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FULL PAPER

Physicochemical characteristic of modified edible film made from gelatine of sea bass (*Lates calcarifer*) residue with palmitic acid and soybean protein isolate treatment

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Abstract

The use of fish waste as raw material in producing gelatin becomes important when it is related to the halal perspective. Sea bass skin contains high protein, especially collagen protein which can be used as the main component of edible film. An edible film made from gelatin of fish skin usually lacks water vapour transmission due to its hydrophilic properties. Modification of formula in producing an edible film from fish gelatin with the addition of lipid such palmitic acid (PA treatment) and soybean protein isolate (SPI treatment) can affect the characteristics of the edible film resulted. This study was aimed to evaluate the chemical (solubility, water vapour permeability) and physical characteristics (thickness, tensile strength, per cent elongation, SEM) of an edible film made from gelatin with the addition of palmitic acid and soybean protein isolate with different concentration. The results showed that edible films with SPI addition yield the best film thickness (<2.5 mm) while PA addition resulted in better tensile strength of the films (>3.92 MPa) according to the Japanese Industrial Standard. The per cent elongation of both films with PA (21.3-34.44%) and SPI (36.06-117.53%) addition decreased with increasing concentration, but SPI generally gave higher elongation compared to PA. The addition of SPI gave lower solubility and higher water vapour transmission values compared to PA, however, the addition of 4.5% of PA gave the lowest water vapour transmission (5.416 g/m^{2}h). Therefore, the best treatment in this study was concluded to be 4% of SPI addition based on the best thickness, per cent elongation, water vapour transmission and solubility results.

1. Introduction

Fish skin contains high protein, particularly collagen which can be used as the main component for an edible film (Arumugam *et al.,* 2018; Govindharaj *et al.,* 2019). Apart from fish skin, gelatine can also be made from fishbone which contained collagen. Gelatine can be utilized as a thickening agent, stabilizer in the food industry and also a potential raw material for the edible film. This tasteless, colourless, solid substance was generated from the hydrolysis of collagen. Collagen is the most abundant protein component of skin, tendons, connective tissue, cartilage, and bones and it is a major type of macromolecule constituent of the animal body (Lv *et al.,* 2019).

Gelatine is known for its film-forming capacity giving transparent films, which make it an excellent edible film component that can fulfil commercial packaging needs (Hosseini and Gómez-Guillén, 2018). Films that can be consumed directly were widely known as edible films, which lately has been used as a food coating and/or packaging. These films can maintain food quality by retaining the transfer of water vapour, gas, odour, and lipid (Bourtoom, 2008), hence also improving the appearance of the product. Gelatine matrix allows

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controlling of bioactive substances released from the packaging film to the coated food during the storage period and thereby slowing down the deterioration of food products. Bioactive peptides from collagen and gelatine with biological properties have become a topic of great interest for healthy food and processing/ preservation industries (Abdelhedi *et al.,* 2019). However, edible films made from fish gelatine has a concern of subpar protection from water due to its hydrophilic tendency, so its usage is still restricted for low water content product.

Previous studies showed that films containing fatty acids presented higher water vapour barrier properties (Fakhouri *et al.,* 2018). Palmitic acid is a long-chain fatty acid with good film-forming characteristics (Pangesti *et al*., 2014) which can be added in edible film production to improve the hydrophobic properties of the film (Pereira *et al.*, 2019) and hence decreasing the water vapour transfer. Apart from palmitic acid, soybean protein isolate was also known to improve the characteristics of edible film. Soybean protein isolate (SPI) is a mixture of proteins with different molecular properties which is the abundant, inexpensive, biodegradable, and nutritional raw material (Galus, 2018). It can protect food products from water vapour and gas, reduce oxygen permeability and improve the functional and mechanical properties of the film (Bai *et al.,* 2012; Alves *et al*., 2017).

