Characteristic study of chitosan addition in Tilapia (Oreochromis niloticus) bone based gelatin film

W Atmaka, B Yudhistira and M I S Putro
Food Science and Technology Program of Faculty of Agriculture, Universitas Sebelas Maret, Indonesia
E-mail: barayudhistira@staff.uns.ac.id

Abstract. Tilapia (Oreochromis niloticus) is one of popular fish species in Indonesia. The high number in tilapia’s production and export of tilapia resulting in the increase of bone fish waste. An attempt to decrease the amount of the aforementioned waste, the fish bones were turned into gelatine. The gelatine produced from this waste can be put to good use by turning it into edible film due to its high water resistance and low tensile strength value. However, in order to make a proper film, both the water resistance and the tensile strength value needs another appropriate additional biopolymer. In this case, the appropriate biopolymer needed both to form the film and to repair its characteristics is chitosan. The purpose of this research is to find out the effect of the chitosan addition on the tilapia bone based gelatine film. The research used several mixtures of gelatine (G) and chitosan (C) with the following ratio: G100:C0 (GC1), G75:C25 (GC2), G50:C50 (GC3), G25:C75 (GC4), G0:C100 (GC5). ANOVA results (P<0.05) shows that the addition of chitosan on the gelatine film affected its characteristics in thickness, solubility, tensile strength, elongation at break, Fourier Transform Infrared (FTIR), color a, and color b but no significant effects on the vapor permeability and color L. The best result is shown on GC2 with thickness 0.119 mm; solubility 74.95%; tensile strength 2.635 Mpa; elongation at break 68.26%; water vapor permeability 5.897 g/h m² and FTIR. The parameters in GC2 shows good compatibility between the two biopolymers.

1. Introduction
Fishes are are one of many popular foodstuffs consumed by the community and also as export commodity. One of many popular fish species are tilapia (Oreochromis niloticus). According to the Data, Statistics and Information Center of Indonesia, in 2011 the number of tilapia production reached 328,473 tons and in 2012 it reached 338,659 tons. The higher the number of the exported tilapia, the higher the amount of its bones wasted. The bone of tilapia is a very good source of protein containing high amount of collagen that can be potential to be turned into gelatin [1]. The skins and the bones of fishes cover 10% to 20% of their total weight [2]. The percentage of gelatin yield on fish bones are generally estimated for around 12%. Therefore, it can be estimated that the expected amount of gelatin produced from 6,703 tons of fish bones are around 804.6 tons [3].

Gelatin is a protein derived from collagen which was extracted from bones [4]. Collagen is the main structural component of connective tissues, it has white color and on fishes, it can be found on their skin, bone and cartilage [5]. Gelatins can be turned into films and coatings with a high optical quality due to its superior gas barrier and mechanical properties on weak moistures which make it possible to be used as a bio-packaging material [6].
Chitosan is a polysaccharide obtained from chitin deacetylation which is generally produced from crustacean skin waste. Chitosan kills bacteria [7], inhibits the growth of spoilage bacteria [8], is not harmful to health [9] and disrupting the activity of the outer membrane of Gram-negative bacteria [10]. Chitosan also functions as antimicrobial agent against the spore germination of Botrytis cinerea, Fusarium oxysporum [11], Penicillium sp [12] and Pseudomonas sp [13].

The purpose of this research is to decrease the amount of tilapia bone waste by turning it into gelatin-based edible film. However, the gelatin itself contains weak vapor permeability and tensile strength resulting on its less-optimal potential as a packaging. Thus, a composition of the gelatin with another renewable film-forming biopolymer is necessary [14]. Chitosan is known to be able to improve the characteristics of the gelatin film. However, its addition to the gelatin as a packaging innovation still lacks of further information. Chitosan and gelatin offers a great potential in completing food nutritive value and are shown to be biodegradable and biocompatible [15]. It is thus, the characterization of chitosan-added tilapia bone gelatin edible film is required in order to observe the characteristics of the edible films.

