

INFLUENCE OF MULTIPLE FREEZE-THAW CYCLES ON PHYSICOCHEMICAL PROPERTIES OF CHICKEN BREAST

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ABSTRACT

Freezing can deteriorate meat quality due to the formation of ice crystals that affect the texture of frozen meat. Meat undergoes undesirable quality changes during the frozen supply chain due to many factors, which often remain unknown due to refreezing. This study was conducted to determine the influence of multiple (four successive) freeze-thaw cycles on texture, water content and lipid oxidation of chicken breast meat. Water contents were estimated by total moisture loss, including thawing loss, drip loss and cooking loss. While physicochemical properties were determined by pH, lipid oxidation, color and tenderness of chicken meat after four days of freezing with a core temperature of $-18^{\circ}\text{C} \pm 2$ (C0-C4). Experimental data were evaluated by one-way ANOVA and post hoc Tukey's test.

The results showed that water holding capacity was significantly decreased with an increase in the number of freeze-thaw cycles through structural changes by forming ice crystals. Pro-oxidant was released due to mechanical damage of the muscle system by ice crystals which potentiated the lipid oxidation and structural denaturation increased the tenderness of chicken meat ($P \leq 0.05$). Color (L^* ; Lightness, a^* ; Redness and b^* ; yellowness) values showed inconsistent change throughout freeze-thaw cycles. This study concluded that multiple freeze-thaw cycles adversely affect the water content and texture of chicken breast fillet which leads to weight loss, tenderness/Juiciness and may drop the customer acceptability.

Keywords: chicken meat, freeze-thaw cycles, water holding capacity, texture, meat quality

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INTRODUCTION

Meat is a component of a balanced diet. It is the edible flesh part of animal's skeleton. It has an excellent nutritional profile and can fulfill consumers' daily protein requirements (De Smet and Vossen, 2016; Pereira and Vicente, 2013). Chicken has a nutritional profile, 75% water, 19% protein, 5% fat, 1% vitamins and other micronutrients, making it a remarkable nutritional source. Though urbanization, economic expansion, industrialization, and the food model have increased meat consumption per capita in recent years, demand for meat and related goods will continue to rise in the coming years (Chartsbin, 2017). To meet this growing demand, the government and the meat industry are now focusing on providing adequate, healthy, and high-quality meat and meat products, both fresh and processed (GOP, 2018-19). In addition, consumer awareness drives the meat industry and regulatory authorities to monitor meat quality for human health by ensuring safety, and animal welfare, and traceability (Steinfeld *et al.*, 2006). To ensure the freshness, quality and safety of meat, it is

subjected to preservation.

Meat is a perishable commodity with a short shelf life and selling times. Therefore, meat preservation in meat supply is of utmost importance for maintaining the quality and safety of meat for transporting meat over long distances without spoiling texture, color and nutritional value after rapid development and development of large markets (Nastasijevi *et al.*, 2017).

Many methods are used to preserve the meat to ensure its better quality and safety (Sultana *et al.*, 2008). Among different preservation methods, freezing is a commonly used and excellent method to preserve meat and meat products (Sung *et al.*, 2013) and it plays an important role in the meat industry to ensure the safety of meat and its products during its supply to all parts of the world (Leygonie *et al.*, 2011). In processing industries, meat is frozen before further processing for the development of different meat products (Srinivasan *et al.*, 1997). Freezing is usually done by two methods, via slow and fast freezing. In both methods, different structural changes occur in muscle tissues due to the formation of

ice crystals which damage the muscle fibers by destroying its protein structure (Ngapo *et al.*, 1999).

During the freezing process, a large number of ice crystals are formed. These crystals cause less damage to the cell membrane. Which increases the exposure of the protein to the ice crystal and causes mechanical denaturation and osmotic removal of water and causes dehydration of muscular structure. (Muela *et al.*, 2010, 2012). Owing to protein oxidation and the mechanical damage of protein, the cellular system lost its integrity. These proteins are responsible for retaining water in muscle structure and affect other attributes of meat quality i.e. tenderness, color and water holding capacity (WHC), etc. (Jeong *et al.*, 2011).

