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FULL PAPER

Characterization on antioxidant and physical properties of gelatin based composite films with incorporation of *Centella asiatica* (pegaga) extract

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Abstract

This study aimed to characterize the influence of *Centella asiatica* at 0.3% and 0.7% on antioxidant activities; mechanical and physical properties of chicken skin gelatin/CMC/ *Centella asiatica* film. Characterization of the blended films with 0.7% *Centella asiatica* extract shows higher antioxidant activities with a total phenolic content of 0.36 mg/g of GAE, DPPH of 89.26%, and reducing power of 0.80 nm compared to 0.3% *Centella asiatica* extract added where the total phenolic content was 0.29 mg/g of GAE, DPPH of 89.26% and reducing power of 0.80 nm. The addition of 0.3% of *Centella* extract provide higher value in tensile strength, elongation at break, melting point and transparency but lower in UV-light penetration and crystallinity of the films. While the addition of 0.7% *Centella* extract contributes to higher value in WVP and puncture test. In conclusion, the incorporation of *Centella asiatica* extracts on film greatly increased antioxidant levels and improved some of the mechanical and physical properties of the film blends.

1. Introduction

In recent years, there has been an increased demand for food packaging that offers an improved shelf life for food products. The most common quality loss in packaged foods is caused by oxidation (Hong and Krochta, 2006). Oxidative processes cause the degradation of meat proteins, pigments, and lipids, limiting shelf life (Liu *et al.,* 2010). Hence, active packaging may carry antioxidants to delay the deleterious effect (Yingyuad *et al.,* 2006).

Currently, many researchers are focusing on packaging films with antioxidant agents from natural sources as alternatives to synthetic antioxidants such as grape seed extract (Moradi *et al.,* 2012), *Zataria multiflora Boiss* essential oil (Moradi *et al.,* 2012), green tea extract (Siripatrawan and Harte, 2010), carvacrol (López-mata *et al.,* 2013). In many kinds of natural extract, *Centella asiatica* contains several active ingredients such as asiaticoside, histidine, brahmoside, brahmonoside, madecassoside, lysine, alanine madecassic acid, riboflavin, threonine, serine, pyridoxine, glutamate, asparate, and vitamin K (Singh *et al.,* 2014). In addition to these active ingredients, it also contains volatile oils such as farnesol and caryophyllene; and also flavonoids such as quercetin, apigenin, catechin, kaempherol and naringin that contribute the high total phenolic contents. Incorporation of antioxidant compounds into films will provide protection to food product from oxidation, enzymatic browning, microorganism's growth, and vitamin losses (Silva-Weiss *et al.,* 2013).

Biodegradable films are generally based on lipids, proteins, and polysaccharides. Previously, studies have shown that one of protein source material that getting high interest nowadays in order to form a packaging and film was gelatin. The use of gelatin in the preparation of edible films or coating has been well studied (Nor *et al.,* 2017; Suderman and Sarbon, 2019). However, several safety concerns and religious issues concerning commercial gelatin (Jridi *et al.,* 2014) have led to the exploration of different alternative substitutes of raw materials for production of gelatin, such as chicken skin (Sarbon *et al.,* 2013) and fish skin (Cheow *et al.,* 2007; Rosli and Sarbon, 2015).

Studies proved that biodegradable films formed by merging selected biopolymers have improvedthe homogeneous structure and better physicochemical properties compared to the films with mono component (Qi *et al.,* 2015). Many researchers on properties of blended film have been conducted such as gelatin-chitosan blended film, cassava starch-wax blended film (Chiumarelli and Hubinger, 2014) and gelatin- soy protein isolate (Cao *et al.,* 2007). Carboxymethyl cellulose (CMC) is a substitute polymer with excellent

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stability, viscosity, availability and biocompatibility and preferably used to blend with gelatin. The addition of CMC to the gelatin based films increases molecular aggregates and modulus of elasticity between gelatin and CMC (Nazmi *et al.,* 2017). Previously, the study on the effect of plasticizer concentration on chicken skin gelatin film characterization has been conducted successfully by Nor *et al.* (2017). However, there is still limited study of the antioxidant and properties of film from chicken skin gelatin/CMC blended film incorporated with *Centella asiatica* extract. Therefore, this study aimed to investigate the effect of different *Centella asiatica* extract levels on antioxidant, mechanical, physical and thermal properties of chicken skin gelatin/CMC blended film as a primary food packaging.