2. Methods
The materials used in this study are the bones of tilapia which are collected from fresh tilapia fillet residuals in MAJU 57 fishing spot of Janti, Polanharjo, Klaten and chitosans. The chemicals used were sorbitols, aquades, NaCl, and 0.85% liquid glacial acetic acids. The instruments used in producing tilapia bone gelatin are a knife, a basin, a pan, beaker glasses, a waterbath, whatman papers, a cabinet dryer, a pHmeter, a scale, and a mixer. The instruments used in producing the edible film are an analytical scale, graduated cylinders, hot plates, a magnetic stirrer, a cabinet dryer, graduated pipettes, propipettes, edible film trays, and a thermometer. The testing instruments used in this research are weighing bottles, an oven, a desiccator, a 0.01 mm Krisbow micrometer, a Lloyd Instrument TA plus texture analyzer, WVTR cups, silica gels, a hygrometer, a Shimadzu IR Prestige21 FTIR Spetrophotometer, and a colorimeter.

The film making procedures are as follow: first, tilapia bone gelatin. The bones of the tilapia were cleansed from both dirt and flesh residuals, then they were degreased in 80°C for 30 minutes. The bones were then re-cleaned to make sure that neither flesh residuals nor any outer high-fat containing surface were left. The clean bones were cut into 3-5 cm pieces and were demineralized in a 4% HCl (v/v) with the ratio of 1:4 (b/v) for 48 hours. The 4% HCl was replaced every 24 hours. After 48 hours of demineralization, ossein was obtained. The ossein was cleansed on flowing water to neutralize its pH (6-7). The ossein of which pH had been lowered was put into a beaker glass, aquades was added with the ratio of 1:3 (b/v). The extraction process was executed in the water bath with the temperature of 85°C for 6 hours. The extract obtained from the process was later filtered using whatman paper and then dried using oven with the temperature of 50°C for 24 hours. Finally, the size of the extract was minimized into powder using mixer, producing powder of gelatin. Second, the gelatin solution. The process of gelatin solution production was started by putting 4 grams of gelatin into a beaker glass placed on a hot plate. Next, 100 ml of aquades was also put into the glass slowly. Then the mixture was stirred using magnetic stirrer (4% gelatin). Finally, 1.2 ml of sorbitol was dissolved into the solution and heated with the temperature of 40°C for 30 minutes. Third, chitosan solution. In producing chitosan solution, the first step was started by putting 3 grams of chitosan into a beaker glass placed on a hot plate followed by 100 ml of 0.85% acetic acid poured into the glass slowly. Then, the mixture was stirred using magnetic stirrer (chitosan 3%). Finally, 1 ml of sorbitol was dissolved into the solution and was heated with the temperature of 40°C for 30 minutes. Fourth, gelatin-chitosan solution. The process of gelatin-chitosan production was started by composing both gelatin solution and chitosan solution on different beaker glasses placed on hot plates with the following ratios: 100%:0%, 25%:75%, 50%:50%, 75%:25%, and 0%:100%. Next, each mixture was stirred using the magnetic stirrer. Finally, each solution was heated within the temperature of 40°C for 30 minutes. The produced solutions later were quenched to the room temperature and after a while, they were dried inside a cabinet dryer within the temperature of 60°C for 24 hours.
The method of the analyses was ran through several tests, namely: thickness test via 0.01 mm Krisbow micrometer [16], solubility test [17], tensile strength and elongation at break test via Lloyd Instrument TA plus texture analyzer [18], vapor permeability test [18], functional groups test via Shimadzu IR Prestige21 FTIR Sptetrophotometer [19], and color test via colorimeter. This research applied randomized complete block design with one factor, namely comparison between addition of gelatin and chitosan solution. The various ratios of gelatin (G) and chitosan (C) mixtures were as follows: G100:C0 (GC1), G75:C25 (GC2), G50:C50 (GC3), G25:C75 (GC4), G0:C100 (GC5). The sampling of each mixture was repeated three times and was analyzed two times. The collected data were later analyzed using one-way ANOVA with 5% significance level and were analyzed further using Duncan’s Multiple Range Test (DMRT) with 5% significance level when significant difference was observed.

