing cause a decline in mineral content and alterations in ion balance due to protein compounds, leading to a reduction in pH. The decrease in pH is influenced by the ability of meat protein to bind H⁺ ions; the lower the protein content, the lower the protein's ability to bind H⁺ ions (Soeparno, 2015). The pH value is influenced by several factors, including genetics, handling processes, slaughtering procedures, post-slaughter handling, and length of storage (Glamoclija et al., 2015).

Variations in meat pH will likely affect the colour and the ability of meat to retain water. The pH value of meat after cutting will undergo a decline from 6.5 to the ultimate pH of 5.5 (Subagyo et al., 2015). By the SNI standard, the pH value of chicken meat falls within the range of 6 to 7 (BSN, 2009).

Water Binding Capacity (WBC)

The results of the analysis of variance indicated that the cooling treatment before frozen storage had a significant effect ($P < 0.05$) on the value of water binding capacity. The length of time spent cooling the thigh meat of broilers in a refrigerator before freezing increased water binding capacity, with the obtained values ranging from 21.77% to 34.60%. Risnajati (2010) discovered that the water-binding capacity of chicken carcasses stored in refrigerators ranged from 36.81% to 28.81%. Similarly, Soeparno (2015) observed that water binding capacity is significantly influenced by the rate and extent of pH reduction, with changes in pH linked to alterations in meat microstructure, including muscle contraction in live animals.

The rate and magnitude of the pH value can influence the water-binding capacity of meat. A reduction in pH will result in a decline in the water-binding capacity of the meat. The findings of this study indicate that meat samples that were not cooled exhibited a higher pH value than meat samples that were cooled for 2, 4, and 6 hours. The cooling durations of 2, 4, and 6 hours resulted in no significant difference in pH, indicating that the cooling duration does not affect the pH value. The water-binding capacity of meat without cooling (P0) was found to be relatively low, as evidenced by the significant amount of water released during the thawing process, which suggests that the ability of protein to hold water is relatively weak. In contrast, the refrigerated meat exhibited a higher water-binding capacity.

The average water binding capacity at 0 hours of cooling (P0) is 26.78%, which is significantly different ($P < 0.05$) from the 2 hours cooling (P1) value of 27.31%. Similarly, the 4-hour cooling (P2) value of 27.84% differs from P0 but is not significantly different from P1. The 6-hour cooling (P3) value of 28.1% is significantly different ($P < 0.05$) from all three previous values.

The value of meat binding capacity is indicated by the amount of meat liquid that is released. In their study, Aberle et al. (cited by Dewi, 2012) observed that during cold storage, there is a degradation of collagen from the

proteins that form the cross-links between meat fibres. They also noted that the primary component responsible for retaining meat water is protein.

Soeparno (2015) stated that the water binding capacity is greatly influenced by the rate and magnitude of the pH decrease, and changes in pH are related to changes in the microstructure of meat, including muscle contractions in living animals. High or low pH values from the isoelectric point of meat protein will increase the water binding capacity because low pH contains a positive charge due to the rejection of myofilaments, so that there is more space for water molecules. A high water-binding capacity value indicates that the meat protein could retain water well. The water binding capacity value is related to the cooking loss value; the higher the water binding capacity value, the lower the cooking loss value, and vice versa. The lower the water binding capacity value, the higher the cooking loss value.

The findings of Zhuang et al. (2013) indicated that the utilisation of cold water instead of cold air for the refrigeration of chicken carcasses resulted in a diminished water-binding capacity. The optimal quality of meat is characterised by an elevated capacity for water binding and a minimal loss of liquid during the cooking process (Rahardjo et al., 2022).

The water-binding capacity value is indicative of the capacity of meat protein to hold water. It is related to the cooking loss value, whereby a higher water-binding capacity value is associated with a lower cooking loss value. Conversely, a lower water binding value is associated with a higher cooking loss value.

Cooking Loss

The analysis of variance revealed that the cooling treatment before frozen storage of chicken thigh meat has a statistically significant impact ($P < 0.05$) on the cooking loss value, indicating that the cooling treatment of broiler thigh meat can effectively reduce the cooking loss value of beef. The average cooking loss value obtained in this study was within the range of 17.20% to 26.60%. This value differs from that reported by Jaelani *et al.* (2014), who observed a cooking loss of 30.61% to 38.23% in broiler meat stored in a refrigerator. The findings of Risnajati's (2010) study revealed that the cooking loss of broiler carcasses stored in refrigerated conditions ranged from 32.24% to 37.10%. The quality of chicken meat is deemed optimal when the weight

reduction during the cooking process is minimal, whereas a greater weight reduction is indicative of inferior meat quality. The risk of losing the food substances contained in meat is closely linked to the amount of weight reduction that occurs during the cooking process.