2. Materials and methods

2.1 Materials

Chicken skin for gelatin production was purchased at TD Poultry Sdn. Bhd. Kuala Terengganu, Malaysia. The fresh *Centella asiatica* was purchased from a local market in Kuala Terengganu, Malaysia. Carboxymethyl cellulose was purchased from Sigma-Aldrich Company Ltd., United Kingdom. All other chemicals used in this study were of analytical grade.

2.2 Methods

2.2.1 Sample preparation

The chicken skins were kept on ice during transport to the laboratory. The visible fat on the skin was removed and rinsed in excessive water in order to remove the impurities. The skin then was oven dried at 55ºC for 24 hrs and ground, then defatted following by Soxhlet method (AOAC, 2006).

2.2.2 Gelatin extraction

Chicken skin gelatin was prepared following the technique as described by Sarbon *et al.* (2013) using acid -alkaline pretreatment. The defatted ground chicken skin was soaked in 0.15% (w/v) of sodium hydroxide, 0.15% (w/v) of sulphuric acid and acid 0.7% (w/v) of citric solution serially. Each soaking treatment with a total time of 2 hrs was repeated three times. Final wash of the skin with distilled water was done in order to remove any residual matter. The solution mixture was extracted in distilled water in water bath at a controlled temperature (45°C) for overnight. The clear extract which is gelatin in solution form was filtered, concentrated by evaporation under low pressure, and freeze-dried to form a gelatin powder.

2.2.3 Preparation of Centella asiatica extract

The *Centella asiatica* extract was prepared following the method used by Reihani and Azhar (2012). The *Centella asiatica* leaves and barks were washed using cleaned water, freeze-dried and ground. Approximately 10 g of dried *Centella asiatica* were weighted and then mixed uniformly in a beaker with 100 mL boiled water for 10 mins using magnetic stirrer. Then, the extracts were filtered with 125 mm filter papers, concentrated by evaporation under low pressure, and freeze-dried. The extraction powder then was kept in a chiller (4°C) before being used.

2.2.4 Development of chicken skin gelatin films

Gelatin film was produced using the casting technique as described by Jahit *et al.* (2016). To prepare film-forming solution (FFS), 3 g of chicken skin gelatin was dispersed in 50 ml distilled water while 3 g of CMC was dispersed in 50 mL distilled water separately. Both solutions then were mixed together, and then 0.78 mL of glycerol were added as plasticizer. Next, *Centella asiatica* extract was added to the chicken skin gelatin/ CMC solution. The following three solutions were prepared: (i) control, without *Centella asiatica extract*; (ii) with 0.3% *Centella asiatica* extract; and (iii) with 0.7% *Centella asiatica* extract. The solutions were heated on the heating mantle with continuous stirring at 45±5°C for 60±5 mins and kept for 5 mins in room condition. A total of 50 g of the solutions in the beakers then were poured onto container in order to control film thickness. They were dried at oven at 45°C until completely dry.

2.3 Antioxidant properties

2.3.1 Total Phenolic content

The total phenolic contents of the blended films were determined with Foline Ciocalteu reagent per Suderman and Sarbon (2019). About 25 mg of each film sample was dissolved in 5 mL of distilled water. Approximately 0.5 mL Folin-Ciocalteu reagent and 7 mL distilled water were mixed with 0.1 mL of the extract solution in the test tube, and stored for 8 mins at room temperature. Next, 1.5 mL distilled water and sodium carbonate (2%, w/v) was added into the same test tube to obtain a final volume of 10 mL. The mixture then was stirred and keeps at room temperature for 2 hrs. Then, absorbance reading of sample mixture at 765 nm against water on a UV spectrophotometer was being taken. The following equation was used to express the results in terms of mg gallic acid equivalents (GAE mg/g) per gram of dried film:

Total phenolic content (mg/g of GAE) = (CV) /M

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

film (g).

2.3.2 DPPH radical – scavenging activity

DPPH test on blended films was conducted following method by Razali *et al.* (2015). Approximately 25 mg of each films sample was dissolved and continuous stir in 5 mL of distilled water. About 3.9 mL of the DPPH solution (0.1 mM in methanol solution) was mixed with 0.1 mL of extract solution, followed by 60 mins incubation room temperature in dark area. The absorbance was measured at 517 nm against pure methanol and the percentage of DPPH radical-scavenging activity was calculated using the following equation:

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where A is absorbance at 517 nm; A_{blank} is absorbance of blank sample which DPPH solution (0.1 mM in methanol solution); and A_{sample} is absorbance of film sample with different extracts concentrations.

