Analysis of histamin content in tuna fish *Thunnus* sp. with Elisa method at fishing port of Belawan, North Sumatra, Indonesia

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**Abstract.** Tuna migration in Indonesian waters is part of the world tuna migration path because the territory of Indonesia is located on the border crossing between the Indian Ocean and the Pacific Ocean. Histamine is a biogenic amine produced through bacterial decarboxylase from amino acid histidine, and is mostly found in large quantities in fish from the family *Scombridae*. Histamine production in fish depends on histidine levels in fish, the presence of decarboxylase enzyme-producing bacteria and environmental conditions. The amount of histamine produced by fish is strongly influenced by temperature, time, and storage conditions and species of fish. This study aims to determine the value of Histamine found in Tuna (*Thunnus* ssp) caught by fishermen. This research was conducted in July 2018 - August 2018. Samples were taken from the catches of fishermen in Belawan randomly, then tested at the Fish Quarantine Center in Medan Fisheries Product Quality and Safety Control I. Histamine test results obtained were around 2.45234 - 13.31231 mg/L. It is concluded that the tuna from Belawan is still safe for consumption.

1. Introduction

Fishery products are one of the mainstays of Indonesia's exports. Considering Indonesia's sea area which consists of Indonesian waters area of approximately 3.1 million km² (territorial sea waters of 0.3 million km² and archipelago waters of 2.8 million km²) and waters of the Indonesian Exclusive Economic Zone (ZEEI) of approximately 2.7 million km² holds many types of fish and other aquatic products that have important economic value. Fisheries product marketing The Indonesian Exclusive Economic Zone directed at the export market has its mainstay products, shrimp and tuna [1]. Several studies on biology of tuna in Indonesia have been reported by several authors, for example, Azwir *et al* [2] has studied about the stomach content of the tuna in Aceh waters; Yusni and Uliya [3] has studied about Endoparasite worms infestation on skipjack tuna *Katsuwonus pelamis* from Sibolga waters, Indonesia. While study on the genetic of tuna from Maluku waters has been reported by Akbar *et al* [4, 5].

The development of the tuna industry in Indonesia is very prospective because fishing areas are available, the market has been established and supported by the Indonesian government's fisheries sector revitalization program. The processing industry in general processes tuna into fresh (cold) fresh products (fresh whole gilled and gutted), frozen products in the form of whole...
grilled (frozen whole gilled and gutted); loin (frozen loin), steak (frozen steak) and canned tuna products [6].

Related to export marketing, product quality and food safety aspects are important factors that must be considered. As can control the risk of histamine. Decline in the quality of tuna can also be known from the histamine levels formed in these fish. Tuna is a group of Scombridae which can produce Scombrototoxin which is a cause of food poisoning because it consumes fish that has produced histamine more than the prescribed standard. Decreasing the freshness of tuna and the formation of histamine are mainly caused by enzymatic and microbiological activities [7].

Fish decaying is causing by microbial activity and enzymes in the body of the fish produces biogenic amine compounds, which are formed from free amino acid decarboxylase processes. The biogenic compounds of amines include histamine, cadaverine, putrescine, tiamin, triptamine, spermidin and derived from free amino acids in the form of histidine, ornitine, lysine, tyrosine, tryptophan and arginine. In addition to decreasing freshness, histamine poisoning, and increasing microbial levels, tuna fish at a certain weight Contamination can occur from ships, demolition, processing sites, or during the distribution chain to consumers of tuna [8]. This study aims to determine whether the fish caught by fishermen have had high histamine value before being marketed, and know what the highest histamine value is in Tuna caught by fishermen in Belawan.

2. Materials and Methods
2.1. Site, time and Data collection
Study was conducted for 4 weeks starting July 16, 2018 until August 10, 2018. Tuna samples were taken randomly from 2 fishermen in Belawan, samples were taken once a week and from each fisherman one Tuna was caught. Fisherman a catches tuna in the Malacca Strait and Fisherman B catches in the waters of the Indian Ocean, both of which land fish in the Belawan Port of North Sumatra. Sample testing was carried out at the Fish Quarantine Center in Medan Fisheries Quality and Safety Control I.

