Quality changes of little tuna fillet (Euthynnus affinis) during chilling temperature storage

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Abstract. Deterioration of fish quality affects the accumulation of metabolites, flavor changes, the formation of volatile components, as well as an increase in the number of bacteria. Chilling temperature storage is the way to maintain the quality of fish. This research was aimed to determine quality changes of little tuna (Euthynnus affinis) through organoleptic test, chemical properties and protein analysis during chilling temperature storage. Observations were conducted every 48 hours for 14 days. The parameters observed were proximate, organoleptic, pH, water-soluble protein, metmyoglobin level, and its molecular weight. Little tuna was still in fresh criteria on the 4th day with organoleptic value of 7 and was spoiled on the 10th day. The chemical composition of the fish changed during storage, increased in moisture content, decreased protein levels, and increased ash content. The values of water-soluble protein decreased during the storage while the metmyoglobin level increased during storage. In conclusion, little tuna suffered a setback in quality during 14 days of chilling temperature storage. The storage time influenced the level of water-soluble protein and the level of metmyoglobin produced.

Keywords: little tuna, metmyoglobin, quality deterioration, water-soluble protein

1. Introduction

Indonesia has a high diversity of marine biological resources including fish. Fish is an important export commodity for Indonesia. The total export volume of Indonesian fishery products reached 974.55 thousand tonnes which valued as much as US $33,602.63 million in 2015 (KKP 2016). Fish protein provides 2/3 of the animal protein requirements needed by humans. Fish protein is easier to digest, however, it is very easily deteriorated. The deterioration process will occur swiftly if the fish were stored in an uncontrolled condition, with improper fish handling, and wrong processing methods which are not keeping the cold chain (Alparslan et al 2012).

Quality deterioration in fish resulting in the accumulation of metabolites, changes in flavor, formation of volatile components, and an increase in the number of bacteria. The more complex changes occur, the lower the freshness of fish will be resulted (FAO 2014). The most important factor in preventing fish deterioration is by controlling the temperature. Pandey et al (2018) stated that the use of low temperature would result in preventing the growth of spoilage bacteria and slowing biochemical processes. The common method is to use a cooling machine or use ice. The facts in the field show that
the use of ice is more usually used by sellers to maintain the freshness of fish, but many are inconsistent in replacing the ice in the fish storage box so that there are puddles of melt ice that immerse the fish. This can affect the quality of fish.

Little tuna (E. affinis) is a type of schooling pelagic fish and categorized as fast swimmer fish (Apriantari et al 2017). This fish species belongs to the Scombridae family and found throughout Indonesian waters. Little tuna has an important economic value because this fish is most caught for consumption by most people in Indonesia.

Little tuna has a high protein content that is 21.6-26.3 g/100 g (Violentina et al 2015) and is a fish that is high in demand by the community because of its protein content which is almost the same as tuna, but the price is more affordable. Little tuna catch value showed an increase in 2013 which was 166,359 tons and increased to 291,863 tons in 2014 (KKP 2015). The high value of consumption and export value makes the quality of tuna fish must be maintained so that fish meat still has good quality in appearance and nutrition when consumed by society.

Research on little tuna previously has been carried out, among others, on the chemical composition of tuna (Intarasirisawar et al 2011), identification of fish bacteria (Violentina et al 2015), characteristics of white meat and red meat of tuna (Chaijan et al 2013), inhibition of histamine formation in tuna fish meat by quercetin (flavonoids) during storage (Prasetiawan et al 2013), the effect of seasonal changes on proximate levels of tuna (Rani et al 2016), study of changes in fish meat color based on myoglobin content (Nurilmala et al 2013), research in autooxidation profiles of tuna myoglobin (Nurilmala and Ochiai 2016), sensitive and high precision method of classification using support vector machine (SVM) models to successfully identify DNA barcode sequences for species of tuna and other fish (Mulyati et al 2016), and research related to the quality of tuna during cold storage has been conducted by Ekasari et al (2017) which showed that the 14-day length of storage greatly influenced the level of TVB-N.