2.3.3 Reducing power

The reducing power was performed according to the method described by Razali *et al.* (2015) with slight modification. Approximately 1.0 mL of sample or the control sample was mixed with 2.5 mL of 10mg/mg potassium ferricyanide and 2.5 mL of 0.2 M phosphate buffer (pH 6.6), prior with incubation for 20 mins at 50° C. The solution then was centrifuged. About 0.5 mL of 0.1% ferric chloride, 2.5 mL deionized water and 2.5 mL of supernatant was mixed together. The absorbance at 700 nm was measured after a 10 mins reaction. A higher reducing power indicated by the higher absorbance.

2.4 Mechanical and physical properties of film

2.4.1 Tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) of the film were determined by using a texture analyser (TA.TX Plus, Stable Micro System, UK) following methods described by Nur Hazirah *et al.* (2016). A film strip with a measurement of 20 mm x 100 mm was prepared by using a cutting blade. The film was then placed onto grip pairs of AT/G probe which was attached to the texture analyzer with 10 kg load cell. The initial gap of 60 mm between the up and down parts of the grip was set. The strip was stretched by the moving at headspace of 100 mm/min until broken. The TS (MPa) was calculated using the following equation:

$$\text{Tensile Strength (MPa)} = \frac{F_{max}(N)}{A_{(m^2)}}$$

Where F_{max} is max load (N) needed to pull the sample apart; and A is cross-sectional area (mm^2) of film sample.

Meanwhile, the elongation at break (EAB) was calculated as follows:

$$\text{EAB (\%)} = \frac{l_{max}}{l_0} \times 100$$

Where l_{max} is the film elongation (mm) at the moment of rupture; and l_o is the initial grip length (mm) of sample.

2.4.2 Puncture strength

The deformation and strength of the films at the breaking point were determined by puncture test. The test was evaluated using an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) instrument. The films were placed in a 5.6 cm in diameter of probe cell. The film was perforated to the breaking point using round-ended stainless-steel plunger 2 mm in diameter, at a crosshead speed of 1 mm/s and a 50 N load cell. Breaking strength was expressed in terms of N and breaking deformation as a percentage, as previously described by Nur Hazirah *et al.* (2016). All determinations were the means of at least three measurements.

2.4.3 Water vapour permeability (WVP)

Water vapour permeability (WVP) was measured by using a modified ASTM method as described by Jahit *et al.* (2016). The films were sealed onto a cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band to hold the films in place. The cups with films were then weighted as initial weight. The cups then placed in desiccators containing distilled water at 30°C. The cups were weighted at 1 hr intervals over 7 hrs of period. Three films were used for WVP determination and the measurement was conducted in triplicate. WVP of the film was calculated as follows:

$$\text{WVP (g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}) = wxA^{-1}t^{-1}\Delta Pa^{-1}$$

Where w is the weight gain of the cup (g); x is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); and ΔPa^{-1} is the vapor pressure difference across the film (Pa)

2.4.4 Thermal properties

The measurement of melting temperature of the film was carried out following the method described by Rosli and Sarbon (2015), with some modifications, using a differential scanning calorimetry (DSC Q2000 Modulated, TA Instrument, USA) equipped with a cooling device (Intercooler II) supported by a Pyris Thermal Analysing System. About 5 mg of films were weighed using the Metler Toledo precision balance (AL 204, Metler-Toledo Ltd., Beaumont Leys Leicester, UK)

and then enclosed in air-tight aluminum pans. The reference was an empty pan sealed with a lid to give a suitable heat capacity. These were analyzed at a heating rate of 10°C/min ranging from 0 – 175°C. The temperature at which one-half of the gelatin film denatured was taken as the top of the peak. The total energy required for denaturing the film (the enthalpy change, ΔH) was measured by integrating the area under the peak. The endothermic peak was selected as the melting temperature for gelatin film and an average reading was taken from three replications.

