Organoleptic Testing and Histopathological Examination of Gills of *Thunnus* Sp. And *Sardinella lemuru* as Raw Materials for Gill Chips

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Abstract. Utilization of tuna (*Thunnus* sp.) and lemuru (*Sardinella lemuru*) in the fishing industry has not been developed optimally and still produces by-products. Fish gills are one of the by-products of fisheries that contain protein, fat, water, ash, and carbohydrates, reaching 15.532%, 1.942%, 69.880%, 10.490%, and 2.156%, respectively. In_Sang Epic is food that utilizes fish gills as its raw material. For consumer safety, the product should undergo an organoleptic test under the SNI 2729:2013 score sheet method. The histopathological examination process which included fixation, tissue processing and embedding, sectioning, staining, mounting, and observation with a microscope. The results of organoleptic test indicated that the gills of tuna and lemuru had average values of 8.3 and 7.8, respectively, which is fulfilling the standards of fresh fish according to SNI 2729:2013. Meanwhile, the result of histopathological from gills of tuna samples were suspected of having necrosis, as characterized by hypertrophy of megakaryolysis, and that those of lemuru were suspected of having autolysis, as characterized by rupture. However, the gills of both kinds of fish were still suitable for use as raw materials of In_Sang Epic because the samples were ineligible to undergo histopathological testing.

1 Introduction

Fish are marine organisms that contain a lot of protein that have functions as a building and regulating agent, helps humans replace damaged body tissue parts, serves as a source of energy, and contains essential amino acids needed by the human body. Each species of fish has a different proportion of protein [1]. Tuna (*Thunnus* sp.), for example, contains about 22.6 - 26.2 g/100 g, while lemuru fish does around 20 g/100 g [2; 3].

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Utilization of tuna and lemuru in the fishing industry has not been optimally developed yet and still leaves by-products in the form of gills, skin, heads, scales, bones, and leftover fish meat, which, as a result, pollute the environment [4]. In fact, fish gills contain protein, fat, water, ash, and carbohydrates of 15.532%, 1.942%, 69.880%, 10.490%, and 2.156%, respectively and can be processed for food that contain good nutrients [5].

Fish gills are the organs responsible for the breathing process in fish. They are the first to come in contact with toxic substances and environmental changes in water. As a result, they are vulnerable to histological changes in their tissue. Toxic substances and environmental pressures in water can cause such changes [6]. In_Sang Epic is a product of utilization of fisheries by-products, namely gills of tuna and lemuru fish, which are processed into thin slices to be mixed with spices. It is crunchy, fragile, and savory. Fish gills, before being processed become In_Sang Epic, should be tested for their freshness through organoleptic testing and histopathological examination.

Organoleptic testing employs the human senses as the main tool for measuring product quality or freshness to detect any spoilage, deterioration, and damage in fresh fish, processed fish products, and other fish-based commodities. Freshness variables include appearance, taste, smell, and texture. In this study, the organoleptic testing would be referring to SNI 2729:2013, using the scoresheet method with a scale of 1-9 [7]. To support the observation of the freshness level of fish gills, histopathological examination was needed by studying abnormal changes in cells or tissues to diagnose any disease in fish. It can serve as a support for a main diagnostic examination or even can be the main one by finding specific cell or tissue changes in certain diseases [8]. It is done as one of the efforts to ensure food safety. According to the Food Law number 7 of 1996, food safety is a condition and effort needed to prevent food from possible biological, chemical, and other contaminants that can disturb, harm, and endanger human health.

In order to ensure the quality of In_Sang Epic products, it is imperative to conduct thorough examinations of the gills of tuna and lemuru fish, which serve as the raw materials for these products. Such examinations include both organoleptic and histopathological evaluations. The results of these evaluations not only serve as a reference for improving the quality of the products, but also guarantee the consistency and reliability of the In_Sang Epic brand.

2 Material and methods

2.1. Samples preparation

The samples of tuna and lemuru gills were each given a test code based on SNI 2346:2015 [9]. Preparation was carried out by presenting each sample in a container that had been given a test code.

2.2. Histopathological solution preparation

All materials of paraffin-embeddedd tissue were prepared as described previously (Table 1) [10]. The materials of paraffin-embeddedd tissue were dissolved in 800 mL distilled water. pH of all solution was adjusted to 7,4, then add water to final volume 1 L.

