Characteristics of Chemical Quality Reduction in Fresh Baung Fish (*Mystus nemurus*) with Different Methods of Death at Room Temperature

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Abstract. Baung fish is a fishery commodity that contains high water content so it is easy to decompose (high perishable product). The purpose of this study was (1) to examine the freshness level of the baung fish, (2) pH level, Total Volatile Base (TVB), and glycogen of the baung fish by different mortality death. This research was conducted using an experimental method, consisting of two steps: (1) preparation and observation of freshness level of fresh baung fish, (2) analysis of chemical reduction of fresh baung fish including pH, TVB, and glycogen content. The treatments of the research were (1) the baungfish died by itself (A1), (2) the fish were stabbed out the medulla oblongata (A2). Chemical reduction of fresh baung fish at room temperature was measured every 4 hours for 12 hours with parameters such as pH, TVB, and glycogen content. The data were analysed descriptively. The results of this research showed that the characteristics of fresh baung fish were move actively, healthy, difficult to catch, the opening and closing movements of the operculum were normal, and normal skin color (not pale). The results of the chemical reduction test revealed that pH were 6.5 ± 0.05 at 0 hours to 7.0 ± 0.20 at 12 hours for treatment A1, while for treatment A2 was 6.3 ± 0.1 at 0 hours to 6.6 ± 0.1 at 12 hours. The TVB test results showed 0.14 ± 0.04 at 0 hours to 31.32 ± 0.09 at 12 hours for A1 treatment. Whereas A2 treatment was 0.10 ± 0.03 at 0 hours to 26.30 ± 1.87 at 12 hours. Furthermore, glycogen levels showed at 6.67% ± 0.03 at 0 hours to 0.35% ± 0.13 at 12 hours for A1 treatment. While the A2 treatment was 7.69% ± 0.12 at 0 hours to 1.15% ± 0.26 at 12 hours.

1. Introduction
Baung fish (*Mystus nemurus*) is a freshwater fish that has high economic value in the Riau area whose production continues to increase. Sungai Paku Village, Kec. Kampar Kiri is famous for its Baung fish cultivation in 2016 with a total production of 8,815 kg [1]. Baung fish has nutritional content, namely water content of 80.3%, protein 17.1%, fat 1.3% and ash content 1.0% [2]. The advantages of Baung fish are not only high protein content, but low fat, delicious and tasty meat taste. The texture of the meat is white, soft and thick fleshy without soft spines.

Fish is consumed by the public in the form of fresh fish. Fresh fish has a weakness, namely it is easy to experience damage or deterioration of quality (high perishable product). However, in choosing fish to be consumed, consumers are very selective in choosing the type of fish as well as the quality of freshness, so that one of the factors that determines the quality of fishery products is the freshness of the fish. The best effort to maintain fish quality is to keep the fish alive, however this is difficult to do without large feed and air supply [3]. Another alternative that can be done is to reduce the temperature of the fish's environment, try to prevent the fish from being stressed before dying or by shutting down immediately after catching. This is done in order to extend the period rigormortis fish [4]. Fish enter phases is faster rigormortis at room temperature and lasts shorter. If this rigorous phase is not maintained for longer, then the decomposition by enzyme activity takes place faster which causes a
very rapid change so that it will enter the phase postrigormortis which indicates that the quality is low and no longer suitable for consumption.

The method of fish death is a method used to determine the steps of decreasing fish freshness that occurred after the fish has died. Fish that have died will experience a decline in quality. fish that have started to decline or fish that are not quickly handled and processed, will affect the quality of the processed products produced and the products will undergo a faster process of decay. Information about changes that occurred after the death of fish with different mortality methods will be a reference in maintaining fish freshness. Determination of the freshness level of fish can be done through chemical parameters such as pH, total volatile base and glycogen. Enzymatic changes produce volatile and foul smelling compounds, so these compounds are used as an index of deterioration of quality. The level of this volatile compound is called TVB which is a test related to determining of pH.

The deterioration of quality by treating of mortality method in several fish has been studied, including the effect of several methods of fish mortality on the quality of snapper [5]. The effect of the fish mortality method and the steps of decreasing fish freshness on the quality of pastatilapia fish [6]. The deterioration of the quality of red tilapia (Oreochromis sp.) During storage at room temperature [7]. However, so far it is still rare to be found the research related to the process of decreasing the quality of fresh fish by different mortality methods in baung fish. Based on this, the authors are interested to conduct the research about the effect of death methods on deterioration of the quality of the baung fish at room temperature.