2.4.5 Film light transmission and transparency

The visible and ultraviolet (UV) light barrier properties of the films were measured using a UV-1700 UV-Visible double beam spectrophotometer (Shimadzu, Kyoto, Japan) following the procedure reported by Jahit *et al.* (2016). Film size of 1 cm x 2 cm was prepared and placed directly into the test cell, with a reference by empty test cell. The absorbance (%) against visible and UV light at selected wavelengths (400, 600, 800 nm) were measured. Film transparency was calculated as follows:

$$\text{Transparency} = -\log T/x$$

Where T is transmission (%) at 600 nm and x is film thickness (mm). Film thickness was measured using Digimatic Micrometer (Mitutoyo, Japan) using the method reported by Li *et al.* (2014). All determinations were recorded as the mean of three measurements.

2.4.6 Microstructure using scanning electron microscopy (SEM)

The scanning electron microscopy (Nova Nano SEM 230, FEI, USA), was used to examine the morphology of the film, per Li *et al.* (2014). Film specimens (2 mm x 2 mm) were fractured by dipping in liquid nitrogen for 2 mins and attached on copper stubs upright to their surface. Samples were gold coated using an accelerating voltage of 30 KV. Samples were observed using magnification from 500 – 1500.

2.4.7 X-Ray diffraction (XRD)

X-ray pattern of chicken skin gelatin/CMC/*Centella asiatica* blended film was analysed using Rigaku X-Ray Diffractometer following a method according to Nur Hazirah *et al.* (2016) with some modifications. The sample was mounted on 2 x 2" glass slide and was secured on the X-ray platform by using tape. This analysis was run with Cu Ka radiation at a current of 30 mA and voltage of 40kV. The sample then was scanned between $2\Theta = 3°$ to 80° with a scanning time 30 mins per running. The tests were conducted in triplicate.

2.5 Statistical analysis

For statistical analysis, one-way ANOVA variance analysis was performed by Minitab 14.0 software and comparisons of means utilized Tukey's test at a confidence level of $p<0.05$. Each analysis was calculated in triplicate.

3. Results and discussion

3.1 Total Phenolic content

Total phenolic contents of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin/CMC film (control film) are presents in Table 1. The total phenolic content values were increased as the concentration of *Centella asiatica* extract in the films increases. *Centella asiatica* incorporated in gelatin/CMC blended film showed higher TP content compared to control films. Chicken skin gelatin film without extract (control film) also showed some antioxidant activity. This may be due to the contribution by amino acid composition of chicken skin gelatin. Chicken gelatin was reported to have high proline, hydroxyproline, glycine in amino acid (Sarbon *et al.,* 2013). Moreover, it also may be due to the reaction of Folin and Ciocalteu reagent with non-phenolic reducing substances and caused the formation of chromogens, which can be detected spectrophotometrically (Siripatrawan and Harte, 2010). Chicken skin gelatin/CMC blended films incorporated with 0.7% *Centella asiatica* extract, possessed higher TP content (0.36 mg/g of GAE) 6 times greater than the control film (0.06 mg/g of GAE). The total phenolic content in the produced film was related to the total phenolic content in the *Centella asiatica* extract showed a strong relationship between the phenolic compound and the antioxidative activity. The phenolic compounds are active hydrogen donors, making them a good antioxidant. The phenolic compounds might be responsible for the oxidative activities of *Centella asiatica* including phenol and flavonoids (Singh *et al.,* 2014).

Table 1. Radical Scavenging DPPH activity and reducing power of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film Formulations	DPPH (%)	Reducing Power (nm)	Total phenolic compound (ml/g of GAE)
Control	41.95 ± 1.96^{c}	0.48 ± 0.01^{c}	0.06 ± 0.01^{c}
0.3 % extract	68.88 ± 0.84^{b}	0.66 ± 0.01^{b}	0.29 ± 0.01^{b}
0.7 % extract	89.26 ± 1.25^{a}	0.80 ± 0.02^{a}	0.36 ± 0.01^{a}

Different superscript within a column with different letters indicate significant difference ($p<0.05$)

