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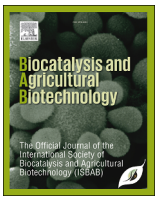

Physical, mechanical and antioxidant properties of chicken skin gelatin films incorporated with virgin coconut oil

N.A.M. Jusoh [a], M.I.N. Isa [b], N.M. Sarbon [a, *]

[a] Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia
[b] Faculty of Science and Technology, Universiti Sains Islam Malaysia, 71800, Nilai, Negeri Sembilan, Malaysia

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ABSTRACT

This study aimed to determine the physical, mechanical, and antioxidant properties of chicken skin gelatin films incorporated with virgin coconut oil (VCO). Prepared chicken skin gelatin films at different VCO concentrations (0–30%) were characterized for their physical (water vapor permeability and light transmission), mechanical (tensile strength, elongation at break), and antioxidant (DPPH scavenging activity and total phenolic content) properties. The tensile strength and elongation at the film's break showed a decreasing and increasing trend with the increase of VCO concentration. The water vapor permeability of films showed no significant difference as the added oil concentration increased. In addition, the films with VCO added showed a good light barrier with a lower light transmission value at a high oil concentration. The incorporation of oil in gelatin films decreases the glass transition temperature (T_g) with the increasing amount of oil. Furthermore, the film's microstructure became rougher and more discontinuous with the addition of oil percentage. The increasing VCO concentration also enhanced the intensity of the Amide A, Amide I, Amide II, and Amide III functional groups. DPPH scavenging activity and total phenolic content in films also increased with VCO concentration. Thus, these results revealed that VCO could be incorporated with gelatin film to make active film packaging or edible film packaging for some food applications for protein-rich food, such as meat or edible packaging for primary packaging.

1. Introduction

Active packaging is packaging in which the package, product, and environment interact together to prolong shelf life and maintain the quality of the product (Prasad and Kochhar, 2014). Active packaging, especially antioxidant packaging, is commonly used to extend a product's shelf life from deterioration, such as nutritional losses and color and flavor changes caused by oxidation (Byun et al., 2010). An active packaging system incorporates natural extracts in food packaging material that can provide antimicrobial and antioxidant properties in the dynamic packaging system and thus prolong the shelf life of the food product (Azman et al., 2022). Some studies reported that the application of synthetic or natural antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and crude extract in packaging film enables retardation of lipid oxidation in food (Barbosa-Pereira et al., 2014; Nazmi and Sarbon, 2020).

Gelatin is an insoluble protein from bones, skin, and connective tissue produced as waste products during slaughter and processing (Said and Sarbon, 2021; Sarbon & Said, 2022). Gelatin can be divided into two types which are type A and type B. Type A gelatin is

* Corresponding author.
E-mail address: norizah@umt.edu.my (N.M. Sarbon).

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obtained from acid-treated collagen meanwhile, type B gelatin is obtained from an alkali-treated precursor. Type A and type B gelatin are commonly derived from pigskin and mammalian animal skin, respectively (Nur Hanani et al., 2014; Said et al., 2021). Gelatin was also reported to have unique functional properties such as gel strength and viscosity, which had been used as an application in food, pharmaceutical, cosmetic, and photographic industries. Recently, gelatin has been used in studies on biodegradable films due to its excellent properties, such as good oxygen barrier, and also is produced in abundant raw materials at a low cost (Cheng and Sarbon, 2020; Lau and Sarbon, 2022). Most gelatin is obtained from mammalian species such as pigskin, bovine hide, and cattle bones. However, the production of gelatin from alternatives to mammalian species is increasing, especially from the by-products of fish skin and chicken skin, as they are abundant at low cost besides contributing to the better physical and mechanical properties of the intelligent packaging films as compared to other oils and gelatin alternative (Sarbon et al., 2013).

Virgin coconut oil (VCO) is a natural oil obtained from the mature kernels of coconut without undergoing chemical refining or heat treatment (a M. Marina et al., 2009). Some studies also reported that VCO possessed good antioxidant capacity and total phenolic compound compared to refined coconut oil due to the removal of coconut testa in the production of VCO. Antioxidant is the main compound in the VCO due to their function as free radical scavengers, reducing agents, quenchers of the formation of singlet oxygen, and complexes of pro-oxidant metals (M. Marina et al., 2009). Moreover, VCO has been used widely in beauty treatments and to treat minor diseases such as skin inflammations and diarrhea due to its antimicrobial and antiviral activity from lauric acid components (Mansor et al., 2012). Therefore, this study aims to determine the effect of different concentrations of VCO on the physical, mechanical, and antioxidant properties of emulsion gelatin-based films.

The novelty of this film is that the chicken skin gelatin used in the film production possessed better properties than other gelatin alternatives. Chicken skin gelatin can have properties similar to bovine gelatin. Meanwhile, incorporating virgin coconut oil with the chicken skin gelatin film contributes to the intelligent packaging films' better physical and mechanical properties than other oils and gelatin alternatives.

