Observation of heavy metal hazard on processed frozen escolar (*Lepidocybium flavobrunneum*) fillets

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Abstract. Heavy metals are one of the most dangerous hazards of food products. This study aims to determine the processing flow of frozen escolar fish fillets by observing the cold chain process. The research methods are carried out by applying cold chain, as well as testing the quality and safety of raw material and final products. The parameters tested were Total Plate Count (TPC), histamine, heavy metal tests, and observation of the application of Hazard Analysis Critical Control Point (HACCP) at PT. XYZ, as well as descriptive data analysis and heavy metal monitoring observations. The results showed the quality of raw materials and final products organoleptic values of 8, TPC of $5.5 \times 10^3$ and $5.5 \times 10^2$, histamine of 2.1 ppm and 2.4 ppm, and heavy metals of raw materials (lead 0.057 mg/kg, cadmium 0.011 mg/kg, mercury 0.037 mg/kg - 0.410 mg/kg). PT. XYZ establishes Critical Control Points (CCP) at the stage of receiving raw materials, metal detecting, and packing and labelling. Supervisor and testing of heavy metal mercury are carried out every three months, lead and cadmium are carried out every six months, on raw materials from each supplier enter the company. Sampling for heavy metal testing was carried out on three size groups, namely size 10-20 kg, size 20-30 kg, and size 30 kg.

In conclusion, the organoleptic value, TPC, histamine and heavy metal (mercury, lead and cadmium) were in accordance with the standard. This is the result of the application of the international standard HACCP, which ensures the quality of frozen fish fillet to provide food quality and safety for consumers.

1. Introduction

One of the fishing exploits in the Indonesian sea is the escolar fish (*Lepidocybium flavobrunneum*), a pelagic fish with a high economic value. Its market price is high due to its belief as a healthy food [1], Escolar fish is a large, deep-sea fish that has a high oil content of about 20% by weight of the fish's flesh and has long been known to have a laxative effect. The main component is more than 90% of the total indigestible wax ester oil [2]. It has also been shown that consumption of escolar fish in the marketing group can cause severe stomach cramps, nausea, and vomiting [3]. Research shows that escolar fish hasn’t been seen as an important fish from a consumer perspective (marketing methods, sensory properties, chemical composition, freshness parameters) [4]
Few types of heavy metals such as mercury, lead, cadmium, and nickel can endanger the health of organisms in minute amounts [5]. Heavy metals are persistent molecules in a way that it accumulates in the food chain and can cause accumulation in the human body [6]. Heavy metals in fish can cause health problems if consumed by humans. To protect consumers from consuming fish, there is a need for quality control to ensure that the fish is safe to consume.

Hazard Analysis Critical Control Point (HACCP) is a system used to estimate the danger and establish a control system that focuses on prevention. HACCP stresses the need for food quality and safety [7]. Therefore, HACCP can be applied to the entire chain process of good processing [8]. An important aspect of HACCP is the determination of the Critical Control Point (CCP). This stage is the key to lower and eliminates hazards that have been identified. While CCP is defined as every step in the process which should be critically monitored, otherwise causing detriment to food safety, spoil, and economic loss [9].

PT. XYZ is a fish processing industry that produces frozen escolar fish fillets. The importance of HACCP application in the fish processing industry correlates to the danger of heavy metals in products to protect consumer health and decrease the rejection of fish exports. The goal of the monitoring is to observe the processing of frozen escolar fish fillets, their quality and safety, and the end-product of the frozen escolar fish fillets, as well as the application of HACCP throughout the entire production process.

2. Materials and methods

2.1. Materials

Escolar fish used in this research comes from the Indonesian Sea (WPP 573) or the Indian Ocean ZEEI in the south of Java Island. An amount of 50 grams of escolar fish is taken from several from sizes 3-10, 10-20, and above 20. Fish samples are chosen randomly then put into styrofoam added with ice gel, then tested from heavy metal content mercury in the laboratory.

2.2. Methodology

2.2.1. Observation of the Frozen Escolar Fish Fillets Processing. Observation of the frozen escolar fish fillet process is done by following the Good Manufacturing Practices (GMP) of the company. At every stage of the process, observation of cold chain and measurement of product central temperature using a digital thermometer that is done six times and in three re-runs (triplicate).