The rising demand for gelatine has led to the research for alternatives to mammalian-derived gelatine, particularly in response to health concerns (mammalian-derived gelatine were believed to be able to transmit pathogenic vectors such as prions) or religious aspects (forbidden to consume of any pork or beef-related products) (Huang *et al.,* 2019; Nilsuwan *et al.,* 2019). One of the potential alternatives was the use of fish waste or residue which referred to parts that have low or no commercial value such as skin, bones, fins, head and guts (Caruso, 2016). Fishing industries generated close to 32 million tonnes of fish waste every year from fish processing, creating a high concern in its waste management and environmental effects such as accumulation of waste sludge and fish parts in near-shore locations, bad odour and unhygienic conditions caused by bacteria and waste decomposition (Joseph *et al*., 2019). The utilization of the waste was limited to fish meal and production of organic fertilizer (Tugiyono *et al*., 2020; Muttharasi *et al*., 2019; Radziemska *et al*., 2019), with the rest disposed back to the ocean. Therefore, using fish waste as the raw material for gelatine production was probable since it is readily available, inexpensive and rich in collagen.

The objectives of this study were (i) to develop an edible film made from sea bass (*Lates calcalifer*) skin-derived gelatine and (ii) to investigate the effect of different palmitic acid and soybean isolate protein concentrations on the physicochemical characteristics of the edible film.

2. Materials and methods

2.1 Materials

Materials used in this study include sea bass skin residues obtained from local markets in Semarang, Indonesia, palmitic acid (98%, Merck, Germany), soybean protein isolate (Merck, Germany), acetic acid (96%, Merck, Germany), glycerol (85%, Merck, Germany) and demineralized water. Equipment used includes an analytical scale (PR124, OHAUS, China), hot plate magnetic stirrer (MS7-H550-S, DLAB, China), and oven (DZF-6020, MTI Corporation, USA). Micrometre screw gauge (125-103, Mitutoyo, Japan) was used to determine film thickness, Synchro-lectric viscometer (RVT, Brookfield, USA) was used to determine the viscosity, film texture was analyzed using TA-XT plus Texture Analyser (Stable Micro System, UK), tensile strength and per cent elongation were determined using Universal Testing Machine (BL-GRS500N, ZwickRoell, US) and film surface morphology was characterized using Scanning Electron Microscope (JSM 6510 LA, JEOL, Japan).

2.2 Methods

This study was done in two steps, the first step was gelatine production using a modified method studied by Trilaksani *et al.* (2012). First, degreasing of the fish skin was carried out by washing and submerging the skins in hot water (70^{o}C) for 1 min. Next, the degreased skins were demineralized by soaking it in 3% acetic acid for 12 hrs with fish to acid weight ratio of 1:4. It was followed by washing with demineralized water until the skin has a pH of 5-6. Collagen extraction was carried out with fish to demineralized water ratio of 1:3 at 70^{o}C for 2 hrs. The extract was filtered and poured into a clean tray and dried at 60^{o}C using the oven for 48 hrs to obtain the gelatine. Laboratory analysis for gelatine covers: yield, gel strength and viscosity.

The second step was edible film production using the modified method of Julianto *et al*. (2011), where 5 g of gelatine was dissolved in 100 mL demineralized water and heated at 85^{o}C using the hot plate magnetic stirrer. Glycerol (0.75 mL) was added into the mixture and various concentrations of palmitic acid (0%, 1.5%, 3% and 4.5%) was also mixed into the mixture. The mixture was heated at 55^{o}C and stirred for 30 mins. The edible

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film mixture was poured into a glass plate covered with plastic and dried at 60°C for 24 hrs before cooled in room temperature. These steps were repeated with soybean protein isolate (3%, 4% and 5%) addition instead of palmitic acid. The edible film was analysed for per cent elongation, solubility, thickness, tensile strength, water vapour transmission, and surface morphology. The study used Completely Randomised design with triplicate for each treatment.

