Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

ADDITION OF GLYCEROL AND SODIUM CHLORIDE INTO *Garcinia atroviridis* CHITOSAN FILM, AND ITS APPLICATION FOR WRAPPING OF CHICKEN MEAT

(Penambahan Gliserol dan Natrium Klorida Pada *Garcinia atroviridis* Filem Kitosan dan Aplikasinya Pada Pembungkusan Daging Ayam)

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Received: 28 February 2021; Accepted: 22 May 2021; Published: xx June 2021

Abstract

Glycerol and sodium chloride (NaCl) have a strengthening effect that can be incorporated into edible films to enhance their mechanical properties. This study evaluates the effects of glycerol (0.5-2.0% v/v) and NaCl (5.0-10.5 mM w/v) on the physical, mechanical, and antimicrobial properties of chitosan film incorporated with *Garcinia atroviridis*. Storage tests were conducted on chicken meat that were wrapped with and without the film. The moisture content, water solubility, and elongation at break of film with 1.5% (v/v) glycerol and 10 mM (w/v) NaCl was the highest. The increment of glycerol and NaCl reduced thickness, tensile strength, and Young's modulus. The total color difference of the film with 0.5% (v/v) glycerol and 0 mM (w/v) NaCl was highest and showed the greatest inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The pH of the control increased whereas the pH of chicken meat wrapped with the film decreased as the number of storage days increased. In conclusion, film with 1.5% (v/v) glycerol and 10 mM (w/v) NaCl might be a suitable film because it demonstrated the highest elongation at break and a prolonged shelf life for the chicken meat of at least 15 days, which was longer than of the control.

Keywords: glycerol, sodium chloride, edible film, chitosan, *Garcinia atroviridis*

Abstrak

Gliserol dan natrium klorida (NaCl), yang mempunyai kesan pengukuhan, dapat dimasukkan ke dalam filem yang boleh dimakan, untuk meningkatkan sifat mekanikal filem yang boleh dimakan. Tujuan kajian ini adalah untuk menilai kesan gliserol (0.5-2.0% v/v), dan NaCl (5.0-10.5 mM w/v) terhadap sifat fizikal, mekanikal, dan antimikrob filem kitosan yang diperbadankan dengan *Garcinia atroviridis*. Ujian penyimpanan dilakukan pada daging ayam yang dibungkus dengan dan tanpa filem. Kandungan kelembapan, kelarutan air, dan pemanjangan pada pemecahan filem dengan gliserol 1.5% (v/v), NaCl 10 mM (w/v) adalah yang tertinggi. Peningkatan gliserol dan NaCl mengurangkan ketebalan, kekuatan tegangan, dan pekali Young. Perbezaan warna keseluruhan filem dengan 0.5% (v/v) gliserol dan 0 mM (w/v) NaCl adalah tertinggi dan ia menunjukkan penghambatan terbesar terhadap *Pseudomonas aeruginosa* dan *Staphylococcus aureus*. Pengawalan pH meningkat sedangkan pH daging ayam yang dibungkus dengan filem menurun ketika hari penyimpanan meningkat. Kesimpulannya, filem dengan gliserol 1.5% (v/v) dan NaCl

10 mM (w/v) mungkin merupakan filem yang sesuai kerana ia mempunyai pemanjangan tertinggi pada pemecahan dan jangka hayat daging ayam yang berpanjangan sekurang-kurangnya 15 hari yang lebih lama daripada kawalan.

Kata kunci: gliserol, natrium klorida, filem boleh dimakan, kitosan, *Garcinia atroviridis*

Introduction

In Malaysia, chicken meat is one of the most consumed foods. Statistics show the poultry consumption per person in Malaysia was approximately 49 kg in 2019 (https://www.statista.com/statistics/756920/malaysia-meat-consumption-per-capita-by-type/). Chicken meat had a shelf life of 6 days when in refrigerated storage at 4 °C. Common pathogens that were reported in chicken meat are *Campylobacter*, *Salmonella*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* [1]. Food packaging was invented to preserve foods from external contamination [2]. Edible packaging is gaining greater interest due to the disadvantages associated with petroleum-based food packaging and how they deplete non-renewable natural resources [3]. This study focuses on consumable films or coatings that are applied onto food products [4]. Edible packaging provides mechanical protection and extends shelf life of foods [5].

Chitosan is widely used to produce edible films because it possesses good antimicrobial and gas barrier properties that give it a film forming ability [6]. Several researchers have found that chitosan has a proven antibacterial effect towards gram-positive and gram-negative bacteria [7-8]. The incorporation of 20% (v/v) *Piper nigrum* leaf extract increased the inhibition zone of chitosan and polyvinyl alcohol blended films against *Escherichia coli* [9]. On the other hand, chitosan film incorporated with grape seed extract extended the shelf life of chicken breast fillets and inhibited lipid oxidation [10]. Additionally, chitosan film incorporated with cinnamon oil inhibited lipid oxidation and reduced microbial growth on chicken breast fillets [11].