Harris hematoxcilin dye was prepared as describe. 5 g of hematoxcilin was dissolved in 50 mL of absolute alcohol, as solution I. Solution II is made by dissolving 100 g of potassium alum using 900 mL of distilled water. Solution I and II were mixed then heated at 80 °C until homogeneous. This mixed was named as solution III. 2.5 g of mercury acid was added on solution III. The solution was stirred while heated at 80 °C. Meanwile, for Eosin dye was

prepared by dissolving 200 mL of eosin Y stock in 600 mL of alcohol 80% and 4 mL of acetic acid 1%, respectively. Eosin Y stock was prepared by dissolving 2 g of eosin crystal in 40 mL of distilled water and 160 mL of alcohol 95%.

Table 1. Solution and buffers for paraffin-embedded tissue.

Material	Final concentration	Amount
NaCl	137 mM	8 g
KCl	2.7 mM	0.2 g
Na_2HPO_4	10 mM	1.44 g
KH_2PO_4	1.76 mM	0.24 g

2.3. Organoleptic testing

The organoleptic testing referred to SNI 2729:2013 with a scoring test, namely by giving scores on the scoresheet according to the level of product quality scaled 1-9 [7]. The results of the organoleptic quality assessment on 12 (twelve) samples from 6 (six) were tabulated, and the quality values were determined by finding the averages in the samples. The spesification for each scale can be seen in SNI 2729:2013 [7].

2.4. Histopathological examination

Formalin fixation, processing, embedding, and sectioning were process described previously [10]. Samples were put into 10% Neutral Buffer Formalin solution for a minimum of 2 days and a maximum of 5 days. The tissues that had been fixed for approximately 42 hours were then sliced using a knife with a thickness of 1 cm. The tissue was taken using tweezers and then put in a tissue cassette and labeled and closed. The next stage used a tissue processing machine for 18 hours, after which the blocking stage was carried out by pouring liquid paraffin into the blocking container, and then placing it in a tissue embedding machine at 5 °C to form solid blocks before cutting. The tissue was incubated with 70%, 80%, 90%, and 96% ethanol and alcohol absolut I, II, III respectively for 2 h. The tissue were placed into xylol I and II respectively for 2 h.

The blocks were cut using a microtome with a thickness of 5 µm, to obtain tissue bands. The cutting results in the form of tissue bands were stretched on the surface of the water in a water bath with a temperature of 40 °C and then attached to object glasses, which were then aerated and stored on a staining rack before staining. The staining of the object glasses was carried out using Hematoxycilin-Eosin (HE) dye. The object glasses to be stained were arranged on a staining rack and then stained sequentially. There were 21 stages of staining. After coloring, the next process was the mounting stage to preserve the tissues that had been colored using entelan. This process was carried out by dripping the object glasses with entelan then covering them with cover glasses. The covered object glasses were then dried beforehand until the entelan were dried and observable under a microscope. The object glasses were then observed using a microscope branded Binocular with magnifications of 1000x.

3 Result and discussion

3.1. Organoleptic test results for tuna and lemuru gills

According to SNI 2729:2013, fresh fish has organoleptic characteristics if the values of appearance, flesh, texture and smell are in the range of 7-9 [7]. In this study, the lowest score for tuna was 8 with less bright dark red or reddish-brown color and a little transparent mucus, while the highest value was 9 with bright dark red or reddish-brown color and very little transparent mucus. For lemuru, the lowest score was 7 with pink or light brown color and little cloudy mucus, while and the highest score was 9 with bright dark red or reddish-brown color with very little transparent mucus. The results of organoleptic testing of the gills of tuna and lemuru by appearance can be seen in Figure 1.

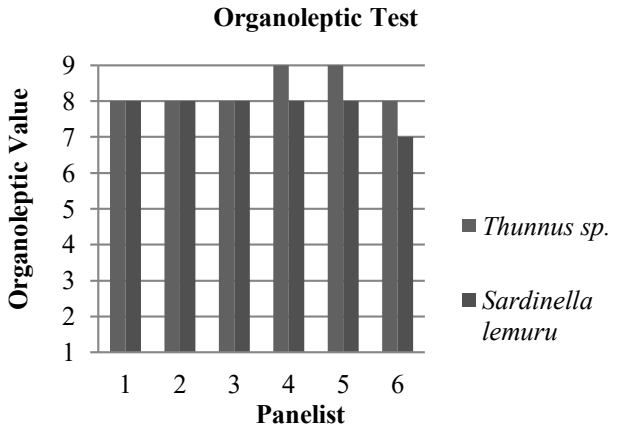

Fig. 1. Organoleptic test results for *Thunnu*s sp. and *Sardinella lemuru.*

The organoleptic test results, through the average calculation of 6 (six) panelists, showed that the gills of tuna and lemuru had average values of 8.3 and 7.8, respectively, both of which were rounded to 8.0 with less bright dark red or reddish-brown color and a little transparent mucus.

