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FULL PAPER

Physicochemical properties of post laying hen breast meat thawed using various methods

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Abstract

The analysis of the impact of various methods of thawing on the physicochemical properties of post laying hen breast meat (*musculus pectoralis superficialis*) was studied. The treatments of various methods of thawing include water immersion (with the temperature measured at 20°C), thawing under tap water (water changes with the temperature measured at 30°C) and the water bath method (with the temperature measured at 40°C). The physical properties such as pH, cooking loss, drip loss, Water Holding Capacity (WHC) and hardness were being evaluated. Meanwhile, the chemical properties such as protein and fat content, protein profile and microstructure were being assessed as well. The analysis revealed significant ($P<0.05$) impacts on the meat quality properties depending on various thawing methods which were being subjected. The practical thawing method is to use water immersion at 20°C, which can be recommended to the public.

1. Introduction

Poultry meat is the most popular protein-rich food due to its affordable price and delicious taste (Buckle *et al.* 2009). Broilers allude to chickens that are grown to produce meat thus it becomes the most common type of chicken to eat, while other types of chickens can still be eaten to fulfil the human diet. Laying chicken is described as the type of hen with the production of eggs about 20% to 25% age in 96 weeks which are ready for harvesting (Gillespie and Flanders, 2010). However, the consumption of laying hen is still very low compared to broilers, due to its small size and tough texture, making it less acceptable to consume.

Post-laying hen meat must be processed before consumption, but some people are unable to do it directly. Thus, preservation methods such as freezing need to be taken to prolong the shelf-life. Freezing is one of the preservation methods that allows meat to maintain its durability by inhibiting the growth of microorganisms and delaying metabolism activities (Oliveira *et al.,* 2015). The optimal freezing temperature in chicken meat is -40°C, where at this temperature, the meat is below the freezing point of liquid in the ambient temperature (Estevez, 2011). The quality and technological appropriateness of meat during freezing and the subsequent freezing stockpiling process relies upon a few variables including storage temperature, storage conditions, and thawing methods. Therefore, frozen meat quality was influenced by freezing and thawing procedures (Akhtar *et al.,* 2013).

The thawing method was done by defrosting the meat to re-establish its quality as near as fresh meat (Augustyńska-Prejsnar *et al.,* 2018). The final quality of meat greatly influences the safety and acceptability in terms of physical quality. However, there was no research yet that focuses on the effect of thawing methods on the physicochemical quality of laying hen breast meat. Therefore, this research aimed to analyse the impact of various methods of thawing on the physicochemical properties of post laying hen breast meat.

2. Materials and methods

2.1 Materials

Post laying hen breast meat was purchased from laying hen husbandry in Kendal Regency. Chemicals used were distilled water, H_2SO_4, HCl 0.1 N, 45% NaOH, MR (Methylene Red) and MB (Methylene Blue) indicators, and ethanol was reagent grade. Meanwhile, the instruments needed were analytical scales (Tanita KD 321, China), centrifuges (K-Centrifuge PLC Series, USA), millimetre tubes, mortars, beaker cups, Ziplock plastic bag (PP), 10 kg freezer box of loads, glass plate,

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oven, desiccator, filter paper Whatman-1 No. 41, analytical scales (Tanita KD 321, China), pH meters (OHAUS ST2100-F, USA), water bath (Memmert WNB14RING, Germany), texture analyser (Brookfield C3, USA), Erlenmeyer tubes, Kjeldahl flasks, Soxhlet, SDS-PAGE, SEM (JEOL JSM-6510LA), water bath (Memmert WNB14RING, Germany), filter papers, knives, freezer and oven.

2.2 Freezing and thawing

Sample preparation was begun by cutting fresh laying hens about 90 weeks old into fillets and put into zip lock bags then were frozen in the storage (–22°C for 24 hrs) and kept under freezing temperatures (-12°C). Three thawing methods were evaluated: T1 (placed into a container containing normal temperature water of 20°C for 13 mins), T2 (thawing under running tap water with the temperature measured at 30°C for 10 mins) and T3 (immersed in a water bath that has been previously filled with water and heated to reach a temperature of 40°C for 8 mins). Control treatment or T0 was prepared by cutting the fresh approximately 90 weeks-old laying hens breast meat into fillets without going through the freezing and thawing process.

