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# Fish gelatin films incorporated with different oils: effect of thickness on physical and mechanical properties

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**Abstract**

Properties of fish gelatin films incorporated with different oils at different thickness investigated. Gelatin films incorporated with all oils resulted in higher elongation at break (EAB) compared to control film, regardless of the oils type ($P \leq 0.05$). Increasing the thickness of gelatin films with oils decreased the solubility value ($P \leq 0.05$) significantly. However, water vapor permeability (WVP) of gelatin films containing oils increased as the thickness of films increased. FTIR spectra showed that incorporation of different oils into gelatin films gave effect on the molecular organization and intermolecular interaction in films matrix particularly at the wavenumber of Amide-I band and 1739-1744 $cm^{-1}$. SEM analysis revealed the addition of oils into gelatin films enhanced the roughness of the film surface and cross-section. An appropriate combination of oils at moderate thickness could improve the mechanical and barrier properties of fish gelatin films thus fulfill the application either as coatings or films.



## Introduction

Demand for food packaging materials that offers biodegradable properties, environmentally safe and good film-forming abilities has increased tremendously. Biodegradable films from proteins have been used as packaging materials mainly due to their abundance, biodegradability, film-forming ability and nutritional qualities. Specific structure of proteins provides a wider range of potential functionalities resulting in various intermolecular bonding (Ou *et al*., 2005; Prodpran *et al.*, 2007).

Gelatin is a proteinaceous material that considered as a 'waste' and obtained from muscle food processing industry including meat, poultry and seafood (Nur Hanani, 2016). Gelatin can be used as a food additive, an edible coating, a film and as an encapsulating agent. Fish gelatin has been reported to have good film forming ability; yet, the film produced has poor water vapor barrier property (Jongjareonrak *et al.*, 2006; Hoque *et al*., 2010; Sahraee *et al*., 2017). This limits the further application of gelatin-based films as food packaging materials. However, properties of these films can be enhanced by adding some substances to the gelatin. Some hydrophobic substances such as oils, fats, waxes and fatty acids have been incorporated into fish gelatin films to improve the water vapor barrier and mechanical properties (Pérez-Mateos *et al*.,2009; Ahmad *et al*.,2012; Tongnuanchan *et al.*, 2012; Arfat *et al*.,2014). Furthermore, the incorporation of oils containing bioactive compounds in gelatin could be beneficial to food packaging industries.

In spite of some research has revealed the improvement of gelatin films added with several of oils, lack of studies relate the effect of thickness in tandem with oil incorporation reported. Physically, adding more oil in the film forming solution will cause the films produced to have thicker films with the same amount of emulsion. Besides addition of the hydrophobic substances, the thickness of films and film-forming dispersion (FFD) also influenced the film performance (Longares *et al*., 2004; Ma *et al*., 2012). Films with different thicknesses have different structural changes affecting the barrier and mechanical properties of those films. Previous studies showed that water vapor barrier and tensile properties were affected by reducing the film thickness. Longares *et al*. (2004) found the linear relationship between thicknesses of protein based edible film with water vapor permeability and elongation.

From our literature studies, there is a little work done regarding the effect of different thickness on the fish gelatin films incorporated with several types

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of oils. Therefore, the purpose of this study was to investigate the physical and mechanical properties of fish gelatin films incorporated with different oils (palm oil, soybean oil, corn oil, olive oil and lemongrass oil) with the thickness ranging from 40 to 80 µm.

## Materials and Methods

*Chemicals*

Fish skin gelatin from warm water fish (~ 240 Bloom) was purchased from Custom Collagen (Addison, USA). Palm oil (PO), corn oil (CO), soybean oil (SO), olive oil (OO) and lemongrass oil (LO) were purchased from Spectrum Chemicals. Glycerol and Tween-20 were obtained from Sigma-Aldrich Co. (St Louis, MO, USA) and were used as a plasticizer and an emulsifier, respectively.

