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Antioxidant, mechanical and physical properties of chicken skin gelatin/CMC film incorporated with *Centella asiatica* extract

N.N.M. Nazmi, N.M. Sarbon*

School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

*Corresponding author: Tel: +609 668 4968

Fax: +609 668 4949

Email address: norizah@umt.edu.my

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Abstract

This study aimed to characterize the antioxidant, mechanical and physical properties of chicken skin gelatin/CMC/*Centella asiatica* blended film. The influence of *Centella asiatica* at 0.3% and 0.7% on antioxidant activities; mechanical properties and physical properties of chicken skin gelatin/CMC/*Centella asiatica* film were investigated. 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. The addition of 0.3% of *Centella* extract provide higher value in tensile strength (5.0×10^{-2} MPa), elongation at break (281%), melting point (131.31 °C), transparency (0.86) but lower UV-light penetration. While the addition of 0.7% *Centella* extract contribute to higher value in WVP (1.13×10^{-4} g $m^{-1}s^{-1}Pa^{-1}$) and puncture test (0.06 N). There are no significant differences between functional groups obtained from this blended film as evaluated by FTIR analysis ($p > 0.05$). Furthermore, XRD analysis showed the addition of extract decrease the crystallinity of film. 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.

Keywords: *Centella asiatica* extract; gelatin; carboxymethyl cellulose (CMC); antioxidant; functionality properties

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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 [1]. Oxidative processes cause the degradation of meat proteins, pigments, and lipids, limiting shelf life [2]. Hence, active packaging may carry antioxidants to delay the deleterious effect [3].

Currently, many researchers are focusing on packaging films with antioxidant agents from natural sources as alternatives to synthetic antioxidants such as grape seed extract, *Zataria multiflora Boiss* essential oil [4], green tea extract [5], carvacrol [6] and citrus essential oil [7]. Essential oil and extracts from numerous plants known to have antioxidant properties which reduced the lipid oxidation thus prolong the shelf-life of foods. Besides, incorporation of essential oil in films may lower the film water vapor permeability [7].

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 [8]. 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 [9].

The increase concerns on bad impact of non-biodegradable petrochemical-based plastics on environment has led to films produced by biopolymers which are nontoxic and biodegradable, while part of them are effective barriers to carbon dioxide and oxygen. Biodegradable films are

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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 [10-12]. However, several safety concerns and religious issues concerning commercial gelatin have led to the exploration of different alternative substitutes of raw materials for production of gelatin, such as chicken bone, chicken skin and fish skin [13-16]. Characterization of chicken skin gelatin was successfully carried out by [15]. The gel strength of extracted chicken gelatin (6.67%, w/v) was significantly higher in bloom value (355 ±1.48 g) compared to bovine gelatin (229 ± 0.71g). In addition, the amino acid composition (pro: 13.4%, hydro: 12.13% and gly: 33.75%) and imino acid (pro and hydro) values which contribute to the chicken gelatin properties were reported to be higher than bovine gelatin (12.66 and 10.67%, respectively) [15].

Studies proved that biodegradable films formed by merging selected biopolymers have improved homogeneous structure and better physicochemical properties compared to the films with mono component [17]. Many research on properties of blended film has been conducted such as gelatin- chitosan blended film [18], cassava starch-wax blended film [19] and gelatin-soy protein isolate [20]. Carboxymethyl cellulose (CMC) is a substitute polymer with excellent stability, viscosity, availability and biocompatibility and preferably used to blend with gelatin. CMC is also inexpensive compared to other polysaccharides. The addition of CMC to the gelatin based films increases molecular aggregates and modulus of elasticity between gelatin and CMC [21]. Previously, study on effect of plasticizer concentration on chicken skin gelatin film characterization has been conducted successfully by [22]. However, there has been little study of the antioxidant and properties of film from chicken skin gelatin/CMC blended film incorporated

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with *Centella asiatica* extract. Therefore, this study aimed to investigate the impact 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. The fresh *Centella asiatica* was purchased from a local market in Kuala Terengganu, Malaysia. Glycerol (LR grade), carboxymethyl cellulose, sodium hydroxide, sulphuric acid and citric acid were 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 dried and grinded, then defatted following by Soxhlet method [23].