The purpose of this study was to determine the freshness level of the baung fish, the pH value, the Total Volatile Base (TVB), and the glycogen of the baung fish with different mortality methods.

2. Materials and Methods

2.1 Time and Place

The research was conducted from April to May 2019. The research was carried out at the Laboratory of Fisheries Product Technology, Fisheries and Marine Integrated Laboratory, Faculty of Fisheries and Marine, Riau University.

2.2 Materials and Tools

The main material of this research is the baung fish (M. nemurus) which is obtained from the baung fish cultivation in Sungai Paku Village, Kampar Kiri District, Kampar Regency, Riau Province. The baung fishes used are the baung fish with a body weight of 150 g / fish. Besides that, chemical materials and tools for pH analysis, Total Volatile Base (TVB) test, and glycogen test are also used.

2.3 Research Methods and Procedures

This research was conducted in two experimental steps, namely: (1) preparation and observation of freshness level of the baung fish, (2) chemical reduction analysis including pH, Total Volatile Base (TVB), and glycogen.

2.3.1 Preparation and observation of freshness level of baung fish (Step 1)

At this step, the baung fishes were selected based on their freshness, whom they were fresh or not stressed by observing their behavior based on the characteristics of healthy fish. After selecting the fish with a high level of freshness, the treatment was given, namely being turned off in different ways, which consisted of A1 (died by itself) and A2 (stabbed in the Medulla oblongata) treatment, this treatment was carried out and observed every 4 hours for 12 hours. The baung fishes that observed every 4 hours separated immediately between meat and liver of the baung fish with other parts.

2.3.2 Analysis of chemical degradation of fresh baung fish (pH, TVB, and glycogen) (Step 2)

After the preparation and observation step of the freshness level of the baung fish, then a chemical reduction analysis was carried out including pH, TVB, and glycogen.
2.3.2.1 pH analysis [8]
A sample of 10 gr was cut into small pieces and homogenized (blended) with 20 ml of distilled water for 1 minute, poured into a 100 ml beaker glass then measured using a pH meter.

2.3.2.2 Analysis Total Volatile Base
The sample was weighed as much as 5 gr, then put into a blender and added 15 ml of 7% TCA ingredient then blended for 1 minute. The ingredient was filtered with filter paper so that the filtrate obtained was clear, then 1 ml of boraxic acid ingredient was put into the inner chamber of the Conway cups. By using another 1 ml pipette, the filtrate is inserted into the outer chamber of the conwaycups, the lid of the conway cup is almost closed, then 1 ml of saturated $K_2CO_3$ ingredient is added to the outer, after that the cup is closed immediately. Previously, the edge of the cup was smeared with Vaseline so that a tight cover is obtained. Then, to make a blank where the filtrate is replaced with a 5% TCA ingredient. The conwaycups were carefully arranged on the shelves then shaken slowly for 1 minute, then incubated at 35°C for 2 hours. After the incubation is complete, the borax acid ingredient in the inner chamber is titrated with a 0.2 N HCl ingredient so that the color of the borax acid ingredient was changed from green to pink (Dirjen Perikanan, 1991).

$$TVB = \frac{ml \text{ titration sample} - ml \text{ titration blank} \times 80mgN}{100 \text{ g sample weight}}$$

2.3.3 Glycogen Analysis [9]
Muscle or liver tissue as much as 100 mg heated in 3 ml of 30% KOH dissolves during 20-30 minutes, then add 0.5 ml of Na 2 SO 4 saturated and 3.5 ml of 95% ethanol is heated to boiling, then the solution is cooled and centrifuged in a cold state, the supernatant is discarded. Glycogen was dissolved in 2 ml of distilled water and re-precipitated with 2.5 ml of 95% ethanol. The supernatant was removed and the glycogen was precipitated for 30 minutes in 2 ml 5 MHCL in a boiling water bath shaker, the hydrolyzate was cooled and neutralized with 0.5 M NaOH, then diluted with distilled water to a known volume, usually 50-100 ml depending on the glycogen content required estimated. 5 ml of neutralized hydrolyzate (containing 15-150 ng glucose) is transferred to the test tube. Then pour 5 ml of standard glucose (111 ng) into the second test tube and 5 ml of distilled water as a blank into the third test tube. The tubes were dipped in cold water and added 10 ml of reagent anthrone and the tube was covered with a marbles glass and heated for 10 minutes in boiling water, then cooled and immediately measured the absorbance at a wavelength of 635 nm, in calorimeters (1 g glycogen = 1, 1 g glucose in hydrolyzate). Glycogen calculation formula:

$$\text{Glycogen (\%)} = \frac{P \times V \times C}{0.01 \times 1000} \times 100\%$$