Information about the chemical changes in little tuna protein during cold temperature storage has never been reported. Considering the high economy value of tuna and the importance of maintaining the protein quality, therefore this study needed to be done in order to obtain information on the chemical changes in little tuna during chilling temperature storage based on organoleptic testing for 14 days (until the sample finally deteriorated), pH testing, proximate analysis, water-soluble protein analysis, metmyoglobin analysis, and protein profile testing by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

2. Research methods

2.1. Materials and tools

The main material used in this study was little tuna obtained from the Fish Auction Place/Temptat Pelelangan Ikan (TPI) Labuan, Banten-Indonesia. The materials used for pH testing were standard buffer pH 4 and 7 and distilled water. Materials used for testing the water-soluble proteins included Bovine Serum Albumin (BSA) (Merck), Coomasie Brilliant Blue (CBB) (Sigma), Orthophosphoric acid (Merck), ethanol (Merck), glacial acetic acid (Merck), NaH2PO4 (Merck), Na2HPO4 (Merck) and acetylacetone (Merck). Materials used for proximate testing include n-hexane (technical), selenium grains (Merck), H2SO4 (Merck), tablets, distilled water, NaOH (technical), H2BO3 (Ensure), HCl (Merck), methyl red, and bromcherosol green, while the ingredients used for testing the myoglobin ratio include distilled water. Materials used for electrophoresis testing include acrylamide, bis-acrylamide, tris-HCl buffer, 10% SDS, 10% APS, TEMED (Merck), standard marker protein (Thermo scientific-USA) size 10-250 kDa.
Tools used for storing fish included styrofoam boxes with the size of 70x40x40 cm for storing fish, sealed polypropylene plastic, thermometers (Thermo Electron, Germany). The instrument used for organoleptic testing was SNI 01-2346-2006 score sheet. The main tools used for analysis were pH meter (WalkLAB TI 9000), cold centrifuge (Beckman J2-21), homogenizer (Nissei AM-3), furnace (Vulcan), fat flask (WTBinder), oven (Karl Kolb), spectrophotometer UV 2500 (Optima), UV-Vis spectrophotometer (Labomed UV-Vis 1259) and SDS PAGE tools (BIO-RAD).

2.2. Methods

The little tuna was caught by one-day fishing fishermen. The fishermen took the fish in the morning and fish were landed at the fish auction place in the afternoon, so, certainly, the fish used for research was still in fresh condition. Fish used in this study consisted of 8 fish. The fish was put in a closed coolbox that contained slurry ice for the transportation process. Fish were transported to the Raw Material Characterization Laboratory, Department of Aquatic Product Technology, Bogor Agricultural University (IPB University). Fish were removed from the coolbox and separated from the melting ice. Fish were weighed and measured morphometrically and determined the percentage of body proportions.

The fish meat was prepared in the form of skin-on fillet. Cutting was done along the back of the meat. Red meat that was still attached to the fish meat was cleaned, so the clean white fish meat obtained. The fish meat was put in a sealed polypropylene (PP) plastic and tightly closed. The fish meat was put into a Styrofoam box size 70x40x40 cm with slurry ice. The ratio of fish and ice was 1:2 with the replacement of ice every 12 hours. The temperature inside Styrofoam was kept cool at 8 ± 2°C. The fish were analyzed for quality deterioration every two days, the analysis carried out consisted of pH testing, water-soluble protein, metmyoglobin levels and analysis of protein profiles with SDS-PAGE. Proximate analysis was performed for 14 days to know the changes in chemical composition occurred during the storage.

2.3. Procedure of analysis

2.3.1. Proximate analysis (AOAC 2005). Proximate analysis was performed to determine the chemical composition of the sample. Analysis conducted included analysis of water content, fat content, protein content, ash content and carbohydrate content which referred to AOAC 2005. Proximate analysis was performed with two replications on the back (skin on fillet) tuna.