Garcinia atroviridis is known as *'*asam keping*'* or 'asam gelugur'. It is commonly used to make pickles, in tea, and as a seasoning in curries. Al-Mansoub et al. [12] found that *Garcinia atroviridis* extract had antioxidant, antimicrobial, and antitumor-promoting properties. According to Basri et al. [13], *Garcinia atroviridis* extracts inhibited gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermis, Bacillus subtilis*) and gram-negative bacteria (*Salmonella enterica, Salmonella typhimurium, Escherichia coli* and *Pseudomonas aeruginosa*). Chitosan film incorporated with *Garcinia atroviridis* extracts showed promising characteristics for use as food packaging [6]. From their study, it was found that the film increased the shelf life of Indian mackerel during storage and inhibited the microbial growth of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. However, the tensile strength and elongation at the break of film were low.

The mechanical properties of packaging materials are important to ensure product safety and security until it reaches the consumer. Glycerol improves the elongation at the break of edible film [14, 15]. Another study, Choi et al. [16] reported that addition of NaCl increased the elongation at break of pea starch films by adjusting formation and strength of intermolecular hydrogen bonds of starch molecules. Further, increasing the NaCl concentration enhanced the hydration effect of chitosan films. More hydrated chloride ions impeded the hydrophobic interactions between chitosan molecules and disrupted hydrogen bonding on the chitosan film [17].

Since studies on enhancing the mechanical properties of chitosan film incorporated with *Garcinia atroviridis* are scarce, the study of addition levels of glycerol and NaCl on chitosan films with *Garcinia atroviridis* can provide further insights into the application of film for chicken meat. This research evaluates the effects of physical, mechanical, and antimicrobial analysis of chitosan film incorporated with *Garcinia atroviridis* by optimizing the additional levels of glycerol and NaCl. The optimized film shall be applied to chicken meat for storage tests. The chicken meat slices will be wrapped

with the optimized chitosan film incorporated with *Garcinia atroviridis* and stored in a chiller at 4 °C for 15 days for further analysis.

Materials and Methods

Materials

Garcinia atroviridis and chicken breast meat (Gallus gallus domesticus) were purchased from a local market (Petaling Jaya, Malaysia). Chitosan analytical grade, glycerol (≥99.5%), and sodium chloride analytical grade were purchased from Sigma-Aldrich (M) Sdn Bhd, Petaling Jaya, Malaysia. Glacial acetic acid (≥99.99%) and Tween-80 analytical grade were purchased from LGC Scientific Sdn Bhd, Malaysia. Ethanol (≥99.8%) (Synertec Enterprise Sdn Bhd, Malaysia), barium chloride (≥99.99%) (Sigma Aldrich, USA), and sulphuric acid (≥96%) (Fisher Scientific, Malaysia), Mueller-Hinton agar (Synertec Enterprise Sdn Bhd, Malaysia), plate count agar (Chemolab, Malaysia), peptone water (Synertec Enterprise Sdn Bhd, Malaysia), stomacher bags (LGC Scientific Sdn Bhd, Malaysia), and petri dishes (90 mm × 15 mm) (Synertec Enterprise Sdn Bhd, Malaysia) were purchased.

Preparation of *Garcinia atroviridis* extracts

The *Garcinia atroviridis* was cut into smaller pieces and dried in an oven (1350 FX, Sheldon Manufacturing, Oregon, USA) overnight at 60 °C. After drying, it was ground into powder with a grinder (8011G, Waring, USA) to increase the total surface area of the *Garcinia Atrovidis* to obtain a higher yield during extraction. The powder was then placed into a sealed pack and kept in a chiller at 4 °C for further analysis.

The extraction was conducted as previously described by Zaman et al. [6] in which 80 g of *Garcinia atroviridis* powder was added to 800 mL of 99.8% (v/v) ethanol in a 1 L beaker. The mixture was then mixed for 24 hours with a magnetic stirrer (MS-H280-Pro, DLAB Scientific, Beijing, China) at 1500 rpm at room temperature. After 24 hours, a vacuum filter (WP6222050, Milipore, MA, USA) and filter paper (Whatman No. 3, Thermoline, NSW, Australia) were used to filter out the residue in the mixture. The filtrate then underwent evaporation using a rotary evaporator (R-000, BÜCHI, Flawil, Switzerland) at 60 °C under vacuum condition and the *Garcinia atroviridis* extracts were poured into a universal bottle and kept at 4 °C until future use.

Preparation of film

Film formation was conducted following the casting procedure of Zaman et al. [6]. To prepare the film solution, 1.5 g of chitosan (CH) powder was added in 100 mL of 1% (v/v) acetic acid to form (1.5%, w/v) CH solution. The CH solution was magnetically stirred for 24 h at room temperature. It was then filtered using a vacuum filter with filter paper to filter out insoluble residue.

For film formation, 25 mL of the CH solution was poured into a 50 mL beaker. After that, 5% (v/v) *Garcinia atroviridis* extract and 0.01% (v/v) Tween-80 were added to the solution with additional levels of glycerol (0.5, 1.0, 1.5, 2.0%, v/v) and NaCl (5, 10, 10.5 mM, w/v) as shown in Table 1. The solutions were homogenized using a homogenizer (HG-15D, Daihan Scientific, South Korea) at 9000 rpm for 4 min. After homogenization, the solution was transferred to a petri dish and placed into the oven for 24 h at 50°C. Once the films were dried, they were peeled and transferred into a zip-lock plastic bag and stored in a desiccator for further analysis.