The cooking loss value at 0-hour cooling (P0) resulted in a count of 21.56, which was significantly different ($P < 0.05$) when compared to the values observed at 2 hours cooling (P1), 4 hours cooling (P2), and 6 hours cooling (P3). The respective values at these time points are 16.86, 16.04, and 15.36. The cooling periods of 2, 4, and 6 hours (P1, P2, and P3, respectively) did not result in significantly different ($P > 0.05$) cooking loss values: 16.86, 16.04, and 15.36, respectively. It is suspected that in meat that was not cooled (P0), ice crystals were formed mainly on the surface of the meat during the freezing process. As a result, a considerable amount of water was released during the thawing process, and a significant loss was also experienced due to the cooking process. The quantity of water released (drip) and the loss due to the cooking process suggest that meat protein is unable to retain water effectively. Meat that has not been cooled (P0) has a high pH and low Water Binding Capacity (WBC). If meat has a low WBC, water loss due to cooking will be higher. The low water loss observed in cooked meat indicates that the nutritional content of the meat is not significantly reduced, suggesting that the quality of the meat is relatively good by the findings of Lapase *et al.* (2016), a reduction in water binding capacity results in an increased rate of cooking loss in meat products, leading to a decline in quality due to the large number of degraded components. Soeparno (1915) in his study suggested that meat with low weight loss due to the cooking process and less loss of nutrients has a relatively higher quality than meat with high cooking loss. Jaelani *et al.* (2014) also indicated that the cooking loss value of broiler meat is influenced by several factors, including cooking temperature, cooking time, livestock age, the ability of meat to bind water, and pH value. The amount of weight loss in the cooking process is found to correlate with the ability of meat to bind water; therefore, a low ability of meat to bind water will result in greater weight loss during the cooking process (Fathurrohman *et al.*, 2021).

Total Bacteria

The results of the analysis of variance indicate that the cooling treatment applied before

the frozen storage of chicken thigh meat had a significant impact ($P < 0.05$) on the total bacterial count. The application of a refrigeration treatment to broiler thigh meat has been found to result in a reduction in the total bacterial value of the meat. The average value of cooking loss obtained in this study was found to be between 6.5×10^4 and 7.6×10^5 . The findings of this study indicate that the duration of meat cooling in a refrigerator is associated with an increase in total bacteria. These findings are higher than those reported by Edi and Rahma (2018), who observed total bacteria in chicken meat stored in refrigerators ranging from 14.4×10^4 to 51.6×10^4 . The findings of Saraswati's research (2015) indicated that the total bacterial count in beef stored in refrigerators ranged from 0.1×10^4 to 3.3×10^5 . The total bacterial results of this study remain below the maximum acceptable limit of bacterial contamination in processed food, as defined by SNI 7388: 2009, which is 5 log CFU/g.

The total bacterial count at 0 hours of cooling (P0) is 6.5×10^4 , exhibiting no significant difference ($P > 0.05$) with 2 hours of cooling (P1), which is 6.1×10^4 . The 4-hour cooling (P2) resulted in a count of 7.4×10^5 , which was not significantly different ($P > 0.05$) when compared to the 6-hour cooling (P3) result of 6.5×10^5 . The 0-hour cooling (P0) result, which was 6.5×10^4 , was found to be significantly different ($P < 0.05$) in comparison to the 4-hour cooling (P2) result, which was 7.4×10^5 . Another significant difference ($P < 0.05$) was also observed between the 2-hour cooling (P1) result, which was 6.1×10^4 , and the 6-hour cooling (P3) result, which was 6.5×10^5 . It was found that psychotropic bacteria were still able to multiply when chicken meat was subjected to long periods of cold storage at refrigerator temperatures. This is because the low temperature of the chicken meat did not prevent the growth of psychotropic bacteria. Pestariati (2002) states that at refrigerator temperatures, the activity of the mesophyll bacterial enzyme system is reduced while psychotropic bacteria metabolize, which can alter the composition of chicken meat and produce metabolic waste that can inhibit the growth of other bacteria.

CONCLUSIONS

The cooling of broiler chicken thigh meat before freezing has been observed to reduce the pH value, enhance water binding capacity, minimise cooking loss, and increase the total bacterial count. Refrigerated storage for up to six

hours can result in pH values, water-binding capacity, cooking loss, and total bacteria that are still within the range of good broiler meat quality.

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