Effect of Pectin on the Characteristics of Edible Film from Pink Ear Emperor (*Lethrinus lentjan*) Gelatin

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Abstract. Edible film is a thin layer used to coat food. One of the biopolymers that can be used as a base for the edible film is fish gelatin. Gelatin can be obtained from the skin of *Lethrinus lentjan*. However, edible film is usually brittle if only gelatin is used, so other materials, such as pectin, are needed to form an elastic film. This study aims to determine the effect of pectin addition on the characteristics of the edible film. The method used in this research was the experimental method with five treatments of pectin concentration (0%, 0.2%, 0.25%, 0.3%, 0.35%). Physical and chemical characteristics (thickness, tensile strength, elongation, water vapor transmission, and moisture content) of edible films were analyzed. The results showed that different concentrations of pectin significantly affected the characteristics of edible film. The best characteristics of edible film (0.12 mm thickness, 15.40 MPa tensile strength, 26.50% elongation, 6.99 gram/m² 24 h water vapor transmission rate, and 8.745% moisture content) were obtained as 0.2% pectin added in making the gelatin-based edible film.

1. Introduction

Pink ear emperor (*Lethrinus lentjan*), a marine fish species, has high economic value. As most pink ear emperor caught was processed as fish fillets, by-products such as skin, heads, and bones are accumulated. Fish by-products, which represent between 25-70% of total fish harvested or caught, are worthy of being valorized [1]. Fish skin, for example, can be a valuable resource for gelatin after soaking it in an acid or alkaline solution. Gelatin is a protein macromolecule that has hydrocolloid properties, can form a thin elastic layer, forming a transparent and strong film. Based on these properties, gelatin can be used as raw material for the production of edible films [2].

Edible films were used as coatings for food components that function as barriers to the migration of moisture, oxygen, carbon dioxide, aroma, and lipids [3]. Other advantages of using edible films are low cost, reducing packaging waste, maintaining the aroma, appearance, and quality of packaged food, and protecting food from contamination [4]. However, if only gelatin is used, the edible film produced has brittle properties. Therefore, to improve the properties of edible films, other materials such as
plasticizers are needed [5]. The function of this plasticizer is to obtain an elastic film, not stiff, and prevent damage to the edible film while wrapping food products. The types of plasticizers commonly used in edible films are glycerol, sorbitol, triethylene glycol sucrose, and polyethylene glycol because of their hydrophilic nature [2].

The addition of sorbitol affects the concentration of soluble solids. Therefore, the higher the sorbitol concentration added will increase the concentration of dissolved solids and the elongation value, and it has good elastic properties [2]. However, adding higher concentration sorbitol will lower the tensile strength. Therefore, it is necessary to add other additives that can increase the tensile strength of edible films, namely by adding pectin. Pectins has been used in food industry as stabilizers, thickening and gelling agents, crystallization inhibitors as well as encapsulating agents [6]. Pectin is also one of the most popular polysacharrides used in the production of edible film or coating [7]. The addition of pectin may increase the thickness, tensile strength, and elongation of the edible film produced. Therefore, this study aims to determine the effect of pectin addition toward the characteristics of the edible film from pink ear emperor (*Lethrinus lentjan*) skin gelatin.

2. Materials and Method

2.1 Materials

*Lethrinus lentjan* skin was obtained from PT Surya Alam Surabaya. The frozen skin was transported to the laboratory using cold box and stored until used in -20°C. Other materials used aquadest, acetic acid, NaOH, sorbitol, pectin, and silica gel. The tools used include hot plate, magnetic stirrer, thermometer, water bath, crushable pliers, glass funnel, tray, oven, blender, coating thickness AMT-15A, cutter, ruler, Universal Testing Machine (UTM), and plastic cup.