2.2.2. Organoleptic Test. Organoleptic testing is done based on SNI 4110:2014 with a scoring test method with a scale of 1-9 for the end products. The test is done by 6 standard panelists in 6 observations and three re-runs (triplicate).

2.2.3. Microbiological Test. Microbiological testing is done on the raw materials and end-products in the Central Laboratory of Fish Production Inspection and Certification, DKI Jakarta. The test involves Total Plate Count (TPC) according to SNI 2332.3.2015. The test for E. coli is done according to SNI 01-2332.1.2015, while Salmonella testing is done according to SNI 01-2332.1-2006. Test for Vibrio cholerae is done according to SNI 01-2332.4-2006.

2.2.4. Histamine Test. Histamine testing is done on the raw materials and end-products in the PT XYC’s internal laboratory, using the biofish method. Testing is done according to the company standard with an amount of 6 observations.

2.2.5. Mercury Heavy Metal Test. Mercury heavy metal testing is done according to the monitoring programmed of PT. XYZ by taking random samples of raw materials and is based on SNI 2354.6-2016 [10].

2.2.6. Observation of the Application of HACCP. Observation of HACCP application in PT XYZ involves the formation of the HACCP team, product description, identification of the consumer base, making a flow-chart, verification of flowchart, hazard analysis, identification of critical points, establishing the limits of critical points, CCP monitoring, correctional protocols, verification, and documentation.
3. **Results and discussion**

3.1 **Frozen Escolar Fish Fillet Production Process**

The production process of frozen escolar fish fillets at PT. XYZ involves receiving raw materials, weighing & measurement, pallet organization, storage, weighing 2, washing, shaping fillet, discarding of innards, bone shaving, discarding of skin and bones, cleaning, washing 2, weighing 3, packaging, weighing 4, freezing, ice coating, weighing 5, packing, vacuuming, ultraviolet treatment, metal detecting, packing and labelling, storage 2.

3.2 **Observation of Cold Chain**

The observation of temperature in the frozen escolar fish fillet production process is shown as below:

![Figure 1. Temperature observation of the frozen escolar fish fillet production process.](image)

Observation of cold chain is done at every stage of the process from receiving raw materials to final product storage. At storage 1, freezing, and storage 2 is done in the observation of cold chain at cold storage temperature and Individual Quick Freezer (IQF) temperature. The company has established a standard production temperature of frozen fish at a maximum of -10 °C, cold storage at a maximum of -21 °C, and the IQF at a maximum of -37 °C ± 2 °C. Based on observation, the company has applied the cold chain process well.

3.3 **Food Quality and Safety**

3.3.1 **Observation of raw materials quality.** Test results on food quality and safety are done as follows:

| Test          | Result | Unit        | Requirement | Method               |
|---------------|--------|-------------|-------------|----------------------|
| Organoleptic  | 8      | -           | Min. 7      | SNI 4110:2014        |
| TPC           | 5,5x10^{3} | colony/g   | <5x10^{4}  | SNI 01-2332.3:2015  |
| E. coli       | <2     | MPN/g       | <2         | SNI 01-2332.1:2015  |
| Salmonella    | Negative | per 25 g   | Negative   | SNI 01-2332.2:2006  |
| V. cholerae   | Negative | per 25 g   | Negative   | SNI 01-2332.4:2006  |
| Histamine     | 2,1    | ppm         | <17        | Biofish              |
| Lead          | 0,057  | mg/kg       | Max. 0,30  | SNI 2354.5:2011     |
| Cadmium       | 0,011  | mg/kg       | Max. 0,05  | SNI 2354.5:2011     |
Test results on food quality and safety as done in Table 1. shows the organoleptic value of the raw material is 8. Total TPC was 5x5 10^3 col/g, histamine value 2.1 ppm, heavy metals (lead 0.057 mg/kg, cadmium 0.011 mg/kg and mercury 0.037 mg/kg- 0.410 mg/kg. This indicates that the raw materials have fulfilled the required standards.