Physical properties of film: Moisture content

The film moisture content was analyzed according to the method described by Lee et al. [3]. Films with a size of 2 × 2 cm were placed in a desiccator overnight. Then, the initial mass of the film was weighed (M_i). The film was then placed into an oven at 90°C until there were no changes in the final mass (M_f). Moisture content was determined using the equation below:

$$\text{Moisture content} = \frac{M_i - M_f}{M_i} \times 100\% \quad (1)$$

where M_i and M_f refer to the mass of the initial dried film and final dried film (g), respectively.

Water solubility

The film water solubility was measured following procedures from Chan et al. [18] with some

modifications. The final mass of the dried films from the moisture content determination were taken as the initial mass (W_1). The dried films were placed into a boiling tube filled with 30 mL of distilled water for 3 h. After immersion, they were taken out and underwent drying again in an oven for 24 h at 60°C. The final dry mass of the film was measured (W_2). The water solubility was determined using the following equation:

$$Water\ solubility = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

where $W1$ and $W2$ refer to the mass of the initial dried film and final dried film (g), respectively.

Color profile

The film color profile was measured according to the method described by Chan et al. [18]. A colorimeter (ColourFlex EZ, Hunterlab, Virginia, USA) was used to determine the color. The values, L^* (luminosity), a^* (green-red) and b^* (blue-yellow) were used to express the film color. The total color difference (ΔE) of the film was determined using the following equation:

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \quad (3)$$

where the color parameters of the white plate are expressed by L, a, and b and the color of the *Garcinia atroviridis* films are expressed as L^*, a^* and b^*.

Thickness

A manual micrometer (QB420100, JY, China) was used to measure the film thickness as described by the method from Kuan et al. [4]. Measurements were made at five random places of the film for which an average value was determined.

Mechanical properties

The measurements of tensile strength and elongation at break were conducted according to the procedures demonstrated by Lim et al. [19] with some modifications. A universal testing machine (CS4921, Lloyd Instrument, UK) was pre-set with the specifications for speed, gauge length, thickness, width, units, and tension mode. Film strips of 20 × 50 mm were held parallel to the grip of the machine. The separation of the grip was adjusted to 30 mm. The speed of the crosshead was adjusted to 20 mm/min. The values from the machine called peak load and peak extension were taken as tensile strength (*MPa*) and elongation at break (%), respectively. Young's modulus was determined using the following equation:

$$Young's\ modulus\ MPa = \frac{Stress}{Strain} \quad (4)$$

where stress is determined as the tensile force (F) / cross-sectional area (A) while strain is the length of film extension (e) / original film length (l_o).

Antimicrobial properties

The film antimicrobial properties were analyzed using the agar disk diffusion method described by Chan et al. [18]. Antimicrobial activities of the film were examined against two bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). The bacterial suspension was prepared with reference to the 0.5 McFarland standard. Then, 100 µL of the suspension was inoculated on a Mueller-Hinton Agar and spread aseptically with a glass hockey stick.

Films were shaped into 6 mm diameter discs and aseptically placed onto the Mueller-Hinton Agar plates that were previously inoculated. The positive control for *Pseudomonas aeruginosa* was Streptomycin (10 mg/mL). For the *Staphylococcus aureus*, penicillin (10 mg/mL) was used as the positive control. Ethanol was used as the negative control. The plates were then put into the incubator at 37°C for 24 h. The inhibition zone diameter including the edible film disk (6 mm) was determined with a vernier caliper.

Storage test of chicken meat

The chicken meat for storage test was prepared according to the method described by Higueras et al. [20]. After purchasing the chicken meat from the market, it was placed in an icebox and brought to the laboratory. It was cleaned with distilled water and lightly tamped with tissue paper. The chicken meat was cut into 2 cm × 2 cm size, 10 ± 1 g, and placed in the chiller at 4°C for further use.

The slices of chicken meat were then wrapped with the optimized film , which was based on the results of a previous analysis. They were then placed into a zip-lock bag. The chicken meat without film was also placed into a zip-lock bag and served as the control in this test. The chicken meat wrapped with film and the control were placed in the chiller at 4°C until analysis. They were stored for 15 days and the analysis on the chicken meats were conducted on day 0, 3, 6, 9, 12, and 15 of storage.

pH changes of chicken meat

The pH of the chicken meat wrapped with film and control were measured following procedures described in Tantasuttikul et al. [21]. The films were peeled off the chicken meat and the chicken meat were put into a stomacher bag. Then, the stomacher bag was filled with 100 mL of distilled water. Then it underwent homogenization for 2 min using a stomacher (BagMixer 400, Interscience, France). The pH reading was measured by dipping the pH meter (pH700, EUTech Instruments, Singapore) into the mixture.

Microbial changes of chicken meat

The total colony count was analyzed according to the procedures outlined in Remya et al. [46] with slight modifications. The films were peeled off the chicken meat and the chicken meat were put into a stomacher bag. Then, 90 mL of peptone water was poured into the stomacher bag, which underwent homogenization for 2 min using a stomacher. The homogenized chicken mixture underwent serial dilutions from 10^{-1} up until 10^{-6} using peptone water. After homogenization, 100 µL of each serial dilution was inoculated on a plate count agar and a glass hockey stick was used to spread the inoculations evenly. The plates were incubated for 24 h at 37°C. After incubation, the colonies were counted with the results expressed as $\log_{10}$ (CFU/mL).