Based on the data, the appearance of the gills, with the specifications mentioned above, was supposed to come from the handling and storage of the samples. This was in accordance with the statement of Ekasari *et al.* [11] that the treatment done for storing the gills can affect their organoleptic specifications. Fresh fish initially has bright red gills. The longer it is stored, the color of the gills will fade to brown, looking a bit slimy, with a more pronounced sour odor. According to Berhimpon *et al.* [12], in the organoleptic test, there are 3 types of quality classes, namely class 1 (<8), class 2 (<7), and class 3 (<5). The gills of tuna and lemuru had an organoleptic value of 8, which is fulfilling the quality standards of fresh fish according to SNI 2729:2013 and suitable to be used as raw materials for In_Sang Epic products.

3.2. Results of histopathological examination of *Thunnus* sp. gills

Gills are the respiratory organs of fish. Healthy gill tissues consist of several arrangements, such as hardened cartilage arches with filaments in them. The gill filaments consist of many lamellae. The lamella margins that are not attached to the gill arches are very thin and are

covered with epithelium and contain capillaries. The lamella structure is composed of thin epithelial cells on the outside, a basement membrane, and columnar cells for support on the inside [13]. Based on microscopic observations, samples of frozen tuna gills obtained from the Kedonganan market were suspected of having necrosis, which was characterized by hypertrophy of megakaryolysis: a state of swollen tissue or increased cell size [14]. This was consistent with previous studies which stated that hypertrophy of megakaryolysis is an early symptom of necrosis.

Necrosis is a decrease in tissue activity that is characterized by the loss of several cell parts one by one from the tissue and brings it to death in a short time [15]. Histological picture of the gills of tuna experiencing hypertrophy of megakaryolysis can be seen in Figure 2.

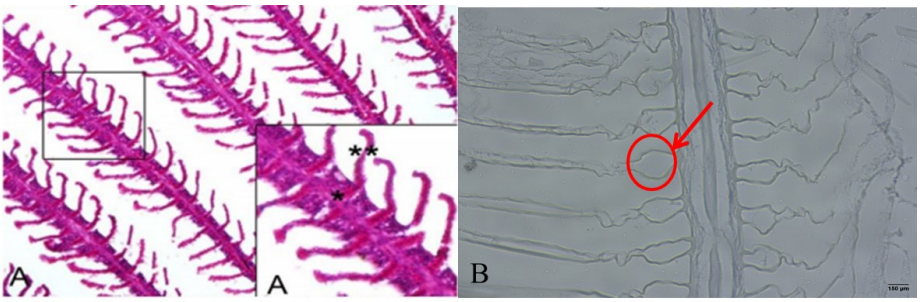

Fig. 2. Histology of *Thunnus* sp. gills: a. Normal gill with primary lamellae (*) and secondary lamellae intact (**), scale bar = 200 µm [16]; b. *Thunnus* sp. suspected of having hypertrophy of megakaryolysis, magnification of 1000x, scale bar = 150 µm.

Based on the histological identification, the frozen tuna gills experienced degradation or changes in their tissue structure due to treatment such as freezing and ice addition. Based on the standards, on longline vessels, tuna must be treated with bulk ice. Along with the development of technology, people use hatches lined with fiberglass material, with a refrigerated sea water (RSW) storage system to maintain fish quality standards, especially for fish freshness [17]. The samples of tuna gills that had undergone a process of freezing or ice addition contained water in the cells that could crystallize when left in freezing temperatures. Water that crystallized in cells could cause swelling or hypertrophy of megakaryolysis. This was in accordance with previous research, which stated that very cold air can cause freezing of cells. The cell membrane will perforate due to ice crystals, bringing the cell to break. Also, a difference in osmotic pressure between intracellular and extracellular can cause the cell membrane to rupture [18]. This statement is also supported by Alif *et al.* [19] who stated that cell swelling occurs due to increased fluid entering from the extracellular into the cell and can cause hypertrophy of megakaryolysis. In this study, the degradation in tuna gills that occurred as a result of the handling method made the samples ineligible for histopathological testing. A sample should undergo a histopathological examination no later than 4 hours after death; it must be necropsied and fixed immediately to get the right test results. Therefore, the tuna fish gills tested were still suitable to be used as raw material for gill chip products (In_Sang Epic).