2.3 pH measurement

the pH of the samples was determined by using a pH meter with a combined glass electrode that had been calibrated previously at pH 4.0 and 7.0. The sample pH was determined by inserting the probe into the sample (Yusop *et al.,* 2010).

2.4 Cooking loss measurement

Each sample that had been thawed was cooked and weighed before and after cooking. The samples were dab dried using tissue before being weighed (Yusop *et al*., 2010). The calculation for cooking loss was measured as follows:

$$\%\ \text{Cooking Loss} = \frac{\text{thawed weight} - \text{cooked weight}}{\text{thawed weight}} \times 100\%$$

2.5 Water holding capacity (WHC) measurement

A total of 2 g of meat cube were laid on the filter paper circles placed between glass plates. The pressure of 10 kg load was put on the glass plates for 5 mins. After that, the meat cube was removed from the filter papers and weighed. Drip loss of meat samples was determined by measuring the difference between initial and final weight. The WHC was presented as the percentage of drip loss relative to the initial sample weight (Hamm, 1961).

2.6 Drip loss measurement

After 24 hrs of post mortem, breast fillet samples were weighed. After that, it was stored in polyethylene trays covered with waterproof plastic film at 3±1°C for 72 hrs and then thawed (Northcutt *et al.,* 1994; Dirinck *et al.,* 1996). The sample was weighed with an analytical balance and the drip loss was calculated as initial weight minus final weight and expressed as a percentage by the formula:

$$\%\ \text{Drip Loss} = \frac{\text{thawed weight} - \text{fresh weight}}{\text{fresh weight}} \times 100\%$$

2.7 Hardness measurement

Hardness was measured by using 2×2×1 cm of the sample meat where it was cut longitudinal. Then the sample was placed in a texture analyser (Warner-Bratzler). The probe would press the sample based on the set measurement.

2.8 Protein content

Protein content was analysed using the Kjeldahl method (AOAC, 2000). It was carried out in three stages, specifically, the destruction stage, where the sample will be destructed with concentrated sulfate (H_2SO_4) origin and selenium as a catalyst in the acidic chamber. Distillation stage, where the ammonium ions were converted into ammonia in the addition of NaOH, and trap N (4% boric acid + 2 drops MRMB indicator), the distillation stage ended when the catcher changes colour from purple to green. The last step was the titration with 0.1 N HCl. The endpoint of titration was marked by a change in solution from green to purple.

2.9 Fat content

Fat content was tested by the Soxhlet extraction method expressed in percentage (AOAC, 2005). The Soxhlet extraction method began with the preparation of filter paper with a size of 11.7×14.5 cm which was dried for 1 hr in an oven at 105°C. The filter paper was put into a desiccator for 15 mins then weighed. The meat sample was weighed 1.5 g (A). After that, it was placed in the middle of filter paper and then folded. The sample was dried in an oven with a temperature of 105°C for 4 hrs later was put into the desiccator for 15 mins and re-weighed to a constant (B). The extracted sample was taken out of the tool and aerated for 30 mins at room temperature, then condensed for 1 hr and was put into the desiccator for 15 mins. The weighing was done again if the weight difference does not exceed 0.2 mg (C).

2.10 Protein profile test

The protein profile was characterized by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel

electrophoresis) which was carried out using standard methods (Hames, 1988). Protein samples were denatured with buffer samples (Tris-Cl 150mM pH 6.8, SDS 6.25%, merk-mercaptoethanol, glycerol 25%, bromophenol blue 2.5 mM) with a protein and buffer ratio of 4: 1 and boiled for 2 mins. Electrophoresis was carried out at a voltage of 150 volts for 45 mins. Coomasie brilliant blue 0.1% was used for staining protein.