2. Materials and methods

2.1. Materials

Fresh chicken skins were purchased from TD Poultry Sdn. Bhd. and chilled in ice during transportation to Universiti Malaysia Terengganu (UMT). Then, chicken skins were washed and weighed. The chicken skins were stored in a freezer at a temperature of −18 °C for further experiments. Meanwhile, virgin coconut (VCO) oil was purchased from Mutiara Dinamik Maju Sdn. Bhd. and stored at room temperature for further experiments. All chemicals used for analysis were of analytical grade.

2.2. Methods

2.2.1. Chicken skin preparation

The chicken skin was prepared according to Sarbon et al. (2013). First, frozen chicken skins were thawed in a chiller (4–5) overnight. Visible fat was removed then chicken skins were rinsed in excessive water to remove impurities before cutting into 2–3 cm pieces. Finally, the chicken skins were defatted using the Soxhlet method (Azman et al., 2022).

2.2.2. Gelatin extraction

Chicken skin gelatin was extracted following Sarbon et al. (2013). Firstly, 15 g of defatted dried chicken skin was mixed with 400 ml of sodium hydroxide (0.15% w/v). The mixture was shaken and stirred slowly for 30 min at room temperature. Then, the mixture was centrifuged using a centrifuge (Multi-purpose Centrifuge 1580R, Korea) at 3500 × *g* for 10 min. The alkaline solution was changed every 30 min three times to remove non-collagenous protein and pigments. Next, the alkaline-treated chicken skin was rinsed with distilled water before mixing with 400 ml 0.15% (v/v) sulphuric acid for 30 min using a magnetic stirrer. The sulphuric acid solution was changed every 30 min three times. This step was repeated with 400 ml of 0.7% (w/v) citric acid solution. The mixture was shaken and stirred lightly for 30 min using a magnetic stirrer, and the citric acid solution was changed every 30 min three times. Then the mixture was centrifuged at 3500 × *g* for 10 min. Then, the pellet was rinsed with distilled water to remove residuals and centrifuged at 3500 × *g* for 15 min. Lastly, the treated chicken skin was mixed with distilled water and heated in a water bath overnight at 45 °C. The mixture was filtered using filter paper before reducing the volume to 1/10 through evaporation under a vacuum at 45 °C. The resultant solution was frozen in the freezer overnight before freeze-drying. Therefore, the obtained dry matter was known as gelatin powder.

2.2.3. Film preparation

Film-forming solutions were prepared using different ratios of gelatin to virgin coconut oil (VCO) (w/w): A (100/0), B (100/10), C (100/15), D (100/20), E (100/25) and F (100/30). Following the method described by Ma et al. (2012a,b), glycerol at 30% (w/w) of total weight as a plasticizer and 100 ml distilled water were added into the film-forming solution. The chicken skin gelatin powder was dissolved in distilled water at 45 °C for 30 min until a clear solution was obtained before VCO was added. The film solution was stirred and heated at a temperature of 45 °C until the oil dispersed. The solution was then cooled to room temperature. Each film solution was filtered to remove air bubbles before being cast on a Petri dish with a diameter of ~8.5 cm and oven dried at 45 °C for 3–4 days. Approximately 25 g of film solution was allocated to each Petri dish to maintain uniform thickness.

2.2.4. Functional properties

2.2.4.1. Tensile strength (TS) and elongation at break (EAB) determination. Tensile strength and elongation at the film's break were measured using a texture analyzer (TA XT Plus- Stable Micro Systems, UK) (Guo et al., 2014). The film was cut into the size of 10 mm × 70 mm. The initial grip separation and mechanical crosshead speed were set at 30 mm and 50 mm/min, respectively. Then, the film strip was mounted between the AT/G probe grip pairs. The film was stretched by moving the upper grid. The following equation calculated the tensile strength and elongation at break:

$$\text{Tensile Strength (MPa)} = \frac{F_{max}}{A}$$

where F_{max} is the maximum load (N) and A is the cross-sectional area (m^2) of the film strip.

$$\text{Elongation at break (\%)} = \frac{L - L_i}{L_i} \text{ x } 100$$

where L is the film elongation (mm) at the moment of rupture and L_i is the initial grip length (mm) of sample.

2.2.4.2. Water vapor permeability (WVP) determination. The film's water vapor permeability (WVP) was determined using the ASTM E96 method, slightly modified by Jahit et al. (2016). The film was sealed over 25 mm × 25 mm of the plastic cup, and 15 g of silica gel (0% RH) was put inside the cup. Together with the film, the cup was weighed (initial weight). Next, the cup was placed in a desiccator containing distilled water at room temperature. Then, the final weight of the cup was recorded every 1 h for 8 h. Finally, WVP was calculated using the following equation:

$$\text{WVP } \left(\text{g mm/m}^2\text{.h}^1\text{.kPa}^1\right) = \frac{w \times x}{A \times t \times P}$$

where w is the weight gain of a cup (g), x is the average film thickness (mm), A is the area of the film surface exposed to the permeant (m^2), t is the time of gain (h), and P is the partial pressure of atmosphere with silica gel and pure water (3.159 kPa at 25 °C).

