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

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Abstract. Meat that has been undergoing the thawing process could affect the quality of a processed meat being produced such as steak. The study aims to know the effect of different thawing method of steak produced from post laying hen breast meat on pH, cooking loss, water holding capacity, hardness, fat content and also protein level, profile and the microstructure. This research was conducted using completely randomized design with 4 treatments and 5 replications T0 as a control, without freezing and thawing treatment; T1 by immersing frozen meat in water at ± 20℃; T2 with water flowing at ± 30℃; T3 with immersing frozen meat in water at ± 40℃ (water bath). The pH, cooking loss, water holding capacity, hardness, fat, crude and dissolved protein content was analyzed using Analysis of Variance (ANOVA) with a 95% significance, furthermore Duncan's Multiple Range Test was utilized to determine the significant difference among results. Protein profile was characterized by SDS-PAGE which was carried out using standard methods while the microstructure was assessed using SEM testing. The research indicated that thawing treatment by immersing the meat in water at 20℃ produced the most optimum physicochemical properties of steak made of post laying hen breast meat.

1. Introduction
Chicken meat is a popular livestock product because of its very high protein content and relatively low price. During this time, chicken meat consumed generally comes from broilers and native chickens. Consumption of purebred chicken per capita/year Indonesian people in 2017 amounted to 5.68 kg per capita/year increased by 573 g (11.2%) compared to the previous year's consumption, while the consumption of native chicken meat 782 grams per capita/year increased by 156 g (24.9%) in the previous year (BPS, 2018). In addition to these two sources, alternative chicken meat can also be obtained from laying hens.

Laying hens are chickens that do not produce eggs. Besides producing eggs, this type of chicken also has the potential of producing meat for consumption after the production period is over (Fenita et al., 2009). The use of laying hens meat is still very less when compared with broilers, whereas every year, the chicken that is rejected by the egg-producing industry is always increasing. Utilizing laying hens is one of the efforts of by-products from the laying hens business that can bring various benefits (Tasse et al., 2015). Laying hens that can be utilized by meat are laying hens that have stopped their productive period. Laying hens are 22-24 months / 88-96 weeks old, and they are no longer producing...
eggs (Purnamasari et al., 2012). The lack of utilization of laying hens meat is caused by tough meat, so it is less acceptable to some consumers. Laying hens have many advantages, including being high in protein and low in fat. The laying hen meat contains 25.4% protein, 56% water, and 3% -7.3% fat, whereas broiler meat contains 18-19% protein, 23% fat, and 3.2% mineral substances (Yahya, 2018). Addressing this issue, meat handling and processing can be modified hence being evaluated to increase the acceptability of processed food made from laying hen meat.

Chicken meat as one of high perishable food usually stores in freezing temperature in order to prolong its shelf life before getting further process. Thawing refers to the defrosting process of frozen meat, releasing the hardness by disclosure to warmth (Akhtar et al., 2013). Research conducted by Augustynska-Prejsnar et al. (2018) concerning the effect of the thawing method on the quality of the breast meat of broiler chickens shows that the physicochemical and sensory properties of frozen stored meat can change depending on the length of freezing storage and the thawing method used. The longer the meat is stored in a frozen condition resulting in increased drip loss and decreased sensory quality. The thawing methods commonly used include refrigerator thawing, cold water thawing, microwave thawing, and thawing at room temperature (Li and Sun, 2002). Physical damage and nutrition lost caused by cell membrane changing during thawing process need to be analyzed in which the thawing method used will result different depends on the meat type (Akhtar et al., 2013). Furthermore, the effect of thawing methods on the quality of the meat can also directly affect the product. One of processed meat that are popular with the public is the steak. Steak is one of processed meat that is sliced thick and served with roasted or grilled cooking method.

This study aims to know the effect of different thawing method of steak produced from post laying hen breast meat on pH, cooking loss, water holding capacity, hardness, fat content and also protein level, profile and the microstructure.

2. Materials and Methods

2.1. Steak Preparation

The study was begun by slaughtering 60 of ±90 weeks old laying hens that immediately filled in the chest for sampling. The next step was frozen the meat in the freezer at -22°C for 24 hours. Some fresh fillet meat directly processed into the steak as a comparison sample (T0). Before testing, the internal temperature of frozen laying hens measured using an infrared thermometer to ensure that the temperature had reached a standard frozen meat temperature. According to the provisions of SNI-3924 (2009), the carcass of meat called frozen if the internal temperature is minimum -12°C. After the temperature was appropriate, the meat began testing. In the T1 treatment, the sample immersed with water at a temperature of ± 20°C. The T2 treatment drained with running water at a temperature of ± 30°C. The T3 treatment of the sample immersed in warm water at a temperature of ± 40°C. The next step, the processed chicken meat processed into steak products. The procedure for making steaks refers to the research of Rasyad et al. (2012) with modification, which was done with marinated meat using 3 grams of each pepper and salt, then fried without oil on non-stick Teflon for about 15 minutes.