3. Result and discussion

| Table 1. Chitosan-added Tilapia Bone Gelatin Edible Film Characteristics Text Result |
|---------------------------------|---------------------------------|------------------|------------------|------------------|
| Edible Film | Thickness (mm) | Solubility (%) | Tensile Strength (Mpa) | Elongation at Break (%) | Vapor Permeability (g/h m²) |
|------------|----------------|----------------|------------------|------------------|------------------|
| GC1        | 0.173±0.060    | 89.52±0.016    | 0.583±0.224       | 254.40±7.762     | 5.987±0.739     |
| GC2        | 0.119±0.016    | 74.95±0.032    | 2.635±0.209       | 68.26±6.459      | 5.897±0.708     |
| GC3        | 0.117±0.021    | 62.51±0.075    | 4.064±1.073       | 35.85±4.384      | 5.966±0.958     |
| GC4        | 0.116±0.027    | 49.31±0.033    | 4.536±0.472       | 21.65±1.417      | 6.865±1.866     |
| GC5        | 0.115±0.015    | 47.40±0.043    | 4.580±0.339       | 13.16±2.037      | 7.204±1.958     |

a, b, c different subscripts showed significant difference at $P<0.05$

3.1. Thickness
The thickness of film determines its capability in preventing any substance exchanges from its surroundings and vice versa [20], affects its biological characteristics and its shelf life [21]. The result of this research is in accordance with the findings of a research conducted [14] in which shows that addition of chitosan on gelatin-based film would significantly lower its thickness. The higher the amount of solid in the solution the thicker the film produced [22].

The result of this research shows that the gelatin-based film tends to be thicker than the chitosan-based film because it holds more solids than the chitosan-based film does. Due to its good physical properties of gel, gelatins are used in food productions in contributing and thicker food textures [23]. The finding of this research shows that the best thickness was resulted in the mixture of GC2 (75% Gelatin 25% Chitosan) with the thickness of 0.119 mm, and overall thickness around 0.173-0.115 mm, which it is stated by JIS (Japanesse Industrial Standard) that the maximum thickness of plastic films food packaging is 0.25 mm. Therefore, the thickness of the gelatin-based film in this research already fulfilled the standard of plastic film food packaging [24].

3.2. Solubility
The solubility of film determines its biodegradability as a packaging. The solubility level of a film depends on the product’s characteristics [25]. Based on the research of the composition of cuttlefish gelatin edible film and chitosan [14], gelatin-based film shows high level solubility of (90.68%), while chitosan-based film only shows lower solubility level (around 50.35%). The solubility of the samples in this research ranged from 89.523% to 47.403%. The best solubility was resulted from the mixture of GC2 (75% Gelatin 25% Chitosan) with the solubility of 74.954%. This value of solubility was close to the value of solubility in the previous research.

There are several factors affecting the solubility of an edible film: hydrophilic and hydrophobic properties, pH, polarity [26] particle size, and molecular structure [27]. This research found that the
more chitosan added to the edible film, the more its solubility decreased. Polysaccharide film has a strong barrier to O₂ and CO₂ due to its tight molecular structure of hydrogen bonding which makes it less soluble [16]. The addition of chitosan will raise water resistance properties which shows interaction between polymers disrupting polypeptide chain inside the gelatin’s matrix. The interaction between the two biopolymers is resulted from electrostatic activity and low solubility is resulted from hydrogen bonding [14].

3.3. Tensile Strength
Film requires an adequate tensile strength and elongation at break in so it can hold external weight, preserving its integrity and may guard the product when applied as packaging [28]. In the previous research, the edible film composed of fish gelatin showed tensile strength at 2.17±0.97 Mpa [29]. This finding was closely related to this research’s in which in the composition of GC2 (75% Gelatin 25% Chitosan), tensile strength was shown at 2.63±0.20 Mpa. As for chitosan was shown at 8.41 Mpa [15] which is far different from this research’s finding in the composition of GC5 (100% Chitosan) where tensile strength was shown at 4.58±0.33 Mpa.