2.11 Microstructure test

Microstructure test (SEM) used samples in the form of frozen laying hen breast meat that had been thawed according to the treatments. The sample was analysed by drying it in an oven at 105°C for 4 hrs. After drying, it was followed by microstructure testing with SEM. The sample was placed on the SEM specimen holder using a carbon twin tip with the cross-section directed vertically upwards for the objective lens. The chamber in the sample was vacuumed up to 10^{-6} torr then operated with standard operating parameters including high voltage 20 kV, spot size 50, and a working distance of 10 mm. The results obtained were presented in the form of a microstructure illustration (Egerton, 2005).

2.12 Statistical analysis

Parametric statistics such as pH value, water holding capacity, drip loss, hardness, cooking loss, protein and fat content were analysed statistically by Analysis of Varian (ANOVA) using the SPSS application.

3. Results and discussion

3.1 pH value

According to the data, every result of the treatment showed the tendency to decrease which was caused by the acceleration of post mortem glycolysis, the transformation of glycogen into lactate acid in muscle. Post-mortem muscle metabolic processes cause the pH to decrease which can predict the final meat quality from the potential lactate formation as a result of lactate accumulation (Dadgar *et al.*, 2012). The thawing treatment at 40°C (T3) showed the lowest pH. High-temperature usage caused protein denaturation in chicken meat and the release of hydrogen ions (Akhtar *et al.,* 2013), therefore pH levels decreased subsequently.

The pH value of meat is a direct reflection of fatty acids thus affecting the physical quality such as tenderness, water holding capacity and colour of meat. The lower the pH, the decrease in meat quality was observed (Toplu *et al.,* 2014). The pH range of non-marinated chicken (raw meat) was 6.0-6.2 (Yusop *et al*., 2010). This statement can be used as a reference for the pH of fresh raw chicken meat, henceforth, between the pH levels of each treatment, T1 showed the optimum pH value since it reaches the highest pH with the nearest characteristic to T0 (fresh chicken meat).

3.2 Water holding capacity

Thawing treatment at 40°C produced the lowest water holding capacity (WHC). The decline of WHC value was caused by the denaturation of protein that changes myofibril and sarcoplasmic protein structure (Karunanayaka *et al.,* 2016). Myosin and actin are the major components of myofibril protein components, myosin begins to denature at 35°C while the actin will start to denature when the denaturation of myosin is complete (Ishiwatari *et al.,* 2013). Subjecting meat to sub -zero temperatures, thawing and then refreezing, mechanically disrupts the muscle cell integrity initiating a series of changes that causes the meat's dependency upon the structure of the myofibrillar proteins, particularly myosin (Ali *et al.,* 2015). In addition, the denaturation of myofibrillar and sarcoplasmic proteins and that water loss in the whole chicken carcass were associated with the protein denaturation due to the rapid glycolysis (Kato *et al.,*2013). pH amount is also taken into account for meat's water-holding capacity (Dyubele *et al.,* 2010). The decrease in pH near the isoelectric point of myofibrillar protein resulted in low WHC due to a reduction in myofibrillar protein's net charge (Jung *et al.,* 2015).

A drip is water that comes out and is not reabsorbed by the fibres during defrosting. Based on Table 1, it was known that the higher the thawing temperature, the higher the drip loss value obtained. Following the previous research, the amount of drip loss that came out of chicken meat would increase with the increasing thawing temperature (Augustyńska-Prejsnar *et al.,* 2018).

Table 1. Measurements for pH, water holding capacity, drip loss, hardness and cooking loss of laying hen breast meat with different thawing methods

Parameters	T0	T1	T2	T3
pH	6.01 ± 0.05^{d}	5.93 ± 0.02^{c}	5.88 ± 0.03^{b}	5.79 ± 0.03^{a}
WHC (%)	50.25 ± 0.46^{d}	40.9 ± 0.72^{c}	39.28 ± 0.78^{b}	35.67 ± 0.41^{a}
Drip Loss (%)	-	0.72 ± 0.30^{a}	1.31 ± 0.59^{ab}	1.8 ± 0.60^{c}
Hardness (gf)	55.96 ± 0.76^{a}	58.82 ± 0.64^{b}	64.84 ± 0.96^{c}	87.4 ± 0.89^{d}
Cooking Loss (%)	38.02 ± 0.97^{a}	39.84 ± 0.57^{b}	41.39 ± 0.71^{c}	59.37 ± 0.41^{d}

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different ($p<0.05$).