*Film preparation*

The preparation of films was conducted by the method as reported by Nur Hanani *et al.* (2012) and Tongnuanchan *et al.* (2013) with slight modification. Gelatin powders were dissolved in deionized water at a concentration of 6% (w/v) to form film forming solution (FFS). Glycerol with 30% (w/w) was added based on gelatin content. Different types of oils were introduced into all gelatin solutions at a concentration of 25% (w/w based on gelatin content). To stabilize emulsion, Tween-20 was added as an emulsifier at 20% (w/w based on oil). Control films were prepared from FFS without addition of oils. All solutions were stirred using a magnetic stirrer hotplate and heated to 80ºC for 30 min. The film forming emulsions (FFE) were homogenized at 24,000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). FFE with different amount were cast into petri dish plate (14 x 14 $cm^2$) to produce films with various thicknesses; 40 µm (8 ml), 60 µm (10 ml), and 80 µm (12 ml). The film samples were conditioned in the humidity chamber at 50 ± 5% relative humidity (RH) and at a temperature of 23 ± 2ºC prior to testing.

*Film thickness*

Film thickness was measured using a hand-held digital micrometer (Mitutoyo, Serial No. 7301, Mitutoyo Corp., Kawasaki-shi, Japan) with measurements were carried out at ten different film locations.

*Mechanical properties*

Mechanical properties of fish gelatin films including tensile strength (TS), elongation at break (EAB) and Young's Modulus (YM) were determined as described by Iwata *et al.* (2000) with slight modification using the INSTRON 4302 Series IX Machine (Instron Co., Massachusetts, USA) equipped with tensile load cell of 1000 N.

*Water solubility of films*

The film solubility was determined according to the method of Nur Hanani *et al.* (2012) by trimming the samples into small strips (2 x 2 $cm^2$) and dried in an oven (Memmert UNB 300, Germany).

*Water vapor permeability (WVP)*

WVP of films were measured using a modified ASTM E-96 standard method (ASTM 1990) according to Nur Hanani *et al*. (2012). WVP of the film was calculated as follows:

$$WVP = w.l.A^{-}.t^{-1}\,\Delta p$$

w is the weight loss of the cup (g); l is the film thickness (mm); A is the exposed area of film ($m^2$); t is the time of gain (s) and Δp is the vapor pressure difference.

*Light transmission and film opacity*

The barrier properties against ultraviolet (UV) and visible light of gelatin films were measured using UV-vis spectrophotometer (Genesys 10-UV-Vis Spectrophotometer, Thermo Scientific) according to the method described by Tongnuanchan *et al.* (2012).

*Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)*

The IR spectra for gelatin films at 60 µm thickness were determined using a Nicolet 6700 FT-IR Spectrometer (Thermo Scientific, USA) equipped with horizontal attenuated total reflectance (ATR) Germanium, (Ge). Before film analysis, a background spectrum using a clean crystal cell was recorded. The spectra in the range of 500 nm to 4000 $cm^{-1}$ with automatic signal gain collected in 32 scans with a resolution of 4 $cm^{-1}$.

*Scanning electron microscopy (SEM)*

Morphology of surface and cross-section of films were visualized using a scanning electron microscopy (SEM) (JEOL JSM 6400, Tokyo, Japan). The samples were mounted on bronze stub by means double-sided tape and were sputtered with gold (Sputter Coater BAL-TEC SCD 005). The photographs were taken at an acceleration voltage of 12-15kV.

Table 1.Mechanical properties, solubility and water permeability of fish gelatin films incorporated with various oils and different thickness.