2.2. Physicochemical Analysis

PH testing had done by adding 45 ml of distilled water to 5 grams of sample. Samples of steak marinated and cooked mashed with a blender, then filtered with filter paper. The filtrate measurement was using a pH meter. The pH measuring device calibrated with buffer solutions with pH 7 and pH 4, then used to measure the pH of the sample (Chan et al., 2011).

Water Holding Capacity (WHC) measurement following Omana et al. (2010) method was done using following method, samples in the form of steak had been marinated and cooked, weighed 0.3 grams. After that the meat was put the between glass plates coated with filter paper, then given a weight of 35 kg for 5 minutes. After that, the area of the wet area formed calculated. The total water content calculated by weighing a sample of steak that has been marinated and cooked weighing 1 gram (x) and then wrapped in filter paper (y) that had been pre-oven for about 24 hours that have known the weight. Samples wrapped in filter paper heated with a temperature of 105°C for approximately 24
hours. After that, the meat sample cooled with a desiccator. Weight of meat sample after roasting was final weight (z).

\[ \text{mgH}_2\text{O} = \frac{\text{wet area}}{0.0948} - \theta \]

Free water content = \( \frac{\text{mg water}}{300} \times x - 100\% \)

Total water content = \( \frac{(x+y)-z}{x} \times 100\% \)

Water Holding Capacity = Total water content – Free water content

The cooking loss test was carried out by weighing the thawed and marinated meat sample as the initial weight. Then the sample was cooked by grilling for 5 minutes using medium heat. After cooling down, the steak samples were weighed as final weight (Soeparno, 2011).

\[ \text{Cooking Loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\% \]

Texture testing of steak samples was carried out in accordance with the research conducted by Han and Bertram (2017) which was conducted using a texture analyzer (Brookfield CT3, USA) with a cylindrical probe 36 mm and setting as follow, Trigger force: 4.5 g, Deformation: 2.5 mm and Speed: 1.0 mm/s.

Crude protein content was analyzed using Kjeldahl method that consists of three main stages, namely digestion, distillation and titration (Rosaini et al., 2015). The analysis began with 0.5 g of sample was mixed with 0.5 g of concentrated sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) 10 ml and selenium as a catalyst. The destruction then was done and completed when the solution turned into transparent and slightly green in colour. The distillation began by adding the solution with 100 ml of distilled water and 40 ml of NaOH 45%, 4% H\textsubscript{3}BO\textsubscript{3} trap 5 ml and two drops of indicator MR and MB. The sample then was titrated using 0.1N HCl until the colour of the solution turned into purple. Each treatment was done in five repetitions. The protein content can be calculated using the following formula:

\[ \text{Protein} \% = \frac{(\text{titrant sample-blank}) \times N \text{HCl} \times 14.008}{\text{sample weight} \times 1000 \times 100\%} \]

Dissolved protein was tested using the Bradford method (Susan et al. 2018) by colourimetry with Coomassie Brilliant Blue (CBB) as an indicator. The colour change was measured using a spectrophotometer visible absorbance at a wavelength of 465-595 nm using a standard solution of Bovine Serum Albumin (BSA).

Fat content analysis was done using the Soxhlet extraction method. The sample was macerated and then dried in an oven, then weighed and wrapped in filter paper and put into the oven for 4 hours at a temperature of 100°C. The sample was introduced to the extraction flask and filled with a solution of ether as much as 2.5-3 times volume of the extraction flask. Then the flask was heated to a temperature of 50°C for approximately 6 hours to extract the fat contained in the sample. Then the sample was dried in the oven for 1 hour 100°C and was weighed. Fat content was calculated with the following formula:

\[ \text{Fat Content} = \frac{\text{Weight B - Weight C}}{\text{weight A}} \times 100\% \]

Where, Weight A= weight after the extraction; Weight B: initial weight; and weight C= weight before extraction.