2.2 Gelatin and Edible film production

Fish gelatin was made with different concentrations of CH₃COOH (0.1 M and 0.05 M) and NaOH (0.1 M and 0.05 M). The making of fish gelatin was done based on previous reported method [29]. The fish skin was doused with warm water, then soaked in an alkaline solution for 1 hour with a ratio of 1:9 (w/v) 2 times, then rinsed to neutralize the pH. Then soaked in an acid solution for 1 hour with a ratio of 1:9 (w/v) 2x, then rinsed and proceed to the gelatin extraction stage using distilled water for four hours with a ratio of 1:9 (w/v) at a temperature of 60-70°C with the water bath. The extraction results were filtered using a white cloth, and the residue was dried using an oven for 48 hours at a temperature of 55°C to form gelatin sheets. The dried gelatin sheets were then grinded using a blender to obtain a powdered gelatin phase. Gelatin quality testing consisted of yield, amino acid, moisture content, ash content, viscosity, and gel strength. A concentration 12.5%, 0.25%, 37.5% gelatin and sorbitol was mostly used on edible films manufacturing. The best formulations of gelatin and sorbitol were used in the second phase of the study.

2.3. Effect of the addition of pectin

The second stage of the research was the manufacture of edible films with the addition of different pectin concentrations, namely P1(0%), P2 (0.2%), P3 (0.25%), P4 (0.3%), P5(0.35%), each repeated as many as four times. The making of edible films referred to previous method [28]. 5 g and 0.37 g of gelatin and sorbitol were added with pectin P1(0 g), P2 (0.2 g), P3 (0.25 g), P4 (0.3 g), P5(0.35 g). Then mixture was put into a 250 ml beaker glass and add 100 ml of distilled water. The solution was heated using a hot plate gastritis stirrer for 20 minutes at a temperature of 70-80°C. Then the solution was printed on a tray and dried using an oven for 10-20 hours at a temperature of 55°C. Finally, the edible film was conditioned at room temperature for 25-40 minutes and placed in a desiccator. Furthermore, the characteristics of edible films were tested in the form of moisture content [6], tensile strength [9], thickness [10], elongation [9], water vapor transmission [9]. Data was analysed using analysis of variance (ANOVA) at the 5% significance level. Statitical software SPSS version 20 was used.
3. Result

3.1 Fish Skin Gelatin Characteristics

The yield of *L. lentjan* skin gelatin can be seen in Table 1. The highest gelatin yield (17.12%) was obtained at a ratio of 0.1 M CH$_3$COOH and 0.05 M NaOH. It was shown that the concentration of acetic acid had a significant effect (P<0.05) on the yield of fish skin gelatin. In addition, the higher the extraction temperature, the higher the yield of gelatin produced because the concentration of acetic acid increases due to higher exposed collagen structure, and higher collagen hydrolyzed. The acetic acid solution can hydrolyze collagen to facilitate its solubility in hot water at the time of gelatin extraction and opened its structure due to some bonds in the protein molecule being released.

| Treatment | Yield (%)       |
|-----------|----------------|
| A (CH$_3$COOH 0.1 M : NaOH 0.05 M) | 17.13±0.83 |
| B (CH$_3$COOH 0.05 M : NaOH 0.1 M) | 14.20±1.37 |
| C (CH$_3$COOH 0.1 M : NaOH 0.1 M) | 12.08±0.85 |

The *L. lentjan* skin gelatin with the highest yield was then tested for its physico-chemical characteristics (Table 2).

| Physico-chemical characteristics | Pink ear emperor skin gelatin | Gelatin Standard$^a$ |
|----------------------------------|------------------------------|---------------------|
| Moisture content (%)            | 7.97±0.31                    | Max. 16             |
| Ash content (%)                  | 0.80±0.07                    | Max. 3.5            |
| Viscosity (cP)                   | 7.50±0.70                    | 2.5-5.5             |
| Gel strength (N)                 | 8.25±0.77                    | -                   |

Annotation : $^a$SNI (1995)

3.1.1 Moisture content

The results showed that the moisture content of gelatin in *L. lentjan* skin gelatin (7.97%) was lower than red snapper skin gelatin (10.19%) [9] and shark skin gelatin (9.33%) [10] but still within the range of moisture content (maximum of 16%) permitted by the Indonesian National Standard (SNI) No. 3735 of 1995 [19] for gelatin products. The moisture content of gelatin had decreased significantly in the alkaline process compared to the acid process because of alkaline curing agents (Ca(OH)$_2$) reaction in breaking down the amino acid structure of skin proteins [14]. This process leads amino acid structure to become very weak until it finally undergoes a denaturation process. The denaturation process causes molecular changes and the amount of water bound becomes weaker and decreases.