| Gelatin-oil Film | Tensile strength (MPa) | Elongation at Break (%) | Young's Modulus (MPa) | Water solubility (%) | WVP ($10^{-9}$ g mm $s^{-1}$ $m^{-2}$ $Pa^{-1}$) |
|---|---|---|---|---|---|
| **Control** | | | | | |
| 40µm | $6.20 \pm 0.36^{c,C}$ | $204.14 \pm 7.24^{b,B}$ | $27.86 \pm 3.93^{b,AB}$ | $60.07 \pm 0.32^{a,A}$ | $3.14 \pm 0.38^{b,A}$ |
| 60µm | $7.84 \pm 0.58^{b,B}$ | $243.34 \pm 29.74^{a,D}$ | $42.77 \pm 11.15^{a,AB}$ | $57.97 \pm 1.60^{ab,A}$ | $6.09 \pm 0.39^{a,A}$ |
| 80µm | $10.45 \pm 0.53^{a,AB}$ | $277.34 \pm 16.92^{a,D}$ | $48.37 \pm 1.70^{a,A}$ | $55.06 \pm 2.14^{b,A}$ | $6.42 \pm 0.18^{a,AB}$ |
| **PO** | | | | | |
| 40µm | $11.21 \pm 1.704^{a,A}$ | $412.45 \pm 37.76^{a,A}$ | $49.58 \pm 16.24^{a,A}$ | $43.71 \pm 1.94^{a,BC}$ | $2.61 \pm 0.23^{b,A}$ |
| 60µm | $10.49 \pm 0.594^{a,A}$ | $340.77 \pm 16.49^{b,BC}$ | $52.14 \pm 15.35^{a,A}$ | $39.3 \pm 2.96^{ab,C}$ | $3.31 \pm 0.42^{b,B}$ |
| 80µm | $11.57 \pm 0.926^{a,AB}$ | $414.10 \pm 19.20^{a,B}$ | $40.48 \pm 9.08^{a,AB}$ | $32.87 \pm 4.44^{b,BC}$ | $10.30 \pm 0.88^{a,A}$ |
| **SO** | | | | | |
| 40µm | $9.04 \pm 1.76^{a,AB}$ | $389.76 \pm 78.32^{a,A}$ | $15.68 \pm 8.10^{b,B}$ | $36.14 \pm 4.82^{a,D}$ | $2.67 \pm 0.22^{c,A}$ |
| 60µm | $9.46 \pm 1.39^{a,B}$ | $430.90 \pm 59.40^{a,AB}$ | $31.38 \pm 2.38^{a,BC}$ | $31.44 \pm 3.28^{a,D}$ | $3.20 \pm 0.22^{b,B}$ |
| 80µm | $9.68 \pm 0.57^{a,B}$ | $477.76 \pm 13.71^{a,A}$ | $30.43 \pm 3.99^{a,BC}$ | $21.79 \pm 1.60^{b,C}$ | $4.74 \pm 0.07^{a,AB}$ |
| **CO** | | | | | |
| 40µm | $8.61 \pm 0.92^{a,BC}$ | $454.95 \pm 18.41^{a,A}$ | $36.36 \pm 12.73^{a,AB}$ | $50.10 \pm 0.85^{a,B}$ | $2.71 \pm 0.51^{c,A}$ |
| 60µm | $10.78 \pm 1.16^{a,A}$ | $309.95 \pm 42.58^{b,CD}$ | $33.73 \pm 7.90^{a,BC}$ | $46.39 \pm 1.11^{b,B}$ | $5.64 \pm 0.13^{b,A}$ |
| 80µm | $9.69 \pm 1.61^{a,B}$ | $343.76 \pm 47.81^{b,C}$ | $35.18 \pm 4.49^{a,ABC}$ | $25.01 \pm 0.45^{c,C}$ | $7.05 \pm 0.52^{a,AB}$ |
| **OO** | | | | | |
| 40µm | $9.96 \pm 0.84^{a,AB}$ | $466.76 \pm 34.85^{a,A}$ | $41.68 \pm 15.05^{a,A}$ | $40.29 \pm 2.76^{a,CD}$ | $2.97 \pm 0.12^{a,A}$ |
| 60µm | $9.31 \pm 0.48^{a,AB}$ | $464.44 \pm 43.04^{a,A}$ | $21.33 \pm 4.77^{ab,C}$ | $33.71 \pm 3.21^{a,C}$ | $3.49 \pm 0.42^{a,B}$ |
| 80µm | $10.47 \pm 0.86^{a,AB}$ | $423.12 \pm 25.71^{a,AB}$ | $33.32 \pm 10.86^{b,BC}$ | $27.97 \pm 2.74^{b,BC}$ | $3.59 \pm 0.70^{a,B}$ |
| **LO** | | | | | |
| 40µm | $10.36 \pm 0.74^{a,AB}$ | $428.44 \pm 29.58^{a,A}$ | $28.31 \pm 10.56^{a,AB}$ | $34.37 \pm 0.43^{a,D}$ | $2.47 \pm 0.05^{c,A}$ |
| 60µm | $10.85 \pm 0.54^{a,A}$ | $355.92 \pm 14.0^{b,BC}$ | $23.16 \pm 10.80^{a,BC}$ | $34.56 \pm 0.28^{a,C}$ | $3.57 \pm 0.28^{b,B}$ |
| 80µm | $11.82 \pm 1.21^{a,A}$ | $345.1 \pm 27.91^{b,C}$ | $23.88 \pm 10.08^{a,C}$ | $34.41 \pm 0.51^{a,B}$ | $4.68 \pm 0.59^{a,AB}$ |