Frozen storage can cause nonmicrobial quality deterioration i.e. in texture, color, tenderness and flavor, due to osmotic removal of water, mechanical damage, solute concentration and myosin denaturation (Offer *et al.*, 1989; Benjakul and Bauer, 2001). It is also stated that the unfrozen water is available in the frozen stage even at -20°C. This unfrozen water also helps physiochemical changes to occur. Lipid oxidation does not completely stop due to the unfrozen water and affects the shelf life of meat and its product. Furthermore, the damage that occurs during freezing depends on many other factors which accelerate the nonmicrobial cellular destruction of meat quality. These factors include storage temperature, fluctuation of temperature and rate of freezing-thawing (Xia *et al.*, 2009).

Thawing is simply a reversal process to freezing, which converts ice crystals into the water. During thawing, many physiochemical changes occur, i.e. lipid oxidation, purge loss, weight loss and microbiological changes, etc. These changes alter meat structure, decrease the quality of meat and cause economic loss (Savage *et al.*, 1990; Xia *et al.*, 2009; Jeong *et al.*, 2011; Mahendra *et al.*, 2018). The global meat export is currently worth more than US\$ 13 billion and freezing plays an important role in ensuring meat safety during the cold supply chain (Leygonie *et al.*, 2012). Freezing is the most convenient and suitable preservation method for the storage of meat for a longer duration and meat transportation. However, on the other hand, due to the fluctuation of temperature during cold chain supply freezing and thawing of meat at restaurants, retailers and meat displayer racks have a ruinous effect on meat quality. This deterioration in meat quality enhanced consumers' rejection. There is much-published information about the effects of frozen storage on the quality of food products. Still, no basic information regarding the effects of the freeze-thawing process on the structures and water contents of chicken breast muscles has been reported. Local processors have no such data. In this context, this study is conducted to evaluate the detrimental changes that occur during multiple freeze-thaw cycles. Our research will be helpful for both the food industry and consumers to control the

quality and enhance eating quality and the monetary value of frozen meat.

MATERIALS AND METHODS

All animal studies were pre-approved by the Institutional Ethical Review Committee, UVAS, Lahore, Pakistan. We collected samples of chicken breast muscle after slaughtering sixty poultry birds reared in controlled poultry shed. Birds were procured from a local commercial poultry farm and slaughtered in the winter season under strict hygienic conditions and the guidelines provided by Pakistan Halal standard (PS:3733: 2019) at the slaughtering facility of the Department of Meat Science & Technology, University of Veterinary and Animal Sciences (UVAS), Lahore. Each bird has an average 2kg weight at the time of slaughtering. Breast samples having approx. 200g±10 weights were separated. Initial data (weight, pH and color) of all the samples were recorded in Meat Tech Lab, Lahore, on the datasheet after removing all visible fat, connective tissues and bone. Samples Weighed by compact digital weighing balance (SF-400, 7000g×1g). The pH of each breast was noted by inserting a calibrated probe of pH meter (WTW®, pH 3210 SET 2, Germany) and values of meat color taken by calibrated Minolta® Chroma meter. All the samples were separately and individually packed into polythene zip and arranged into five groups (C0 = fresh/control, C1 = Cycle 1, C2 = Cycle 2, C3 = Cycle 3, C4 = Cycle 4). Each group represented each cycle. The first cycle evaluated on the zero-day was a control group remaining groups were kept in a horizontal freezer. The core temperature of samples was -18°C ±2, which was measured through a digital food probe thermometer (TP101®, Cixi Sinco, China). After four days, all samples were thawed at 4 °C temperature for 12 hours. After thawing, the second group or second cycle was evaluated for pH, thawing loss, cooking loss, drip loss, thiobarbituric acid reactive substances (TBARS), color and shear force value of meat and the remaining three groups (C2, C3 and C4) were again subjected to freeze and then thawed for 12 hours.