3.1.2 Ash

In this study, the ash content of *L. lentjan* skin gelatin (0.8%) was higher than red snapper skin gelatin (0.4%) [12]. The low ash content of red snapper skin gelatin is presumably due to a large amount of dissolved minerals in the washing process. Although it has lower ash content, it is still within the range of ash content permitted by the Indonesian National Standard (SNI) No. 3735 [19] for gelatin products maximum of 3.25%.

3.1.3 Viscosity

Viscosity is one of the main criteria in determining the feasibility of gelatin for industrial needs[13]. In this study, *L. lentjan* skin gelatin (*Lethrinus lenjant*) was 7.5 cP. These results were higher than shark skin gelatin (3.34 - 4.35 cP) [13]. However, it does not meet the standard requirements for gelatin based on the Gelatin Manufacturers Institute of America (GMIA) (type A 15.0 – 75.0 cP and type B
20.0 – 75.0 cP). The high value of viscosity is related to the average molecular weight and molecular distribution of gelatin, while the molecular weight of gelatin is directly related to the length of the amino acid chain, which longer amino acid chain leads to higher viscosity [15]. Lower concentration of acid causes lower viscosity value because of uncompleted hydrolysis process formed a short amino acid chain. The viscosity of the gelatin is also highly dependent on the concentration of dissolved gelatin and the temperature of the extraction process.

3.1.4 Gel strength
Gel strength is an essential standard in determining the best treatment in gelatin extraction process. This is because gelatin must be able to convert liquids into solids or change the shape of the sol into a gel reversibly [16]. In this study, the average gel strength of L. lentjan skin gelatin (8.25 N) was higher compared to previous studies (0.667 – 1.467 N) [14]. The different strength of the gel was due to differences in the amino acid content of the skin, especially hydroxyproline and proline, whose perform as gelatin gel stabilizer.

3.1.5 Amino Acid
The amino acid composition of L. lentjan skin gelatin showed in Table 3. The highest amino acids yields are found in glycine (21.9%) and proline (12.44%). Similar results were found in mackerel skin gelatin (glycine 18.41%, and proline 9.13%) [15]. The highest amino acid content in milk shark skin gelatin and commercial bovine gelatin are glycine and proline [18]. The glycine and proline in milk shark fish skin are 11.29% and 14.91%, whereas, in commercial beef gelatin was 9.04% and 11.88%. Glycine and proline have an important role in the physical characteristics of gelatin. Glycine in gelatin has an important role in the water binding mechanism [20]. Based on these data, L. lentjan skin gelatin has a high amino acid content glycine and proline that can affect the gel strength and viscosity of the gelatin.

| Amino acid      | Pink ear emperor skin gelatin (%) | Mackerel skin gelatin(%) | Commercial gelatin (%) |
|-----------------|----------------------------------|--------------------------|------------------------|
| L-Tirosine      | 0,6                              | 0,4                      | 1,99                   |
| L-Leusine       | 2,32                             | 1,91                      | 1,09                   |
| L-Proline       | 12,44                            | 9,13                      | 11,88                  |
| L-Histidine     | 0,85                             | 0,48                      | 1,89                   |
| L-Theorine      | 2,9                              | 2,46                      | 1,48                   |
| L-Asam Aspartat| 4,35                             | 4,69                      | 2,68                   |
| L-Lisine        | 3,93                             | 3,7                       | 2,09                   |
| L-Glisine       | 21,9                             | 18,4                      | 9,04                   |
| L-Arginine      | 9,47                             | 6,53                      | 1,62                   |
| L-Alanine       | 9,16                             | 8,87                      | 1,21                   |
| L-Valine        | 1,95                             | 1,6                       | 1,29                   |
| L-Isoleusine    | 0,89                             | 1,06                      | 1,31                   |
| L-Fenilalanine  | 2,51                             | 1,72                      | 2,17                   |
| L-Asam Glutamat| 8,47                             | 8,39                      | 4,47                   |
| L-Serine        | 3,02                             | 2,32                      | 2,16                   |