2.3.2. Organoleptic test (BSN 2006). Organoleptic testing is a subjective method of testing using the five senses. The organoleptic testing method was carried out using a score sheet based on SNI 01-2729.1-2006 about fresh fish. The test was carried out by 30 trained panelists. It was conducted at intervals of observation, which was every 2 days for 14 days in chilling storage (8 ± 2°C). Organoleptic testing was done by descriptive method on each observation. Organoleptic tests carried out included meat appearance, odor, and texture. Based on the data obtained, then the freshness of fish would be categorized as fresh if it had organoleptic values ranging from 7-9, categorized rather fresh if it had organoleptic values of 5-6, and categorized as not fresh or deteriorated if it had organoleptic values of 1-3.

2.3.3. Test of pH (Apriyantono et al 1989). The measurement of pH values was done using a pH meter. The instrument used for testing the pH value was calibrated first using a standard buffer of pH 4 and 7. Fish meat as much as 10 grams were crushed and homogenized with distilled water as much as 90 mL using homogenizer. Homogeneous meat was then measured using a pH meter that was previously calibrated. The electrode was dipped in the mixture and the pH value could be read on display.
2.3.4. Analysis of water soluble proteins (Ren et al 2008). Samples of 10 grams were homogenized with 40 mL phosphate buffer pH 7.5 (15.6 mmol/L Na$_2$HPO$_4$ and 3.5 mmol/L KHPO$_4$) and then centrifuged at 5,000 g for 20 minutes at 4°C. The supernatant was taken and the residue was extracted with 40 mL phosphate buffer then homogenized again. The results were centrifuged at the same speed, temperature and time as before. The two products were combined and added with ratio of 1:1 acetone solvent with the extract. The extract was incubated for one night at a temperature of -20°C. The precipitate from the extract was taken by centrifugation with the same speed, time, and temperature. The precipitate was taken and dissolved in a 1:4 phosphate buffer. The extract was tested by Bradford method.

2.3.5. Bradford analysis (Bradford 1976). The Bradford test was carried out to determine the protein concentration in the sample with Bovine Serum Albumin (BSA) as standard. The Bradford solution is made by mixing 0.01 g of Coomassie Brilliant Blue (CBB) with 5 mL 96% ethanol, then adding 10 mL of 85% phosphoric acid solution and distilled water until the volume reached 250 mL. The Bradford solution was then filtered using filter paper. The determination of dissolved protein by the Bradford method was done using a spectrophotometer. Sample as much as 0.1 mL (extraction results) added 5 mL Bradford solution then homogenized. The absorbance value was determined by a UV-2500 spectrophotometer at a wavelength of 595 nm.

2.3.6. Metmyoglobin content analysis (Chow et al 2009) modified. Samples of 1 gram of fish were homogenized with 7 mL cold distilled water, then put in a 50 mL centrifuge tube, then centrifuged at 3000 g for 15 minutes. The extract was taken using a syringe, then the sample was filtered using a 0.2 µL filter paper. The solution obtained was measured by its absorption using a UV-VIS spectrophotometer with a wavelength of 380-780 nm, with a scale of 1.00. The calculation of metmyoglobin levels was determined using the formula 1.

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\text{Concentration (mg/100g) = \left(\frac{\text{Absorbance 540 x 2 x 16,000}}{11300}\right) x 100}
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2.3.7. Analysis of molecular weight distribution with SDS-PAGE (Nurilmala 2017). SDS-PAGE analysis refers to Nurilmala et al. (2017). The gel was made by mixing acrylamide, bis-acrylamide, tris-HCl buffer, SDS 10%, APS 10%, and TEMED. This research used 15% separating gel and 3% stacking gel. The process of forming the separating and stacking gel took 60 minutes. The completely solidified gel was then inserted into the chamber and filled with electrophoretic running buffer.

Samples were mixed with 1: 2 (v/v) buffer and heated at 95°C for 5 minutes. A 5 µL sample and 10 µL protein marker were put into the well. Electrophoresis was carried out at 150 volts for 3 hours. The marker used was a standard protein marker (Thermo scientific-USA) with a size of 10-250 kDa. Electrophoresis was stopped when dye migration reached the ± 1 cm limit from the bottom of the gel. The gel staining process used CBB. Destaining was done by soaking in 25% methanol and 10% acetic acid solution to clarify the protein bands. Protein bands showed the molecular weight profile and were measured by Photocapt software.