The test results have fulfilled the standards because, during the reception of raw materials, the fish are found with good quality and is handled properly, as well as the processing is done quickly, cleanly, and meticulously, kept in the cold chain without microbial contamination. The application of GMP is done well where each employee uses the proper work apparel and clean tools to avoid bacterial contamination.

Mercury testing is done because mercury is a prevalent heavy metal that causes export rejection in fishing. Heavy metals testing is done using the Atomic Absorption Spectrophotometer (AAS) according to SNI 2354.6-2016. The testing result of mercury in raw materials are as follows:

| Observation | Size | Mercury Content (mg/kg) |
|-------------|------|-------------------------|
| 1           | 4    | 0.401±0.010             |
| 2           | 10.1 | 0.322±0.026             |
| 3           | 20.7 | 0.346±0.020             |
| 4           | 5.8  | 0.037±0.023             |
| 5           | 10   | 0.047±0.015             |
| 6           | 24.7 | 0.045±0.018             |

A sampling of heavy metal mercury is done with three group sizes which are size 3-10, size 10-20, and size 20 up. The group is determined by observing the heavy metal content representing each size of escolar fish received by the company, which are sorted into sizes of small, medium, and large. According to research done[11], mercury content on escolar fish (L. flavobrunneum) is affected by the size (weight) of the sample. In Table 2, we can see that the mercury testing shows compliance with the standard based on the Commission Regulation (EC) No. 1881/2006, which is a maximum of 1.0 mg/kg.

Mercury in escolar fish is due to the fish’s nature as a predator. Escolar fish are predators and in the food chain, lies in the peak of the chain. As a predatorial fish, escolar fish warrants the potential of accumulating heavy metal hazard in its flesh. According to [12], escolar fish (L. flavobrunneum, family Gempylidae) are big predatorial fish with dark colours, found in deep cold waters (under 200 m) at daylight and in warmer waters at night-time. Maximum accumulation of Hg through bioaccumulation is found in predatorial fish at the highest tropic of the food chain, depending on the size and lifespan of fish [13].

### 3.3.2 Observation of Final Product Quality

The quality of the final product is found as follows:

| Test          | Result | Unit   | Requirement | Method         |
|---------------|--------|--------|-------------|----------------|
| Organoleptic  | 8      | -      | Min. 7      | SNI 2696:2013  |
| TPC           | 6.5 x10^2 | colony/g | <5x10^4     | SNI 01-2332.3:2015 |
| E. coli       | <2     | MPN/g  | <2          | SNI 01-2332.1:2015 |
| Salmonella    | Negative | per 25 g | Negative   | SNI 01-2332.2:2006 |
| V. cholerae   | Negative | per 25 g | Negative   | SNI 01-2332.4:2006 |
| Histamine     | 2.4    | ppm    | <17         | Biofish        |

The quality of the final product at Table 3. shows the organoleptic value of raw material 8. Total TPC 6.5x5 10^2 col / g, histamine value 2.4 ppm, this indicates that the final product has met the required standards.
This indicates that the final product is according to the required standard. The testing has fulfilled standards due to correct processing according to the protocol, GMP has been applied well.

3.4 Observing the Application of HACCP

![Diagram of HACCP process]

**Figure 2. Application of HACCP**

3.4.1 *Formation of the HACCP Team*. Formation of the HACCP team at PT. XYZ is organized into several divisions or fish processing units.

3.4.2 *Product Description*. Product description at PT. XYZ has been comprised of complete information.

3.4.3 *Identifying the Indication of Use*. Frozen escolar fish fillets are marketed towards all ages except for babies under 6 months old and those allergic to escolar fish.

3.4.4 *Assembling the Flow Chart*. Flow chart of the production process of frozen escolar fish fillets at PT. XYZ consists of 25 step processes.

3.4.5 *Flow Chart Verification in the Field*. Based on verification in the production room, PT. XYZ has done the process according to the flow chart in the HACCP manual.
3.4.6 Hazard Analysis. Hazard analysis has been done at all stages of the process in a satisfactory manner. Hazard analysis is done at 3 stages of the process with high identified risks which are the reception of raw materials, metal detecting, and the packaging & labelling processes.