Thawing loss: All frozen samples were kept in a chiller having 4°C for 12 hours. After thawing, the respective cycle proceeded for further evaluation and the remaining cycles were again subjected to freeze in the freezer having -18°C. Thawing loss was expressed as a percentage of initial weight before freezing.

$$\text{Thaw loss (\%)} = \frac{(\text{sample weight before freezing (g)} - \text{sample weight after thawing (g)})}{\text{sample weight before freezing (g)}} \times 100 \text{ (Honikel, 1998).}$$

pH and Color measurement: pH was measured by inserting a calibrated probe of pH meter (WTW®, pH 3210 SET 2, Germany) into breast samples just after thawing, while initial readings of fresh samples were

taken just after 2 hours of slaughter. Samples were subjected to color blooming after thawing of the respective freeze-thaw cycle. The color was assessed for L^* (Lightness), a^* (Redness), b^* (Yellowness).

After thawing, samples were wrapped in a tray with oxygen-permeable PVC-film and put in air at 4°C for an hour for blooming purposes. Then, color was measured using a calibrated Minolta® Chroma meter from the skin side of breast samples. Value was recorded at three different locations of a sample and the result was expressed as an average of the three values described by (Vieira and Fernandez, 2014).

Drip loss: Drip loss was calculated by a hanging method. After completion of thawing, samples were suspended individually at 4°C in inflated polythene bags to avoid physical contact with the bag's walls for 24 h. After 24 h, samples were gently blotted dry and weighed; drip loss was calculated as the percentage of weight lost (Honikel, 1987).

$$\text{Drip loss (\%)} = \frac{[(\text{sample weight (g)} - 24 \text{ hrs. after sample weight (g)}) / \text{sample weight (g)}] \times 100.}$$

Cooking Loss: Before cooking, the Sirloin samples were weighed using a compact digital weighing balance (SF-400, 7000g×1g). After that, samples were individually packed in plastic bags and placed in a water bath (Memmert®, WNB45, Germany) operated at 80°C for cooking purposes until the samples attained a core temperature of 72°C. The sample took approximately 45-50 minutes to attain a core temperature of 72°C. The temperature of the sample was measured by placing a food probe thermometer (TP101®, Cixi Sinco, China) in the center of the sample during cooking (Liu *et al.*, 2015). After that, cooked samples were cooled down until the samples attained room temperature (Shanks *et al.*, 2002). The cooking loss was calculated based on the difference between sample weight before and after cooking by the following formula (Liu *et al.*, 2015).

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

Lipid oxidation: Lipid oxidation was calculated by measuring Malon-di-aldehyde as described by (Ohkawa *et al.*, 1979). Standard was prepared from 1,1, 3, 3-tetraethoxypropane of various concentrations 0.00, 1.25, 2.5, 5.0, 10.0, 25.0 and 50 μ mole per L. 1 g of sample was taken then it was homogenous with the help of homogenizer in 10 mL of KCl buffer. The reaction mixture contains sample 200 μ L, 8.1% aqueous TBA solution 1500 μ L and 700 μ L distilled water added in the mixture and heated up for one hour in 95°C. After heating for one hour, cool down it with water, then 3mL n-butanol and 1mL distilled water was added and shake it vigorously, mixture was then centrifuged at 4000rpm for 10 min and absorbance was measured at 532nm.

Shear Force Values: The shear force value of sirloin samples was calculated using Warner Bratzler shear force machine /Texture analyzer (TA. XT plus® texture analyzer, UK). After cooking, samples were cut approximately 1×1cm² (Height × width) along the parallel axis of muscle fiber. The shear force value was estimated, cut strips were placed at the right angle under the V-Slot blade (Barbut *et al.* 2005). The amount of force required to cut fiber was taken in Newton. Minimum 5 shear force values were taken from each sample (n=5).

Statistical Analysis: The collected data were analyzed by the general linear model under one-way ANOVA by Microsoft-excel and GraphPad Prism 7. The groups were further analyzed and compared by the post hock test i.e. Tukey test.

RESULTS AND DISCUSSION

Influence of multiple freeze-thaw cycles on pH of chicken meat: There is no significant difference was observed after subjected to multiple freeze-thaw cycles. The mean pH value of C0 was 5.936 and the mean value of cycle four (C4) was 5.893. When it compared with all treatment cycles (C1, C2, C3 and C4), which was not a significant change (Table 1). In the previous study, pH of chicken meat was evaluated throughout the freeze-thaw cycles. The pH data of chicken breast were inconsistent, and it was evaluated that pH of chicken remained unchanged in the first five cycles. However, it showed pH decreased in the sixth cycle compared to the first/control group (Ali *et al.*, 2015).