2.2.4.3. Light transmittance and transparency determination. The light barrier properties of the film were measured according to Hosseini et al. (2013). The film was cut into 10 mm × 50 mm and placed directly into the test cell. The transmittance (%) against UV and visible light at a selected wavelength between 280 and 800 nm was measured using a UV–Vis spectrophotometer (UVmini-1240, Japan). An empty test cell was used as the reference. The following equation calculated the transparency of the film:

$$\text{Transparency} = -\log T_{600}/x$$

Where T_{600} is the transmittance at 600 nm and x is the film thickness (mm).

2.2.4.4. Thermal properties determination. Thermal properties (glass transition) of the film were measured according to Hosseini et al. (2013) using a differential scanning calorimeter (DSC) (Q2000 TA Instrument, USA). About 3 mg of the film was weighed and put into aluminum pans. The pan was then hermetically sealed before being heated over 25–150 °C at a scanning rate of 5 °C/min. Meanwhile, an empty aluminum pan was used as a reference. T_g value was measured at the changing phase of the solid to liquid state. The glass transition (T_g) temperature was obtained from the thermograms.

2.2.4.5. Film morphology determination. The morphology of the film produced was investigated following Tongnuanchan et al. (2015) using a scanning electron microscope (SEM) (Tabletop Microscope TM1000, Japan). The film was fractured under liquid nitrogen before visualization. Then, the film was mounted on copper stubs perpendicularly to its surface and coated with gold to make the sample conductive. The image was captured at an acceleration voltage of 15 kV with a magnification range of 1500 to 3000X.

2.2.4.6. Functional groups determination. The functional group of the film was determined according to Tongdeesoontorn et al. (2011) using an FT-IR spectrometer (Nicolet iS10 Thermo Scientific, USA). Briefly, the film was cut and placed on the attenuated total reflection (ATR) crystal surface for scanning. FTIR spectrum was set at a range of 4000-400 cm^{-1} with 32 numbers of scans and 4 cm^{-1} resolutions. The peak of Amide A, Amides I, II, and III were defined.

2.2.5. Antioxidant properties

2.2.5.1. DPPH scavenging activity. The antioxidant activity of the film was measured according to the method by Dashipour et al. (2015) with a slight modification. Firstly, 25 mg of the prepared film was dissolved into 5 ml of distilled water and stirred. Then, 0.1 ml of film extract solution was mixed with 3.9 ml of 2,2-diphenyl-1-picryl hydrazyl (DPPH) solution (0.1 mM in methanol solution). Then, the solution was vortexed and incubated in a dark place at an ambient temperature for 30 min. Methanol was used as a blank. The absorbance of the resulting solution and blank sample was measured at 517 nm using a spectrophotometer (UV–Visible Spectrophotometer Spectroquant® Pharo 300, EU). Therefore, the percentage of DPPH free radical scavenging activity was determined according to the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \text{ x } 100$$

where Abs_{blank} is the absorbance value of the methanol solution DPPH, meanwhile Abs_{sample} is the absorbance of the sample extract.

2.2.5.2. Total phenolic assay. Total phenolic content was measured according to Dashipour et al. (2015). Firstly, 25 mg of the prepared film was dissolved into 7 ml of distilled water and stirred. Then, 0.1 ml of film extract solution was mixed with 7 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent before storing the solution for 8 min at room temperature. The mixture was added with 1.5 ml (2% w/v) of sodium carbonate solution and distilled water until the final volume reached 10 ml. The mixture was placed for 2 h at room temperature. After that, the absorbance was measured at 765 nm using a spectrophotometer (UV–Visible Spectrophotometer Spectroquant® Pharo 300, EU). Then, the gallic acid solution was prepared at the specific concentration range same as the range for constructing the calibration curve. Therefore, the concentration of total phenolic compounds in the sample was expressed as gallic acid equivalents (GAE mg/mg) per gram of dried film. The total phenolic compound was calculated as follows:

$$\text{Total phenolic (mg GAE/g sample)} = \frac{C \times V}{M}$$

C is the concentration of gallic acid obtained from the standard calibration curve (mg/mg), V is the volume of film extract (ml), and M is the weight of dried film (g).

2.3. Statistical analysis

All the analysis was performed in triplicate, and the data were presented by (mean ± standard deviation). Then, the data obtained were analyzed using one-way Analysis of Variance (ANOVA) of Minitab-14.0 software. Fisher's Test compared the means with a confidence level of $p < 0.05$.

