The effect of various concentration of tilapia (*Oreochromis* sp.) surimi for edible coating on the shelf-life of *Pangasius* sp. fillets

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Abstract. *Pangasius* sp. fillets prone to deterioration the quality that will affect the appearance and the shelf life of fillets. The effort to extend the shelf life of fish fillet that is by using an edible coating. Surimi can be used as a protein-based edible coating because they have superior inhibitory and mechanical properties compared to the polysaccharides based material. Surimi can be made from freshwater tilapia (*Oreochromis* sp.) fish. The experimental design used was Completely Randomized Design (CRD) with five treatments of surimi (0 gr, 2 gr, 4 gr, 6 gr, 8 gr) with four replications. The results showed that *Pangasius* sp. fillets with an edible coating 8 gr surimi have the highest value in the organoleptic test. The pH testing on *Pangasius* sp. fillets with edible coating 2 gr, 4 gr, 6 gr, and 8 gr surimi from the 0th hour to 18th hour have increased but slower than *Pangasius* sp. fillets without edible coating surimi. The best value of Total Plate Count (TPC) test is in edible coating 6 gr and 8 gr surimi as it is in accordance with SNI 2696:2013 at room temperature storage until the 18th hours.

1. Introduction

*Pangasius* sp. is a freshwater fish commodity with high potentials. This fish is much in demand by the community and has a wide export market [1]. Indonesian Fishery Statistics shows that the number of the national production increased from 229,267 tons in 2011 to 347,000 tons in 2012 and 410,883 tons in 2013 [2]. These data indicate that has an important role in national fishery industry.

There has been effort to develop of products in the form of fillets similar to what is known in the global market as a dory fillets [3]. is not scaly, has less spinal bones and reddish-white flesh, and is easy to peel, so it is relatively easy to make good fillet products from it [4].

The problems that often arise in the development of fish fillets are that they quickly decline in quality. fillets are prone to damage and deterioration that will affect the appearance and shelf life of the fillets. This is due to the characteristics of that contain high protein and water and the pH of its meat is close to neutral [5]. According to Killoincceker *et al.* [6], edible coating can be used to extend the shelf life and maintain product quality. Edible coating is formed directly on packaging materials and food products, which serve as a barrier to moisture, lipid, and gas and it can improve texture of the food products [7]. Edible coating is important for food products that are easily damaged, such as seafood, because it can inhibit the growth of microorganisms on the surface of the products [8].
Surimi is a hydrocolloid that can be used as an ingredient to make protein-based edible coating [9]. Protein-based films and coatings have superior inhibitory and mechanical properties compared to the homopolymer polysaccharides-based materials since proteins contain 20 different types of amino acids that produce more varied functional characteristics, such as the high molecular bonding [10]. Surimi can be made from freshwater fish such as from fillets of tilapia (Oreochromis sp.) since the flesh is white and the side of the body is thick so it is suitable to be made into fillets (boneless cutlets). According to Yoon et al. [11], tilapia has been used to produce surimi due to its good ability to form gel. There have not been many studies performed on surimi-based edible film or coating and its application, including edible film from Alaskan Pollack surimi [9] and from red snapper fillets [12]. Most edible films or edible coatings that have been produced are from marine fish, thus this study was aimed to use surimi from fresh water tilapia fillet. Therefore, the objective of this study was to determine the effect of various concentration of tilapia surimi tilapia for edible coating on the shelf life of fillets.

2. Methodology
2.1. Materials and chemicals
The tools used to produce surimi were knife, cutting board, muslin cloth, stirrer, food processor, kitchen scale, analytical balance, plastic tray, cooler box and equipments used for testing total volatile base (Conway Cup), pH (pH meter), and total plate count (beaker glass, measuring cup, hotplate, magnetic stirrer, tissue paper, gloves, mask, analytical balance, test tubes, test tube shelf, spoon, petri dish, Bunsen burner, inoculation needle, autoclave, spatula, pH meter, oven, incubator, heat-resistant plastic, paper label, knife, lighter, thermometer, vortex, Drigalski spatula, cotton, and clamp). For making edible coating from tilapia surimi, several equipments were used: hot plate, measuring cup, magnetic stirrer, pipette, analytical balance, pH meter, coating container, and glass bottle. The ingredients used in this study were tilapia, water, ice, cryoprotectant (sodium tripolyphosphate), NaOH 1 M, saline, distilled water, 70% alcohol, 95% alcohol, and plate count agar (PCA).