2.2.2. Gelatin extraction

Chicken skin gelatin was prepared following the technique as described by [15] using acid–alkaline pretreatment. The defatted grinded 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.

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Each soaking treatment with a total time of 2 h 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 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 [24] with modification. The *Centella asiatica* leaves and barks were washed using cleaned water, freeze dried and grinded. Approximately 10 grams of dried *Centella asiatica* were weighted and then mixed uniformly in a beaker with 100 ml boiled water for 10 min using magnetic stirrer. Then, the extracts were filtered with 125mm 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 [25] with slight modifications. 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

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heated on heating mantle with continuous stirring at 45±5° C for 60 ± 5 min and kept for 5 min in room condition. 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 it completely dry.

2.3. Antioxidant properties

2.3.1. Total Phenolic (TP) Content

The total phenolic contents of the blended films were determined with Foline Ciocalteu reagent per [26]. About 25 mg of each film sample was dissolved in 5 ml of distilled water. 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 min at room temperature. Next, 1.5 ml distilled water and sodium carbonate (2%, w/v) were added into same test tube to obtain a final volume of 10 ml. The mixture then was stirred and keeps at room temperature for 2 h. 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:

$$\text{Total phenolic content (mg/g of GAE)} = (CV)/M$$

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 is based on the electron donation abilities or hydrogen atom, and was assessed by measuring the bleaching of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) from purple to clear solution. DPPH test on blended films was conducted following method by [27]. Approximately

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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 min 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 method described by [27] with slight modification. Approximately 1.0ml of sample or the control sample was mixed with 2.5 ml of 10mg/mg potassium ferricyanide and 2.5ml of 0.2 M phosphate buffer (pH 6.6), prior with incubation for 20 min at 50°C. The solution then was centrifuged. About 0.5ml of 0.1% ferric chloride, 2.5ml deionized water and 2.5 ml of supernatant was mixed together. The absorbance at 700nm was measured after a 10 min 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 was determined by using a texture analyser (TA.TX Plus, Stable Micro System, UK) following methods described by [28].

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A film strip with measurement of 20 mm x 100 mm was prepared by using a cutting blade. The film then were placed onto grip pairs of AT/G probe which was attached to the texture analyzer with 10 kg load cell. 60 mm of initial gap 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. 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, A is cross sectional area (mm^2) of film sample.

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

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

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 was 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 2mm 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 [28]. All determinations were the means of at least three measurements.

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203 2.4.3. Water Vapour Permeability (WVP)

204 Water vapour permeability (WVP) was measured by using a modified ASTM method as
205 described by [25]. The films were sealed onto a cup containing silica gel (0% RH) with silicone
206 vacuum grease and a rubber band to hold the films in place. The cups with films were then
207 weighted as initial weight. The cups then placed in desiccators containing distilled water at 30°C.
208 The cups were weighted at 1 hour intervals over 7 hours of period. Three films were used for
209 WVP determination and the measurement was conducted in triplicate. WVP of the film was
210 calculated as follows:

211 $$WVP\ (g\ m^{-1}s^{-1}Pa^{-1}) = wxA^{-1}t^{-1}\Delta Pa^{-1}$$

212 Where:

213 w is the weight gain of the cup (g),

214 x is the film thickness (m),

215 A is the exposed area of film (m^2),

216 t is the time of gain (s),

217 ΔPa^{-1} is the vapor pressure difference across the film (Pa)