3.2 Antioxidant properties

3.2.1 DPPH radical scavenging activity

Data on antioxidant activities of chicken skin gelatin film (control) and chicken skin gelatin/CMC film incorporated with *Centella asiatica* extract are shown in Table 1. The DPPH values of blended films with 0.7% extract added were significantly ($p<0.05$) higher as compared to blended film with 0.3% extract added and control film. The results show that the addition of *C.asiatica* extract into gelatin based film possessed higher scavenging activity on DPPH radical. The antioxidant activities of the *C.asiactica* plant are mainly due to phenolic compounds including flavonoids, phenolic acid, and tannins (Zainol *et al.,* 2003). These phenolic compounds can interrelate with protein through chemical cross-linking interaction. Phenolic compounds are significant to antioxidant because their redox potentials will able them to act as a metal chelator, reducing agents, singlet oxygen quenchers and hydrogen donor (Chew *et al.,* 2011). The compounds usually interact via covalent interactions. The covalent interaction between protein and phenolic compounds occur through oxidation of phenolic compounds to radicals (Simpson *et al.,* 2012). With antioxidant properties, the film with the addition of antioxidant might provide benefits as packaging able to delay or inhibit oxidation (EÇA *et al.,* 2014). The addition of *Centella asiatica* extract will give antioxidant properties to chicken skin gelatin/CMC blended film by reducing the DPPH radical activity (Suderman and Sarbon, 2019).

3.2.2 Reducing power

Similar to the DPPH radical scavenging activity, films blended with *Centella asiatica* extract showed higher value in reducing power compared to the control (without extract), as shown in Table 1. The increased in the concentration of *Centella asiatica* extract significantly increased reducing power ($p<0.05$). The ability to reduce ferric ion (Fe^{3+}) of the blended film with 0.7% *Centella asiatica* extract added was higher than blended films with 0.3% *Centella asiatica* extract added and control films ($p<0.05$). This was similar to the finding by Moradi *et al.* (2012), which found that chitosan film's reducing power value was increased by adding grape seed extract and Zataria multiflora Boiss essential oil. The amount of added antioxidant additives generally is proportional to the degree of the antioxidant power of edible film. This blended film incorporated with *Centella asiatica* extract can play a role of an electron or hydrogen donors, which could terminate the radical chain reaction by reacting with free radicals and convert them to more stable products (Suderman and Sarbon, 2019).

3.3 Mechanical and physical properties

3.3.1 Tensile strength (TS) and elongation at break (EAB)

The tensile strength (TS) of chicken skin gelatin film (control) and chicken skin gelatin/CMC film incorporated with *Centella asiatica* extract are shown in Table 2. Films Incorporated with *Centella asiatica* extract were significantly ($p<0.05$) higher in tensile strength as compared to control film. The increased film tensile strength with *Centella asiatica* extract added is attributed to the polyphenolic compounds which contain many hydrophobic groups, which can form hydrophobic interaction with the hydrophobic region of gelatin molecules. Hydrogen acceptors of gelatin molecule able to combine with Hydroxyl groups of polyphenolic compounds via hydrogen bonds (Hoque *et al.,* 2011). Furthermore, *Centella asiatica* contained a lot of polyphenolic compounds. Because of that, *Centella asiatica* via hydrophobic interaction and hydrogen bonds could interact with gelatin thus leading to film strengthening (Rasid *et al.,* 2018). Polyphenol-protein interactions had improved mechanical properties of gelatin films through incorporation with cinnamon extracts (Hoque *et al.,* 2011).

The elongation at break (EAB) of chicken skin gelatin film (control) and chicken skin gelatin/CMC film incorporated with *Centella asiatica* extract are shown in Table 2. The EAB for blended films fused with 0.3% *Centella asiatica* extract was increased from 223.05% to 281%. However, the EAB was apparently reduced to 271.17% when the concentration of 0.7% *Centella asiatica* extract was added. The EAB is reflected in the flexibility of the film. The higher elongation values at breaking point may be related to flexibility. Increase in

Table 2. Tensile strength, elongation at break, puncture test, water vapor permeability, melting point and glass transition of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film formulations	Tensile strength (MPa)	EAB (%)	Puncture Test (N)	WVP × 10^{-4} ($g\ m^{-1}s^{-1}Pa^{-1}$)	T_m (^oC)	ΔH (j/g)
Control	$3.0 \times 10^{-2} \pm 0.01^b$	223.05 ± 3.84^c	0.06 ± 0.0^a	1.03 ± 0.00^a	124.38^c	0.10 ± 0.01^c
0.3 % extract	$5.0 \times 10^{-2} \pm 0.00^a$	281.00 ± 0.00^a	0.05 ± 0.0^a	1.11 ± 0.00^a	131.31^a	0.63 ± 0.00^b
0.7 % extract	$4.5 \times 10^{-2} \pm 0.00^a$	271.17 ± 2.12^b	0.06 ± 0.08^a	1.13 ± 0.00^a	130.11^b	1.26 ± 0.01^a

Different superscript within a column with different letters indicate significant difference ($p<0.05$)

concentration of extract might cause an increase in pore sizes of the films and creating possible rapture points, thus leads to decreased of EAB (Rasid *et al.,* 2018; Nazmi and Sarbon, 2019).