3. Results and discussion

3.1. Functional properties

3.1.1. Tensile strength and elongation at break

Table 1 shows the tensile strength (TS) of chicken skin gelatin films with different percentages of virgin coconut oil (VCO). The tensile strength (TS) of packaging materials is important in determining the protection and tampering resistance of food packaging (Said and Sarbon, 2022). Tensile strength value for film formulation A, B, C, D, E and F were 0.70, 0.48, 0.46, 0.43, 0.41 and 0.29 MPa, respectively. This result indicated that the inclusion of VCO in gelatin film decreased the film's tensile strength. Wittaya (2012) reported that the lower tensile strength of emulsion film is caused by the plasticizing effect of oil added to the film. Virgin coconut oil is more likely to disrupt protein-protein interaction in the film network, promoting the film matrix's discontinuity (Tongnuanchan et al., 2015). Therefore, the lack of continuity in the film network impacted the film's tensile strength. Similarly, bovine-hide gelatin film with carvacrol also showed lower tensile strength as carvacrol concentration increased (Kavoosi et al., 2013). Including hydrophobic substances such as essential oils into gelatin composite films reduced the TS value, as these substances may hinder or interfere with protein-protein interaction in the film network. This leads to the discontinuity of the film matrix and lack of cohesive structure integrity of the film network and, thus, lowers the strength of films (Said and Sarbon, 2022).

Table 1 presents the elongation at break (EAB) of chicken skin gelatin film with different percentages of VCO. The addition of VCO revealed an increase in the trend, which is 117.47, 123.48, 134.01, 157.58, 164.22, and 183.57% for film formulations A, B, C, D, E, and F, respectively. These results indicated that chicken skin gelatin film without adding oil was more firm and less extensible than the film incorporated with oil. Similarly, with tensile strength, the interaction between gelatin molecules in these emulsion films was also disrupted due to oil molecules acting as plasticizers. The results of EAB were supported by a study conducted by Tongnuanchan et al. (2015), as they found that gelatin films incorporated with palm oil increased EAB's value as the concentration of palm oil increased. In addition, the plasticizing effect of oil in emulsion film is due to the oil concentration and chemical composition of oil, mainly linoleic (C18:2) and oleic (C18:1) acids (Galus et al., 2016). As M. Marina et al. (2009) reported, virgin coconut oil contained higher linoleic and oleic acids, which were 6.65 and 3.53, respectively. Therefore, these fatty acids led to a higher plasticizing effect in the film, thus lower tensile strength with a simultaneous increase in elongation at the break of films.

Table 1
Tensile strength, elongation at break, water vapor permeability, glass transition temperature, DPPH scavenging activity and total phenolic content of chicken skin gelatin films incorporated with VCO at different levels.

Films formulation	Tensile Strength (MPa)	Elongation at Break (%)	Water Vapor Permeability (g mm/m^2 h kPa)	Glass transition temperature (T_g) (°C)	DPPH Scavenging Activity (%)	Total Phenolic Content (mg GAE/g sample)
A (0% VCO)	0.70 ± 0.12^{a}	117.47 ± 8.37^{a}	4.49 ± 0.95^{a}	52.53 ± 0.09^{a}	10.50 ± 0.53^{d}	2.29 ± 0.21^{ab}
B (10% VCO)	0.48 ± 0.03^{a}	123.48 ± 7.76^{a}	3.80 ± 0.64^{a}	50.22 ± 0.07^{a}	14.37 ± 0.59^{c}	2.00 ± 0.08^{b}
C (15% VCO)	0.46 ± 0.08^{a}	134.01 ± 19.99^{a}	3.73 ± 0.40^{a}	49.60 ± 0.05^{ab}	16.07 ± 0.47^{b}	2.19 ± 0.05^{b}
D (20% VCO)	0.43 ± 0.06^{b}	157.58 ± 16.33^{b}	3.47 ± 0.62^{a}	48.69 ± 0.00^{ab}	16.10 ± 0.76^{b}	2.22 ± 0.19^{b}
E (25% VCO)	0.41 ± 0.06^{b}	164.22 ± 15.26^{b}	3.27 ± 0.61^{a}	46.85 ± 0.06^{b}	16.71 ± 0.38^{a}	2.43 ± 0.10^{a}
F (30% VCO)	0.29 ± 0.19^{b}	183.57 ± 15.52^{b}	2.90 ± 0.71^{a}	45.63 ± 0.04^{b}	17.39 ± 0.54^{a}	2.61 ± 0.15^{a}

Different letters ($^{a\text{-}d}$) in the same column indicate significant differences ($p < 0.05$).
* Mean ± SD (n = 3).

Based on the tensile strength and elongation at break analysis of the film produced, the increasing concentration of VCO lowered the film's tensile strength and is associated with higher elongation at the film's break due to the oil disruption in protein-protein interaction, which leads to the discontinuity of film network. The discontinuity of the film was also confirmed by the increased roughness of the film structure when the concentration of VCO added was increased. This study found that increasing VCO content in gelatin film led to more extensible films.

3.1.2. Water vapor permeability

Table 1 presents the water vapor permeability of chicken skin gelatin film incorporated with different levels of VCO. Water vapor permeability of emulsion film decreased as the percentage of VCO increased from 4.49, 3.80, 3.73, 3.47, 3.27, and 2.90 g mm/m^{2}h kPa for each film formulation A, B, C, D, E, and F, respectively. This finding revealed that the increasing oil concentration added to the emulsion film resulted in lower water vapor permeability. The presence of the hydrophobic phase due to the inclusion of oil influenced the hydrophilic phase, limiting the water vapor permeation into the film (Tongdeesoontorn et al., 2012). Besides, the film components' hydrophilic-hydrophobic ratio also impacted the emulsion films' water vapor diffusion to lower the water vapor permeation rate (Pires et al., 2013). The incorporation of corn oils was revealed to increase the hydrophobic phase of the polymer and reduce the film's tendency toward water uptake capacity. Moreover, a higher oil concentration inhibited active groups' availability from interacting with water (Binsi et al., 2013).