2.3 Water vapour transmission

The water vapour transmission test was carried out based on ASTM (1993) by placing the sample to cover the permeable cell tube with 4 cm in diameter. Inside the permeable cell, silica gel was added into the (0% RH). Then the cell was covered with film and placed in a desiccator which has been filled with saturated NaCl (70% RH) at a temperature of 30°C. Water vapour transmission rate was calculated by weighing the permeable cell with 1-hour interval monitoring by using screw gauge micrometre.

$$WVTR = \frac{Slope}{Sample\ area\ (m^2)} = kgf/cm^2$$

2.4 Per cent elongation

The per cent elongation was determined based on tensile strength following ASTM (1993). Per cent elongation was calculated using a formula as follow:

$$Elongation\ (\%) = \frac{Final\ film\ length - initial\ film\ length}{initial\ film\ length} \times 100\%$$

2.5 Thickness

The thickness of the edible film was measured by using screw gauge micrometre (0.001 mm precision) with 5 different locations on four sides and centre of the edible film (2×5 cm) as shown in Figure 1. Thickness was calculated for the average of five measurements.

$$Thickness\ (mm) = \frac{A + B + C + D + E}{5}$$

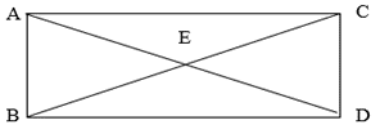

Figure 1. Diagram of thickness calculation

2.6 Tensile strength

Tensile strength was determined by using texture analyzer TA-TX. Edible film of 5×2 cm was measured for its thickness by screw gauge micrometre, then it was placed on the grip. Tensile strength was calculated by formula as follows (Wibowo *et al*., 2016):

$$\Sigma = \frac{N}{T \times L} = Mpa$$

2.7 Yield

The yield of the gelatine was determined from the gelatine obtained and the raw material used based on AOAC (2005). The following equation was used to calculate:

$$Yield(\%) = \frac{Dry\ gelatine\ weight\ (g)}{Wet\ fish\ skin\ weight\ (g)} \times 100\%$$

2.8 Viscosity

Gelatine mixture (6.67%) was prepared by dissolving gelatine produced in demineralized water. The viscosity of the gelatine was measured using Synchro-lectric viscometer at 60°C with stirring speed of 60 rpm and 1 spindle. Viscosity value was measured in centipoise (cP) unit (British Standard 757, 1975).

2.9 Gel Strength

Gelatine mixture (6.67%) was prepared by dissolving gelatine in demineralized water at 80°C for 15 mins. The mixture was poured into Standard Bloom Jars (d: 58-60 mm, h: 85 mm), left for 2 mins to settle before put into the Texture Analyzer TA-XT machine. The gel strength was measured at 0.5 mm/s probe speed with 4 mm depth. The gel strength value was measured in bloom unit (British Standard 757, 1975).

3. Results and discussion

3.1 The characteristics of gelatine made from sea bass skin

Parameter observed in this study was the quality of gelatine which include yield, viscosity and tensile strength. The quality of gelatine made from sea bass skin can be seen in Table 1.

Table 1. Quality of gelatine made from sea bass skin

Parameter	Value
Yield (%)	20.036±0.037
Viscosity (cP)	3.40±0.043
Gel strength (g bloom)	138.980±15.973

The yield resulted from the treatment was considered affected by a treatment applied on the sample such as the washing, neutralisation of gelatine extract and incomplete hydrolysis process. In addition, the temperature of extraction can also affect the yield. Generally, the yield of gelatine made from fish skin is relatively low in the range of 6-19% (Kusumaningrum *et al*., 2018) which was due to the loss of collagen during the washing process, incomplete hydrolysis process and also the collagen dissolving during acid or alkali soaking treatments.

The average viscosity of gelatine made from sea bass skin treated by acetic acid fall into the standard range as

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the viscosity standard for food gelatine is 2.5-4.5 cP (Suptijah *et al.*, 2013). Acetic acid treatment disrupted the non-covalent bonding in the protein (indicated by the swelling of fish skin), exposing the collagen into the environment which increases the solubility and easier for it to be extracted. Subsequently, the acidic treatment also broke down the intermolecular crosslink in triple helix fibres collagen structure, resulting in single chains of amino acids (α, β and γ) which have higher molecular weight compared to the collagen structure (See *et al*., 2015). The viscosity of gelatine is related to the average molecular weight of the amino acid chains. Hence, a higher concentration of acid will encourage the hydrolysis of the links yielding more gelatine and resulted in highly viscous gelatine (See *et al*., 2015; Yudhistira *et al*., 2019).