Different superscript letters (a,b,c,d) indicate significant differences (P≤0.05) in the same column under the same type of oil with different thickness including control. Different superscript letters (A,B,C,D) indicate significant differences (P≤0.05) in the same column under the same thickness including control.

*Statistical analysis*

Statistical analyses were performed using one-way analysis of variance (ANOVA) and Tukey's multiple test using Minitab 16 Software. Level of significance was set for P≤0.05.

## Results and Discussion

*Mechanical properties*

Films incorporated with PO, CO and LO showed higher values (P≤0.05) of TS compared to control film (Table 1). Different components in PO (carotenes, tocopherols, tocotrienols, sterols, etc.) and LO (myrcene, citronellal, geraniol, neral, limonene, citral, etc.) might interact with gelatin at different degree, thus give different impact to TS of films. This is in agreement with Ahmad *et al.* (2012) who stating that different compound of lemon grass oil in gelatin films has caused higher TS compared to film with bergamot oil. Also, viscoelasticity of PO, CO and LO might contribute to its high TS compared to the other oils. The proper viscoelasticity facilitated the forming and stability of the smaller oil droplets during emulsion, thus contributing to strong interaction of oil and gelatin (Xiao *et al.*, 2016; Nur Fatin *et al.*, 2017). Furthermore, the effect of lipid addition on the mechanical properties of film depends on both the characteristics and its capacity to interact with the protein matrix. Among all incorporated films at the thickness of 60 µm, film with OO has the lowest TS and highest EAB. OO has high concentration of oleic acid content compared to other oils. Fabra *et al.* (2010) showed that oleic acid interacts with the protein matrix forming bonds through polar groups, where the interaction balances in the protein network acting as plasticizer has been modified and increasing the film flexibility.

Increasing the thickness of control films has shown a significant increase in TS values of films (P≤0.05). However, there are no significant differences (P>0.05) of TS values observed when the thickness of the composite films increased, regardless of oils. Yet the trend increased. Thicker films caused the polymer matrix become denser and higher in inter and intra molecular interactions and consequently more resistant to rupture (Mali *et al.*, 2005).

The incorporation of oils into gelatin films showed higher (P≤0.05) EAB compared to control film at 60 µm thicknesses. This result might be due to the homogenization condition of FFE have lead the small lipid particles embedded in the protein network, which seemed to have some plasticizing effect, thus contributed to more stretchable films. Atarés *et al.* (2010) found that increasing cinnamon oil content into SPI film had led to more extensible films. Adding the thickness of control films from 40 to 60 µm has increased (P≤0.05) the EAB value significantly. Nonetheless, the increase of composite films thickness resulted lower EAB values except for films with SO. Janson and Thuvander (2004) also found the similar effect probably due to large difference in the thickness.