The lowest drip loss value was obtained by treatment of T1 with a value of 0.72, followed by T2, and the highest drip loss value was obtained by T3. The lowest drip loss showed by T1 indicated that the nutrients in post laying hen breast meat can be maintained. Denaturation of myofibril and sarcoplasmic proteins can decrease or even eliminate the functionality of protein in water binding capacity and facilitate the loss of sarcoplasmic constituents that dissolve from muscle cells into the extracellular space therefore more drip comes out of meat (Nkukwana *et al.,* 2015).

3.4 Hardness

The lower the hardness, the better the texture of the meat. T1 gave the lowest point of hardness while T3 gave the highest point of hardness. The hardest meat achieved by T3 was attributed to the meat contraction because of shock temperature due to thermal treatment. Thermal treatment affects the tissue, which in turn influences the hardness of the foal meat since changes in the cutting force are closely related to the myofibril contraction and degree of collagen denaturation (Lorenzo *et al.,* 2015). Meat contraction causes sarcomere shortening, thus the shorter the sarcomere, the tougher the meat. The shortening of sarcomere during thawing may cause a lot of water to come out thus the meat will become harder (Xia *et al.,* 2009). The more water that comes out of the meat, the meat will be drier and tends to be harder with lower elasticity (Öztürk and Serdaroğlu, 2015).

3.5 Drip loss

Thawing treatment of frozen meat can cause water loss during cooking. One of the main problems caused by meat thawing and cooking is the excessive release of liquid and consequently, nutrient loss, damaging processing profitability and consumers' deception (Oliveira *et al.,* 2015). According to the above results, higher temperature gives higher drip loss. When meat is cooked, the linkages between the proteins dissolve causing excess free water to escape and the solids to become edible. Variation of the water content in the meat during cooking affects meat juiciness while dripping loss from meat tissue after cooking was generally attributed to the denaturation of myofibrillar proteins and it was strictly related to water holding capacity (Chiavaro *et al.,* 2009). Thawing of frozen meat causes significant quality deterioration, due to the formation of large extracellular ice crystals, lipid oxidation, protein oxidation, and protein denaturation that affects each function (Xia *et al.,* 2012). Increased myosin denaturation may also construe the higher the drip loss of meat samples (Xia *et al.,* 2010). Another factor that affects drip loss is fat intramuscularly, it inhibits or reduces meat juiciness which comes out during heating, even if the meat contains bigger intramuscular fat, the meat will still lose fat in greater amounts (Lawrie, 2003).

3.6 Protein and fat content

The different treatments of thawing method in frozen laying hen breast meat had a significant influence on its fat and protein content ($p<0.05$). There was a protein content decrease on laying hen breast meat with T1, T2, and T3 treatment compared to T0 as portrayed in Table 2. The thawing process not only affected the decrease in protein content but also influenced the quality of the protein in the post laying hens due to the protein oxidation. This is due to the release of pro-oxidants that increase the oxidation potential of proteins, one of which is iron which is soluble in drips. When a protein undergoes oxidation; thus, there will be amino acid destruction, protein degradation, increased surface hydrophobicity, fragmentation, and cross-linking to proteins (Leygonie *et al.,* 2012).

The treatment with the lowest percentage of protein content was T3, with a protein content of 23.75%. The thawing process with higher temperatures caused some liquids to come out of the meat. Drips released by the meat were caused by the inability of the muscles to absorb the water that came out. Drip loss will cause the meat to be less acceptable due to the loss of components such as amino acids or nucleotides, hence making the meat to be less tasty (Oliveira *et al.,* 2015). Drips that come out during the thawing process have the potential to reduce protein levels in the post laying hen breast meat since some nutrients dissolved with water. T1 showed the optimum thawing method with high levels of protein, indicated by an average protein was approximately 39.26%. Thawing at a low temperature will cause fewer drips because the ice crystals in frozen meat are not melted completely. It was in agreement with Ozogul (2019) reporting that a proper thawing temperature ranges between 12-25°C, which can be done with air and water media.