2.2. Tools and Materials
The tools used in the histamine test were Coolbox, Blender, Knife, Sudip, Digital Scales, 15 ml Tube, 1000 µl Micro Pipette, Mikrotip, Vortex, Sentrifuge, Well, Rack, and Microplate Reader ELISA method (Enzyme Linked Immuno Sorbent Assay). The material used in the Histamine test is Tuna Meat, tuna sample (Certified referenced material), 9 mL Methanol and tissue.

2.3. Procedures
2.3.1. Tuna Sample Preparation
1. Meat Tuna parts that are more susceptible to histamine are cut into pieces and chopped using a blender then stored full in a 50 mL tube.
2. Homogeneous samples were then weighed using 1 gram digital scales inserted into a 15 mL tube.
3. Then 9 mL of Methanol were added to the sample.
4. The sample is vortexed for 1 minute then left for 5 minutes. Then vortex again for 1 minute.
5. The sample was centrifuged with 4000 rpm for 5 minutes.

2.3.2. ELISA Procedures. A fast, sensitive and specific method of analysis, which is an enzyme linked immunosorbsent assay (ELISA). The advantage of this analysis technique is that it is very sensitive and specific to use antibodies. In addition, the analysis time is fast, both in single and multiple instances. The use of this tool can be aimed at sharing tests to analyze the availability and concentration of an ELISA sample in the prepared sample. The steps of the ELISA procedure are as follows:
1. Samples of Tuna and CRM are taken as many as 100 μl each with Micropipette which is placed on the well of the Histamine test.
2. Added 200 μL of Reaction Mix to each well.
3. Shaken the sample for 1 minute then incubated the sample with a tissue wrapped for 1 hour at room temperature and dark conditions.
4. Read on an ELISA reader with 450 nm

3. Results and Discussions

3.1. The Histamine Test of Histamine Test

Results in Tuna caught by Belawan fishermen were tested at the Medan I KIPM Laboratory, with the ELISA method showing that fluctuating results every week can be seen in Table 1. Table 1, values levels histamine captured by Fisherman A, landed in Belawan, was found to be of higher value, reaching 13.31231 (µg / g) while the catch by fisherman B in Belawan reached the highest value of only about 11.38765 (µg / g). The test results of Histamine on tuna catch showed that in the fourth week the Histamine value was quite high compared to the previous week.

The Histamine Concentration in Tuna catches by fishermen who made arrests in the Malacca Strait, obtained the highest value of 13.31231 (µg / g) and the catch was carried out in week IV. The lowest histamine content obtained from Fisherman A is 6.08864 (µg / g) captured in the second week. On fishermen B the content of Histamine Tuna caught from the Indian Ocean to Belawan the highest concentration is 11.338765 (µg / g) in week IV. The lowest concentration is obtained in the first week of 2.45234 (µg / g).

| No. | Weeks of | Code Sample | Sample Weight (g) | Absorbance | Histamine Concentration (µg / g) | Certificates (mg / g) | FAO (mg / kg) |
|-----|----------|-------------|-------------------|------------|----------------------------------|----------------------|--------------|
| 1.  | I        | A           | 1.0237            | 0.189      | 12.15945                         |                      |              |
|     |          | B           | 1.0057            | 0.211      | 6.51787                          |                      |              |
|     |          | CRM         | 1.0102            | 2.139      | 167.764                          |                      |              |
| 2.  | II       | A           | 1.0091            | 0.138      | 6.08864                          |                      |              |
|     |          | B           | 1.0301            | 0.102      | 2.45234                          |                      |              |
|     |          | CRM         | 1.0435            | 2.175      | 54.77553                         | 0.5 to 30            | 20 -50       |
| 3.  | III      | A           | 1.0178            | 0.287      | 10.04725                         |                      |              |
|     |          | B           | 1.0198            | 0.177      | 11.09916                         |                      |              |
|     |          | CRM         | 1.0521            | 2.195      | 96.6222                          |                      |              |
| 4.  | IV       | A           | 1.0049            | 0.229      | 13.31231                         |                      |              |
|     |          | B           | 1.0351            | 0.210      | 11.38765                         |                      |              |
|     |          | CRM         | 1.0201            | 2.189      | 211.8534                         |                      |              |
Figure 1. Histamine Test Concentration Diagram in Tuna with the ELISA Method