Table 2.Light transmittance and transparency value of fish gelatin films incorporated with various oils and different thickness.

| Gelatin-oil film | Light transmission (%) at different wavelength (nm) | | | | | | Opacity value |
|---|---|---|---|---|---|---|---|
| | 200 | 280 | 400 | 500 | 600 | 800 | |
| **Control** | | | | | | | |
| 40µm | 0.031 | 0.049 | 68.10 | 71.61 | 72.99 | 74.91 | $1.43 \pm 0.12^{b,C}$ |
| 60µm | 0.026 | 0.044 | 72.89 | 76.66 | 77.49 | 78.79 | $1.85 \pm 0.35^{ab,C}$ |
| 80µm | 0.028 | 0.041 | 72.76 | 75.90 | 76.83 | 78.26 | $2.44 \pm 0.30^{a,D}$ |
| **PO** | | | | | | | |
| 40 µm | 0.080 | 0.097 | 37.06 | 43.42 | 47.09 | 52.03 | $6.46 \pm 0.057^{b,A}$ |
| 60 µm | 0.061 | 0.055 | 25.90 | 31.63 | 34.96 | 39.48 | $6.91 \pm 0.81^{ab,A}$ |
| 80 µm | 0.022 | 0.048 | 21.36 | 27.62 | 31.72 | 37.64 | $7.75 \pm 0.462^{a,B}$ |
| **SO** | | | | | | | |
| 40 µm | 0.055 | 0.076 | 55.13 | 62.52 | 65.85 | 69.05 | $2.76 \pm 0.150^{b,C}$ |
| 60 µm | 0.045 | 0.069 | 44.22 | 52.49 | 56.16 | 60.54 | $3.82 \pm 0.944^{b,B}$ |
| 80 µm | 0.034 | 0.062 | 43.84 | 54.98 | 60.09 | 66.19 | $5.52 \pm 1.158^{a,C}$ |
| **CO** | | | | | | | |
| 40 µm | 0.057 | 0.099 | 46.55 | 52.17 | 55.31 | 58.88 | $4.34 \pm 0.391^{b,B}$ |
| 60 µm | 0.034 | 0.087 | 47.85 | 54.91 | 58.91 | 63.65 | $4.04 \pm 0.652^{b,B}$ |
| 80 µm | 0.030 | 0.750 | 33.76 | 40.66 | 45.02 | 51.02 | $6.25 \pm 0.707^{a,BC}$ |
| **OO** | | | | | | | |
| 40 µm | 0.052 | 0.077 | 39.42 | 44.23 | 46.63 | 49.93 | $6.65 \pm 1.15^{b,A}$ |
| 60 µm | 0.046 | 0.064 | 8.38 | 14.97 | 21.51 | 33.76 | $7.32 \pm 0.65^{b,A}$ |
| 80 µm | 0.040 | 0.043 | 18.10 | 22.20 | 24.83 | 28.56 | $11.04 \pm 0.02^{a,A}$ |
| **LO** | | | | | | | |
| 40 µm | 0.070 | 0.081 | 40.5 | 69.17 | 77.71 | 81.75 | $1.95 \pm 0.417^{a,C}$ |
| 60 µm | 0.063 | 0.078 | 24.62 | 61.67 | 75.88 | 80.58 | $2.00 \pm 0.219^{a,C}$ |
| 80 µm | 0.042 | 0.061 | 18.69 | 60.63 | 78.55 | 83.68 | $2.73 \pm 0.549^{a,D}$ |

Different superscript letters (a,b,c,d) indicate significant differences ($P \leq 0.05$) in the same column under the same type of oil with different thickness including control. Different superscript letters (A,B,C,D) indicate significant differences ($P \leq 0.05$) in the same column under the same thickness including control.

YM is an indicator of film rigidity. No significant difference was observed for films with oils and control, irrespective of oils used. Nonetheless, film incorporated with PO had the highest values, 52.14%. This might be due to high saturated fatty acid (40.9% of palmitic acid) content in palm oil that cause the film more rigid and stiffen.

*Film solubility*

Table 1 shows the solubility of fish gelatin films incorporated with different types of oils. Film solubility is the measure of the water resistance and integrity of film (Rhim *et al*., 2000). Adding oils into the films has decreased ($P \leq 0.05$) the solubility significantly, regardless the thickness. The addition of oils as hydrophobic substance into fish gelatin films could have lowered the solubility as supported by Ahmad *et al.* (2012). The films possibly have high-stable protein-lipid polymer network since they did not break apart after immersed into water for 24 hours. Increasing the thickness of composite films contributed to decreasing solubility ($P \leq 0.05$) except for films with LO. The result suggested that the thicker the films will cause the non-polar oils interacted stronger with hydrophobic domain of gelatin, subsequently reduced the solubility. Comparing the films with oils at intermediate thickness, 60 µm, film incorporated with SO was less soluble, meanwhile film with CO has high solubility, 46.39%.