Statistical analysis

Results were expressed as mean ± standard deviation of three determinations. The results were statistically analyzed using Minitab version 17 ($p \leq 0.05$) (Minitab, USA). The comparison of means was performed by one-way analysis of variance followed by Tukey's test. The Paired T-test was conducted to analyze results of total colony count and pH reading between the chicken meat wrapped with film and chicken meat without film (control).

Table 1. Formulation of chitosan film incorporated with *Garcinia atroviridis* extract

Film Formulation	Glycerol Concentration (%)	Sodium Chloride Concentration (mM)
A	0.0	0.0
B	0.5	0.0
C	1.0	5.0
D	1.5	10.0
E	2.0	10.5

Results and Discussion

Physical properties of films

Moisture content

Dehghani et al. [22] showed that low moisture content is usually favorable for edible films as it reduces the risk of microbial growth. Table 2 presents the moisture content of each chitosan film with *Garcinia atroviridis* extract with different addition levels of glycerol and NaCl.

The increment of glycerol and NaCl increased the moisture content of the film from 15.42% to 27.29%. This might be due to the glycerol's hygroscopicity whereby it tended to absorb and retain moisture from its surrounding [23]. Additionally, as a humectant, the

hydroxyl groups in glycerol formed hydrogen bonds in the water [24]. The addition of glycerol also elevated the viscosity and allowed for film matrix interaction with water to increase [25]. These results are also similar to Peng et al. [26] and Dick et al.[27] in which the increment of glycerol caused the moisture content of a film to increase.

Water solubility

Water solubility is an important attribute because it determines biodegradability, integrity, and water resistance [28]. Table 2 shows that the increment of additional levels of glycerol and NaCl increased the water solubility of the films from 46.40% to 59.30%. The increment in water solubility might be due to the hydrophilic behavior of glycerol. The incorporation of glycerol increased the affinity of the film matrix to bind to water thus increasing the solubility of the film [29].

Additionally, Yang et al. [30] suggested that glycerol increased the hydrogen bonds in the chitosan film matrix and leads to a higher availability for it to interact with water molecules. Furthermore, it could be explained that glycerol disrupted the chitosan network interaction density. The presence of a polar molecule, glycerol would increase the interaction with water. It caused a decrease in intermolecular forces by interacting with the functional groups of the chitosan, causing an increase in solubility. This led to the elevation of the water solubility of the film [23]. This trend of increasing water solubility was also observed by Cerqueira et al. [28], whereby the water solubility of chitosan films increased from 51.86% to 69.94% when the glycerol was increased from 0.5% (v/v) to 2.0% (v/v).

Color profile

Color plays a significant part in the consumer's perception of food as it is one of the first characteristics that the customer will evaluate [4]. Figure 1 shows the appearance of the films. As seen in the figure, the films were yellowish-brown in color. According to Zaman et al. [6], the yellowish-brown film color was mainly due to the presence of pigments in *Garcinia atroviridis* called xanthonoids.

The overall color profile of the films is best described using the total color difference (ΔE) (Table 3). The range of the ΔE of the films was from 0.00–1.37. The results showed that the ΔE of the film with 0.5% (v/v) glycerol and 0 mM (w/v) NaCl was highest. However, there was no significant difference between the color profile of the films. The ΔE of the film reduced when the addition level of glycerol and NaCl increased. This could be attributed to the fact that glycerol and NaCl was colorless and it did not have the color imparted into the film. Peng et al. [26] and Dick et al. [27] observed the increment of glycerol had not changed the ΔE of film in combination with tea polyphenols. Another factor to consider is the concentration of chitosan used that influences the color of the edible film. However, in this study, the concentration of chitosan was kept constant. Therefore, it did not influence the color of the films.

Thickness

Thickness is a key characteristic in an edible film because thicker films provide more security for the food product. Figure 2 shows the results of the film thickness analysis. The thickness of the films ranged from 0.097–0.122 mm. The thickness value of film with 2% (v/v) glycerol and 10.5 mM (w/v) NaCl was the lowest. The concentration of NaCl in the film with 2% (v/v) glycerol and 10.5 mM (w/v) allowed the permeation of hydrated chloride ions (Cl^-) into the chitosan films, which disrupted the hydrogen bonds and hydrophobic interactions. This led to the loss of cohesion capacity of chitosan films. As a result, water molecules were squeezed out from hydrated ions and reduced the film's thickness [17]. Similar results were reported for chia mucilage films [27].

Mechanical properties of film

Tensile strength measures the amount of tensile stress the film can withstand until it breaks. Conventional standards state that food packaging should have a tensile strength greater than 3.5 MPa to be deemed as satisfactory [31]. Table 4 presents the tensile strength of the films was in the range of 4.03–9.24 MPa. Hence, based on this standard, all the film formulations in this study fulfilled this criterion.

Further, the tensile strength reduced as the additional levels of glycerol and NaCl increased. The increment in the additional levels of glycerol in the film caused a

plasticizing effect for which the chitosan network chains became weaker [31]. Furthermore, more mobile regions were created due to the interference caused by glycerol. High additional levels of glycerol elevated the film water content. This could be attributed to the hygroscopic behavior of glycerol that led to the reduction in the interactions between adjacent macromolecules [29].