2.2. Surimi manufacturing from tilapia fillets
Total volatile base (TVB) analysis and pH measurement were performed before surimi production to know the freshness level of tilapia that was used. The manufacture of surimi from tilapia fillets used methods modified from [13]. Tilapia Fillet (skinless) was grinded until smooth and crushed using a food processor to produce meat without bones, spines, and dirt. After that, the fish meat was washed twice using cold water (±5 °C) and 0.3 % (w/w) salt solution. First immersion with cold water (water: meat = 3:1) was performed for 10 min to clean dirt that was still attached to the meat as well as to dissolve sarcoplasmic protein. The second immersion was with 0.3 % (w/w) salt solution (salt: meat = 3:1) for 10 min and then it was filtered with a muslin cloth. After the process, cryoprotectant (0.3% sodium tripolyphosphate) was added and mixed using a food processor. Cryoprotectant was added to prevent protein denaturation in frozen surimi. Surimi produced was stored in polyethylene plastic at -20°C.

2.3. The production of edible coating surimi solution
The production of edible coating was modified from [14]. First Frozen tilapia surimi of was thawed for 20 min. After that, it was weighed as much as 0 g, 2 g, 4 g, 6 g, and 8 g. The surimi was then dissolved in 1 M NaOH until the pH reached 11 and heated with 150 mL distilled water on a hot plate. The solution was homogenized and heated to a temperature of ± 55 °C. Next, it was filtered using muslin cloth and a filtrate of surimi solution was obtained.

2.4. Application of edible coating surimi tilapia fillets.
First, fillet (7x3 cm) was soaked in 50 mL edible coating solution obtained in 2.3 mL for 30 s on each side, then it was drained and left to dry for 30 min. After that, fillets were stored at room temperature for 18 hand observed every 6 h [15].

3. Characterization of edible coating surimi

3.1. Total plate count
Microbial testing was done by determining the total plate count (TPC) (Nugraheni, 2013). The maximum limit of microbial contamination in fish fillets according to SNI 2696: 2013 is 5x10⁵ colony/g. TPC was performed by creating a multilevel dilutions. One g of fillet was grinded gin nine mL of sterile saline with a ratio of 1: 9; thus, a 10⁻¹ dilution was obtained. next, 1 mL of 10⁻¹ dilution was taken using a sterile volumetric pipette and inserted into a reaction tube containing 9 mL of sterile saline and homogenized to obtain a 10⁻² dilution. Further dilutions were prepared (10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵) [16].

Sterile PCA media that had been cooled to 50 °C was inserted into 15 mL petri dishes. While pouring the medium, the lid must not be opened too wide to avoid contamination. The Petri dishes were then moved on the table in a circular motion mimicking the number eight, and after that it was left to solidify (Fardiaz, 1993). TPC calculation was done by spread plate method by taking 1 mL of the diluted sample with a sterile volumetric pipette, and then it was transferred to the surface of the media and flattened it using sterile Drigalski spatula. The petri dish was incubated at 37 °C for 24 h n reversed position [16].

3.2. pH measurement
Measurement of pH of fillet meat was performed by dissolving 3 g in 50 mL sterile distilled water. Sample was homogenized and the then the pH was measured using a pH meter. Determination of pH is done after pH meter is calibrated first. After that, the electrodes are rinsed with distilled water and dried. The electrode is dipped in the sample solution, and the pH measurements can be set. The electrode is left immersed for a while until a stable reading is obtained, then the sample pH can be recorded.

3.3. Organoleptic test. Organoleptic testing is a way of testing using the human senses as a primary tool in assessing the quality of fish and fresh fishery products based on SNI 2696: 2013. There were 30 untrained panelists from Fisheries and Marine Faculty of Airlangga University. The trait of organoleptic testing is subjective because it only relies on the sense and sensibility of panelists [17].

4. Results and Discussion
Total Volatile Base (TVB) of the raw material of tilapia fillets was 20.07 mgN/100g with pH of 6.97. The value of TVB within the range of 20-30 mgN/100g indicates that the raw material is still fresh and in the edible boundaries [18]. Tilapia (Oreochromis sp.) fillets showed a neutral pH that would help to create high gel strength because the myosin is easily soluble [19].