*Water vapor permeability*

No significant effects of WVP were observed for the films with oils and control at 40 and 80 µm, respectively (Table 1). This means types of oil used do not affect the water barrier of the films. However, at 60 µm, WVP values were decreased ($P \leq 0.05$) when the oils were added to the solution except for the films with CO. The hydrophobic substance, in this case oils could increase the hydrophobicity of films, thereby reducing the water vapor migration through the films. Basically, the blend films based on protein have the decreased WVP with increasing content of lipids or hydrocarbon. The result was in agreement with Ma *et al*. (2012) who reported that the inclusion of olive oil decreased the WVP significantly in gelatin films compared to control film. Similar result was obtained by Tongnuanchan *et al.* (2013), where the WVP of gelatin films incorporated with different root essential oils (ginger, turmeric and plai) decreased gradually with increasing concentration of oils. CO showed the highest WVP compared to the other oils probably due to the hygroscopic nature of the oils used which gave different effect on WVP of films where CO has been reported to contain relatively low level (<15%) of saturated fatty acids content (Robert, 2002). Besides, CO also has high degree of unsaturated fatty acids, specifically linoleic acid (C18:3) at 58% (Robert, 2002) that might cause the films have poor water vapor barrier. This result was supported by Tanaka *et al.* (2001) who found that the effect of reducing WVP was dependent upon the low degree of unsaturation of C18 fatty acids. Oils have different ability to attract water to the film network and the interactions of oil components with some hydrophilic protein domains could promote the decrease in the hydrophobic character of film matrix (Ahmad *et al*.,

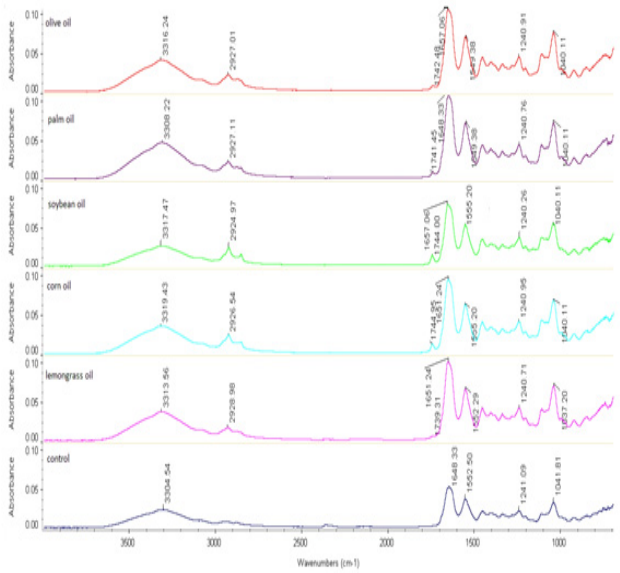


Figure 1.ATR-FTIR spectra of fish gelatin films incorporated with different vegetable oils

2012). Moreover, the possible formation of laminar-like structures during film drying, as took place in CO incorporated film, and the reduction of the particle size of CO aggregations to increase the tortuosity factor in the continuous matrix could be the factor of the higher WVP (Fabra *et al*., 2010).

The results also showed WVP of the control films increased (P≤0.05) when the thickness increased. A similar trend was also found in films with PO, SO, CO and LO. As the film thickness increases, the film provides increased resistance to mass transfer across it, so the equilibrium water vapor partial pressure at the inner surface increases (Longares *et al.*, 2004).

*Light transmission and opacity*

The transmission of UV light was low for all films incorporated with oils, regardless the thickness and types of oils (Table 2). The results suggested that the incorporation of oils into the films could lower the light transmission and improve light barrier properties. Film at the thickness of 80 µm showed the lowest light transmission (%) at almost all wavelengths for all types of oil. This could be due to the light scattering effect at the interface of vegetable oils droplets imbedded in the film matrix (Tongnuanchan *et al*., 2012). The light transmittance for each film also increased as the wavelength increased. This is correlated with the lower light transmission of the films. Meanwhile, the opacity values of gelatin composite films with higher thickness from 40 to 80 µm had increased significantly (P≤0.05) compared to control film, indicating the films were opaque except for films with LO.

*Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy*

FTIR spectra have been used to monitor the

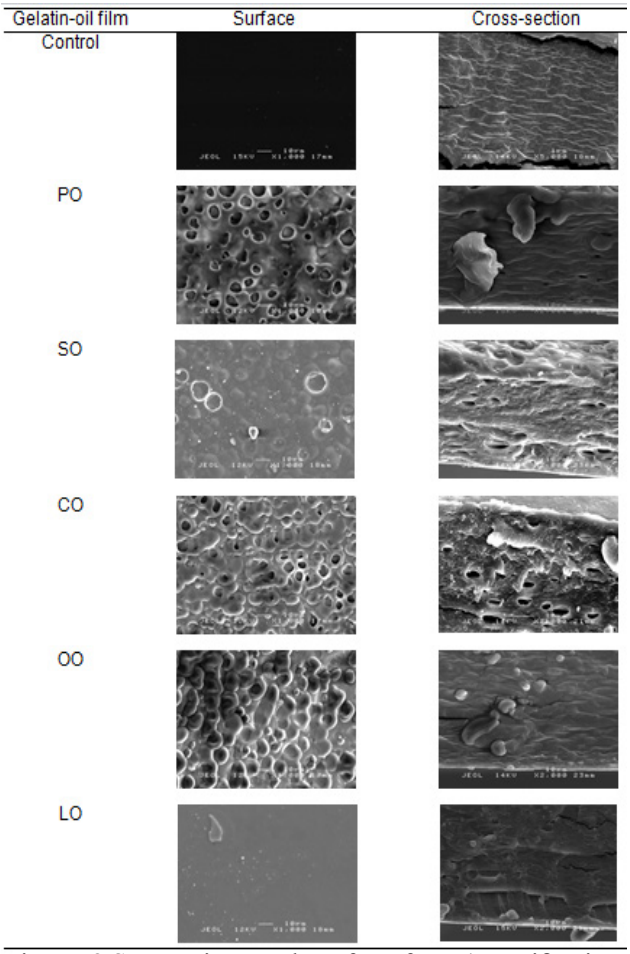


Figure 2.SEM micrographs of surface (magnification: 1000x) and cross section (magnification: 2000x) of films from fish skin gelatin incorporated with different oils (60 µm).

functional groups and structural changes of film samples at molecular level through a detailed spectral analysis (Ahmad *et al.* 2012). As shown in Figure 1, the major absorption peaks of gelatin films were found at 1648-1657 $cm^{-1}$ (amide I), 1549-1555 $cm^{-1}$ (amide II), 1240 – 1241 $cm^{-1}$ (amide III), 3304-3319 $cm^{-1}$ (amide A) and 2924-2928 $cm^{-1}$ (amide B). All the films incorporated with oils showed higher absorbance compared to control film, which was the characteristic to the saturated fatty acids (Alexa *et al*., 2009).

Amide-I band illustrating the C=O stretching vibration where, control film, PO, SO, CO, OO and LO incorporated gelatin films displayed the amide-I bands at the wavenumbers of 1648.33, 1648.33, 1657.06, 1651.24, 1657.06 and 1651.24 $cm^{-1}$, respectively. Different conformation and orientation of polypeptide chains as affected by incorporation of oils showed the spectral differences between different films samples. Spectral result showed that the peak of gelatin films incorporated with SO and OO had shifted to the higher wavenumber at this band compared with other films. This might be due to the interaction of gelatin network with SO and OO had produced high amount

of low molecular weight components, which C=O reactive group could be more exposed and become more reactive between α-chains (Kittiphattanabawon *et al.*, 2010). Besides that, narrow absorption, normally centered on 1650 $cm^{-1}$ is indicative of olefinic unsaturation (C=C) (John, 2000). This justifies the result of film with OO that possessed higher wavenumber at this band, related to the high oleic acid content (55-85%) in OO. Meanwhile for amide II (illustrating the bending vibration of N-H groups and stretching vibration of C-N groups) bands were observed at the wavenumber of 1549.38 – 1555.20 $cm^{-1}$ with SO and CO incorporated gelatin film having the highest frequencies.