Table 2 shows the higher temperature used in the thawing reduce fat content in laying hen breast meat as T0 treatment showed the highest fat content followed by T1, T2 and T3 treatment respectively . The level of fat lost after the thawing process might be due to the changes in tissue that causes water and fat to come out. The change in muscle structure in the meat during the frozen storage process causes the formation of cavities that cause water and fat to come out during the thawing process (Sigurgisladottir *et al.,* 2000). Damage to the muscle structure of the flesh due to ice crystals allows the release of pro-oxidants which cause fat to oxidize during thawing (Akhtar *et al.,* 2013). The presence of pro

Table 2. Protein and fat content of laying hen breast meat with different thawing methods

Parameters	T0	T1	T2	T3
Protein Content	39.26±0.70[a]	33.23±0.81[b]	32.67±0.75[b]	23.75±0.80[c]
Fat Content	3.59±0.54[a]	2.52±0.86[b]	2.49±0.85[b]	1.39±0.41[c]

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different ($p<0.05$).

-oxidants such as oxidized fats, free radicals, and oxidative enzymes will affect the occurrence of protein oxidation. Some protein derivatives that can bind to the fat substrate that trigger oxidation includes carbonyl forming compounds. One of the fat substrates that react with protein derivatives and form carbonyl groups (ketones and aldehydes) is malondialdehyde (Xiong, 2000). The loss of fat with this lipoprotein and also the occurrence of compound oxidation allow a change in sensory quality namely flavour and aroma.

3.7 Protein profile

The results of SDS-Page post laying hen breast meat with various thawing treatments can be seen in Figure 1 where the detection of 5 bands with different molecular weights. The bands with a molecular weight of 25 kDa, 36 kDa, 39 kDa, 47 kDa, 58 kDa, and 60 kDa were detected. The proteins in breast chicken include Phosphopglycerate mutase (25 kDa), Glyceraldehyde phosphate dehydrogenase (36 kDa), Aldolase (39 kDa), Enolase (47 kDa), Phosphoglucose isomerase (58 kDa), and Pyruvate kinase (36 kDa), Aldolase (39 kDa), Enolase (47 kDa), Phosphoglucose isomerase (58 kDa), and Pyruvate kinase (36 kDa) 60 kDa) (Mudalal *et al*., 2014). The presence of bands that appeared vague or unclear on the protein profile sequence was due to adjacent bands having the same amount of amino acids, but among them, there were extracts of amino acid residues that cause the band's position to change. The adjacent bands have the same amount of amino acids but one of them has an extract of amino acid residues so that the position of the ribbon is slightly different.

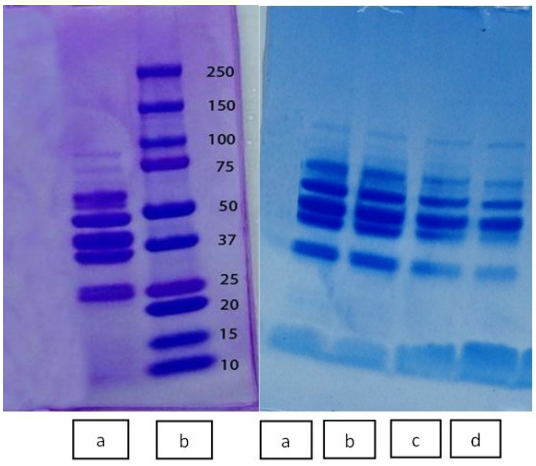

Figure 1. (a) Protein profile of laying hen breast meat without freezing and thawing treatment, (b) protein profile of laying hen breast meat with thawing treatment immersed in water at temperature 20°C, (c) protein profile of laying hen breast meat with thawing treatment submerged under running water at temperature 30°C, (d) protein profile of laying hen breast meat with thawing treatment immersed in water bath with temperature of 40°C.