3.3.2 Puncture test

Puncture test is a measure of the resistance of the film to be perforated. When packed product has protuberances, the film should show good biaxial mechanical properties in order to maintain integrity. Puncture test was determined the force at the breaking point of the film. Table 2 shows the result of puncture force on the chicken skin gelatin film and chicken skin gelatin film blended with CMC/*Centella asiatica* extract. The addition of *Centella* considerably did not affect the puncture force of chicken skin gelatin film. The results suggest that the chain length of gelatin may determine the interactions between phenolic compounds in herb extracts and protein. Gelatin with higher chain length (without hydrolysis), more likely provided a more reactive group for interaction with phenolic compounds via hydrophobic interactions and hydrogen bonds, leading to film strengthening. As a result, the interconnection between gelatin molecules was more noticeable. This is similar to the findings of Kanatt *et al.* (2012) as results on puncture strength of chitosan-based film with and without extract were not significantly different ($p>0.05$). This result proved that the addition of *Centella asiatica* will maintain the puncture force and at the same time benefit other properties of chicken skin gelatin/CMC blended film.

3.3.3 Water vapour permeability (WVP)

The water vapour permeability (WVP) values of the blended films are important measures for the applications of packaging materials. Table 2 shows the WVP of control film and blended films incorporated with 0.3% and 0.7% *Centella asiatica* extract. Blended films with 0.3% and 0.7% *Centella asiatica* extract were not significantly higher in WVP value as compared to control film ($p > 0.05$). This was in the same agreement with Kanatt *et al.* (2012), which found that incorporation of plant extracts did not significantly ($p>0.05$) changed the WVP in bovine-hide gelatin films. The permeable characteristics of film were affected by the structural/ morphological characteristics of the polymeric matrix, the chemical nature of the macromolecule, the degree of cross-linking, and the chemical nature of the additives. The chemical nature of *Centella aciatica* did not significantly affect the cross-linking and polymetric matrix of the blended film. WVP value of film should be as low as possible since the main function of a food packaging is often to decrease moisture transfer between two components of a heterogeneous food product, or between the food and the surrounding atmosphere. This study found that the addition of *Centella asiatica* extract still will maintain the lower WVP value which is desirable in film packaging, besides improved other properties of the blended film. The WVP of composite films depends on the hydrophobic-hydrophilic ratio of the film constituents. High degrees of hydrogen bonding exhibit by highly polar polymers, resulting in elevated WVP values.

3.3.4 Thermal properties of blended gelatin films with Centella extracts

The melting temperature (T_m) values of chicken skin gelatin/CMC blended film with *Centella asiatica* extract and control film were presented in Table 2. The addition of *Centella asiatica* extract to the blended film increased the T_m value to a concentration of 0.3%. However, the addition of *Centella* extract up to 0.7% had decreased the melting temperature. The chicken skin gelatin/CMC blended film with 0.3% *Centella* extract showed the highest T_m value as compared to 0.7% extract and control film. In contrast, the transition enthalpy (ΔH) of blended film with 0.7% extract (1.26 J/g) was the highest as compared to 0.3% extract (0.63 J/g) and control (0.10 J/g). The higher melting point values for blended film of 0.3% *Centella* extract added indicated that cross-linking enhance by the presence of the phenolic compound in *Centella* extract and it might contribute to lower molecular mobility. These findings were supported by the TS value obtained in this study where the TS value of 0.7% extract added was lower than 0.3% extract added in the films. The higher melting point, due to the chain rigidity, may result from the phenolic compound and the intensity of both intermolecular and intramolecular interactions, including difficulty to internal rotation along the macromolecular chain. The melting point blended film of 0.7% extract shows significantly lower than blended film of 0.3% extract ($p<0.05$). The reduction in melting point of film may be due to the increase of OH group in phenolic compound into film matrix as concentration of the centella extract was increased to 0.7%. For transition enthalpy, the lowest enthalpy was found in the control film followed by 0.3% extract film and 0.7% extract film ($p<0.05$).