218

219 2.4.4. Thermal Properties

220 The measurement of melting temperature of film was carried out following the method
221 described by [14] with some modifications, using a differential scanning calorimetry (DSC
222 Q2000 Modulated, TA Instrument, USA) equipped with a cooling device (Intercooler II)
223 supported by a Pyris Thermal Analysing System. About 5 mg of films were weighed using the
224 Metler Toledo precision balance (AL 204, Metler- Toledo Ltd., Beaumont Leys Leicester, UK)
225 and then enclosed in air-tight aluminum pans. The reference was an empty pan sealed with a lid

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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. Structural properties by Fourier Transforms Infrared Spectroscopy (FTIR)

Infrared spectra of the films were measured using an FTIR spectrometer (Nicolet, Thermo Electron, USA), according to [28]. The sample scanning frequencies were in range of 650 to 4000 cm^{-1} with spectra resolution of 4 cm^{-1}. The measurements were performed at room temperature. The interaction between CMC, glycerol, gelatin, and *Centella asiatica* extract were determined through the spectra thus obtained. The data were collected in triplicate and were averaged. The peaks of amide I, amide II, amide A, and phenol compound were identified via software and assigned according to the literature values.

2.4.6. 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). 1cm x 2 cm film size was prepared and placed directly into the test cell, with a references by empty test cell. The absorbance (%) against visible and UV light at selected wavelength (400, 600, 800 nm) were measured. Film transparency was calculated as follows:

$$\text{Transparency} = -\log T/x \quad (2)$$

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Where T is transmission (%) at 600 nm and *x* is film thickness (mm) [29]. Film thickness was measured using Digimatic Micrometer (Mitutoyo, Japan) using the method reported by [30]. All determinations were recorded as the mean of three measurements.

2.5. Microstructure Using Scanning Electron Microscopy (SEM)

The scanning electron microscopy (Nova Nano SEM 230, FEI, USA), was used examine the morphology of the film, per [30]. Film specimens (2 mm x 2 mm) were fractured by dipping in liquid nitrogen for 2 minutes 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.

2.6. 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 [28] with some modifications. The sample was mounted on 2x2 inch 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 30mA and voltage of 40kV. The sample then was scanned between $2\Theta = 3°$ to $80°$ with a scanning time 30 min per running. The tests were conducted in triplicate.

2.7. 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.

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3. Results and Discussion

3.1. Total Phenolic (TP) content

TP 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. Table 1 showed that the TP content values were increased as the concentration of *Centella asiatica* extract in the films increases. *Centella asiatica* incorporated in gelatin/CMC blended film showed a higher TP contents 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, hyrdroxyproline, glycine in amino acid [15]. 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 [5] 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 produced film was related to the total phenolic content in the *Centella asiatica* extract showed a strong relationship between 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 [8]. Similar findings by [31] reported that total phenolic content of tuna – fish films was increased with the addition of oregano and rosemary extract. However, total phenolic compound of blended films with 0.3% and 0.7% *Centella* extract was lower as compared to film incorporated with-grape seed extracts (72 mg/g of GAE) [4]. The small amount

of TP content for film with *Centella asiatica* extract as compared to other antioxidant blended film, perhaps because of dissimilarities in extraction procedures and/or raw materials.

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 [32]. 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 metal chelator, reducing agents, singlet oxygen quenchers and hydrogen donor [33]. The compounds usually interact via covalent interactions. The covalent interaction between protein and phenolic compounds occur through oxidation of phenolic compounds to radicals [34]. With antioxidant properties, film with addition of antioxidant might provide benefits as packaging able to delay or inhibit oxidation [35]. The addition of *Centella asiatica* extract will give antioxidant properties to chicken skin gelatin/CMC blended film by reducing the DPPH radical activity.