Microstructure analysis was done by using a Scanning Electron Microscope. It started by putting a sample on the SEM specimen holder using carbon double tip with a cross-sectional portion is directed vertically upwards or the objective lens. Space on up to 10-6 torr vacuum samples was then operated
as standard operating parameters which include high voltage 20 kV, spot size 50, and work distance of 10 mm. Results were presented in the form of images of microstructure and composition data samples (Sujatno et al., 2015).

2.3. Data analysis
The pH, cooking loss, water holding capacity, hardness, fat, crude and dissolved protein content was analyzed using Analysis of Variance (ANOVA) with a 95% significance, furthermore Duncan's Multiple Range Test was utilized to determine the significant difference among results. Microstructure were presented in figure and then were analyzed descriptively.

3. Results and Discussion

| Parameter                | pH     | Cooking Loss (%) | Water Holding Capacity (%) | Hardness (gf) |
|--------------------------|--------|------------------|----------------------------|---------------|
|                          | T0     | T1               | T2                         | T3            |
| pH                       | 7.21±0.25b | 7.17±0.16b | 7.00±0.09ab | 6.93±0.08a  |
| WHC (%)                  | 37.93±0.88d | 34.63±0.95c | 15.93±0.63b | 7.43±0.97a  |
| Cooking Loss (%)         | 26.09±0.68a | 30.78±0.85b | 34.88±0.90c | 36.07±0.88d  |
| Hardness (gf)            | 228,90±0.89a | 280,10±0.65b | 429,00±0.79c | 570,20±0.83d |

* a-c different superscript letters indicate significant differences (P <0.05). T0: control treatment, fresh laying hen meat; T1: thawing with immersing frozen meat in water at ± 20℃; T2: thawing with water flowing at ± 30℃; T3: thawing with immersing frozen meat in water at ± 40℃(water bath).

**pH.** The highest pH treatment value was the T0 treatment, with a pH of 7.21, and the lowest pH treatment was the T3 treatment, with a pH of 6.93. The higher the temperature of the thawing water used, the more substances dissolve, causing a change in pH. According to Leygonie et al. (2012), during the thawing process, frozen meat lost minerals and small protein compounds as exudates, which changed the balance of ions in meat, and resulted in a pH decrease. The temperature used during thawing can also cause denaturation of proteins, causing a lower pH. The finding is consistent with the opinion of Leygonie et al. (2011) that the occurrence of protein denaturation caused the release of hydrogen ions and a pH decrease.

Frozen meat has an increased concentration of solute because the liquid lost from the meat tissue results in a decrease in pH. Following the opinion of Akhtar et al. (2013), freezing causes loss of fluid from the meat tissue, increasing the concentration of solutes, which results in a pH decrease. The pH value for all treatments ranging from 6.93-7.21 can be caused by the addition of other ingredients, such as salt, during the cooking process. The finding is in line with studies conducted by Laksmi et al. (2012) that the mixing of ingredients created a new hydrogen balance point in the product that affects the pH of the product produced.

**Water Holding Capacity.** The highest water holding capacity at T0 treatment was 37.93%, and the lowest water holding capacity at T3 treatment was 7.43%. The higher the water temperature in the thawing process, the lower the water holding capacity of the laying chicken meat steak. The high temperature at the time of thawing allowed the denaturation of proteins. Protein denaturation is the loss of higher structural properties by the disruption of hydrogen bonds and other secondary forces that require protein molecules. Following the opinion of Risnajati (2010), the denaturation of proteins caused damage to the protein component of myofibrils, thereby reducing the water-binding capacity of meat. Water molecules that are bound to meat are very dependent on protein groups. This finding is consistent with the opinion of Purnamasari et al. (2012) that the component of meat to bind water closely related to the binding capacity of protein because the meat component to bind water molecules was very dependent on the protein groups, especially hydrogen bonds in proteins.
The water capacity of the steak from fresh meat was higher than the water capacity of the steak from frozen meat by the thawing method. Due to protein meat strength, the water-binding function has damaged due to freezing. This finding is consistent with the opinion of Ali et al. (2015) stated that the same phenomenon could be explained by a lower pH level when meat frozen and refreshed many times. Meat pH is also one of the factors that affect the holding water capacity. This finding is consistent with studies conducted by Agustina et al. (2012) that water-bound originally released the water as when the pH increased.

**Cooking Loss.** The treatment with the highest percentage of cooking losses was T3 with 36.07%, caused by a high thawing temperature so that more water came out. Following the opinion of Biyatmoko and Sulaiman (2018), cooking losses are affected by the temperature where the higher the temperature, the greater the level of liquid flesh lost until it reaches a constant level. Besides, another factor influence cooking losses is the water holding capacity. Thawing with a higher temperature makes it easier for water to come out, denaturing the protein contained in meat, thereby reducing the water holding capacity. Following the opinion of Rompis (2015), the percentage of cooking losses influenced by the amount of cellular membrane damage, the amount of water that comes out of meat, the meat shelf life, protein degradation, and the meat ability to bind water.