Several studies showed that the increment in the additional levels of glycerol reduced the tensile strength of the film [33, 34]. Apart from that, the results from the this study showed an improvement in tensile strength when compared to the results of Zaman et al. [6]. The authors detected that at 5% (v/v) addition level of *Garcinia atroviridis* extract, the tensile strength was 3.28 MPa, which was lower than for films in this study. This indicated that the addition of glycerol and NaCl improved the tensile strength of the film.

Elongation at break is the film's capability to resist changes in shape without breaking. For edible films, a high elongation is favorable as it enhances the ability of the film to wrap and package food. Table 4 shows the range of the elongation at break was from 59.32–123.35%. The film with 1.5% (v/v) glycerol and 10 mM (w/v) NaCl had the highest elongation at break. Additionally, the increment in additional levels of glycerol and NaCl in the film showed increments on the elongation at break except for the film with 2% (v/v) glycerol and 10.5 mM (w/v) NaCl.

The increment in elongation of the film can be due to the interactions of the glycerol with the chitosan chains. The plasticizing effect of glycerol reduced the acting forces between the molecules leading to the sliding of the polymer chain. Thus, the flexibility and ductility of the film were increased [33]. Apart from that, the addition of NaCl enhanced elongation of the film. The NaCl ions may cause an increase of electrostatic repulsion among chitosan chains leading to the formation of stretched chitosan chains from coiled chitosan chains [35]. Prateepchanachai et al. [48] demonstrated that salt produces a counter-ion effect that depresses the third electroviscous effect and renders the chitosan molecules more flexible with a random, coiled shape. Coiled molecules will result in less inter-molecular entanglements. The ability of the film to stretch was increased, increasing the elongation of the film. Th results of this study are similar to Liu et al. [36] in that the elongation of chitosan-starch blended films had an incorporation of 2.5-10% (v/v) glycerol increased from 13.5-18.0%, accordingly.

However, the elongation of the film decreased from 123.35% to 70.45% when the additional levels was increased to 2% (v/v) glycerol and 10.5 mM (w/v) NaCl. This was probably associated to the anti-plasticizing effect exhibited by glycerol at high additional levels [36]. Souza et al. [38] found that at high additional levels, plasticizers exhibited strong interactions with biopolymers. This led to the decrement in the macromolecular mobility and elongation at break of the film. This explained why, at higher plasticizer concentrations, such as the anti-plasticization phenomena were associated with stronger interactions between plasticizer and chitosan molecules, which impedes macromolecular mobility. When the amount of plasticizer molecules in a plasticized chitosan-based film exceeded a critical value, anti-plasticization occurred. As a result, the film's elongation at the break was reduced [47].

Young's modulus measures the ability of a film to withstand elastic deformation when under elongation or compression. Table 4 shows that the range of the values of Young's modulus was from 0.046–0.146 MPa. The increment in additional levels of glycerol and NaCl decreased the Young's modulus of the film. The decreasing trend of Young's modulus might be due to the effect of glycerol in increasing the elasticity of the film and reducing the interactions among the chitosan chain. This resulted in the film becoming less stiff [32]. Similarly, Kusumaningtyas et al. [39] detected that the increment in glycerol lowered the Young's modulus of films as well.

Antimicrobial properties of film

Figures 3 and 4 show the inhibition zone of the different film formulations on selected pathogens. The findings showed that film with 0.5% (v/v) glycerol and 0 mM (w/v) NaCl, which showed the highest inhibition zone

against *Pseudomonas aeruginosa* (10.50 mm) and *Staphylococcus aureus* (11.00 mm).

The inhibition zone of the films ranged from 9.67–10.50 mm for *Pseudomonas aeruginosa* and 10.17–11.00 mm for *Staphylococcus aureus*. The results indicated that the films exhibited antimicrobial properties towards these pathogens. The antimicrobial properties of the film could be attributed to the chitosan film and the *Garcinia atroviridis* extract. Chitosan has been widely known to exhibit antimicrobial properties because it possessed charged groups and has ionic interactions with bacterial cell walls. The antibacterial action of chitosan was done via the binding of the amino group with the membrane of the bacterial cell. This led to the disruption of the cell membrane, followed by the intracellular leakage that led to cell death [8].

The extracts from *Garcinia atroviridis* are known to exhibit antibacterial properties against various bacteria due to flavonoids and other phenolic compounds [13]. Zhang et al. [40] demonstrated that chitosan film with 10% (w/v) mangosteen rind powder was able to inhibit *Salmonella* and *Staphylococcus aureus*. Additionally, Zaman et al. [6] observed that chitosan film incorporated with 5% (v/v) *Garcinia atroviridis* extract was able to inhibit *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Table 2. Moisture content (%) and water solubility (%) of the different formulations of chitosan films incorporated with Garcinia atroviridis extract

Film Formulation	Moisture Content, (%)	Water Solubility (%)
A	15.42 ± 1.84^{a}	46.40 ± 1.31^{a}
B	18.64 ± 2.10^{b}	49.60 ± 1.94^{ab}
C	22.31 ± 1.96^{c}	52.13 ± 2.43^{b}
D	24.15 ± 1.64^{c}	55.77 ± 1.64^{c}
E	27.29 ± 1.37^{d}	59.30 ± 2.04^{d}

Results are presented as mean value ± standard deviation of three replicates.
Different letters superscripted denote significant difference ($p \leq 0.05$) between samples for each parameter in the same column.