The average seabass fish skin tensile strength with a soaking time of 12 hrs was 138.980 g bloom. This value complies with the gelatine standard of the Gelatine Manufacturers Institute of America, which stated the gel strength of commercial gelatine is in the range of 50-300 g bloom (ASTM, 1993). The longer time of soaking results in excessive hydrolysis of protein collagen and gelatine, therefore, producing oligopeptide (Trilaksani *et al.,* 2012). In addition, some of the amino acids remain and do not run out during the washing process, thus lowering the gel strength.

3.2 Characteristics of edible film

3.2.1 Thickness

The thickness of edible film with palmitic acid addition is depicted in Figure 2. The smallest thickness in this study was found on 0% palmitic acid treatment (0.107 mm), while the highest thickness was found on 4.5% palmitic acid treatment (0.321 mm). Increasing the thickness of the edible film as the concentration of palmitic acid increases was due to the increasing amount of soluble solute present in the film. Moreover, the combination of glycerol and palmitic acid can form a good homogenous mixture, which would consequently increase the thickness (Sanyang *et al.,* 2016).

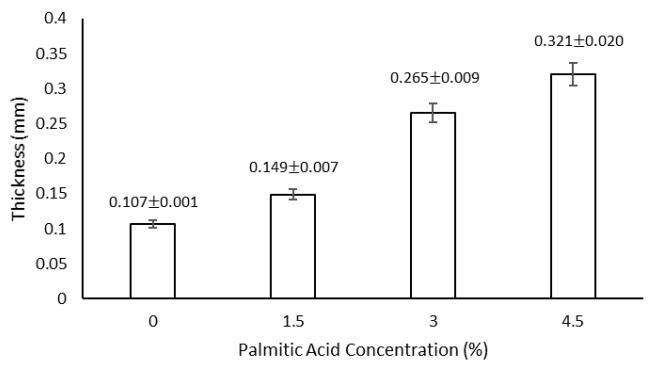

Figure 2. Thickness of edible film from seabass fish skin gelatine with different palmitic acid concentration

The thickness of control edible film and edible film from seabass fish skin gelatine with soybean protein isolate (0%, 3%, 4%, and 5%) addition and 0.7% glycerol as plasticizer gave thickness of 0.132-0.264 mm. The highest thickness was found in edible film with glycerol and soybean protein isolate 5% addition while the lowest was the edible film without the addition of soybean protein isolate. Figure 3 depicts the thickness of edible film from sea bass fish skin and soybean protein isolate addition.

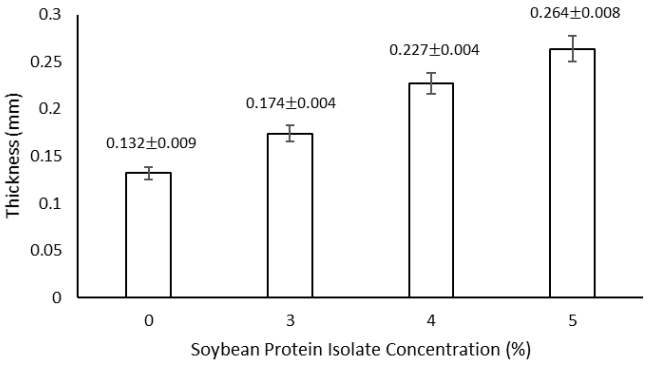

Figure 3. Thickness of edible film from sea bass fish skin gelatine with different soybean protein isolate concentration

The thickness of the edible film increases with increasing soybean protein isolate addition (SPI). This finding was corroborated with the study carried out by Nandane and Jain (2018) which showed that increasing SPI concentration with constant plasticizer concentration gave increasing edible film thickness ranging from 143.162-163.529 μm. Increasing the SPI concentration would provide more protein (solute) in the mixture which increases the chance of interactions between the molecules to yield film-building polymer matrices (Salimah *et al*., 2016; Wulandari *et al*., 2018). Conversely, lower SPI concentration can reduce the distance of intermolecular chain polymer bounds which reduces the elasticity and water bonding capacity, resulting in the decrease of thickness (Sudaryati *et al.*, 2010).