3. Results and discussion

3.1. Morphometric and proportion of little tuna

Little tuna is a type of pelagic fish and fast swimmers that live in groups. Tuna has a wide distribution area, generally inhabiting coastal and oceanic waters (Aprientari et al 2017). Measurement results can be seen in table 1.
Table 1. Little tuna morphometrics.

| Parameter          | Value          |
|--------------------|----------------|
| The total weight (gram) | 1,350.00 ±14 |
| Total length (cm)   | 40.14±0.8     |
| Standard length (cm)| 37.34±1.4     |
| Height (cm)         | 19.93±0.2     |
| Width (cm)          | 9.71±0.2      |

Eight tuna were used in the study with the average weight was 1,350±14 g. Morphometric measurement results showed different results in each part observed. The measurement results were specific for each type of fish. Morphometric differences in tuna can be caused by differences in growth in each individual tuna. Female fish are usually heavier than male fish despite having the same age and length. Seasonal variations in size and volume occur when the gonads are in the process of development, then shrink back immediately after laying eggs. Fish growth rates depend on the food available in the water in which they live so fish of the same age and species caught in different waters may vary in weight and length (Irianto and Giyatmi 2009). The proportion of little tuna fish can be seen in figure 1.

Figure 1 shows that the tuna meat had the highest percentage value, which was 40.87±0.42%. The lowest proportion of skin yield was 5.2±1.3%. Pianusa et al (2015) stated that the portion of tuna that could be consumed ranges from 45-50%. Little Tuna is a pelagic fish from the Scombridae family which has a high economic value and is a relative of tuna and skipjack. Tuna is widely used by people in Indonesia to process meat. Utilization of tuna fish meat is not only limited to being used as boiled fish and smoked fish, but there are many people who process it into processed products, such as nuggets, dumplings, and fillets. The results showed that tuna fish waste in the form of the head, bones, viscera, and skin reached 45.13%. Kittiphattanabawon et al (2005) explained that tuna fish waste in the form of heads, bones, viscera, and skin reached 50-70%. However, waste from tuna can be further utilized as a product of high economic value. Liu et al (2012) explained that the skin does have the lowest proportion of other body parts, but in its use the skin can be used as a source of collagen. Thiansilakul et al (2011) explained that the head of tuna fish can be used as a source of fish oil because it contains high fatty acids. Nurhayati et al (2013) research results showed that fish viscera can be used as a basic ingredient in enzymatic production of peptones.

3.2. Organoleptic test of little tuna
The organoleptic test conducted included meat appearance, odor, and texture. Based on the obtained data, the fish will be categorized as fresh if it got an organoleptic score of 7-9, rather fresh 5-6, not fresh 1-3 (BSN 2006). The results of the organoleptic test can be seen in figure 2 and table 2.
The organoleptic score of tuna was decreasing along with the length of storage time. Observation results on day 0 and day 2 showed that fish were still in fresh condition with very bright meat appearance, specific species, the texture is still solid and still elastic when pressed with a finger, meat is difficult to tear from the skin, and the smell is still very fresh. Observation on the 4th day showed the characteristics of less bright meat appearance, a rather stiff texture and rather dense when pressed with a finger, and a neutral odor. These observations were in accordance with Alparslan et al (2012) which stated that the results of sensory assessment of Seabass (Dicentrarchus labrax) until the 4th day of storage still showed good fish condition. Organoleptic observations indicated that tuna fish still have good quality in this observation, and were included in the criteria of fresh fish because they have organoleptic values ranging from 7-9 (BSN 2006).

![Little tuna appearance during chilling temperature storage.](image)

**Figure 2.** Little tuna appearance during chilling temperature storage.

Organoleptic observations on the 6th day showed characteristics of dull meat appearance and brownish color rather clearly visible, a bit soft texture and less elastic when pressed with a finger and smelled sour, organoleptic values on the 6th day were 6, while the organoleptic observation on the 8th day showed organoleptic score of 5 with the characteristics of a dull meat appearance and the color of the meat changed to brown, the texture was getting soft and the sour odor smelled stronger. Little tuna still had
pretty good quality in this observation, despite the deterioration of quality. Tuna on this observation was included in the criteria of rather fresh fish because it has organoleptic values ranging from 5-6 (BSN 2006).