3.3.5 Light transmission and transparency

Transparency and light transmission at selected wavelengths of all films are shown in Table 3. Light transmission of all films tested was insignificant at 200 nm. In the UV range of 280 nm, films added with *Centella asiatica* extract significantly exhibited low UV light transmission (0.3% extract, 0.01; 0.7% extract, 0.02) compared to control film (0.42) ($p<0.05$). Films with a lower UV light transmission value possess a good

Table 3. Light transmission on chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film Formulations	Wavelength (nm)								Transparency value
	200	280	350	400	500	600	700	800	
Control	0.00 ± 0.00^{a}	0.42± 0.00^{a}	4.07 ± 0.00^{a}	5.28 ±0.00^{a}	6.21 ± 0.00^{b}	6.64 ± 0.00^{b}	7.12 ± 0.00^{b}	7.43 ± 0.00^{b}	0.82 ± 0.00^{b}
0.3 % extract	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.03 ± 0.00^{b}	1.97 ± 0.00^{c}	6.64 ± 0.00^{a}	7.69 ± 0.00^{a}	9.13 ± 0.00^{a}	9.24 ± 0.00^{a}	0.86 ± 0.00^{a}
0.7 % extract	0.00± 0.00^{a}	0.02 ± 0.00^{b}	0.06 ± 0.00^{b}	2.60 ± 0.00^{b}	4.35 ± 0.00^{c}	5.14 ± 0.00^{c}	5.83 ± 0.00^{c}	5.96 ± 0.00^{c}	0.71 ± 0.00^{c}

Different superscript within a column with different letters indicate significant difference (p<0.05)

barrier of UV penetration through the film. The alignment or arrangement of polymer in the film most likely governed the light transmission of film. Non-uniformities in the composition of the material of transparent material could cause significant changes in optical properties (Ahmad *et al.,* 2012).

Transparency values of control film and blended films with *Centella asiatica* extract were presents in Table 3. The results show that the transparency value of blended film at 0.3% of *Centella asiatica* extract was the highest followed by control and blended film with 0.7% extract added. High transparency value indicated high film opacity, which improved light barrier properties. These findings are similar to a study by Suderman and Sarbon (2019), who found that increase of film opacity caused by incorporation of *Centella asiatica* extract, thereby improving the properties of the films as a light barrier. However, when the incorporation of *Centella* extract is increased into 0.7%, the transparency value decreased, even lower than both control and 0.3% extract film. This might be probably due to properties of *Centella asiatica* which is hygroscopic thus increased the amount of water content in the film. The high amount of unbound water molecule inside the film matrices making light can penetrate through the film, thus reduced the opacity of films.

3.4 Scanning electron microscopy (SEM) analysis

Table 4 presents the cross section and surface morphology of blended film added with *Centella asiatica* extract and control film. For control film, the surface morphology of the film was fairly bumpy and rough. However, the film added with the extract showed a smooth and more homogeneous surface. This finding was similar to Tongnuanchan *et al.* (2012), in which films added with essential oils showed a smooth surface. This observation might due to the intermolecular interactions and entanglement between gelatin and extracts resulting in more homogenous surface. It also indicated that film forming solution had no collapse of emulsion occurred during dehydration due to the stable emulsion system of film forming solution (Tongnuanchan *et al.,* 2012). The micrographs of cross – section showed films blended with extract exhibited smooth matrix morphologies with a few cracks, and not much different as compared to the control film. However, for blended film with 0.7% extract, the crack was not so obvious as compared to other film. This indicates that the gelatin, glycerol and *Centella* extract mixed well in the film forming solution.