Influence of multiple freeze-thaw cycles on color of chicken meat: Color is an important attribute of meat quality. Freeze-thaw significantly affects the lightness of meat color, which shows that its quality deteriorates as freezing and thawing are increased. The lightness of meat color values increases with the influence of multiple freeze-thaw cycles. The change in the lightness of color values was significant ($P \leq 0.05$) when the mean value of C0 was compared with C1, C2, C3 and C4. While the differences among the mean values of a^* and b^* from C1, C2, C3 and C4 were not significant (Table 1). Color categorized by L^* value (lightness), a^* value (redness) and b^* value (yellowness) angle in a recent study. L^* value of chicken meat increased significantly up to the fourth treatment (C4) compared with fresh/control group C0. While a^* values remain essentially unchanged when compared with the control group. b^* remained unchanged up to C4 when it compared with C0. A minor change was observed when the b^* value of treatment first (C1) compared with the b^* value of treatment four (C4). In previous studies, L^* , b^* values increased with a decrease in a^* value Thanonkaew *et al.*, (2006). While Yu *et al.* (2005) evaluated that a^* values decreased with the

increase in the number of cycles and the values of b^* increased with the effect of repeated freeze-thaw cycles.

Influence of multiple freeze-thaw cycles on thawing, drip and cooking loss of chicken meat: Thawing loss was significantly increased from 0.01% to 10% throughout freeze-thaw cycles C0 to C4 ($P \leq 0.0001$). Thawing loss of fresh / control group C1 was noted as 0.01% and continuously increased until the fourth treatment or cycle four (C4) (Table 2). In the current study, thawing loss of chicken meat is significantly increased by the influence of multiple freeze-thaw cycles. Thawing loss of chicken meat was significantly increased from 2.9% to 10% throughout freeze-thaw cycles C1 to C4 ($P \leq 0.0001$). Xia *et al.*, (2009) explained freeze-thaw cycle affects the drip loss and increased thawing loss by the formation of ice crystals which causes denaturation of protein, so repeated freeze-thaw cycle potentiate drip losses

The influence of multiple freeze-thaw cycles accelerated drip loss. It was significantly high with the number of cycles ($P \leq 0.0002$). The mean values were highly significant when compared from C0 to C4 mean values were 4.143 to 8.418, respectively (Table 2). When the mean of cycle one (C1) was compared with the mean of cycle two (C2) and C2 compared with C3, the values were not significant, but it showed a remarkable difference when it was compared with C0 ($P \leq 0.0002$). A significant change was also observed between the mean of C1 and C4. Ali *et al.*, (2015) explained that repeated freeze-thaw cycles initiate a high rate of proteolysis, lipid and protein oxidation, leading to an increase in drip loss which causes a reduction in the water holding capacity. In the present study, drip loss of chicken meat accelerated with the influence of multiple freeze-thaw cycles. Drip loss was significantly increased with the number of cycles ($P \leq 0.0001$). This increasing trend could be due to the increase of lipid oxidation and lowering of pH, which destroys the protein structure.

Cooking loss of chicken meat was significantly influenced by multiple freeze-thaw cycles. Cooking loss was increased from 14.99% to 24.09%, which was a remarkable difference when the mean of C0 compared with C4 ($P \leq 0.0002$) (Table 2). Initially, there was no remarkable increase in the mean values of C0 and C1. The significance level gradually increased with the increase in the number of cycles. The prominent change was found between C1 and C2, and a highly significant difference was found in C0 compared to C2, C3 and C4. In the present study, cooking loss of chicken breast samples was significantly increased from 14.99% to 24.09% from C0 to C4, respectively ($P \leq 0.0002$). Cooking loss was slightly increased from the fresh/control group to the first treatment C1, but this slight change was not significant. While cooking loss

showed an increased pattern when initial fresh group C0 compared with C2, C3 and C4 ($P \leq 0.0002$). In the previous studies, Vieira *et al.*, (2009) explained that cooking loss is chemically bounded water and some part of its melted fat. While Rahman *et al.*, (2014) observed that cooking loss increase with the increase in freeze-thaw cycles.