Table 2. Organoleptic results of little tuna during chilling temperature storage.

| Day- Observance | Meat Texture | Odor | Score |
|-----------------|--------------|------|-------|
| 0               | Very bright appearance, specific meat type can be seen clearly | Solid and still elastic when pressed with a finger, the flesh is difficult to tear from the skin | Very fresh odor, specific | 9 |
| 2               | Bright appearance, specific meat type can be seen clearly | A little dense, elastic when pressed with a finger, the flesh is difficult to tear from the skin | Fresh odor, specific | 8 |
| 4               | Less bright, specific meat type still can be seen clearly | A bit stiff, rather dense, the flesh is still difficult to tear from the skin | Neutral | 7 |
| 6               | Dull appearance and brownish color rather clear | A bit soft, less elastic when pressed with a finger and a bit easy to tear the skin | Slightly sour odor | 6 |
| 8               | Dull appearance and brownish color rather clear | A little soft when pressed with a finger and a little easy to tear the skin | Strong sour odor | 5 |
| 10              | Very dull appearance and brownish color | Soft, not elastic, a mark was left when pressed with a finger and meat was easily torn/separated from the skin | Strong ammonia odor, strong sour odor and very stink | 3 |
| 12              | Very dull appearance, very brown | Very soft, not elastic, a mark was left when pressed with a finger and meat was easily torn/separated from the skin | Clearly very stink | 1 |
| 14              | Very dull appearance, very brown | Very soft, not elastic, a mark was left when pressed with a finger and meat was easily torn/separated from the skin | Clearly very stink | 1 |

The results of observations on the 10th day showed dull and brownish meat appearance, soft texture and the meat was easy to tear, also began to stink, organoleptic showed a score of 3, while the observations on the 12th and 14th day showed characteristics of meat appearance were very dull and brownish color, texture was very soft and finger marks did not disappear when pressed and there was a very foul odor with organoleptic score of 1. The fish on this observation was included in the criteria of fish that are not fresh because it has organoleptic values ranging 1-3 (BSN 2006).

Fish on the 10th day until the final storage showed that it was no longer suitable for consumption because it had a soft texture and gave off a foul and rancid odor. Rayeni (2016) stated that changes in fish meat texture during storage is a major problem in consumer acceptance. The decomposition process of protein in fish meat will trigger the formation of volatile base compounds, such as ammonia which can cause changes in taste, texture, and appearance of fish (Irianto and Giatami 2009). Rancidity that arises in the deterioration/decay phase is thought to occur due to oxidation of fat by oxygen and air. This is in accordance with the statement of Ridwansyah (2002) which stated that the foul odor caused in the
deterioration phase by the content of unsaturated fatty acids undergoing the oxidation process. Rayeni (2016) stated that rancidity can be inhibited by minimizing the free air, especially in the storage of processed products.

3.3. Chemical changes of little tuna

3.3.1. Proximate analysis. The proximate analysis of the sample was done on fresh fish that was observed on day 0 and fish on day-14 observation. Proximate analysis conducted in this study included water content, ash content, fat content, protein content, and carbohydrate content. The results of the proximate analysis can be seen in table 3.

| Chemical Composition | Storage Day 0 | Storage Day 14 | Storage Day 0* | Storage Day 14* |
|----------------------|--------------|---------------|---------------|---------------|
| Moisture (%)         | 73.14±0.07   | 77.09±0.17    | 74.77         | 75.21         |
| Ash (%)              | 1.32±0.01    | 0.27±0.51     | 1.96          | 1.11          |
| Fat (%)              | 0.08±0.01    | 0.33±0.02     | 5.75          | 3.45          |
| Protein (%)          | 22.97±0.06   | 18.09±0.44    | 19.46         | 15.31         |
| Carbohydrate (%)     | 2.46±0.13    | 4.20±0.58     | -             | -             |

*Pandey et al. 2018

The proximate content of fish can vary depending on the type of fish, sex, age, environment and season (Pandey et al 2018). Table 3 shows the chemical composition of tuna before and after storage. The value of the chemical composition changes with the length of time of storage. The water content of little tuna increased by 5.4% after cold temperature storage. This is similar to the study of Pandey et al (2018) who examined proximate levels of mackerel with 12 days of storage, that there was an increase in water content during storage.