The SDS-Page results indicated that the thawing treatment affected the protein components in the meat with the presence of different protein bands in each treatment. Meat with thawing temperatures of 20°C showed clearer protein bands compared to protein bands in laying hen breast meat which was at 30°C and 40°C. The bands that fade after heating were caused by heating which protein denaturation thus the molecular weight gradually decreased. Protein in food that was heated would have a lower molecular weight because the protein was denatured and broken down into smaller structures (Murphy and Marks, 2000). The heat treatment that was applied during the thawing process, caused protein denaturation and thus affected the protein fraction such as actin, myosin, tropomyosin, and others. An increase in thawing temperature can trigger an increase in cooking loss and increase myosin denaturation (Xia *et al.,* 2010). The results of SDS-Page showed that there was a change in the structure of the band on the protein of T3 meat.

3.8 Microstructure

The muscle structure of the post-laying hens as seen on Figure 2 showed a difference in each treatment. The T1 treatment appeared to have not undergone rolling or wrinkling texture, whereas in T2 began to experience shrinkage and rolls appeared in the muscle fibres, and in the T3 treatment appeared more rolls and wrinkles in the muscle fibres of the post laying hens. In contrast to the form of muscle fibres at T0 (control) which has smooth muscle fibres without wrinkles and rolls. The structure of muscle fibres due to the treatment of thawing with water flowing (T2) shows less muscle fibre damage compared to the treatment of thawing T3, but worse when compared to thawing soaked with water (water immersion) (T1). Thus, the existence of different thawing methods will affect the quality of the microstructure and the quality of the gel from meat (Li *et al.,* 2019). The process of thawing at 40°C with the highest temperature caused the most significant destructive effect on the microstructure of the post laying hen breast meat. This is in accordance with the previous experiment that states the high-temperature thawing method can cause a massive damage effect on the muscle fibres and increase the damage of the muscle fibre

bundle compared to the low-temperature thawing method (Xia *et al*., 2012).

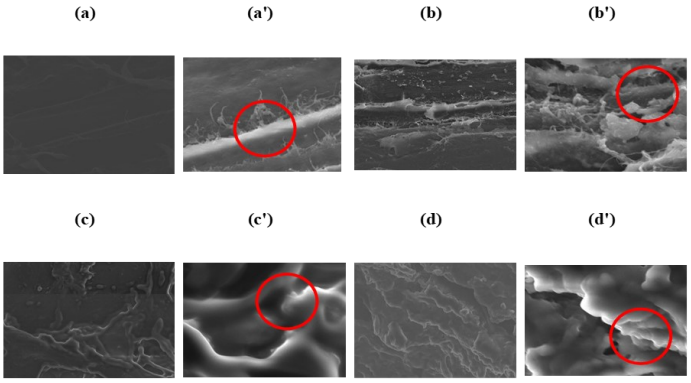

Figure 2. (a) Microstructure of muscle fibres of fresh meat (control) (b) Microstructure of muscle fibres after thawed with water immersion at a temperature of 20°C (c) Microstructure of muscle fibres after thawed with water flow at a temperature of 30°C (d) Microstructure of muscle fibre after thawed with warm water immersion with a temperature of 40°C (water bath).

The difference in muscle fibres in the post laying hens might be due to the freezing and re-thawing process which causes protein damage. The occurrence of different conditions in different muscle fibres is caused by the muscle undergoing a freezing cycle and experiencing myofibrillar protein damage caused by ice crystals during freezing and thawing (Jeong *et al.,* 2011). The effect of the thawing method which showed the differences in microstructure of post laying hens meat was caused by protein dissociation during thawing, which can trigger protein denaturation. Protein dissociation during thawing can trigger depolymerization of actin and actomyosin, resulting in fractures or broken non-covalent bonds that lead to protein denaturation (Zhu *et al.,* 2015).

4. Conclusion

Thawing methods under various temperatures in the range of 20°C up to 40°C resulted in the changes in the post laying hens' breast meat physicochemical qualities. The result indicated that there were significant ($P<0.05$) impacts on the meat quality properties depending on various methods of thawing which were being subjected. Practical application of the thawing method using water immersion (with the temperature measured at 20°C) was recommended to the public refers of the physicochemical properties of post laying hen breast meat.

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