The results agreed with a study by Pérez-Mateos et al. (2009). They reported that the incorporation of sunflower oil in cod gelatin films decreased the water vapor permeability of the film from 0.43 to 0.32 g mm/m^{2}h kPa at 0–1% of sunflower oil. Other than that, Galus and Kadzinska (2016) also reported that whey protein films with walnut oils showed an excellent water vapor barrier at higher concentrations of walnut oil (1.0%) which is from 17.3 to 8.8 g mm/m^{2}h kPa. The water vapor permeability of emulsion film is influenced by the types of lipids used and the particle size distribution that is primarily correlated with homogenization methods and conditions (Galus and Kadzinska, 2016). Therefore, incorporating VCO in chicken skin gelatin film showed a good water vapor barrier compared to the chicken skin gelatin film without adding VCO due to the minimization of active groups to interact with water. In comparison, gelatin film's low water vapor permeability is considered good enough at 8.713×10^{-3} g mm/m^{2}h Pa (Tyuftin et al., 2022). And the result obtained in this study is still lower than this value, indicating a good value was obtained. This means that Formulation F is suitable for food that is sensitive to moisture.

3.1.3. Light transmission and transparency

Table 2 shows the light transmission of chicken skin gelatin films with different levels of virgin coconut oil at wavelengths 280, 350, 400, 500, 600, 700, and 800 nm. Films with low UV light transmission value are a better barrier to UV penetration through the film. At a wavelength of 280 nm, the light transmission of film formulations A, B, C, D, E, and F decreased from 53.80, 46.83, 45.57, 44.33, 36.77, and 20.07 nm, respectively. The gelatin-based film had a lower barrier ability against UV light as it lacked aromatic amino acids such as tyrosine and tryptophan, which is 1.22% for tyrosine and 1.01% for tryptophan (Sarbon et al., 2013) that functioned as a light absorber in films (Ma et al., 2012a,b). Meanwhile, benzene rings from antioxidant compounds such as polyphenols (caffeic acid, *p*-coumaric acid, and ferulic acid) in lipids contributed to strong UV absorption (Li et al., 2014). Therefore, adding oil to the film might enhance the UV light transmission of emulsion film due to the benzene ring in the oil added.

Light transmission in the visible range (350–800 nm) of gelatin film with the addition of VCO also showed a decreased trend with increasing VCO concentration. This result proves that the inclusion of VCO in chicken skin gelatin film has the potential as a good light barrier. The decrease in light transmission might be due to the light scattering of lipid droplets dispersed throughout the protein network (Tongnuanchan et al., 2013). This result corresponded with gelatin film incorporated with plai essential oil that showed the visible light transmission to decrease at a wavelength of 350–800 nm with the increasing amount of plai essential oil (Tongnuanchan et al., 2013). In addition, oil droplets localized in the film matrix and the alignment of polymer in the film network caused less light transmission (Tongnuanchan et al., 2014). These findings indicate that gelatin proteins from chicken skin exhibited good UV-barrier properties due to the presence of aromatic amino acids that absorb UV light. The low light transmission is better. However, the light transmission for gelatin film is considered good enough at 85.62 nm (Tew et al., 2017). In comparison, the light transmission for the commercial film is considered good enough at 670 nm (Kwon et al., 2018). The result obtained in this study is still lower than this value, indicating that a good value was obtained in this current study.

Table 2
Light transmission and transparency of chicken skin gelatin films incorporated with VCO at different levels.

Films formulation	Light Transmittance at different wavelength (nm)						Transparency value
	280	350	400	500	600	700	
A (0% VCO)	53.80 ± 4.85^{a}	85.80 ± 8.65^{a}	84.47 ± 4.34^{a}	90.80 ± 2.66^{a}	93.10 ± 2.10^{a}	93.60 ± 1.65^{a}	0.23 ± 0.12^{d}
B (10% VCO)	46.83 ± 3.66^{ab}	65.87 ± 11.61^{b}	72.07 ± 6.05^{ab}	80.80 ± 4.10^{b}	83.67 ± 3.46^{b}	84.77 ± 2.99^{a}	0.35 ± 0.05^{cd}
C (15% VCO)	45.57 ± 2.51^{ab}	58.27 ± 0.81^{bc}	65.27 ± 4.63^{b}	70.53 ± 1.92^{c}	72.70 ± 2.03^{c}	73.33 ± 2.12^{b}	0.56 ± 0.067^{cd}
D (20% VCO)	44.33 ± 5.11^{b}	55.13 ± 11.64^{bc}	57.93 ± 8.99^{bc}	62.30 ± 5.21^{d}	67.10 ± 6.94^{c}	70.37 ± 8.49^{b}	0.67 ± 0.16^{c}
E (25% VCO)	36.77 ± 3.69^{b}	41.83 ± 6.21^{c}	46.00 ± 4.88^{c}	49.30 ± 2.31^{e}	51.10 ± 2.15^{d}	51.27 ± 1.12^{c}	1.10 ± 0.09^{b}
F (30% VCO)	20.07 ± 0.90^{c}	27.20 ± 3.29^{c}	29.80 ± 3.44^{d}	34.80 ± 2.91^{f}	37.03 ± 0.67^{e}	38.00 ± 1.31^{d}	1.45 ± 0.24^{a}