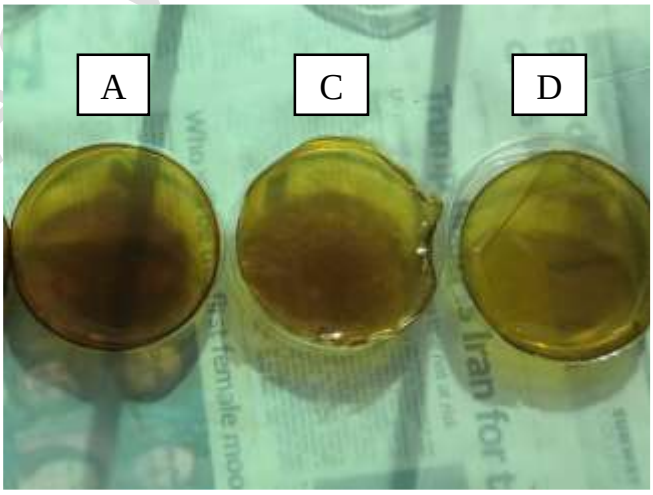

Figure 1. The appearance of the films made from formulation A, C and D

Table 3. Color parameters of the different formulations of chitosan film incorporated with *Garcinia atroviridis* extract

Film Formulation	L*	a*	b*	ΔE
A	8.50 ± 1.03^{ab}	0.39 ± 0.65^{a}	8.70 ± 0.62^{ab}	0.00 ± 0.00^{a}
B	8.30 ± 0.64^{a}	-1.43 ± 0.40^{b}	8.37 ± 1.46^{a}	1.37 ± 0.93^{b}
C	9.19 ± 0.72^{ab}	0.77 ± 0.94^{a}	8.40 ± 0.67^{a}	0.78 ± 0.61^{a}
D	8.46 ± 0.21^{ab}	1.20 ± 0.38^{a}	9.16 ± 0.51^{b}	0.27 ± 0.91^{a}
E	9.31 ± 1.07^{b}	1.12 ± 0.25^{a}	8.81 ± 0.06^{ab}	0.63 ± 0.78^{a}

Results are presented as mean value ± standard deviation of three replicates.
Different letters superscripted denote significant difference ($p \leq 0.05$) between samples for each parameter in the same column

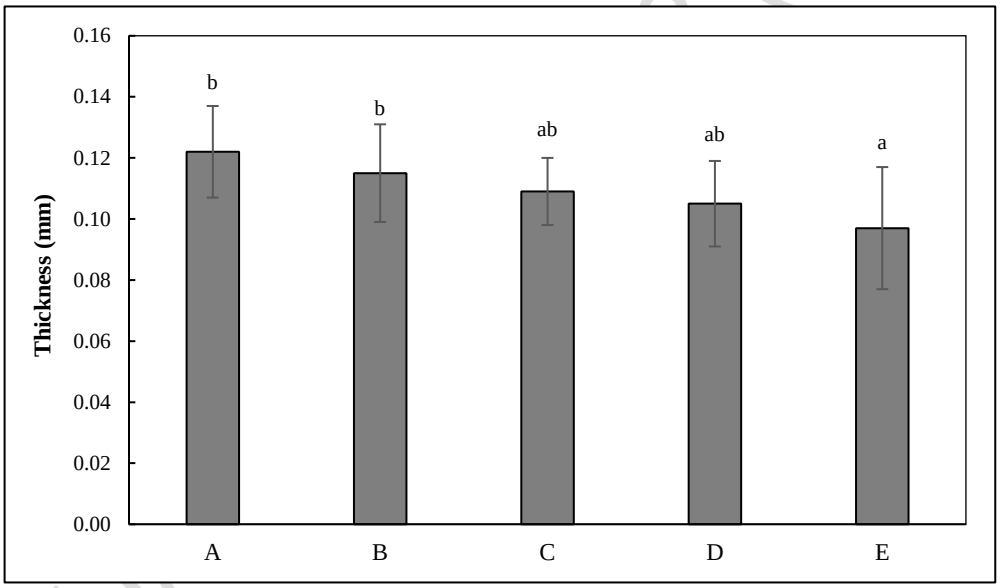

Figure 2. The thickness (mm) of the different film formulations of chitosan film incorporated with *Garcinia atroviridis* extract. A represents film containing 0% glycerol and 0 mM NaCl; B represents film containing 0.5% glycerol and 0 mM NaCl; C represents film containing 1.0% glycerol and 5 mM NaCl; D represents film containing 1.5% glycerol and 10 mM NaCl; and E represents film containing 2.0% glycerol and 10.5 mM NaCl. Error bars indicate mean ± standard deviation of three replicates. Bars with different letters denote significant difference ($p \leq 0.05$) between samples.