Histamine content Tuna caught by Belawan fishermen tested is known that every week, the value of Histamine in Tuna fish is around 2.45234 - 13.31231 mg / L which means that it is quite fluctuating even though the difference in value is not too far away. The histamine content can be different due to the presence of several internal and external factors, such as the levels of hisdin and bacteria in tuna, and the condition of the waters traversed by the tuna. This is in accordance with [8] who say that histamine production in fish depends on histidine levels in fish, the presence of decarboxylase enzyme-producing bacteria and environmental conditions. The amount of histamine produced by fish is strongly influenced by temperature, time, and storage conditions and species of fish.

On histamine testing of Tuna caught by Belawan fishermen at the Medan I KIPM Laboratory, the ELISA method used 12 samples. The use of these tools and methods makes it easy to process and read histamine content in tuna. The capacity of reading samples can be read as many as 96 samples and can be read at once. This is consistent with [1] who say that analytical methods are fast, sensitive, and specifically the enzyme linked immunosorbent assay (ELISA). The advantage of this analysis technique is that it is very sensitive and specific to use antibodies. In addition, the analysis time is fast, both in single and multiple instances.

The value of absorbance of Histamine in Tuna obtained from the ELISA method is around 0.138-2189. This method relies on spectra of colors that have a high degree of accuracy. The color spectra produced by each sample will be different and this is what shows histamine levels in Tuna fish. This is in accordance with [9] who said that this method is based on the use of enzymes to detect sample bonds to antigens (Ag) and antibodies (Ab). The enzyme will convert colorless substrate (chromogen) to a colored product. This change shows the sample bond to Ag: Ab. The color spectra are read the absorbance at a certain wavelength. This absorbance value will be inversely proportional to the histamine concentration contained in it.

Histamine content Tuna caught by Belawan fishermen tested obtained 54.77553 - 211.8534 mg / Lth that the value is still in the value of CRM (Certified referenced material) which is 5-50 mg / kg, which means that tuna histamine levels are tested has been correct and has accurate values. This is in accordance with [10], who said that analytical quality control can be done by means of Certified Reference Materials (CRM) or by means of skills testing programs. These activities are very important to obtain formal recognition of the technical competence of the laboratory and they are also important to confirm reliability and accuracy.

Histamine content Tuna caught by Belawan fishermen tested found that values 2.4-13.4 (µg / g) were still below the maximum limit of black in content in consumption fish, which was...
below the value of 20 mg / L. This is in accordance with [11] who said that histamine poisoning will arise if someone consumes fish with histamine 50 mg / 100 g fish content. Fish with histamine content of more than 20 mg / 100 g of fish should not be consumed because it can cause poisoning in the human body.

The limit value of histamine content in tuna suitable for consumption is around 20 mg / L to 100 mg / L. If the histamine content is above that value can cause human poisoning which is usually characterized by itching or rash, nausea and diarrhea. This is in accordance with [12] who said that fish with histamine content of more than 20 mg / 100 g of fish should not be consumed. Symptoms of mild poisoning are, rash, flushing, burning and red face. Symptoms of moderate poisoning are, persistent redness of the skin (flushing), urticaria / skin lesions, headaches, anxiety (anxiety), nausea, vomiting, diarrhea. Symptoms of severe poisoning, hypotension, respiratory tract disorders, and respiratory failure.

4. Conclusion
The Histamine content in tuna fina caught by Belawan fishermen was ranged between 0.123 to 0.211 µg / L. This value is still below the maximum limit of black in content in consumption fish according to FAO, and therefore it is concluded that the tuna from Belawan is still safe for consumption.

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