Different letters ($^{a\text{-}d}$) in the same column indicate significant differences ($p < 0.05$).
* Mean ± SD (n = 3).

Table 2 also shows the transparency value of chicken skin gelatin film by adding VCO at different levels. As expected, the transparency of produced film increased with increased VCO concentration. The transparency values for film formulation A, B, C, D, E and F were 0.23, 0.35, 0.56, 0.67, 1.10 and 1.45, respectively. Thus, the result indicated that increasing VCO caused lower film transparency. These were due to the inclusion of oil in the film produced, which enhanced the turbidity of the film as oil droplets distributed throughout the film matrix hence decreasing the transparency of the film (Tongnuanchan et al., 2015). This finding agrees with a study conducted by Tongnuanchan et al. (2014), in which fish gelatin film added with lemon essential oil recorded a reduction in transparency. Film transparency is influenced by coloring components contained in oil which is associated with decreased film networks due to the interruption of oil droplets in polymer chains (Tongnuanchan et al., 2013). Therefore, adding virgin coconut oil to chicken skin gelatin decreased the light transmission and transparency value due to the distribution of oil droplets throughout the film matrix. Thus, it can be seen that chicken skin gelatin films have better visible light barrier properties and can achieve lower oxidation rates.

3.1.4. Thermal properties

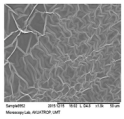
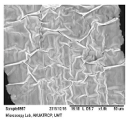
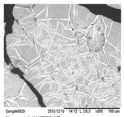
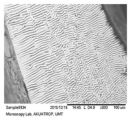
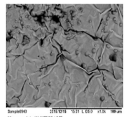
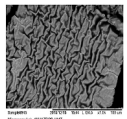
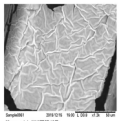
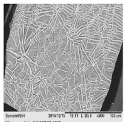
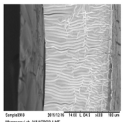
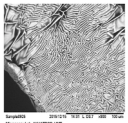
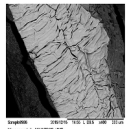
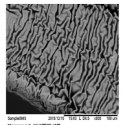
Table 1 shows the glass transition temperature (T_g) of chicken skin gelatin film incorporated with virgin coconut oil at different levels. The T_g values of chicken skin gelatin film produced were 52.53, 50.22, 49.60, 48.69, 46.85, and 45.63 °C for film A (0% VCO), B (10% VCO), C (15% VCO), D (20% VCO), E (25 % VCO) and F (30% VCO), respectively. Oil added to the film produced might interrupt the protein-protein chain and result in structural relaxation, reducing the T_g values. Besides that, the plasticizer effect on oil might also reduce the T_g value and mobility of protein chains in film networks (Tongnanchuan et al., 2015). Subsequently, the polymer matrix becomes less dense, and the mobility of polymer chains increases. This result agrees with Tongnuanchan et al. (2015), who reported that the film with additional palm oil revealed a decrease of T_g from 41.02 to 35.85 °C when palm oil was added to 75%. The increasing amount of plasticizer promoted a lower T_g value of gelatin film (Ghanbarzadeh and Oromiehi, 2008). In conclusion, T_g values of chicken skin gelatin film with the addition of VCO decreased as the oil concentration increased.

3.1.5. Film morphology

Table 3 presents the scanning electron microscope (SEM) micrographs of the surface and cross-section of chicken skin gelatin films incorporated with virgin coconut oil at different levels. Film formulation A (0% VCO) showed a smoother and continuous surface compared to film formulation with the addition of virgin coconut oil. Meanwhile, films incorporated with virgin coconut oil had discontinuous (crack), rough surfaces with bulky particles. Tongnuanchan et al. (2015) stated that the bulky surfaces of emulsion films were due to the non-homogenized oil droplets that floated on the upper surface of the film during the drying process. Besides that, the increasing amount of oil also limited the capacity of the film matrix to hold the oil droplets in place. The film matrix These correspond to the fact that the low water vapor permeability of film incorporated with oil as the hydrophobic nature of bulky particles on the film surface provided a barrier for water vapor permeation, which is 2.90 g mm/m2h kPa at a higher concentration of VCO (30%).