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318 3.2.2. Reducing power

319 Similar to the DPPH radical scavenging activity, films blended with *Centella asiatica*
320 extract showed higher value in reducing power compared to the control (without extract), as
321 shown in Table 1. The increased in concentration of *Centella asiatica* extract significantly
322 increased reducing power ($p < 0.05$). The ability to reduce ferric ion (Fe^{3+}) of blended film with
323 0.7% *Centella asiatica* extract added was higher than blended films with 0.3% *Centella asiatica*
324 extract added and control films ($p < 0.05$). This was similar with the finding by Moradi et al.
325 (2012), which found that chitosan film's reducing power value were increased by adding grape
326 seed extract and Zataria multiflora Boiss essential oil. The amount of added antioxidant additives
327 generally is proportional to the degree of antioxidant power of edible film [31]. This blended
328 film incorporated with *Centella asiatica* extract can play a role of an electron or hydrogen
329 donors, which could terminate the radical chain reaction by reacting with free radicals and
330 convert them to more stable products.

331

332 *3.3. Mechanical and Physical properties*

333 3.3.1. Mechanical properties

334 3.3.2. Tensile Strength (TS) and elongation at break (EAB)

335 The tensile strength (TS) of chicken skin gelatin film (control) and chicken skin
336 gelatin/CMC film incorporated with *Centella asiatica* extract are shown in Table 2. Films
337 incorporated with *Centella asiatica* extract were significantly ($p<0.05$) higher in tensile strength
338 as compared to control film. The increased film tensile strength with *Centella asiatica* extract
339 added is attributed to the polyphenolic compounds which contain many hydrophobic groups,
340 which can form hydrophobic interaction with the hydrophobic region of gelatin molecules.

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Hydrogen acceptors of gelatin molecule able to combine with Hydroxyl groups of polyphenolic compounds via hydrogen bonds [36]. 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. Polyphenol-protein interactions had improved mechanical properties of gelatin films through incorporation with rosemary, oregano, cinnamon extracts, and borage [37; 31; 36].

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 to the flexibility of film. The higher elongation values at breaking point may related with flexibility. Increase in concentration of extract might cause an increase in pore sizes of the films and creating possible rapture points, thus leads to decreased of EAB [38].

3.3.3. Puncture Test

Puncture test is a measure of the resistance of the film to be perforated. When packed product has protuberances, film should show good biaxial mechanical properties in order to maintain integrity. Puncture test were determined the force at the breaking point of the film. Table 2 showed the result of puncture force on the chicken skin gelatin film and chicken skin gelatin film blended with CMC/*Centella asiatica* extract. There is no significant difference in puncture force value between control film (0.6 N) and blended film incorporated with 0.3% extract (0.5N) and blended film incorporated with 0.7% extract (0.6 N) ($p>0.05$). The addition of

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Centella considerably did not affect the puncture force of chicken skin gelatin film. The results suggest that the chain length of gelatin may be determine the interactions between phenolic compounds in herb extracts and protein. Gelatin with higher chain length (without hydrolysis), more likely provided more reactive group for interaction with phenolic compounds via hydrophobic interactions and hydrogen bonds, leading to film strengthening. As a result, interconnection between gelatin molecules was more noticeable. This is similar with the findings of [39], as results on puncture strength of chitosan based film with and without extract were not significantly different ($p>0.05$). [31] also found that the film with addition of plant extracts did not significantly ($p>0.05$) adjust the puncture force for any of the blended gelatin film compared to the control film. 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.

3.3.4. Water vapour permeability (WVP)

The water vapour permeability (WVP) values of the blended films are important measures for the applications of packaging materials. One of food packaging function is to minimize moisture transfer between surrounding atmosphere and food product. Low WVP give a wide application of the composite packaging film, especially in highly humid environments [17] Table 2 shows mean values of 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 (1.11×10^{-4} g $m^{-1}s^{-1}Pa^{-1}$ and 1.13×10^{-4} g $m^{-1}s^{-1}Pa^{-1}$, respectively) as compared to control film (1.03×10^{-4} g $m^{-1}s^{-1}Pa^{-1}$) ($p > 0.05$). This was in the same agreement with [31] and [39], which found that incorporation of plant extracts did not