According to JIS, the minimum value of tensile strength which can be classified as food packaging film is 0.39 Mpa [24]. The tensile strength of the samples on this research ranged from 0.58 Mpa to 4.580 Mpa, meaning that they already fulfilled the minimum standard of food packaging film. The addition of chitosan on film causes increment on its tensile strength, while gelatin-only-based film does not. Tensile strength and elongation at break are usually related to micro structure of the film network and intermolecular interaction [14].

Compositions of protein with different ratios of polysaccharide may improve tensile strength. In the composition of gelatin-chitosan film, the addition of chitosan improves significant tensile strength compared to the gelatin alone [29]. The level of chain elongation and sequence of amino acid residue may affect the mechanical properties of protein-based film [30]. This condition results on the strengthening of the interaction between gelatin and chitosan due to hydrogen bonding and complex polyanion forming between the gelatin and the chitosan [31]. Chitosan with higher molecular weight or deacetylation degree may contribute more reactive groups in one chain to interact with the gelatin through the hydrogen bonding which results on film strengthening [32].

3.4. Elongation at Break
Elongation at break is a parameter which is inversely proportional to tensile strength. The lower the value of tensile strength, the higher the value of elongation [24]. In the previous research, edible film composed of fish gelatin showed elongation at 82.64±20.11% [29] which is far different from the result of this research in which elongation was shown at 254.40%. As for chitosan the elongation at break was shown at 19.55% [15] which is close to the result of this research in the composition of GC4 (25% Gelatin 75% Chitosan) in which elongation at break was shown at 21.65%. The elongation at break of the samples in this research ranged from 254.49% to 21.65%. The finding of this research shows that the best elongation was resulted in the mixture of GC2 (75% Gelatin 25% Chitosan) with the elongation at break of 68.260%.

According to JIS, the minimum value of elongation at break which can be classified as food packaging film is 70% [24]. Therefore, only the mixture of GC1 managed to fulfill the standard. However, the mixture of GC2 was also close the standard value with the elongation at break of 68.260%. Gelatin-based film is more flexible yet fragile compared to chitosan-based film. Thus, the composition of gelatin and chitosan will strengthen gelatin-based film and strengthen chitosan-based film [29]. The elasticity of film depends on its material which can be crystals or amorphous solids. Gelatin is a more amorphous material compared to chitosan. The elasticity of film composed of gelatin and chitosan is improved due to the lowering of its crystallinity that makes the composite film more amorphous compared to the chitosan film. The lowering of the composite film’s crystallinity is mainly caused by damaged hydrogen bonding on the molecule of chitosan. Groups of amino on the hydrogen bonding of chitosan and groups of carboxyl on gelatin formed complex gelatin-chitosan
polyelectrolytes. This form shatters the hydrogen bonding of amino groups and hydroxyl groups on the chitosan and results on the forming of amorphous structure of complex polyelectrolyte, causing improvement of value of elongation at break [33].

3.5. Water Vapor Permeability

Water vapor permeability consists of dissolution process and active diffusion in which vapor dissolves on one of either side of the film and then diffuses on the other side of the film. The resistance to water vapor transmission rate is determined by the thickness of the film, temperature, and water vapor partial gradient pressure [16]. The result of this research shows higher water vapor permeability than that of the result of the research conducted [24], in which is stated that according to JIS, the maximum value of water vapor transmission rate which can be classified as food packaging film is 7 g/m²/day. As for commercial LDPE (Low-density polyethylene) has the value of water vapor permeability of 9.14 g/m s and HDPE (High-density polyethylene) has the value of water vapor permeability of 2.31 g/m s [23]. Generally, protein and polysaccharide-based film has a high value of water vapor transmission rate [16]. High value of water vapor transmission rate affects the packaged product shelf life; the higher the product’s water vapor transmission rate, the less durable the product will be.