The cross-section of the control film also showed a smoother and more compact structure than other films with the addition of virgin coconut oil. The technical significance of the cross-section of the material may provide a view of the internal structure of that material. It does not exist before the material is cut off at right angles to an axis. For the cross-section morphology, the homogenous matrix of films is an excellent sign of their structural integrity and rigidity (Farahnaky et al., 2013). However, emulsion films displayed rough and discontinuous systems with an increasing VCO percentage. Discontinuity of the structure is related to the formation of two phases which are lipid globules and protein components in the film matrix (Valenzuela et al., 2013). Galus and Kadzinska (2016) reported that the lack of integrity between oil droplets and protein networks was due to the focusing of oil droplets on the airside of films which are associated with the emulsion behavior of protein that is not effective in stabilizing oil droplets in the film matrix. Moreover, the interaction of protein molecules was reduced due to the interruption of oil droplets in protein alignment, indicating the film structure's roughness (Tongnuanchan et al., 2015). Therefore, adding a different percentage of VCO on chicken skin gelatin films affects the surface and cross-section appearance of the film.

Table 3
SEM micrographs of surface and cross-section of chicken skin gelatin films incorporated with VCO at different levels.

Position	Film formulation					
	A (0% VCO)	B (10% VCO)	C (15% VCO)	D (20% VCO)	E (25% VCO)	F (30% VCO)
Surface						
Cross-section						

3.1.6. Functional group properties

Fig. 1 presents the Fourier transform infrared (FTIR) spectra of chicken skin gelatin incorporated with virgin coconut oil at different levels. The FTIR spectra pattern indicated several functional groups detected in each film formulation which are Amide A (3288.39–3290.37 cm^{-1}), Amide I (1632.74–1633.31 cm^{-1}), Amide II (1547.19–1548.43 cm^{-1}), Amide III (1235.77–1237.51 cm^{-1}) and carbonyl group 1740.40 cm-1 for film formulation D, E, and F.

Amide I was detected due to the C = O stretching or hydrogen bonding coupled with COO. The absorption peak of Amide I represented the coil structure of gelatin in film (Jongjareonrak et al., 2008). Meanwhile, the slight changes in Amide I of emulsion films might be due to the different percentages of VCO added. The Helix coil structure of gelatin might interact with oil components, thus reforming their secondary structure (Jongjareonrak et al., 2008). Amide II was detected due to the bending vibration of the N–H groups and the stretching vibration of the C–N groups. Jongjareonrak et al. (2008) reported that the Amide II band is encouraged by hydration, thus resulting in secondary structure change. However, since the analysis was performed in a dry state, the result might be due to adding VCO in chicken skin gelatin films. Other than that, the vibrations in-plane of C–N and N–H groups of bound amide or vibrations of CH2 groups of glycine that exist in gelatin was represented as Amide III in all the film formulation. Tongnuanchan et al. (2012) also found similar bands of Amide I, II, and III at wavenumber 1630.44, 1542.61, and 1237.53 cm-1, respectively, in all fish gelatin films incorporated with citrus essential oil.

Amide A was found to correspond to the NH- stretching coupled with hydrogen bonding. Amide A of film formulation A (0% VCO) shifted to higher wavenumbers as the amount of oil was added differently. Meanwhile, the amplitude of Amide A in emulsion films decreased with increasing VCO percentage. This finding revealed that the protein-protein interaction, especially with the existing hydrogen bonding, was interrupted by the components of the oil added (Tongnuanchan et al., 2015). Furthermore, strong peaks were found at wavenumber 1740.40 cm^{-1} of gelatin films with the addition of virgin coconut oil at levels 20–30%, which indicates oil compounds in films produced. The peak could be observed due to the higher concentration of oil added to films. The peak represented the C = O stretching vibration of aldehyde or ester carbonyl groups which is associated with increasing hydrophobicity of gelatin films with the addition of oil (Tongnuanchan et al., 2013). In addition, Binsi et al. (2013) reported that FTIR spectra of chitosan film incorporated with virgin coconut oil showed a strong peak at wavenumber 1743 cm^{-1} with the increasing amount of VCO at 1 ml/g chitosan, which was almost similar with film formulation F. The result is also supported by several gelatin films with oil, such as cod gelatin with 0.3% of sunflower oil (1744 cm^{-1}) (Pérez-Mateos et al., 2009) and fish gelatin with 20% of olive (1747 cm^{-1}) (Ma et al., 2012a,b).

3.2. Antioxidant properties

3.2.1. DPPH scavenging activity

Table 1 shows the DPPH scavenging activity values of chicken skin gelatin films incorporated with virgin coconut oil at different levels. Antioxidant activity on DPPH scavenging of chicken skin gelatin films showed increased VCO concentration. This result indicated that increasing VCO in the gelatin-based film increases antioxidant activity value. The antioxidant content of virgin coconut oil was initially related to the phenolic content of the oil (Dashipour et al., 2015). According to previous reports, virgin coconut oil is higher in ferulic acid (5.05 mg/kg oil), vanillic acid (2.08 mg/kg oil), and others such as caffeic acid, syringic acid, and *p*-Coumaric acid. So, these phenolic acids of VCO might promote more significant antioxidant activity in films as VCO added increases. Besides that, residues of amino acids in gelatin, such as glycine and proline, might also contribute to higher antioxidant activity on DPPH scavenging activity (Tongnuanchan et al., 2013). As expected, glycine and proline values in chicken skin gelatin which are 33.70%