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387 significantly ($p>0.05$) changed the WVP in either tuna-skin or bovine-hide gelatin films. The
388 permeable characteristics of film were affected by the structural/morphological characteristics of
389 the polymeric matrix, chemical nature of the macromolecule, degree of cross-linking, and
390 chemical nature of the additives. The chemical nature of *Centella aciatica* did not significantly
391 affect the cross linking and polymetric matrix of the blended film. WVP value of film should be
392 as low as possible, since a main function of a food packaging is often to decrease moisture
393 transfer between two components of a heterogeneous food product, or between the food and the
394 surrounding atmosphere. This study found that the addition of *Centella asiatica* extract still will
395 maintain the lower WVP value which is desirable in film packaging, besides improved others
396 properties of blended film. The WVP of composite films depends on the hydrophobic-
397 hydrophilic ratio of the film constituents. High degrees of hydrogen bonding exhibit by highly
398 polar polymers, resulting in elevated WVP values.
399
400 3.3.5. Thermal Properties of blended gelatin films with Centella extracts
401 The melting temperature (T_m) values of chicken skin gelatin/CMC blended film with
402 *Centella asiatica* extract and control film were presents in Table 2. The addition of *Centella*
403 *asiatica* extract to the blended film increased the T_m value to a concentration of 0.3%. However,
404 the addition of *Centella* extract up to 0.7% had decreased the melting temperature. The chicken
405 skin gelatin/CMC blended film with 0.3% *Centella* extract showed the highest T_m value as
406 compared to 0.7% extract and control film. In contrast, the transition enthalpy (ΔH) of blended
407 film with 0.7% extract (1.26 J/g) was the highest as compared to 0.3% extract (0.63 J/g) and
408 control (0.10 J/g). The higher melting point values for blended film of 0.3% *Centella* extract
409 added indicated that cross-linking enhance by the presence of phenolic compound in *Centella*

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extract and it might contribute to lower molecular mobility. This findings was supported by the TS value that 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 [40]. The number of hydrogen bonds reduced with a synchronized increase in the extent of covalent cross-linking also will increase the thermal stability of film [41]. The melting point blended film of 0.7% extract show 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 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 ($p<0.05$).

3.3.6. Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) technique was used in order to identify the structural properties of the film produce as the effect of interactions of different molecules between chicken skin gelatin, CMC and *Centella asiatica*. Table 3 presents FTIR spectra of blended films incorporated with 0.3% and 0.7% *Centella asiatica* extract and control film. The peak of Amide I, Amide II, Amide A and phenol band were observed.

The Amide II is representing arising from stretching vibrations of C–N groups and bending vibration of N–H groups. In addition, amide II peak in chicken skin gelatin/CMC blended film was shifted to the lower wavelength from 1562.98 cm^{-1} to 1551.94 cm^{-1} when 0.3% *Centella* extract was added and from 1562.98 cm^{-1} to 1551.41 cm^{-1} for 0.7% *Centella* extract

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433 addition. The interactions of *Centella asiatica* polyphenolic compounds with hydroxyl and
434 amino groups in gelatin and also glycerol in film matrix might cause the decrease in Amide II
435 peak due to particular arrangement in the films. This finding was in the same agreement with
436 study by [5].

437 Amide A represents N–H stretching vibration [42]. The addition of 0.3% of *Centella*
438 *asiatica* extract into chicken skin gelatin/CMC blended film as shown in Table 3, the O-H
439 stretching peak shifted toward lower wavelength from 3291.85 to 3290.54 cm^{-1}. The shift to the
440 lower wavelength was observed when the concentration of *Centella* extract added was increased
441 to 0.7%, which is from 3291.85 to 3287.99 cm^{-1}. This shift into a lower wavelength caused by
442 weaker hydrogen bonds acting on the –OH groups of film. This was because of the
443 intermolecular interaction between carboxyl group from CMC and hydroxyl group from *Centella*
444 *asiatica*, thus reduced amount of hydrogen bond that can act on free hydroxyl group. Moreover,
445 covalent bonding and hydrogen bonding could form from polyphenols, thus occupy the
446 functional group of gelatin matrix, and subsequently lower the free hydrogen group which can
447 form hydrophilic bonding with water.