The increase in water content is related to the freshness of fish, fish that deteriorated in the quality will experience an increase in water content (Purwaningsih 2010). The decrease in pH will also affect the physical properties of the meat. The rapid rate of decrease in muscle pH will result in a low ability to bind water in the material or water holding capacity (WHC) due to the increased contraction between the actomyosin formed. This causes free water to easily enter the fish muscle tissue. Gultom et al (2015) stated that the high water content in fresh fish indicates that water is bound in a tissue of an ingredient. The water content is very influential on the texture of the ingredients where most of the fresh ingredients contain 70% or more water. Water content in fish bodies generally ranges from 70-80%.

Protein levels have decreased during the 14 days of storage. The results showed that on the initial day (day 0) of observations, the tuna protein levels were 22.97±0.06%, observations on the 14th day showed a decrease in fish protein levels to 18.09±0.44%, while the protein levels of fish in the study of Pandey et al. (2018) also experienced a decline. The decrease is caused by the work of proteolytic enzymes that can break down fish muscle tissue into simpler compounds and degrade muscle tissue in the fish's body.

Ash content during storage also decreased. The observation result on the initial day showed that the ash content of tuna was 1.32±0.01% and the result of ash content of tuna after 14 days of storage was 0.27±0.5%. Ash content in the study of Pandey et al (2018) also decreased from 1.96% to 1.11%. The difference in the decrease in ash content can be influenced by differences in the size of the fish used and the ratio between meat and bone. Ash content contained in fish bodies is influenced by mineral content found in live fish habitat (Suwandi et al 2014).
The results of the proximate analysis of little tuna during storage showed that the fat content of day 0 was 2.46±0.13%, while storage on day 14 showed a fat content of 4.20±0.58%. Fish are classified as low-fat fish if they contain less than 2% lipids, medium fat fish contain 2-5% lipids and high-fat fish contain lipids above 5% and very high-fat fish if they contain 20% lipids (Irianto and Giyatmi 2009). The results showed that little tuna included in fish which had relatively low-fat content and high protein content. Noordiana et al (2011) stated that meat fat content correlates directly with the total microbes that grow in the fish meat.

3.3.2. Degree of acidity (pH) and protein analysis. The degree of acidity (pH) was one of the indicators used to determine the level of freshness of fish. Protein characteristics based on water-soluble protein levels and metmyoglobin content were also used to determine the quality of fish. The pH value, water-soluble protein and metmyoglobin contents of little tuna during storage can be seen in Table 4.

| Observation (Storage Time) | pH       | Water-Soluble Protein Content | Metmyoglobin Content |
|---------------------------|----------|-------------------------------|----------------------|
| Day 0                     | 6.25±0.02b | 25.22±0.78d mg/mL             | 39.01±0.55%a         |
| Day 2                     | 6.36±0.02cd | 24.11±2.35d mg/mL             | not tested           |
| Day 4                     | 6.28±0.06bc | 19.39±2.50bc mg/mL            | 40.59±0.03%b         |
| Day 6                     | 6.22±0.02b  | 18.56±0.78bc mg/mL            | not tested           |
| Day 8                     | 6.10±0.02a  | 16.89±3.14ab mg/mL            | 55.27±1.12%c         |
| Day 10                    | 5.99±0.02b  | 15.22±2.35b mg/mL             | not tested           |
| Day 12                    | 6.07±0.01d  | 13.28±2.74ab mg/mL            | 57.10±0.02d          |
| Day 14                    | 6.88±0.03c  | 7.97±0.74a mg/mL              | not tested           |

Note: Different letter in superscript shows significant differences (p<0.05)

Tuna on Day 0 had a pH of 6.25±0.017. The pH of fish could be depended on the glycogen content of the fish (Eskin 1990). Alparslan et al (2012) stated that the initial pH of Seabass (Dicentrarchus labrax) during cold storage was around 6.43. The fish meat pH after death is around ≥6.00, but this value can change according to the storage condition (Khidhir 2011). Table 4 showed that the pH value during storage changed, but not too significantly.