Annotation : **Gunawan et al., (2017)**

3.2 Edible Film from Fish skin Gelatin
The analysis was conducted to find the best concentration of sorbitol plasticizer in the manufacture of L. lentjan skin gelatin edible film based on the physical characteristics. Our results showed that the
best thickness was found in formulation 3 (Figure 1). The concentration 37.5% Sorbitol plasticizer showed the highest thickness value (0.1 mm), while the lowest was found in 12.5% Sorbitol (0.07 mm). Increment on thickness value effects on concentration of soluble solids. A higher concentration of sorbitol causes a higher concentration of dissolved solids that were resulting thicker edible film [1]. The results of this first stage research (extracted with 0.05M NaOH: 0.1M CH₃COOH and 37.5% sorbitol) used as edible film formulation in the next stage.

![Figure 1. Effect of different sorbitol concentrations on thickness](image)

**Figure 1.** Effect of different sorbitol concentrations on thickness

### 3.3 Characteristics of Edible Film

This step aims to determine the best pectin concentration to be applied to manufacture edible film gelatin from *L. lentjan* skin based on physical and chemical analysis (thickness, tensile strength, elongation, water vapor transmission and moisture content).

#### 3.3.1 Thickness

Thickness is one of the physical properties that can affect tensile strength and water vapor transmission rate of edible films. The thickness of the edible film is also in accordance with the packaged product in order to protect the product properly. The results showed that different pectin concentrations had a significant effect on the thickness of *L. lentjan* skin gelatin edible film (P<0.05). the relation between *L. lentjan* skin gelatin edible film thickness and different pectin concentrations are shown in Figure 2.

![Figure 2. Effect of different pectin concentrations on edible film thickness](image)

**Figure 2.** Effect of different pectin concentrations on edible film thickness

Based on Figure 2 the *L. lentjan* skin gelatin edible film thickness were between 0.10 – 0.18 mm. The highest thickness was found in 0.35% pectin (0.18 mm), while the lowest was in 0% pectin (0.10 mm). The different pectin on concentrations causes different thickness levels. The increase in the
pectin concentration leads to an increase in the number of the total dissolved solids in the film solution, resulting in an increase in the thickness of the film formed [21].

3.3.2 Tensile Strength
Tensile strength is the maximum tensile force that the film can stand before breaking or tearing. The ANOVA test showed that different pectin concentrations significantly affected the tensile strength of the L. lentjan skin gelatin edible film (P<0.05). Relation between average tensile strength of L. lentjan skin gelatin edible film and different concentrations of pectin are showed in Figure 3.

![Figure 3. Effect of different pectin concentrations on edible film tensile strength](image)

Based on Figure 3, it was found that the higher concentration of pectin, the higher tensile strength of the L. lentjan skin gelatin edible film. The highest value tensile strength was found in 0.35% pectin (25.08 MPa), while the lowest was in 0% pectin (12.41 MPa). Our result corroborates a previous study by [22] that showed an increase in green grass jelly pectin concentration causes an increase in edible film tensile strength (0.70 to 2.53 MPa). An increase in concentration of green grass jelly pectin leads stronger interaction force between the molecular matrix in the edible film, thereby increasing the strength of the resulting edible film. The greater the concentration added, the film’s tensile strength also increased due to the stronger interactions between glucomannan polymers [23].

3.3.3 Elongation
Elongation is the length at the breaking point expressed as a percentage of its original length. The ANOVA test showed that different pectin concentrations had a significant effect on the elongation of the L. lentjan skin gelatin edible film (P<0.05). The relation between elongation of L. lentjan skin gelatin edible film and different concentrations of pectin are shown in Figure 4.
Figure 4. Effect of different pectin concentrations on edible film elongation

Figure 4 shows that different pectin concentrations cause different percent elongation. The elongation percentage of *L. lentjan* skin edible film were between 14.48 to 29.25%. The highest percent elongation was found in 0% pectin treatment at 29.25%, while the lowest was in the 0.35% pectin treatment at 14.48%. According to the Japanese Industrial Standard (1975) [24], the minimum edible film percent elongation is 5%. The higher pectin concentration cause film to become easier to tear. A previous study on raja bulu banana edible film has a similar result. An increase in pectin concentration leads decrease upon elongation value from 15.43% to 8.97% [25].