448 Meanwhile, the band of Amide I and also phenol group for blended films with *Centella*
449 *asiatica* extract show in no shiftment as compared to the control films. The Amide I represent in
450 plane NH bending modes, C=O stretching vibration coupled with CN stretch and CCN
451 deformation. From FTIR analysis, it is evident that polyphenols *Centella asiatica* extract could
452 form covalent and hydrogen bonding with functional group of chicken skin gelatin/CMC blended
453 film matrix. As a result, it will enhance antioxidant activity and also improve the mechanical
454 properties of the blended film.

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456 *3.4. Light transmission and transparency*

457 Optical properties are essential to define the ability of films and coatings to be applied
458 over a food surface, since these affect the appearance of the coated product, which is an
459 important factor in quality [43]. Transparency and light transmission at selected wavelengths of
460 all films are shown in Table 4. Light transmission of all films tested was insignificant at 200 nm.
461 In the UV range of 280 nm, films added with *Centella asiatica* extract significantly exhibited
462 low UV light transmission (0.3% extract, 0.01; 0.7% extract, 0.02) compared to control film
463 (0.42) ($p<0.05$). Films with a lower UV light transmission value possess a good barrier of UV
464 penetration trough the film. This finding was in the same agreement with study conducted by
465 [10]. Packaging film's function is act as a shield to food from effect on UV radiation and light
466 penetration [44], as it can cause oxidative deterioration of packaged foods, leading to nutrient
467 losses, discoloration and off- flavors [45]. The alignment or arrangement of polymer in film most
468 likely governed the light transmission of film. Non-uniformities in the composition of the
469 material of transparent material, could cause significant changes in optical properties [46].

470 Transparency values of control film and blended films with *Centella asiatica* extract were
471 presents in Table 4. The results show that the transparency value of blended film at 0.3% of
472 *Centella asiatica* extract was the highest followed by control and blended film with 0.7% extract
473 added. High transparency value indicated high film opacity, which improved light barrier
474 properties. These findings are similar to a study by [37], who found that increase of film opacity
475 caused by incorporation of borage extract, thereby improving the properties of the films as light
476 barrier. However, when incorporation of *Centella* extract are increased into 0.7%, the
477 transparency value decreased, even lower than both control and 0.3% extract film. This might be
478 probably due to properties of *Centella asiatica* which is hygroscopic thus increased the amount

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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.

3.5. Scanning Electron Microscopy (SEM) Analysis

Table 5 presents the cross section and surface morphology of blended film added with *Centella asiatica* extract and control film. For control film, surface morphology of film was fairly bumpy and rough. However, the film added with the extract showed smooth and more homogeneous surface. This finding was similar to the agreement by [7],which is films added with essential oils showed a smooth surface. This observation might due to the intermolecular interactions and entanglement between gelatin and extracts resulting 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 [7]. The micrographs of cross – section showed films blended with extract exhibited smooth matrix morphologies with a few crack, 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.

3.6. X-Ray Diffraction (XRD) analysis

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 [47]. 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°,

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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 mean, it demonstrated a more amorphousness structure than the control film. Lack of re-crystallization during film production was the reason of amorphous character of the films. This phase obtained may be due to the increased 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 between gelatin and CMC and extract leads to their good compatibility [48] 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.

Conclusions

In conclusion, the antioxidant mechanical and physical properties of chicken skin gelatin/CMC blended films incorporated with *Centella asiatica* extract have been successfully evaluated. 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-

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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. Good interactions between functional groups of chicken skin gelatin and CMC have been verified by FTIR. The addition of extract, however, decreases the crystallinity of the film, confirming 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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Tables

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
0.3 % extract	68.88 ± 0.84^{b}	0.66 ± 0.01^{b}	0.29
0.7 % extract	89.26 ± 1.25^{a}	0.80 ± 0.02^{a}	0.36

$^{a-c}$ mean within a column with different letters are significant difference ($p < 0.05$)