3.2.2 Tensile strength

The tensile strength of edible film with palmitic acid addition is presented on Figure 4, with values ranging from 3.52-10.91 MPa. The highest tensile strength value was found on edible film without the addition of palmitic acid (10.910 MPa), while the lowest was found on the highest palmitic acid addition (3.520 MPa). Tensile strength in edible films is one of the important properties in packaging and coating of products, as it affects the flexibility of the film; high tensile strength would give lower flexibility and low tensile strength would give higher flexibility (Krishna *et al*., 2012). Edible film with high tensile strength would be more suitable for products requiring stiffer packaging, while edible film with lower

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tensile strength would be more suitable for products requiring flexibility in its packaging (Katili *et al*., 2013). With that said, the strength of the packaging should be specific and/or complement the properties of the food product.

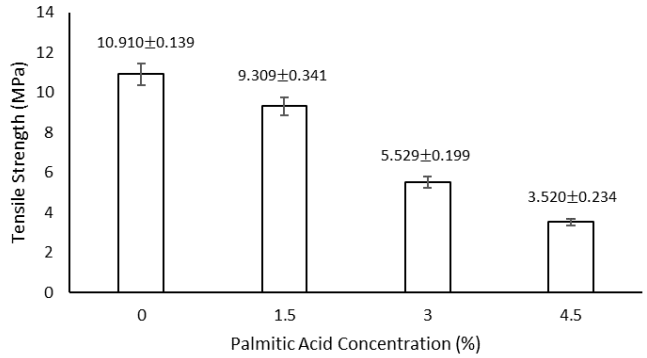

Figure 4. Tensile strength of edible film from seabass fish skin gelatine with different palmitic acid concentration

Tensile strength results of control edible film and edible film made from seabass fish skin gelatine with the addition of SPI (0%, 3%, 4% and 5%) and 0.7% glycerol was in the range of 5.130-2.502 MPa as shown in Figure 5. The highest tensile strength was found in an edible film without the addition of SPI (5.130 MPa) while the lowest strength was found in edible film with 5% SPI addition (2.502 MPa).

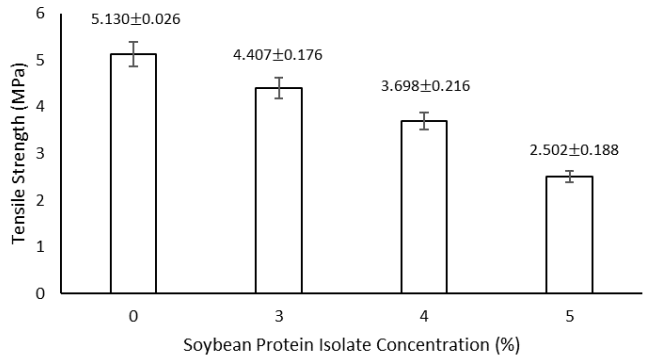

Figure 5. Tensile strength of edible film from seabass fish skin gelatine with different concentration soybean protein isolate

Based on the tensile strength test results of the edible film, it can be inferred that the values tend to decrease. This decrease is suspected due to the increase of palmitic acid and SPI concentration added to the edible film. The constant addition of glycerol plasticizer compared to the increasing concentration of palmitic acid and SPI also played a role, leading to less protein/fat-glycerol attraction, hence promoting the formation of weaker hydrogen bonds between the protein-protein and fat-fat molecules (Sanyang *et al*., 2015). Subsequently, the domination of strong hydrogen bonds produced by protein-protein and fat-fat intermolecular interaction over the protein-glycerol or fat-glycerol attraction will reduce the tensile strength of the edible film (Bai *et al*., 2012).

From the graph, it could also be concluded that edible film with palmitic acid addition has higher tensile strength compared to the edible film with SPI addition. Incorporation of lipids (palmitic acid) into the edible film led to a more compact structure of the film polymer network due to the strong interaction of crosslinking effect between the polymer and lipid particles, decreasing the free volume and mobility of the polymer molecules hence resulting in a higher tensile strength (Galus and Kadzinska, 2016).