Influence of multiple freeze-thaw cycles on lipid oxidation of chicken meat: Initially, the lipid oxidation of chicken meat significantly remained the same when the mean values of fresh / control (C0) were compared with cycle first (C1). Due to the influence of multiple freeze-thaw cycles, lipid oxidation started increasing with a remarkable difference compared with cycle 0 ($P \leq 0.0001$). The mean values were highly significant when C0 compared with C2, C3 and C4 (Figure 1). In previous studies, repeated freeze-thaw cycles increase lipid oxidation and its affected physiochemical qualities of meat (Jin-ping *et al.*, 2012). Normally, freeze-thaw cycles accelerate the oxidation due to the formation of ice crystals which destroy the cell membrane. Due to the destruction of the cell and muscle system, pro-oxidants are released, speeding up lipid oxidation. So, multiple freeze-thaw cycles became the reason for lipid oxidation (Benjakul and Bauer, 2001).

Ali *et al.*, (2016) also found the same results an increase in the number of cycles accelerates lipid oxidation. In a recent study, it was observed that multiple freeze-thaw cycles accelerated the lipid oxidation in chicken meat. Lipid oxidation was increased significantly the number of freeze-thaw cycles increased in chicken meat ($P \leq 0.0001$). These results are also supported by previous research studies discussed above.

Influence of multiple freeze-thaw cycles on the tenderness of chicken meat: The tenderness of chicken meat was gradually increased with the influence of multiple freeze-thaw cycles. The mean shear force value of fresh / control samples was 34.75 ± 1.252 showed decreasing pattern ($P \leq 0.0001$). The overall P value was ≤ 0.0001 among the mean of all the cycles. The statistical comparison of the mean values of C2 and C3, C3 and C4 showed no difference (Figure 2). The tenderness of the chicken showed an increasing pattern throughout the freeze-thaw cycles. Its share force values decreased from 34.75 newtons to 18.01 from Fresh/control group C0 to 4th cycle C4. Many research studies concluded that multiple freeze-thaw cycles reduce the share force value of meat and increase the tenderness of meat (Farouk *et al.*, 2004; Lagerstedt *et al.*, 2008). Repeated freeze-thaw cycles result in the denaturation of the membrane and decrease its strength by the action of ice crystals (Liu *et al.*, 2010).

Table 1: Influence of multiple freeze-thaw cycles on pH and color of Chicken meat.

Parameters	C0	C1	C2	C3	C4	Level of significance
pH	5.94±0.12	5.95±0.11	5.85±0.11	5.91±0.09	5.89±0.10	0.07
L*	56.68±2.42 ^a	59.31±3.08 ^b	59.11±0.98 ^b	60.53±1.59 ^b	61.18±1.68 ^b	0.001
a*	12.09±1.08	12.34±1.09	12.26±1.12	11.9±0.87	11.31±0.87	0.06
b*	14.55±2.07	13.68±1.7	14.2±1.17	15.28±1.52	15.88±1.35	0.08

C0 = fresh/control, C1 = Cycle 1, C2 = Cycle 2, C3 = Cycle 3, C4 = Cycle 4. Meat color was elaborated by L, a*, b*. L* shows lightness of meat color.