3.3. Results of histopathological examination of *Sardinella lemuru* gills

Based on the microscopic observation, the samples of lemuru gills obtained from the Kedonganan market were thought to have undergone autolysis, namely the process of tissue reshuffling by enzymes derived from the fishery products themselves [20]. The autolysis in the samples was characterized by rupture, namely damage to the filament structure, or loss

of the epithelial layer [21]. An overview of the results of histological examination of the healthy and ruptured gills of lemuru can be seen in Figure 3.

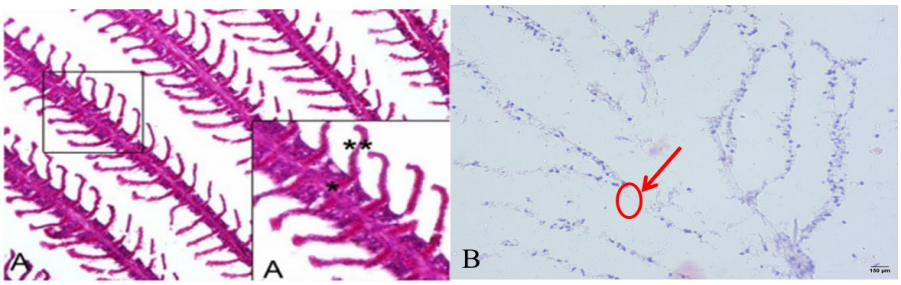

Fig. 3. Histology of *Sardinella lemuru* gills: a. Normal gill with primary lamellae (*) and secondary lamellae intact (**), scale bar = 200 µm [16]; b. Rupture gill from *Sardinella lemuru*, magnification of 1000x, scale bar = 150 µm.

Histopathologically, frozen lemuru gills experienced degradation or changes in tissue structure due to the application of cold chain at the time of fish capture. Tani *et al.* [22] stated that the application of cold chain aims to maintain the quality lemuru and keep them in cold conditions (low temperatures), which can result in tissue degradation or autolysis. This is in accordance with previous studies which stated that tissue degradation due to the autolysis process is characterized by cell damage that occurs and the secretion of enzymes that can destroy cell structures [23]. The autolysis phase occurs after the rigor mortis phase. When the fish dies, the organic compounds in its muscles are decomposed by enzymes that are still active in the tissues. In the early stages, glycogen is hydrolysed to lactic acid, which accumulates in the muscles and causes a decrease in pH. When glycogen decreases, lactic acid accumulation in the muscles becomes not much. Lemuru, when still alive, its meat has a pH value of around 7.0; After death, it drops to a pH range of 5.8 to 6.2. This condition stimulates enzymes that hydrolyse organic phosphates. The phosphate that is first decomposed is creatine phosphate by the formation of creatine and phosphoric acid, followed by a decrease in adenosine triphosphate (ATP) [24]. Microscopically, the characteristics of cells that undergo autolysis look pale, the cell wall boundaries are missing, and there are cells that do not absorb colour [25]. Based on the results of histopathological examination, the gills of lemuru, which were suspected of experiencing autolysis, were considered quite fresh and fit to eat [24]. Therefore, lemuru gills are still suitable to be used as raw material for gill chip products (In_Sang Epic).

4 Conclusions

Organoleptic values of both tuna (*Thunnus* sp.) and lemuru (*Sardinella lemuru*) met the organoleptic quality standards which set that the minimum value is 7. Meanwhile, the histological examination of fish gill samples showed that necrosis was found in tuna, as characterized by hypertrophy megakaryolysis, and autolysis was found in lemuru. However, the gills of tuna and lemuru were still suitable to be used as raw materials for In_Sang Epic products.

We thank Balai Karantina Ikan, Pengendalian Mutu dan Keamanan Hasil Perikanan (BKIPM) Kelas I, Denpasar, Bali, for histopathology analysis.

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