3.2.3 Percent elongation

Per cent elongation of the edible film with palmitic acid addition is depicted in Figure 6, with results in the range of 21.30-34.44%. The highest elongation value was found on edible film with the highest palmitic acid addition (34.44%), while the lowest elongation value was found on edible film without the addition of palmitic acid (21.30%). The elongation values increase with increasing palmitic acid concentration due to the decreasing of the intermolecular bonds between the polymer chains and the formation of weaker hydrogen bonds which led to increasing elasticity (Sanyang *et al*., 2015).

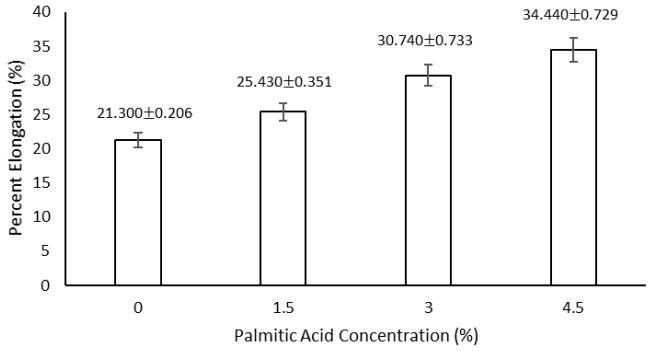

Figure 6. Percent elongation of edible film from sea bass fish skin gelatine with different palmitic acid concentration

Figure 7 shows the per cent elongation of the edible film with the addition of SPI results in the range of 36.063-117.51%. The highest elongation was found in the edible film without SPI addition (117.51%), while the edible film with the highest SPI addition has the lowest elongation of 36.063%.

Generally, the edible film with palmitic acid addition has lower elongation values compared to the films with SPI addition. Tensile strength and elongation value are inversely proportional since high tensile strength gave more structured and ordered molecules which led to lower flexibility. This statement was also supported by Chakravartula *et al*. (2019), who stated that edible films with high tensile strength would give lower values of

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elongation at the break due to compatibility and chemical synergetic interaction between components. The significant contrast of values between the tensile strength and elongation results for both edible films with palmitic acid and SPI addition was also due to the same reasons above, where films having little tensile strength is easier to deform and meant requiring higher strain to be broken down (Galus and Lenart, 2013).

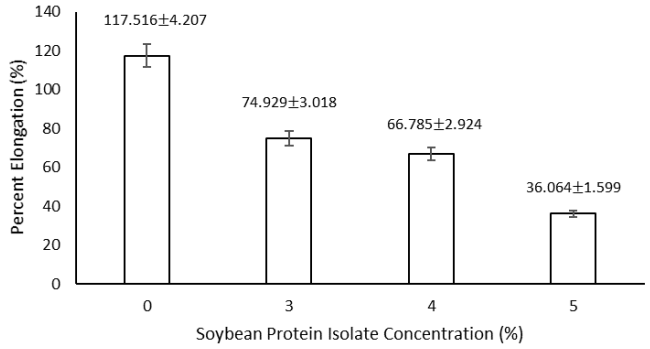

Figure 7. Percent elongation of edible film from sea bass fish skin gelatine with different concentration soybean protein isolate

3.2.4 Water vapour transmission rate

The water vapour transmission rate in this study decreases with the increasing addition of palmitic acid as indicated in Figure 8. The lowest value was found on film with 4.5% palmitic acid concentration, while the highest value was on 0% palmitic acid concentration. From these results, it can be inferred that the addition of palmitic acid can decrease the water vapour transmission rate. Palmitic acid has a hydrophobic property that will inhibit the rate of water vapour transmission on edible film. Increasing palmitic acid concentration added will result in a film with a low water vapour transmission rate (Seyedi *et al*., 2015). The fatty acid has a hydrophobic characteristic, consequently, it will inhibit the water vapour transmission rate. Therefore, increasing the concentration of palmitic acid added will result in a smaller rate of water vapour transmission.