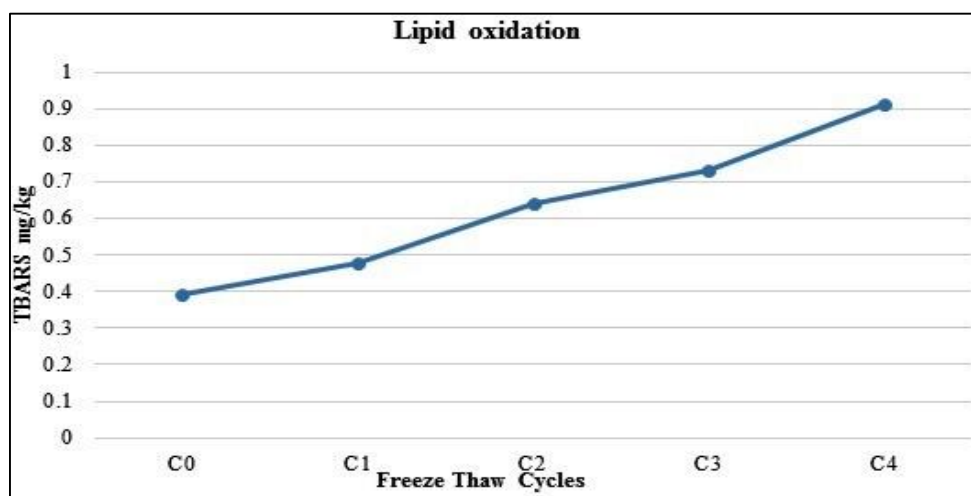
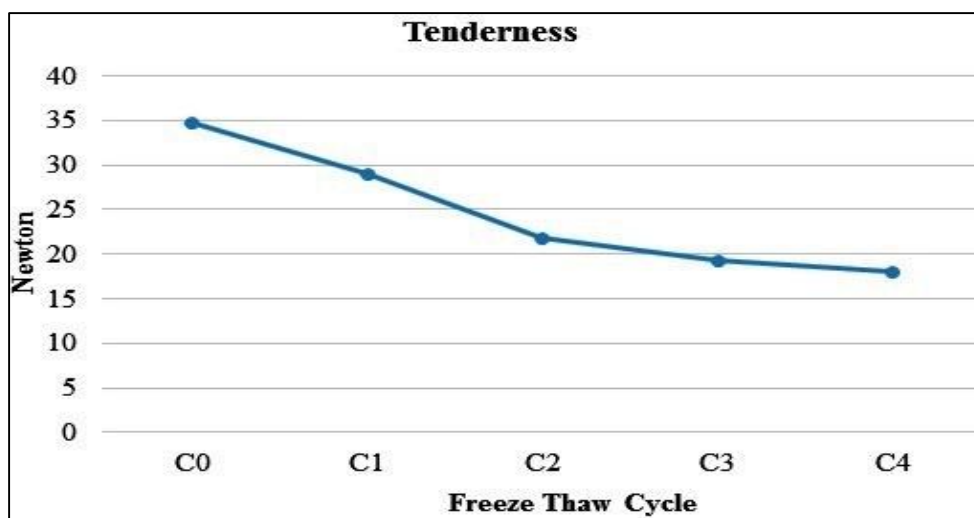
*Means having different superscripts are highly significant $P \leq 0.05$ among others in a row

Table 2: Influence of multiple freeze-thaw cycles on water content of Chicken meat.

Parameters	C0	C1	C2	C3	C4	Level of significance
Thawing loss	0.01	2.98±0.68 ^a	5.86±0.98 ^b	8.28±0.76 ^c	10±0.59 ^d	0.0001
Drip loss	4.14±0.042 ^a	6.61±0.56 ^b	7.81±0.55 ^{bc}	7.70±1.1 ^{bc}	8.41±0.74 ^c	0.0002
Cooking loss	14.99±3.03 ^a	16.03±1.16 ^a	20.77±2.30 ^a	22.32±0.81 ^b	24.09±1.05 ^c	0.0002

*C0 = fresh/control, C1 = Cycle 1, C2 = Cycle 2, C3 = Cycle 3, C4 = Cycle 4.

*Means having different superscripts are highly significant $P \leq 0.05$ among others in a row

**Figure 1: Influence of multiple freeze-thaw cycles on Lipid oxidation of Chicken meat.****Figure 2: Influence of multiple freeze-thaw cycles on Tenderness of Chicken meat**

Conclusion: This study reveals the effect of different freeze-thaw cycles on physicochemical changes in chicken meat. It was found that multiple freeze-thaw cycles adversely affect the water content and texture of the chicken breast fillet. The results are helpful to determine the quality and structural changes taking place in white meat during different freezing and thawing cycles. It is recommended to avoid refreezing over and over again. Fast freezing should be used instead of slow freezing for the preservation of chicken meat.

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