Information:
- $P$ = Volume of the titration
- $C$ = Spectrophotometer value
- $V$ = 0.005

2.4 Data Analysis
The data obtained were tabulated and then descriptive data analysis was carried out.

3. Results and Discussion
3.1 Preparation and observation of freshness level of baung fish
The baung fish used in this study were obtained from the aquaculture pond fish in Sungai Paku Kampar Village. The general morphology of the baung fish is that it is long, smooth and not scaly, the head is rough and depressed with three pairs of tentacles around the mouth and and a pair of breathing holes, the length of the maxillary barbel almost reaches the anal fin. On the pectoral and dorsal fins there are patil spines. The top of the head and body are blackish brown to the middle of the body and
white towards the bottom, this morphology is in accordance [10]. In full, the baung fish used as raw material can be seen in Figure 1.

![Figure 1. Raw material for baung fish (M. nemurus)](image_url)

Baung fish before being given the treatment is selected first based on the high level of freshness based on the characteristics of fresh fish, which is seen from the movement of active healthy fish, difficult to catch, normal operculum openings (not fast and slow), normal skin is not pale. The results of these observations are in accordance with the characteristics of healthy fish movement according to [11]. Which from the movements are active, difficult to catch by hand and responsive to external stimuli. According to [12], the characteristics of fish that experience stress are swimming to the surface to take in oxygen accompanied by fast movement operculum, movement becomes passive and fish reflexes decrease. Meanwhile, fish that experience stress, the body color of the stressed fish will turn dark or pale [13].

The baung fish that have been selected based on their freshness are then treated with different death methods consisting of two levels, namely treatment A₁ (dead by itself) and A₂ (pricked part Medulla oblongata) this treatment was carried out and observed for 12 hours with a time interval of 4 hours. The baung fish that had been treated every 4 hours were immediately separated from the meat and liver of the baung fish from the other parts.

### 3.2 Analysis of chemical degradation of fresh baung fish (pH, TVB, dan glycogen)

Chemical content analysis carried out in this study aims to determine the pH value, value Total Volatile Base and the glycogen content of the baung fish with two different treatment methods of death every 4 hours.

### 3.3 pH Analysis

The results of the analysis of the pH of the fish with two treatment methods of death can be seen in Table 1.
Table 1. The results of the analysis of pH values of the baung fish every 4 hours

| Treatment | Observation time (Hours) |
|-----------|-------------------------|
|           | 0          | 4            | 8            | 12           |
| A₁        | 6.5 ±0.05  | 5.6 ±0.66    | 4.8 ±0.83    | 7 ±0.20      |
| A₂        | 6.3 ±0.1   | 6.5 ±0.1     | 5.8 ±0.25    | 6.6 ±0.1     |

Based on the value of pH in Table 1, it can be known that the best treatment is A₂ treatment, that is, use the death method of fish by piercing part of medulla oblongata, where A₂ treatment is slower to change than A₁ treatment. A₁ value is higher at 12 hours of observation, it is indicating that the fish is not fresh too because pH is higher or bases than fresh fish meat. This is caused by compounds of bases appear such as ammonia, trimethylamine and others volatile compounds [14].

Based on the test results above that treatment of A₁ has changes faster than A₂ treatment, this is because baung has got pressure and action before the fish is dead [15]. Which is the fish die then glycogen will be hydrolyzed to lactic acid, so that pH of fish will be decreases but with longer save of that, it will be increases. Effect from time of the save in increases it can involve its protein and derivarat will straggling in microbiologist or enzymatic which is derivative of bases, so that it can cause pH value to increase again.

3.4 Analysis of Total Volatile Base (TVB)

For observe of fish freshness level, it can use TVB test, that determination of TVB test intend to determine amount volatile bases compounds. Its result can be seen on Table 2.