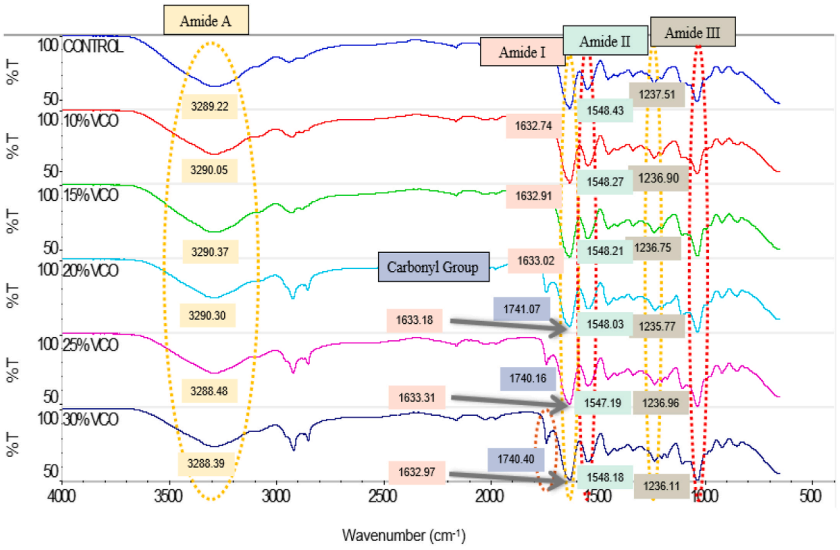


Fig. 1. FTIR spectra of chicken skin gelatin films incorporated with VCO at different levels.

and 13.42%, respectively, were higher hence promoting a more significant percentage of antioxidant activity in films produced (Sarbon et al., 2013). The results agree with the study on fish skin gelatin film combined with plai essential oil by Tongnuanchan et al. (2013), where the antioxidant activity was increased from 0.95 to 1.90% as the amount of plai essential oil increased at the level of 25–50%. Moreover, the main reason for antioxidant activity in emulsion film is the reactivity of hydroxyl groups present in different percentages of oil added that acted as pro-oxidants, influencing the antioxidant action (ECA and Sartori, 2014). Thus, the result showed that an increasing amount of VCO in different film formulations contributed to the different percentages of DPPH scavenging activity in films.

3.2.2. Total phenolic content

Table 1 presents the total phenolic content of chicken skin gelatin films incorporated with virgin coconut oil at different levels. The total phenolic content of chicken skin gelatin films increased with increasing VCO concentration. Total phenolic content for film formulations A, B, C, D, E, and F were 2.29, 2.00, 2.19, 2.22, 2.43, and 2.61 mg GAE/g, respectively. Film formulation A (0% VCO) showed a lower amount of phenolic content because of the ability of some amino acid residues in gelatin (tyrosine and histidine) to reduce the Folin−Ciocalteu reagent in the sample (Liu et al., 2015). Meanwhile, the increasing amount of total phenolic content from 2.00 to 2.61 mg GAE/g sample indicated that the interaction of different film extracts and the Follin-Ciocalteau reagent had released a higher amount of free phenolic compound. Little saturation of protein-polyphenol interaction caused the free phenolic compound presented in the film matrix to increase as the amount of oil increased (Gomez-Estaca et al., 2009).

This finding correlates with Gomez-Estaca et al. (2009). They reported that bovine-hide gelatin with the addition of oregano extracts had higher phenolic content (1673 μg/g of the film) at 0.3g/100 ml of oregano extract compared to 1.25g/100 ml of oregano extract, which was recorded phenolic content of 5819 μg/g of the film. Moreover, the result obtained correlates with the DPPH scavenging assay that resulted in higher antioxidant activity as the phenolic compounds can promote additional free radical-scavenging activities due to their reactivity as hydrogen or electron-donating agents and also metal ion-chelating properties Haddar et al. (2012).

4. Conclusion

Formulated chicken skin gelatin film incorporated with virgin coconut oil (VCO) showed a decrease in tensile strength while increasing elongation at break with the increasing amount of virgin coconut oil. Meanwhile, light transmission of films decreased with the addition of virgin coconut oil was associated with the roughness of the film surface and cross-section. The increasing amount of VCO in gelatin film also enhanced the intensity for the functional group (Amide A, Amide I, Amide II, and Amide III) and lowered the T_g of film during heat treatment. Regarding antioxidant properties, DPPH scavenging activity and total phenolic content increased as the level of VCO increased in the gelatin film. The optimal formulation was Formulation F with 30% VCO, which provides the lowest light transmission and water vapor permeability. Furthermore, the low light transmission will indicate a good light barrier. Adding VCO improved the functional and antioxidant properties of chicken skin gelatin films that are suitable as food packaging films.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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