3.3.4 Water Vapor Transmission Rate

The water vapor transmission rate is the movement of water vapor in a specific unit of time through a unit area at a certain humidity temperature. The water vapor transmission rate is also the amount of water vapor that passes through a film surface per unit area of time [1]. The results of the analysis of variance (ANOVA) showed that different pectin concentrations had a significant effect on the water vapor transmission of the *L. lentjan* skin gelatin edible film (P<0.05). The relation between average water vapor transmission of *L. lentjan* skin gelatin edible film and different concentrations of pectin are shown in Figure 5.
Based on Figure 5, it was found that the higher concentration of pectin, the lower vapor transmission rate of the *L. lentjan* skin gelatin edible film. The vapor transmission rate of *L. lentjan* skin gelatin edible film were between 194.8 – 182.88 gram/m² 24 h. The highest value tensile strength was found on 0.35% pectin (182.88 gram/m² 24 h), while the lowest on 0% pectin (94.8 gram/m² 24 h). *L. lentjan* gelatin edible film showed higher water vapor transmission than another film in previous studies. The highest water vapor transmission of edible film with 10% grapefruit albedo pectin (91.44 grams/m² 24 h) while the lowest found in 30% grapefruit albedo pectin (33.12 grams/m² 24 h) [21]. However, the water vapor transmission rate of *L. lentjan* skin gelatin edible film was appropriate to the Japanese Industrial Standard (1975) [24] (<200 grams/m² 24 h).

3.3.5 Moisture content

Moisture content greatly affects how long a food product will last on the shelf, because it affects on physical, chemical, chemical activity, microbiological decomposition and enzyme activity, especially for unprocessed food [27]. Analysis of variance (ANOVA) on moisture content showed that different pectin concentrations had a significant effect on the moisture content of the *L. lentjan* skin gelatin edible film (P<0.05). The relation between *L. lentjan* skin gelatin edible film moisture content and pectin concentration was shown in Figure 6.

![Figure 6](image_url)

**Figure 6.** Effect of different pectin concentrations on edible film moisture content

Based on Figure 6, the increase in the concentration of pectin causes a decrease in the moisture content of the edible film. The 0.2% pectin concentration has the highest moisture content of 10.12%, while the lowest water was on 0.35% pectin concentration. In previous studies, the highest moisture content value in edible film from corn starch was 11.801% and the lowest value was 14.306% [10]. The highest moisture content of edible film was found in 0.2% pectin at 10.89%, and the lowest water in 0.3% was 8.745%. However, the moisture content of the *L. lentjan* skin gelatin edible film is lower than [21], which has a moisture content of 10-30%.

The best treatment was determined by analyzing physical parameters and chemical parameters (thickness, tensile strength, elongation, water vapor transmission, and moisture content), followed by De Garmo (1984) method [27] (Table 4). It showed that the most beneficial treatment for physical and chemical parameters was found in 0.2% pectin with 0.12 mm thickness, 15.40 MPa tensile strength, 26.50% elongation, 167.76 grams/m² 24 h water vapor transmission rate and10.12% moisture content. It was to edible film standard in Japanese Industrial Standard (1975) [24] (thickness <0.25 mm, tensile strength >0.3 MPa, elongation >5%, a water vapor transmission rate <200 grams/m² 24 h) and (moisture content 10-30%) [21].
Table 4. De Garmo analysis result (best treatment)

| Characteristic                  | Results            | Standard          |
|---------------------------------|--------------------|-------------------|
| Thickness                       | 0.12 mm            | <0.25 mm*         |
| Tensile strength                | 15.40 MPa          | >0.3 MPa*         |
| Elongation                      | 26.50%             | >5%*              |
| Water vapor transmission        | 167.76 gram/m² 24 h| >200 gram/m² 24 h*|
| Moisture content                | 10.12%             | 10-30%**          |

Source: *Japanese Industrial Standart (JIS)
**Syarifudin dan Yunianta (2015)

4. Conclusion

Study finding indicates pectin concentration different significantly affect thickness, tensile strength, elongation, water vapor transmission and moisture content (p<0.05). Lentjham fish skin gelatin edible film with 0.2% pectin has met the Japanese Industrial Standard (1975) and the SNI standard.

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