Cooked meat loss could also be influenced by pH values. The lower the pH, the more the shrinkage of cooked meat will increase. pH in meat influences the interaction style of actin and myosin proteins affected the water holding capacity of the meat. The water contained in meat is mostly stored in myofibrils and between actin and myosin protein filaments. If the volume of myofibrils increases due to the amount of water stored, the cooking loss of meat will decrease. Following the opinion of Hatta et al. (2011), changes in volume influenced by interaction styles in proteins. Protein interaction style influenced by meat pH. This finding is in line with the opinion of Soeparno (2015). There is an excess of positive charge resulted in myofilament rejection and gave more space for water molecules at lower pH levels than the isoelectric point of meat proteins.

**Hardness.** The texture test profile used was hardness, which is the maximum strength needed to be able to compress an object until it reaches deformation (Wardhani et al., 2017). If a solid material is given a stress load, one or more dimensions will change. This dimensional change results in deformation. All treatments had a significant difference with the lowest level of violence, which was T0 treatment and the highest level of violence, which was T3 treatment. This finding is consistent with studies conducted by Xia et al. (2009), meat given thawing treatment has a higher level of violence compared to fresh meat (meat that is not frozen). Hardening occurs because of the meat sarcomeres, about 40% of the initial length during the thawing process.

The meat sarcomere length is a critical factor in tenderness. This is consistent with the opinion of Bahtiar and Abustam (2014), meat with shorter sarcomeres had a higher level of orthodoxy than those whose sarcomeres do not experience shortening. Erbjerg and Puolanne (2017) add that due to freezing, ice crystals form and penetrate the sarcoplasmic reticulum. The melting ice crystals or refreshing process (thawing) using increasingly high temperatures, then allowed the rapid release of Ca$^{2+}$ into the sarcoplasm and caused the accumulation of Ca$^{2+}$ to the level where contractions occur. Thus, the shortening of sarcomeres length increased with increasing thawing temperature.

### Table 2. Chemical Analysis of Steak Made of Post Laying Hen Breast Meat

| Parameter          | T0 (a) | T1 (b) | T2 (c) | T3 (d) |
|--------------------|--------|--------|--------|--------|
| Crude Protein (%)  | 65.80 ± 0.29 | 65.84 ± 0.53 | 60.40 ± 0.88 | 54.83 ± 0.42 |
| Fat Content (%)    | 3.52 ± 0.34 | 2.50 ± 0.39 | 2.50 ± 0.39 | 0.8 ± 0.15 |

* a, b, c different superscript letters indicate significant differences (P <0.05). T0: control treatment, fresh laying hen meat; T1: thawing with immersing frozen meat in water at ± 20℃; T2: thawing with water flowing at ± 30℃; T3: thawing with immersing frozen meat in water at ± 40℃ (waterbath).
**Crude protein Content.** The result showed that there was a significant difference in each treatment, thereby proving that the thawing process may affect the chemical properties of processed meat, including proteins. This is following the opinion of Li and Sun (2002) states that thawing can cause physical and chemical changes and tissue damage. The drips falling from the meat thawing process most commonly in the treatment of 40 °C water bath for the highest temperature. According to Sari (2019) Protein in frozen meat will shed in drips when it does thaw, as stated by Wulandari et al. (2019), protein will decrease due to the hydrophilic nature of the protein so that it will come late in drips. While in the water immersion treatment with a temperature of 20 °C did not produce a tangible effect on levels of protein than a steak from fresh meat. In accordance with Diana et al. (2018), the higher the temperature, the more the thawing of ice crystals in frozen meat melt and vice versa. Thawing process can also affect the quality of the protein, which is caused by the protein oxidation reaction. The release of pro-oxidants such as iron participating in drips which increases the protein potential undergoes oxidation. According to Leygonie et al. (2012) Protein oxidation cause the destruction of amino acids, protein degradation, increase in surface hydrophobicity, fragmentation and crosslinking proteins. Beside the thawing process, the freezing process can also affect the quality of the protein. The process of freezing and thawing meat will reduce the quality of the meat itself (Krai et al. 2016). During the freezing process, ice crystals can damage the microstructure of meat and cause intracellular ion to migrate into the extracellular follows so that the protein can be denatured.