The decrease in pH value in tuna during storage is caused by the accumulation of lactic acid in fish meat. The situation when muscle tissue becomes supple in fish after death is biochemically characterized by a decrease in ATP and keratin phosphate. The energy in fish muscle tissue is obtained anaerobically from the breakdown of glycogen which produces ATP and lactic acid which causes a decrease in pH (Eskin 1990).

Generally, spoiled fish have a higher pH value (alkaline) than those of fresh fish. This is caused by the process of autolysis in fish meat which results in the breakdown of enzymes. Alparslan et al (2012) in their study stated that the pH of Seabass (Dicentrarchus labrax) during cold storage was 6.72 on the 12th day. The increase in pH value in this study could be caused by the process of autolysis in fish meat, namely the decomposition of proteins by enzymes and the accumulation of volatile bases.

The results showed the amount of water-soluble protein in tuna was decreasing along with the storage time. This is thought to be due to the nature of sarcoplasmic proteins which are easily dissolved in water. Sarcoplasmic protein decreases with the degradation of fish muscle tissue. Gandotra et al (2012) stated that fish stored at 4°C for 21 days storage had a protein reduction of 54.30%. Aufborg et al (2005) stated that sarcoplasmic protein decreases during cold temperature storage in Psseta maxima or turbot fish.

Sarcoplasmic proteins are present in the fluid and between muscle fibers and encompass many metabolic
enzymes that can reduce the functional stability of proteins during storage (Park and Lin 1996). A decrease in sarcoplasmic levels is caused by enzymatic and microbiological activities that occur in the body during deterioration process (Suwandi et al 2014). Sarcoplasmic or myogenic proteins consist of albumin, myoalbumin, and myoprotein. Sarcoplasmic content in fish meat varies depending on the type of fish depending on the fish habitat. Gultom et al (2015) stated that the myogenic content in fish muscles depends on the species, but is generally higher in pelagic fish when compared to demersal fish. The presence of water-soluble protein in fish meat to be made into fish gel products will have a detrimental effect on making gel (ashi) because it inhibits the formation of gels (Irianto and Giyatmi 2009).

On the other side, myoglobin is a globular protein that functions as a place to store oxygen in muscle tissue and helps in the process of respiration. Myoglobin levels can change with the duration of storage. Zhu and Brewer (2007) stated that the color of meat can be determined primarily from the concentration and content of myoglobin. The forms of myoglobin derivatives are deoxymyoglobin, oxymyoglobin, and metmyoglobin. Deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb) can change in fish meat tissues. Deoxymyoglobin and oxymyoglobin are easily oxidized to metmyoglobin and cause changes in the color of the meat.

Myoglobin is a heme protein that binds to iron (Fe). The color of myoglobin depends on the state of the heme and iron bond (Fe⁡²⁺ or Fe³⁺) and the type of molecule that binds to iron. If the freshly-cut meat looked purple, it is caused by deoxymyoglobin which has no ligands attached to Fe²⁺. Deoxymyoglobin will easily bind to oxygen, which will cause the flesh to colored cherry red. Over time, oxymyoglobin will continue to oxidize (Fe²⁺ turns into Fe³⁺) and metmyoglobin is formed which is shown by brown color (Motoyama et al 2010).

Metmyoglobin accumulation in fish meat is the main factor causing color changes (red to brown). The ratio of oxymyoglobin is inversely proportional to the ratio of metmyoglobin. Oxymyoglobin can indicate the quality parameters of tuna meat that is still fresh. Nurilama et al (2013) stated that the levels of myoglobin in tuna will be damaged at 40°C and pH 6 which will cause decay in the structure. The stability of the linkage between the heme and the globin decreases at acidic environment so that the unstable regions will expose the heme to the medium, resulting in increasing metmyoglobin ratio (Nurilmala and Ochiai 2016).