Gelatin-based edible film has lower water vapor permeability than chitosan-added edible film, as stated [34], the more polysaccharides added to a film, the weaker its barrier toward water vapor permeability and oxygen will be. It occurs due to the gelatin-based film thickness which is thicker than chitosan-based film [24]. In addition, the low water vapor permeability of a fish gelatin-based film is due to its high hydrophobicity of which the amount of its proline and hydroxyproline decreases by [35]. Chitosan-based film is also polysaccharide which tends to be more useful as sacrificing agent than as barrier because it absorbs water and thus gives a temporary protection to the product [23]. The use of chitosan to block the water vapor transmission has an optimal value which in this research was shown in the mixture of GC2 where chitosan functioned as sacrificing agent and the composition of gelatin was higher than chitosan. In the other mixtures, the water vapor permeability was shown growing due to the composition of chitosan which temporary blocked the water vapor transmission and the low composition of gelatin which resulted on higher water vapor permeability.

3.6. FTIR Test

Fourier Transform Infrared (FTIR) is one of the methods to detect compound molecular structure through the functional group identification of its composition. The chemical structure, molecular shape and functional group of the tested sample function as the basic shape of a spectrum which later was obtained from the result of the analysis [36]. Theoretically, hydrogen bonding or other chemical polymer interaction causes shift in peak position [37]. FTIR is useful in completing the characterization of composite film microstructure due to its function as an evaluation instrument to the film inter-component interaction.

Gelatin has similar structure which generally owned by protein, namely carbon, hydrogen, hydroxyl group, (OH), carbonyl group (C=O), and amine group (NH). The result of the analysis on the mixture of GC1 using via FTIR showed a stretching vibration of OH group with the absorption peak of 3424.76 cm⁻¹. The presence of OH group was strengthened by absorption in the region of amide II (1538.30-1320.33 cm⁻¹) which exhibited bending vibration of OH group. The absorption peak in the region of 2921.32-2852.84 cm⁻¹ showed stretching vibration of CH group which was bonded to NH group in the amide region. Stretching vibration of C=O group (amide I) was shown on the absorption peak in the region of 1632.81 cm⁻¹. The absorption peak region of 1271.14 cm⁻¹ showed amide III (CN stretching and NH bending) which was the absorption region of gelatin functional group.
In previous research, gelatin powder showed wavelength of amide A group around 2930 cm$^{-1}$. Absorption peak of 1565-1644 cm$^{-1}$ in the region of amide I, absorption peak of 1560-1335 cm$^{-1}$ in the region of amide II, and absorption peak of 670-1240 cm$^{-1}$ in the region of amide III was shown in the absorption region of gelatin functional group [38]. In the other hand, gelatin powder made of sharpnose stingray (*Himantura gerrardi*) skin soaked in 4% HCl showed absorption peak of 1647 cm$^{-1}$ which exhibited stretching of C=O [39].

![Infrared Spectrum of Gelatin-based Edible Film (GC1)](image1)

**Figure 1.** Infrared Spectrum of Gelatin-based Edible Film (GC1)

In previous research, chitosan exhibits amide I bonding on the absorption of 1637 cm$^{-1}$, indicating the presence of acetyl group (C-N), and the region of 3500-3400 cm$^{-1}$ is the absorption band to the stretching vibration of NH and OH group of chitosan compound [40].

**Figure 2.** Infrared Spectrum of Chitosan-based Edible Film (GC5)

![Infrared Spectrum of Chitosan-based Edible Film (GC5)](image2)

**Figure 3.** Infrared Spectrum of Gelatin-Chitosan-based Edible Film (GC3)

**Figure 2** represents the result of the FTIR test on the chitosan-based gelatin film mixture of GC5. The absorption peak of this film was shown in the region of 3445.01 cm$^{-1}$, exhibiting stretching vibration of OH and NH group. The presence of OH group was strengthened by absorption in the region of amide II (1552.76-1333.83 cm$^{-1}$) which exhibited bending vibration of OH group. The absorption peak in the region of 1640.53 cm$^{-1}$ exhibited stretching vibration of C=O group (amide I) and the absorption peak in the region of 2954.11-2853.81 cm$^{-1}$ exhibited stretching vibration of CH group. In previous research, chitosan exhibits amide I bonding on the absorption of 1637 cm$^{-1}$, indicating the presence of acetyl group (C-N), and the region of 3500-3400 cm$^{-1}$ is the absorption band to the stretching vibration of NH and OH group of chitosan compound [40].