3.5 X-Ray diffraction (XRD) analysis

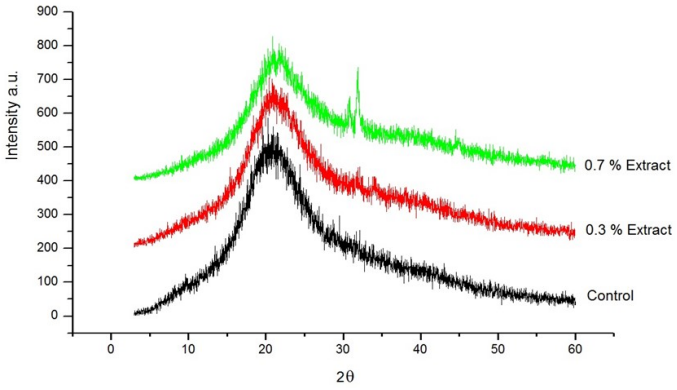
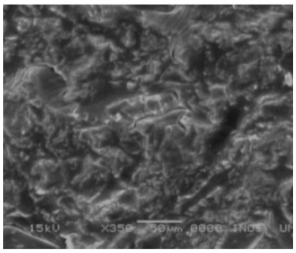
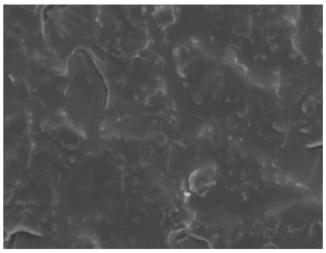
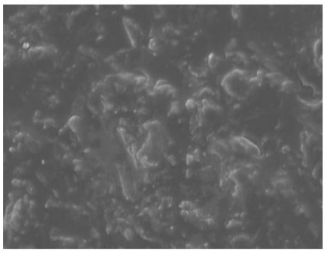
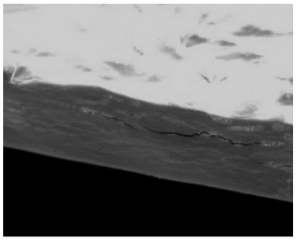
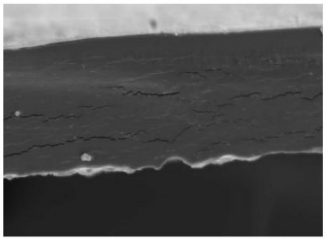
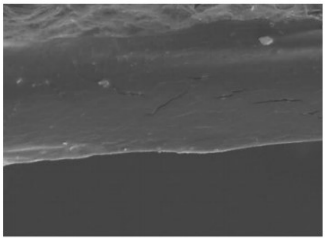


Figure 1. X-Ray Diffractogram of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film).

X-ray diffraction (XRD) was used in order to investigate the crystallinity of structure and evaluate the compatibility of each material in blended film production (Nazmi *et al.,* 2017). Figure 1 showed the diffractogram pattern of control and films with *Centella asiatica* extract. The diffractogram pattern showed peaks at 2θ = 20° for all films. Diffractogram patterns were slightly similar for all film but with different intensities. The control film which showed stronger reflections at 20°, with higher intensity substantially compared to the intensity of the blended film with 0.3% and 0.7% *Centella asiatica* extract at same reflection area. Thus, the crystalline structure of gelatin/CMC blended film was progressively reduced by the addition of *Centella asiatica* extract, which means, it demonstrated a more amorphousness structure than the control film. Lack of re -crystallization during film production was the reason for the amorphous character of the films. This phase obtained may be due to the increase of moisture in the films contributed by *Centella asiatica* extract which is high hygroscopic properties, preventing the formation of semi-crystalline regions. The amourphous phase of the composite film implies that the hydrogen bonding

Table 4. SEM micrographs of the surfaces and cross sections of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film	Control	0.3 % extract	0.7 % extract
Surface			
Cross section			

between gelatin and CMC and extract leads to their good compatibility. There is another peak observed at 2θ= 30° -35°, of blended film with 0.3% and 0.7% *Centella asiatica* extract. The peak was obviously observed at diffractogram of film with 0.7% *Centella asiatica* extract as compared with 0.3% *Centella asiatica* extract, however, control film was not seen in diffractogram of control film. The appeared diffraction peak might show that with the addition of *Centella asiatica* extract to the film, the film was in a semi crystalline state. Thus, it may be concluded that increasing levels of *Centella asiatica* extract resulted in decreased crystallinity of blended film.

4. Conclusion

In conclusion, the antioxidant activity of chicken skin gelatin/CMC blended film increased with increasing amounts of extract. *Centella asiatica* addition into chicken skin gelatin/CMC blended film greatly increased their extensibility, transparency and tensile strength, while reduced UV- light penetration trough the blended films. Although the water vapour permeability of control film is lower than blended film with *Centella asiatica* extract, the existence of extract improved the thermal stability of the film. The addition of extract, however, decreases the crystallinity of the film, confirmed by XRD analysis. The effect and interactions of gelatin, glycerol, CMC and *Centella asiatica* extracts on the properties of active gelatin-based films show that extracts association on film greatly influenced the properties of the films blends.

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