Amide-III (presented the vibrations in plane of C-N and N-H groups of bound amide as well as vibrations of $CH_2$ groups of glycine backbone and proline side-chains of gelatin molecules) bands were observed at the wavenumber of 1240.26 – 1241.09 $cm^{-1}$. It was shown that the peak of control film was lower than the other films. The peak around 1740 $cm^{-1}$ was attributed to ester carbonyl functional group of the triglycerides. There was no peak observed in control film since there was an absent of oil.

All films had similar peaks at amide-A region represented the NH-stretching coupled with hydrogen bonding, which indicated the present of nitrogen in gelatin as a protein biopolymer. The highest wavenumber can be seen in CO incorporated film, while control film showed the lowest wavenumber. The peak around 2925 $cm^{-1}$ is attributed to the symmetric stretching vibration of the aliphatic CH2 group (Vlachos *et al*., 2006). The peak heights denoted the percentage of the hydrogen-carbon bond coupled by cis-double bond (=CH) which represent triglyceride functional groups present in the oil. The peaks were higher in films added with oils, in comparison with the control film. Also, gelatin film incorporated with LO had higher wavenumber at this band compared to others probably due to the indicative for the absent of aromatic compound (Edwin *et al*., 2012).

The peak around 1040 $cm^{-1}$ was found in all film samples, corresponding to the present of OH-group contributed by glycerol which added as a plasticizer (Bergo and Sobral, 2007). Therefore, incorporation of oils into gelatin films affected the molecular organization and intermolecular interaction in film matrix.

*Scanning electron microscopy (SEM)*

SEM images (Figure 2) show that control film has a smooth surface compared to other films. This indicates that there is homogenous protein network present in control film without lipid. Besides, smooth and continuous surface also can be seen in LO incorporated film, where LO might be evaporated during drying, thus lead to the micro-pores formation throughout the films. Similar images had been reported by Tongnuanchan *et al.* (2012) whereby fish gelatin films incorporated with citrus essential oils have continuous surface as the FFS had the stable emulsion system and no collapse of emulsion occurred during FFS dehydration. LO also might be more likely to localized inside the film network, whereby no oil droplet on the surface of film was noticeable (Tongnuanchan *et al*., 2014). This is contradicted with the other films incorporated with PO, SO, CO and OO which SEM images showed discontinuous surface with heterogeneous distribution of oils. Nevertheless, SO incorporated film has less oil droplets agglomerates on the surface compared to PO, CO and OO. This could be attributed by the protein-lipid interaction and the emulsion system in the films that not well-homogenized. Films incorporated with PO and CO have the most cavities and porous. This uneven structure could be related to the differences of WVP obtained in films.

From cross-section images, control film has more homogeneous structure compared to oil incorporated films. Protein bonding was suggested being well-interacted to each other without disruption of oil droplet. Similar image can be seen in the film incorporated with LO where the structure was more compact and smoother than the other oils. However, the addition of oils into gelatin films enhanced the roughness of the film cross-section. CO incorporated film showed the most porous structure for cross-section image and might be the reason of high WVP value compared to the other oil incorporated films at 60 μm.The surface of films incorporated with OO showed oil droplets were full and compact to each other, whilst the cross-section showed lack of pin hole or crack. This structure could prevent the process of water passage through the film.

## Conclusion

Incorporation of different oils into gelatin films gave significant effect on physical and mechanical properties whereby highest TS, EAB and YM values were found in films incorporated with LO, OO and PO, respectively. WVP of gelatin films also reduced with the oil added. However, increasing films thickness has caused an increase in the WVP, regardless the oil types. This study has provided additional evidence with respect to thickness effect towards physical and mechanical properties of gelatin composite films.

Also, use of appropriate oils at moderate thickness is crucial to ensure films produced can fulfil future applications either as coatings or packaging films.

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