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 (^{o}C)	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}

$^{a-c}$ mean within a column with different letters are significantly difference ($p < 0.05$)

Table 3: FTIR band of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film Formulations	Amide I (cm^{-1})	Amide II (cm^{-1})	Amide A (cm^{-1})	Phenol (cm^{-1})
	C=O stretching	Bending vibration N-H group, stretching vibration of C-N group.	Stretching vibration of C-N bands and N-H groups of bound amide, vibration of C-H groups of glycine	Hydroxyl group(-OH), C-O stretching
Control	1635.64 ± 0.00^{a}	1562.98 ± 0.00^{a}	3291.85 ± 0.00^{a}	1242.80 ± 0.00^{a}
0.3 % extract	1635.64 ± 0.00^{a}	1551.94 ± 0.00^{a}	3290.54 ± 0.00^{a}	1242.80 ± 0.00^{a}
0.7 % extract	1635.64 ± 0.00^{a}	1551.41 ± 0.00^{a}	3287.99 ± 0.00^{a}	1242.80 ± 0.00^{a}

^{a} mean within a column with no different letters are not significantly difference ($p < 0.05$)

Table 4: 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}

$^{a-c}$ mean within a column with different letters are significantly difference ($p < 0.05$)

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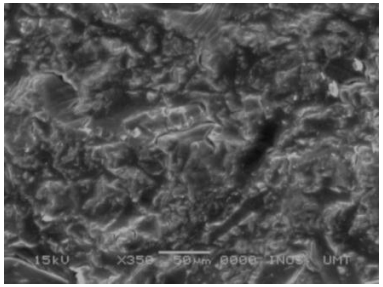
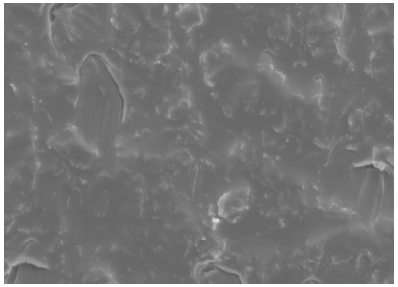
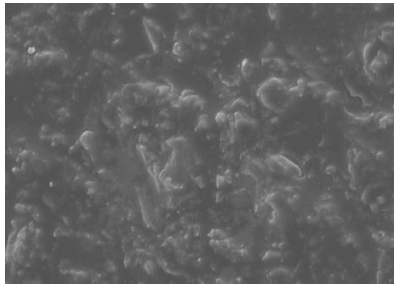
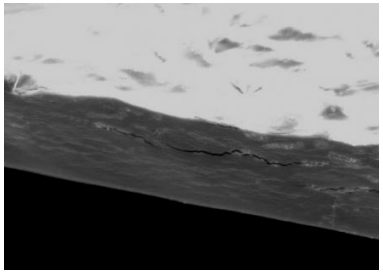
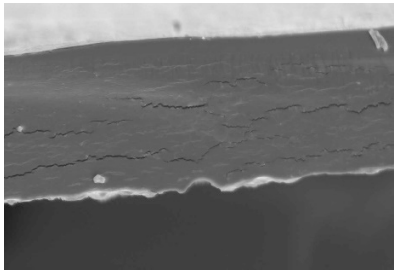
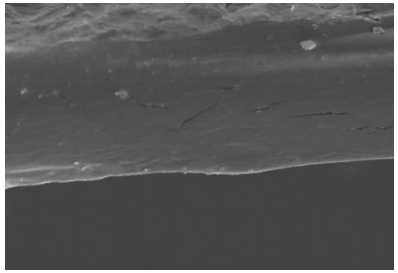
Table 5: SEM micrographs of the surfaces and cross sections of chicken skin gelatin/CMC film incorporated with different concentration of *Centella asiatica* extract and chicken skin gelatin film/CMC (control film)

Film	Control	0.3 % extract	0.7 % extract
Surface			
Cross section			

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List of Figure

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).

Figure 1