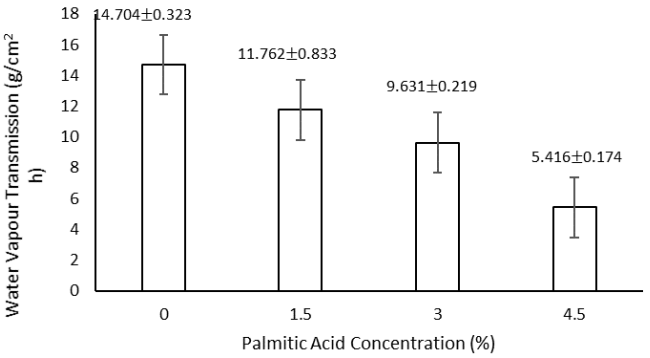

Figure 8. Water vapour transmission rate of edible film from seabass fish skin gelatine with different concentration of palmitic acid

In this study, it could be seen from Figure 9 that the addition of SPI in the edible film gave the average water vapour transmission of 8.910-15.473 g/m^2 h. The edible film which was made without the addition of SPI has the highest water vapour transmission of 15.473 g/m^2 h, whereas film with the addition of 5% SPI has the lowest value 8.910 g/m^2 h. Low water vapour transmission value indicates edible film could protect food material against water vapour, consequently, the product could have a longer shelf life. The water vapour transmission rate on the Japanese Industrial Standard has a maximum value of 10 g/m^{2}h (Shinta *et al.,* 2016). Therefore, it could be concluded that from the study results of edible film production with the addition of 3%, 4% and 5% SPI had met the requirements, while film without SPI addition has not yet met the requirements because the water vapour transmission value was below the given standard.

Water vapour transmission rate results in this study are not only influenced by the concentration of palmitic acid but also by edible film raw material and film thickness. Increasing film thickness will give a lower water vapour transmission rate. The edible film made from gelatine tends to has a high water vapour transmission rate because of its hydrophilic nature. Water vapour transmission rate depends on the ratio of hydrophilic and hydrophobic material in the biofilm formula (Herliany *et al.,* 2013). Generally, biofilm made from protein and polysaccharides has a high water vapour transmission value. Protein is a polar polymer with a big number of hydrogen bonds, therefore resulting in water adsorption in high humidity.

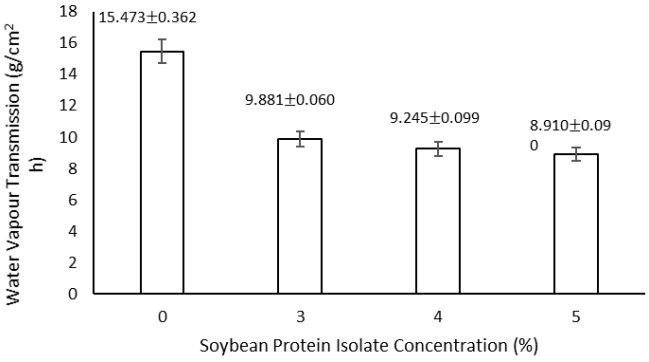

Figure 9. Water vapour transmission rate of edible film from sea bass fish skin gelatine with different soybean protein isolate concentration

3.2.5 Solubility

Figure 10 shows the average value of edible film solubility with palmitic acid addition tested on this study is 54.666-92.306%, with the lowest value on the addition of 4.5% palmitic acid (92.306%) and the highest on without addition of palmitic acid (54.666%). These results were in accordance with research carried out by

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Julianto *et al*. (2011), which stated palmitic acid is soluble in organic polar and non-polar solvents. The solubility of fatty acid in water is influenced by the length of the C chain. The longer the C chain, the fatty acid will be more difficult to dissolve in water, hence the lower solubility value (Khuwijitjaru *et al*., 2002).