Treatments with edible coating application were found to provide higher organoleptic value than control treatment without edible coating within similar storage time. This shows that the application of surimi as edible coating could maintain the quality of fillets. Organoleptic tests performed included appearance, odor, and texture. Appearance is an important organoleptic parameter because it is the sensory trait that will be seen first by the consumers [20]. The value for appearance given to all samples (control, 2 g, 4 g, and 6 g edible coating) was 7 in a hedonic scale. Meanwhile, the value given to treatment with 8 g surimi was 9 (table 1). Treatment with 8 g surimi could form good edible coating. Edible coating from surimi can cover the surface of the fillet perfectly and when applied, it can produce clear, transparent, shiny, and bright fish fillets [12].
At the end of the organoleptic test, the appearance value given to control treatment was 5 as it began to change its color and became dull. Meanwhile, the value given to other treatments with edible coating was 7 (table 2). This is probably due to the non-enzymatic browning reaction or Maillard reaction that may occur during storage. Maillard reaction describes the brownish reaction between reducing sugars and amino acid groups. Surimi, in the preparation for edible coating, was first dissolved with NaOH to reach pH 11. The pH value has a significant effect on Maillard reaction. The browning level increases with increasing pH [21]. The Maillard reaction has an optimum pH above 7. This alkaline condition induces the degradation of myofibrillar protein, leading to the availability of free amino groups for the browning reaction [22].

At the end of the organoleptic test, control sample received the score 3 for odor (acidic, little smell of ammonia, rancid). Meanwhile, other treatments were given the score of 5 (musty, slightly rancid) (table 2). Coating layer proved to be able to slow the emergence of off odor caused by the microbial decomposition of protein. Lipid oxidation in fish meat can cause unwanted rancid odors and carbonyl compounds resulting from the lipid oxidation can damage amino acid causing the formation of brown pigment and striking rancid odor [23]. However, if the organoleptic value is adjusted to the SNI 2696:2013 standard fillet was scored 7, the fillet coated with edible coating surimi cannot be accepted by consumers until the 18 h at room temperature storage.

Good quality Fish fillets have a solid, compact, and elastic texture. When it has declined in quality, the texture becomes soft and crumbling [20]. This study indicated that the score for texture of control sample at the beginning of organoleptic testing was 7 (solid, less compact, less elastic). The score given to other treatments were 9 (solid, compact, and elastic) (table 1). At the end of organoleptic testing, control sample was scored 3 (soft, inelastic, and watery). Meanwhile, the score for other treatments was 5 (somewhat soft, less elastic, and slightly watery) (table 2). The organoleptic value of fillet tended to decrease during 18 h of the texture of the fish to be not compact, soft, and tender, due to the autolysis process that causes changes in fish meat [24].

### Table 1. Organoleptic Average Value Result of Early (Pangasius sp.) Fillets.

| Organoleptic Test | Edible coating 0 gr surimi | Edible coating 2 gr surimi | Edible coating 4 gr surimi | Edible coating 6 gr surimi | Edible coating 8 gr surimi |
|-------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Appearance        | 7                           | 7                           | 7                           | 7                           | 9                           |
| Odor              | 9                           | 9                           | 9                           | 9                           | 9                           |
| Texture           | 7                           | 9                           | 9                           | 9                           | 9                           |

### Table 2. Organoleptic Average Value Result of Final (Pangasius sp.) Fillets.

| Organoleptic Test | Edible coating 0 gr surimi | Edible coating 2 gr surimi | Edible coating 4 gr surimi | Edible coating 6 gr surimi | Edible coating 8 gr surimi |
|-------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Appearance        | 5                           | 7                           | 7                           | 7                           | 7                           |
| Odor              | 3                           | 5                           | 5                           | 5                           | 5                           |
| Texture           | 3                           | 5                           | 5                           | 5                           | 5                           |
Degree of acidity (pH) is one indicator that is measured to determine the level of the fishery products freshness chemically. Generally, every microorganism has the optimum pH range for its growth that is usually between 6.5 and 7.5 [21]. Fillet had fluctuating pH values at 0, 6, 12, and 18 h storage. Control sample increased its pH in 0 to 12 h. Meanwhile, the pH of other treatments was also increased (table 3), but in a slower rate because the fillets were protected by surimi edible coating with stable gel, so the process of protein degradation became slow due to the strong bond in gel such as by disulfide bonds that was involved in the stability of the film matrix from surimi [25].