The highest crude protein content at T1 treatment was 65.84% followed with the lowest crude protein content at T3 treatment was 54.83%. The research conducted by Syam (2013) noted that the protein levels in fresh culled laying hens meat ranged from 21.90 to 25.75%. This suggests that the protein content of the overall treatment was higher than the protein content of fresh meat of culled laying hens. This is due to the decrease in the water content of the processing of steak. According to research conducted Dwiloka et al. (2006) beef steak has protein content of 70%. Where, according to Soeparno (2015), fresh beef has a protein content of 16-22%. The heat used during the processing will cause the protein to become denatured, thus decrease its ability to bind water. Erfiza et al. (2018) states that the protein denaturation will facilitate the nitrogen atom on the protein to be destructed, diminishing the protein ability to binding water.

**Dissolved Protein.** The results of the analysis presented in Table 3 show that the thawing method has a significant effect (P <0.05) on the dissolved protein content of the laying chicken steak. It can be seen that the dissolved protein content decreases with the high thawing temperature. This indicated that the thawing temperature positively correlated with the dissolved protein loss. The damaged structure of the meat causes this phenomenon at a relatively high thawing temperature due to shock temperature. The number of drips or liquid in the meat that decays during the thawing process, where the drips contain nutrients in the meat including dissolved protein, which is a collection of amino acids that have short chains so that they are easily digested in the body. This is in accordance with the opinion of Hendrarti and Adiwinarto (2018) which states that the more drips lost while thawing, the higher the decrease in water-soluble nutrients. Supported by the opinion of Purwoko and Handajani (2007) that dissolved protein is an oligopeptide or short-chain protein that is easily absorbed by the digestive system.

**Fat content.** The fat content in the steaks in each thawing treatment indicated a significant difference (P <0.05) with the fat content of the T3 treatment was below the average fat content of the laying hen meat. According to Yahya et al. (2018), fat content of laying hens ranged from 1.3 to 7.1%. The thawing process will dissolve the drips in the meat. Drips are water produced from refreshing ice crystals in meat that is formed during the freezing process. With the freezing and thawing processes, it will dissolve several components which can increase the rate of fat oxidation. Kılıç et al. (2016) and Benjakul and Bauer (2001) stated that the release of pro-oxidants such as iron could occur during the freezing and thawing processes.
The microstructural quality of meat will indirectly affect the physicochemical quality. The microstructure of meat can be affected by pre-processing such as freezing and thawing and processing itself, such as the cooking process. In Figure 1, with a magnification of 100x, it can be seen that the structure of each treatment showed a difference. In the T1 treatment, the texture or pores were still visible that were not wide open with the uniformity of pores that were still regular. Meanwhile, T2 began to experience damage to the pores of the flesh, it was seen by the opening of the pores. Likewise, with the T3 treatment which was entirely open and the shape of the pores of the meat microstructure was not uniform, it is different from steaks from fresh meat which still have tight and uniform pores. The difference in the quality of the microstructure indicated the influence of different thawing methods. It is in accordance with the opinion of Li et al. (2019) which stated that the existence of different thawing methods would affect the microstructure quality and gel quality of the meat.

The more chapped pores, the easier the drips fall on the meat causing the nutritional decrease and dehydration of meat. As seen in Figure 2, laying hen steak microstructure with a magnification of 1000x, the T1 treatment still has water spots in the relatively tight pores. Meanwhile, in the T2 and T3 treatments, there were no water spots because the pores were wide open due to higher thawing temperatures. Supported by Sign and Heldman (2001) statement that thawing can cause the intracellular water content becomes extracellular permanently, resulting in damage to muscle cells and dehydration of meat. Therefore, the temperature during thawing needs to be considered so that it does not cause damage to muscle tissue due to the shock temperature from freezing to higher temperature. The more damaged the texture of the muscle fiber tissue, the lower the ability of meat to bind water; hence the protein can dissolve with water or drips during thawing. The water content can also affect the level of the tenderness of the meat. This is in accordance with the opinion of Chandirasekaran and
Thulasi (2010) which stated that damage to the muscle structure has a negative effect on the physical quality of processed meat, such as juiciness and texture. Airlangga (2016) stated that the higher the water content in the meat, the softer the texture of the meat. 

4. Conclusion
The higher the temperature used during thawing, the lower the physicochemical quality and microstructure of the meat made from post laying hen breast meat. Treatment by immersing the meat in the water at 20°C produced the most optimum final quality as near as using fresh meat.

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