Table 2. Result of TVB analysis of baung fish every 4 hours

| Treatment | Observation time (Hours) |
|-----------|-------------------------|
|           | 0            | 4            | 8            | 12           |
| A₁        | 0.14 ±0.04   | 10.79 ±3.70  | 15.82 ±1.05  | 31.32 ±0.09  |
| A₂        | 0.10 ±0.03   | 8.74 ±1.25   | 14.40 ±1.57  | 26.30 ±1.87  |

The value of baung’s TVB in A₁ treatment (die by itself) on 0’s hour is 0.14 mg/100 g, while a2 treatment (stabbed to death) is 0.10 mg/100g. At 4’s hour, A₁ TVB’s value is 10.79 mg/100 g and A₂ is 8.74 mg/100 g. Then at 8’s hour, A₁ TVB’s value is 15.82 mg/100 g and A₂ is 14.40 mg/100 g. TVB’s value at 12’s hour is 31.32 mg/100 g and A₂ is 26.30 mg/100 gram. This value is indicating that the fish is very fresh. The fresh fish has TVB’s value is 10 mg/100 g or less [12]. TVB’s value will be more increasing along with quality deterioration phase [18]. Decreased freshness of fish because of microorganism activities and autolysis enzyme that produces volatile base compounds found on fish.

Based on TVB’s value, it known that great treatment is treatment with stabbed to death of fish on Medulla oblongata (A₂) that this treatment when fish has dead, it didn’t many bring out energy, so quality deterioration more less than A₁. Fish TVB’s value will be increasing along length of keeping time. The increasing caused by autolysis activity and putrefactive bacteria on keeping time. On protein enzymatic process will be struggled to be simple compounds, such as; peptide, amino acid and ammonia. Besides hydrolysis of protein will establish purine base and pyrimidine [19]. During the storage process due to protein degradation and its derivate it produces a number of volatile bases, namely ammonia, histamine, H₂S and trimetilaminare smelling foul [20].

3.5 Analysis Glycogen

Glycogen is a carbohydrate store in the form of glucose in the body which functions as a source of energy. The glycogen value of the fish with different mortality methods during 12 hours of storage can be seen in Table 3.
Table 3. Average glycogen value of the fish every 4 hours

| Treatment | Observation time (hours) |
|-----------|-------------------------|
|           | 0           | 4           | 8           | 12          |
| A_1       | 6.67% ±0.03 | 2.35% ±0.09 | 1.37% ±0.20 | 0.35% ±0.13 |
| A_2       | 7.69% ±0.12 | 6.07% ±0.07 | 2.72% ±0.33 | 1.15% ±0.26 |

Based on the table, it can be seen that the glycogen content of baung fish at 0 hours observation, treatment A_1 is 6.68% and A_2 is 7.69%. In the 4 hours observation, A_1 was 2.35 and A_2 was 6.07. The 8-hour observation was A_1 1.37% and A_2 2.72% while the glycogen contained in the 12-hour observation was A_1 1.37% and A_2 2.72%. Judging from Table 2, that the best treatment is the treatment of dead fish by stabbing the parts Medulla oblongata. This explains that the longer the storage of the baung fish the glycogen value in the fish is decreasing, in the above results it is also seen that the difference in glycogen content between the two treatment methods of death, in which A_1 treatment is faster and has a greater decrease in glycogen content than A_2 treatment. Fish that are not killed immediately will move a lot so that the glycogen in the meat is reduced, the lactic acid is produced less and the fish freshness period is reduced. In general, the liver can store glycogen by 5-8% [18].

4. Conclusions and Recommendations

4.1 Conclusions

From the results of the tests that have been carried out, it can be concluded that:

1. The characteristics of fresh fish used in this study are active healthy fish movement, difficult to catch, normal operculum opening (not fast and slow), normal skin is not pale.

2. The test results of chemical quality degradation of fresh baung fish include the pH value 6.5 ± 0.05 at 0 hours to 7.0 ± 0.20 at 12 hours for treatment A_1. Meanwhile, treatment A_2 is 6.3 ± 0.1 at 0 hours to 6.6 ± 0.1 at 12 hours. The TVB test results showed 0.14 ± 0.04 at 0 hours to 31.32 ± 0.09 at 12 hours for treatment A_1. Meanwhile, treatment A_2 is 0.10 ± 0.03 at 0 hours 26.30 ± 1.87 at 12 hours. Furthermore, glycogen levels showed 6.67% ± 0.03 at 0 hours to 0.35% ±0.13 at 12 hours for treatment A_1. Meanwhile, treatment A_2 was 7.69% ± 0.12 at 0 hours to 1.15% ± 0.26% at 12 hours.

4.2 Recommendations

Based on the research results, it is necessary to carry out further research regarding similar studies with different fish species with histopathological testing.

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