3.4.6.1 Receiement of Raw Materials. Escolar fish is predatory in nature, causing heavy metal accumulation from the prey it consumes. According to [14], heavy metals enter organisms through their food chain, because nearly 90% of toxins or heavy metals (mercury) enter through food. Heavy metals that enter the body of escolar fish cannot be broken down and will accumulate continuously. [15] stated that the accumulation of heavy metals in the bottom of the waters may enter the food chain. According to [16], heavy metals cannot be decomposed or degraded like other organic pollutants in exposure to sun or heat. Heavy metals are accumulated in landfills and leach to sediments, but can never be fully removed and remains a continual hazard in the future. While according to [17], heavy metals in animals cannot be removed from their bodies and will accumulate, therefore accumulating continuously in the food chain.

Based on the abovementioned literature, the high potential of heavy metal contents in escolar fish is deemed as a medium hazard with a medium severity, due to the accumulation of heavy metals in the body and not contributing directly towards death. However, the hazard of heavy metals itself is deemed significant when causing health problems if consumed. Preventive measures are done to obtain supplier approval and a monitoring program for testing heavy metals in accredited laboratories is done once every three months for mercury and once every six months for lead, mercury, and cadmium.

3.4.6.2 Metal detecting. Physical hazards that can occur is the contamination of metal traces in the product, its severity is high, and its probability is low, but its hazard is deemed significant. This is due to the dangers caused if heavy metals are consumed by humans.

3.4.7 Identification of CCP. Heavy metal is a hazard, which is deemed significant because it cannot be reduced or eliminated and can go over the critical points without follow-ups to render its content. Therefore, heavy metals are designated as CCP. This is in accordance with a research by [18] on the live mantis shrimp process, [9] on live tiger snail, and [19] frozen whole cuttlefish, the states of heavy metals inspection are determined as CCP, on the receipt raw materials with of potential hazard.

3.4.8 Determination of Critical Points. The critical point for heavy metals is determined by the company for Pb at maximum 0.30 mg/kg, Hg at maximum 1.00 mg/kg and Cd at a maximum 0.05 mg/kg. This is already according to the standard upheld. According to SNI 7387:2009 about the maximum heavy metal contamination of food is held at a maximum: 0.4 mg/kg for Pb, 1.0 mg/kg for Hg, and 0.5 mg/kg for Cd. According to Commission Regulation (EC), No 1881/2006 for the species L. flavobrunneum, the maximum Pb contaminant on fish muscle is 0.30 mg/kg for Pb, 0.05 mg/kg for Cd, and 1.0 mg/kg for Cd.

3.4.9 Establishment of Monitoring Procedure. Heavy metals are monitored in an accredited external laboratory. Sampling is done with 20 influx of raw materials from each supplier or every 20 tons of raw materials input per supplier. Sampling is done to know the heavy metal content on escolar fish for Pb, Hg, Cd for every 6 months once and monitoring of Hg every 3 months once.

3.4.10 Correctional Protocol. The critical point of heavy metals if exceeded should be corrected by re-testing the samples at the final product at the same lot. If it still has not fulfilled the standard, then the product should be held back and not exported, then filing a complaint to the supplier should be done.

3.4.11 Establishment of Verification Procedures. Verification procedures by PT. XYZ has been found satisfactory. Verification is done through calibration, testing, training, reviews, and auditing.
3.4.12 Documentation and Recording. The documentation and recording system of HACCP application in PT. XYZ has been done well and administratively controlled.

4. Conclusion
Production of frozen escolar fish fillets is made from 25 step processes, in which the cold chain application is found according to the company’s standard. Quality and food safety testing show that the organoleptic, TPC, histamine, and heavy metal values are in accordance with the established standards. The company implements the CCP when receiving raw materials, metal detection, as well as packaging and labelling as quality control during processing. This is the result of the application of international standard HACCP that carries out quality control and food safety for heavy metal hazards, in the escolar fish fillet production chain, from receiving raw materials, handling, and processing, distribution, and marketing, to consumers.

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