The level of metmyoglobin on day 0 was 39.01 ± 0.55%. The increase of metmyoglobin continued during storage until the observation on day 12 was 57.10 ± 0.20%. These results were in accordance with research conducted by Zapata et al (2011) which stated that the content of myoglobin in meat will decrease with increasing time after the fish die, while the metmyoglobin content increases. The brown color will be seen more clearly if the content of metmyoglobin in fish reaches 50%. Wodi et al (2014) stated that there was a change in the color of the flesh from red to brown due to the oxidation of oxymyoglobin to metmyoglobin. Little tuna stored for 9 days showed that all parts of the meat have been degraded, oxidation has occurred, red pigment turned to brown, and this is an indicator of the lower level of freshness of the fish.

3.3.3. Protein molecular weight. Protein molecular weight was measured using SDS-PAGE. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a gel electrophoresis technique for separating charged proteins based on their ability to move in electric currents (Hames 2002). The distribution of molecular weights from the SDS-PAGE test results on a crude extract of little tuna during chilling temperature storage can be seen in figure 3.
Figure 3 shows that little tuna protein bands changed during storage until the end of the observation. The protein bands at day 0 and day 2 were still thick, especially at a molecular weight of 71-86 kDa. Observations on day 4 to day 10 showed protein breakdown so that the protein bands had begun to separate. The breakdown of these proteins was related to the decrease in pH which activated the cathepsin enzyme thus it could break down protein components. Nurhayati et al (2010) stated that the highest activity of the cathepsin enzyme is in the rigor mortis phase so that the protein bands would separate and look thinner.

Protein bands on day 0 showed a protein molecular weight of 188.76 Da, the band could still be observed until the 12th day, while observations on the 14th day showed the band was no longer visible. The band is thought to be a heavy chain myosin (MHC). Research conducted by Ladrat et al (2003) stated that the heavy chain myosin protein is in molecular weights around 200 kDa. The heavy chain myosin protein plays a role in gel formation in surimi. The band observed on the 14th day showed a protein molecular weight of 157.77 kDa. The difference in molecular weight of myoglobin protein band on the 14th day is possible because on the 14th day a protein denaturation has occurred due to a decrease in the pH of fish meat. Thiansilakul et al (2011) stated that low pH can reduce the stability of myoglobin haem and increase autoxidation.

Myoglobin molecular weight ranges from 14-18 kDa. Myoglobin in fish is generally smaller than mammals. Thiansilakul et al (2011) stated that the stability of myoglobin varies, depending on the species due to different amino acid sequences and globin secondary structures. Chow et al (2009) stated that the globular protein myoglobin has a small molecular weight of 17 kDa found in tilapia fish meat. Observation on day 0 showed the presence of bands showing myoglobin protein. The band remained visible until the 12th day of observation, whereas on the 14th day the band showed the myoglobin protein was completely faded. Observation on the 14th day showed that the protein band were thinner at 18-33 kDa molecular weight, but formed a protein band with 11 kDa molecular weight. This could indicate that the protein has been degraded. Thiansilakul et al (2011) stated that the molecular weight of goldfish myoglobin stored for nine days at pH 6.0 was 16 kDa.
Albert et al (2002) stated that the area formed by the protein band shows the content or volume of proteins that have the same molecular weight which is in the same band position. Roy et al (2012) stated that the principle of movement of charged molecules, is charged molecules can move freely under the influence of an electric field, molecules with the same charge and size will accumulate in the same or adjacent area or band.

4. Conclusion

Little tuna has deteriorated during the 14 days on chilling temperature. Changes in pH value occurred during the storage period. The storage time influenced the level of water-soluble protein and the level of metmyoglobin produced. The longer the storage time, the more water-soluble protein decreased, while the level of metmyoglobin increased. Protein breakdown occurred on the 4th day due to changes in pH so that visible protein bands that gather began to separate.

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