Table 3. Average Result of pH (Pangasius sp.) Fillets.

| Observation time | pH Treatment | Average±SD |
|------------------|--------------|-------------|
|                  | A            | B           | C            | D            | E            |
| 0 Hour           | 6.54<sup>a</sup> | 6.77<sup>ab</sup> | 6.93<sup>a</sup> | 6.95<sup>a</sup> | 6.91<sup>a</sup> | 6.82±0.171 |
| 6 Hours          | 6.89<sup>a</sup> | 7.29<sup>b</sup> | 7.35<sup>b</sup> | 6.83<sup>ab</sup> | 7.15<sup>ab</sup> | 7.10±0.233 |
| 12 Hours         | 6.98<sup>a</sup> | 7.05<sup>a</sup> | 7.14<sup>a</sup> | 7.09<sup>a</sup> | 7.10<sup>a</sup> | 7.07±0.060 |
| 18 Hours         | 6.91<sup>a</sup> | 6.85<sup>a</sup> | 7.00<sup>a</sup> | 7.01<sup>a</sup> | 6.88<sup>a</sup> | 6.93±0.071 |

Note: A). Control without edible coating, B). edible coating 2 gr surimi, C). edible coating 4 gr surimi, D). edible coating 6 gr surimi, E). edible coating 8 gr surimi. The notation shown with different superscript letters in the same column shows the comparison between treatments having significant differences (P <0.05).

However, pH of treatments with edible coating (2 g, 4 g, 6 g, and 8 g surimi) decreased after h18 h storage because protein-based edible coatings are a high barrier to oxygen and carbon dioxide. According to Aryanta [26], pH reduction in Pangasius sp. is caused by lactic acid accumulation that affects the increasing growth of lactic acid bacteria (BAL) due to reduced oxygen content. Moreover, protein-based edible coatings may inhibit bacterial activity, resulting in inhibition of production of non-protein nitrogen content that can lead to accumulation of bases [27].

The pH value of control treatment as well as treatments with edible coating increased with increasing storage time due to protein degradation and production of their derivatives by microorganisms that produce volatile bases, such as trimethylamine, dimethylamine, and ammonia. In addition, the increasing pH was caused by the alkaline condition of surimi filtrate that reached pH 11 due to the addition of NaOH. NaOH is added to surimi solution to dissolve the myofibrils protein. Microorganisms are the main cause of food damage. The growth of microbes in food will cause damage and quality deterioration. Total plate count (TPC) is one of the microbiological parameters to determine the level of deterioration. The TPC value of fillet decreased in Treatment D (edible coating with 6 g surimi) and E (edible coating with 8 g surimi) at 18 h of room temperature storage (Table 4). If adjusted to SNI 2696: 2013, treatment E (edible coating 8 g surimi) and D (edible coating 6 g surimi) had been able to inhibit the growth of microorganisms in lower than the maximum limit of 5x10<sup>5</sup> colonies/g up to 18 h of storage. Thus the fillets were still edible until h18 h of storage at room temperature. The increased surimi concentration added in the edible coating was able to increase the amino acid concentration and the bond in the film structure. According to Junianto [28], the bond in the structure of a film or coating is influenced by the molecular weight and the length of the amino acid chain. Greater length of amino acid chain will make the molecular weight larger, so the layer of film or coating that is generated will be tighter.

The results showed that surimi edible coating had stable gel strength so that when it was applied to the Pangasius sp. fillet, it could stick and cover the fillet surface properly due to the myofibril fish protein that has the ability to form a stable three-dimensional gel network [11]. The coating of Pangasius sp. fillets by surimi edible coating may affect the oxygen content present in the product. Edible coating surimi can inhibit the products from being in contact with oxygen, so that oxidation process gets inhibited. Moreover, protein-based edible coating is rich in amino acids that have good antioxidant properties such as cysteine and aromatic amino acids [29].
Edible coating from tilapia surimi can slow down the shelf life of *Pangasius* sp. fillets at room temperature storage. The higher the surimi concentration, the higher the organoleptic value of the fillet and the lower the Total Plate Count value, which was in accordance with SNI 2696:2013. The highest scores of organoleptic testing for appearance, odor, and texture were obtained by sample of fillets with 8 g of surimi edible coatings. Control treatment increased its pH continuously from 0 h to 12 h. Meanwhile, the pH of treatments with edible coating was fluctuating but it tended to increase and approach the alkaline condition slower. The Total Plate Count of samples with 6 and 8 g of surimi edible coating after 18 h of storage at room temperature was in accordance with SNI 2696: 2013 with the value 2.85x10^5 and 1.9x10^5, respectively. Further research on utilization of surimi wastewater as the base material for protein-based edible coating could be of interest.

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