Table 4. Mechanical properties of the different film formulations of chitosan film incorporated with *Garcinia atroviridis* extract

Film Formulation	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
A	9.24 ± 0.77^{d}	59.32 ± 2.50^{a}	0.156 ± 0.011^{c}
B	7.44 ± 0.55^{c}	93.77 ± 2.85^{c}	0.080 ± 0.005^{b}
C	6.17 ± 0.72^{b}	109.63 ± 3.36^{d}	0.056 ± 0.006^{a}
D	5.66 ± 0.64^{b}	123.35 ± 3.73^{e}	0.046 ± 0.054^{a}
E	4.03 ± 0.62^{a}	70.45 ± 1.69^{b}	0.057 ± 0.009^{a}

Results are presented as mean value ± standard deviation of three replicates

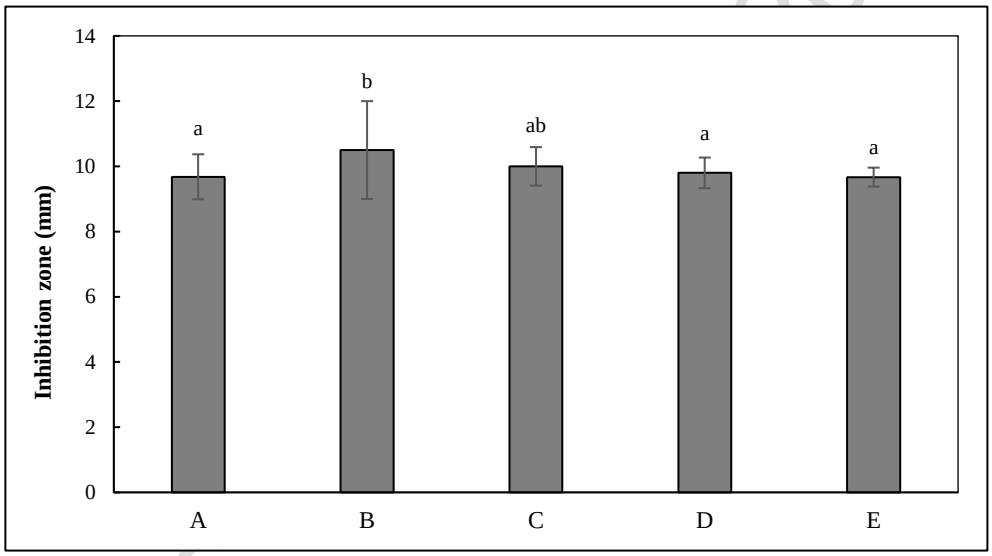

Figure 3. The antibacterial activity of the different film formulations of *Garcinia atroviridis* extract incorporated into chitosan film on *Pseudomonas aeruginosa*. A represents film containing 0% glycerol and 0 mM NaCl; B represents film containing 0.5% glycerol and 0 mM NaCl; C represents film containing 1.0% glycerol and 5 mM NaCl; D represents film containing 1.5% glycerol and 10 mM NaCl; and E represents film containing 2.0% glycerol and 10.5 mM NaCl. Error bars indicate mean ± standard deviation of three replicates

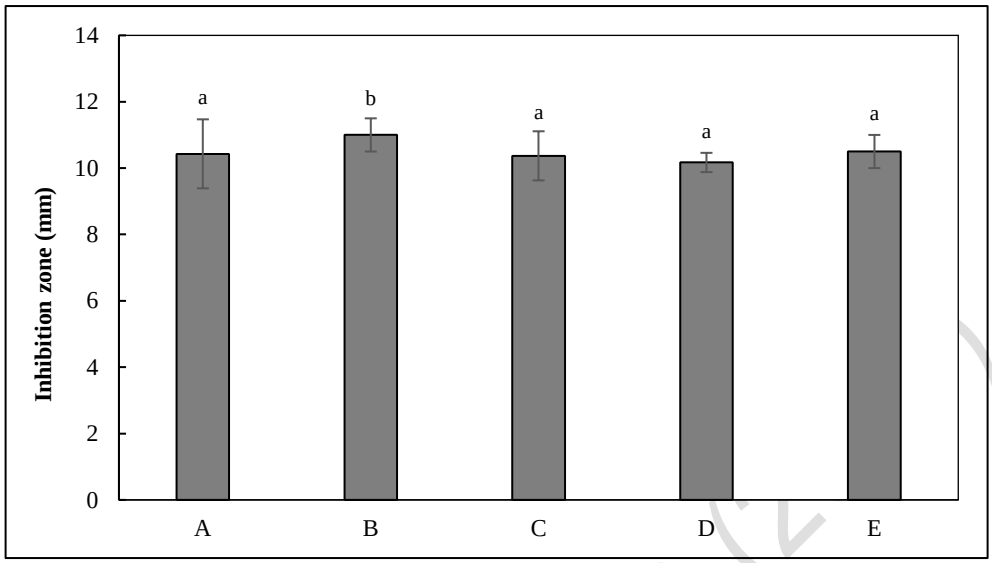

Figure 4. The antibacterial activity of the different film formulations of *Garcinia atroviridis* extract incorporated into chitosan film on *Staphylococcus aureus*. A represents film containing 0% glycerol and 0 mM NaCl; B represents film containing 0.5% glycerol and 0 mM NaCl; C represents film containing 1.0% glycerol and 5 mM NaCl; D represents film containing 1.5% glycerol and 10 mM NaCl; and E represents film containing 2.0% glycerol and 10.5 mM NaCl. Error bars indicate mean ± standard deviation of three replicates

Storage test of chicken meat: Optimized film

The film formulation that was used for the storage test was chosen through the evaluation of physical, mechanical, and antimicrobial properties of the films. Each film formulation showed antimicrobial properties. Hence, films with optimum additional levels of glycerol and NaCl were selected based on its mechanical properties. The elongation at break of the film with 1.5% (v/v) glycerol and 10 mM (w/v) NaCl was the highest (123.35%) and had an acceptable tensile strength (5.66 MPa) and Young's Modulus (0.046 MPa). The film with incorporation of 1.5% (v/v) glycerol and 10 mM (w/v) NaCl was applied to the chicken meat for the storage tests.