**Figure 3** represents the result of the FTIR test on the gelatin-chitosan-based film mixture of GC3. The absorption peak of this film was shown in the region of 3411.26 cm$^{-1}$, exhibiting stretching vibration of OH and NH group. The absorption peak in the region of 1645.35 cm$^{-1}$ exhibited stretching vibration of C=O group (amide I) and the absorption peak in the region of 2954.21-2853.81 cm$^{-1}$ exhibited stretching vibration of CH group.

In the region of 1653.07-1463.07 cm$^{-1}$ (the absorption peak of amide I and amide II), compared to gelatin-based and chitosan-based edible films, a change of the gelatin’s polypeptide chain structure occurred [14], which was due to the addition of chitosan. Gelatin-chitosan-based edible film
experienced intensity increment in the region of 1700-1500 cm\(^{-1}\) [41]. This statement was in accordance with the finding of this research in which in the region of 1653.07-1463.07 cm\(^{-1}\), an increment of vibration intensity emerged in the samples of GC1 and GC5. Such pattern occurred due to the electrostatic interaction between amino group and carbonyl group in the formation of polyelectrolyte complex.

3.7. Color

| Table 2. Chitosan-added Tilapia Bone Gelatin Edible Film Color Test Result |
|---------------------------|------------------|------------------|
| **Edible Film** | **L** | **a** | **B** |
| GC1 | 45.91\(^{a}\) ± 2.222 | 2.38\(^{b}\) ± 0.499 | -0.80\(^{b}\) ± 0.317 |
| GC2 | 48.13\(^{b}\) ± 3.763 | 1.74\(^{ab}\) ± 0.272 | 3.77\(^{b}\) ± 1.062 |
| GC3 | 46.62\(^{a}\) ± 5.062 | 1.9\(^{ab}\) ± 0.530 | 3.03\(^{b}\) ± 0.674 |
| GC4 | 47.55\(^{b}\) ± 5.704 | 1.78\(^{ab}\) ± 0.577 | 4.82\(^{b}\) ± 1.817 |
| GC5 | 44.69\(^{a}\) ± 3.798 | 1.24\(^{a}\) ± 0.745 | 11.38\(^{a}\) ± 4.864 |

The color of the edible film is an important factor in its use as packaging when covering the surface of the product. The color of the edible film is expected not to disrupt the original color of the packaged product in order to make it attractive to the consumer. The data of collected colors in the research is shown in Table 2. The result of this research is in accordance with the previous research which shows that chitosan film has lower values of L (lightness) dan a (redness) than gelatin film and composite film do, but has higher value of b (yellowness) which shows that the color of chitosan tends to be yellow [14].

| Table 3. Edible Film Effectiveness Test Result |
|----------------|----------------|----------------|
| **Edible Film** | **Effectiveness Value** |
| GC1 | 0.736 |
| GC2 | 0.527 |
| GC3 | 0.487 |
| GC4 | 0.324 |
| GC5 | 0.226 |

4. Conclusion
Chitosan addition on the tilapia bone-based gelatin edible film affected its characteristics in thickness, solubility, tensile strength, elongation at break, Fourier Transform Infrared (FTIR), Color A, and Color B but no effect on the vapor ability and Color L. The best result for gelatin-chitosan film is shown on GC2 with thickness 0.119 mm; solubility 74.95%; tensile strength 2.635 Mpa; elongation at break 68.26%; water vapor permeability 5.897 g/h/m\(^2\); and FTIR shows good compatibility between the two biopolymers.

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