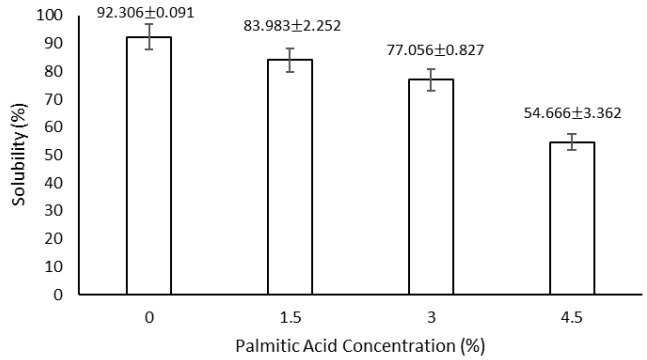

Figure 10. Solubility of edible film from seabass fish skin gelatine with different palmitic acid concentration

The solubility of the edible film with SPI addition showed a decreasing trend (Figure 11), with the highest solubility (31.670%) without the addition of SPI and the lowest solubility (22.800%) with 5% SPI addition. SPI addition will produce cross-linking covalent bonds during the edible film formation and as SPI concentration increases, the crosslinking degree will also increase which induced the formation of polymers with high molecular weight. Polymers with high molecular weight are difficult to be dissolved in water, due to the strong inhibition of the interaction between the polymer chains and water by the highly crosslinked polymers, hence the decreasing solubility of the edible film as the SPI concentration increases (Wittaya, 2012; University of Southern Mississippi, 2020).

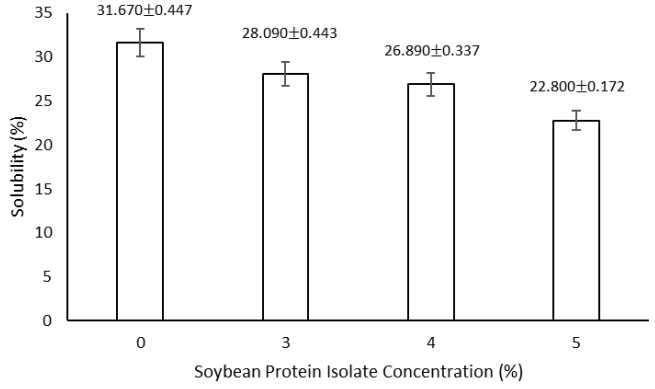

Figure 11. Solubility of edible film from seabass fish skin gelatine with different soybean protein isolate concentration

Solubility will influence the application of the edible film. The edible film with high solubility means it has low protection against water, therefore could only be used in products with low water content. Whereas edible film low solubility could be used in food with high water content because of its difficulty in dissolving. For the purpose of edible food wrapper, a high solubility property in the edible film is needed (Diova *et al.,* 2013).

4. Conclusion

The quality of gelatine made from sea bass fish skin through hydrolysis with acetic acid in this study has successfully created and met the regulated gelatine standards, with its viscosity of 3.40 cP and gel strength of 138.980 g bloom. The addition of palmitic acid (PA) and soybean protein isolate (SPI) on edible film from seabass fish skin gelatine has a significant influence on the characteristics of edible film. Based on the results, edible films with SPI addition yield the best film thickness (<2.5 mm) while PA addition resulted in better tensile strength of the films (>3.92 MPa) as per the Japanese Industrial Standard due to the strong crosslinking effect between the polymer and lipid particles. Meanwhile, the per cent elongation of both films with PA (21.3-34.44%) and SPI (36.06-117.53%) addition decreased with increasing concentration, but SPI generally gave higher elongation compared to PA due to the lower tensile strength found in SPI films which are easier to deform.

Water vapour transmission is one of the key parameters in edible film. It was found that the addition of SPI gave higher water vapour transmission values compared to PA due to the hydrophilic nature of the protein. The addition of 4.5% PA gave the lowest water vapour transmission (5.416 g/m^{2}h) among all the films because of the hydrophobic nature of lipids which improved the water barrier and resistance in the film. Based on the solubility results, edible films with PA addition (92.306-54.666%) are easier to dissolve in water compared to SPI films (31.670-22.800%). Therefore, it was concluded that the best film is the 4% SPI addition based on its thickness, elongation value, water vapour transmission and solubility results which all met the standard regulations and gave the best values for each property. Subsequently, the use of this edible film is suitable for food products that contain high water content and requiring packaging and/or coating with high flexibility.

Conflict of interest

The authors declare no conflict of interest.

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