pH changes of chicken meat

Figure 5 presents the pH values of the chicken meat wrapped with the film and chicken meat without film (control) during storage. The pH of the chicken meat at the beginning of the storage test was 5.86. The pH of the control increased as the number of storage days increased whereas the pH of the chicken meat wrapped with film decreased as the number of storage days increased (Figure 5). The increment in the pH value of the control might be caused by the accumulation of amines and ammonia due to the growth of bacteria. The bacteria broke down amino acids, which produced an accumulation of ammonia that increased the pH value of the chicken meat [41]. The studies on chicken breast meat storage have shown that pH and bacterial growth had a positive correlation [42]. Further, it was suggested by Bazargani-Gilani et al. [43] that the rise in pH value of chicken meat during storage because of a rise in volatile bases caused by the activity of endogenous enzymes such as protease and lipase.

Krishnan et al. [41] showed that the pH of raw chicken meat increased after 15 days of storage at 4 °C, which agrees with our findings. On the other hand, the decrease of the pH value of the chicken meats wrapped with film may be caused by the reduction of bacterial activity and the production of volatile base compounds in the chicken meats [44].

Microbial changes of chicken meat

The total colony count of the chicken meat wrapped with film and control during storage was calculated and expressed in log (CFU/mL) as shown in Figure 6. The black dotted lines in the line graph represents the safe microbial limits for consumption of chicken meat, which was less than log 6.0 [10]. The chicken meat was considered to be unsafe for consumption when its total colony count exceeded log 6.0. The total colony count of the chicken breast meat at beginning of the storage test was log 3.48.

The total colony count increased as the number of storage days increased for the controls and the chicken meats wrapped with film. The total colony count for the controls reached a value of log 8.17 after 15 days of storage. Conversely, the chicken meats wrapped with the film reached a total colony count of log 6.10 after 15 days. The total colony count for the chicken meats wrapped with the film was lower than the control for all days of the storage period.

In addition, from Figure 6, the colony-forming unit (CFU) for the control exceeded log 6.0 on the 6th day of storage; whereas, the chicken meats wrapped with the film only exceeded log 6.0 on the 15th day of storage. Hence, we deduced that the control was safe for consumption up to day 5 of storage while the chicken meats wrapped with the film were safe for consumption up to day 14 of storage. It can be concluded that the chitosan film incorporated with 5% (v/v) *Garcinia atroviridis* extract can extend the shelf life of chicken meat from 5 days to 14 days when stored at 4°C. The ability of the film to prolong the shelf life of the chicken meat might be due to the presence of antimicrobial properties in the film. Figures 3 and 4 show that the films inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Basri et al. [13] showed that *Garcinia atroviridis* extract can inhibit the growth other pathogenic bacteria such as *Bacillus subtilis*, *Salmonella enteritidis* and *Escherichia coli*. Hence, the films can reduce the total colony count of the chicken meat and consequently extended its shelf life. Several studies have similarly reported on the ability of edible films to increase the shelf life of food products. Chitosan film incorporated with clove essential oil and nisin increased the shelf life of pork patties from 6 days to 12 days [45]. In another study, the shelf life of Indian mackerel fish was extended when the fish was wrapped with chitosan film incorporated with 5% (v/v) *Garcinia atroviridis* [6].

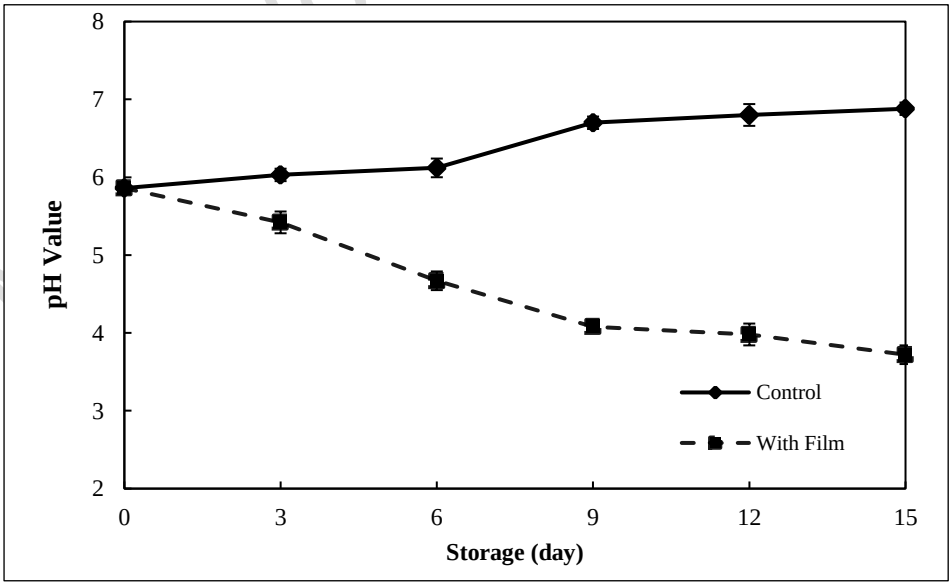

Figure 5. Changes in pH value throughout the storage period

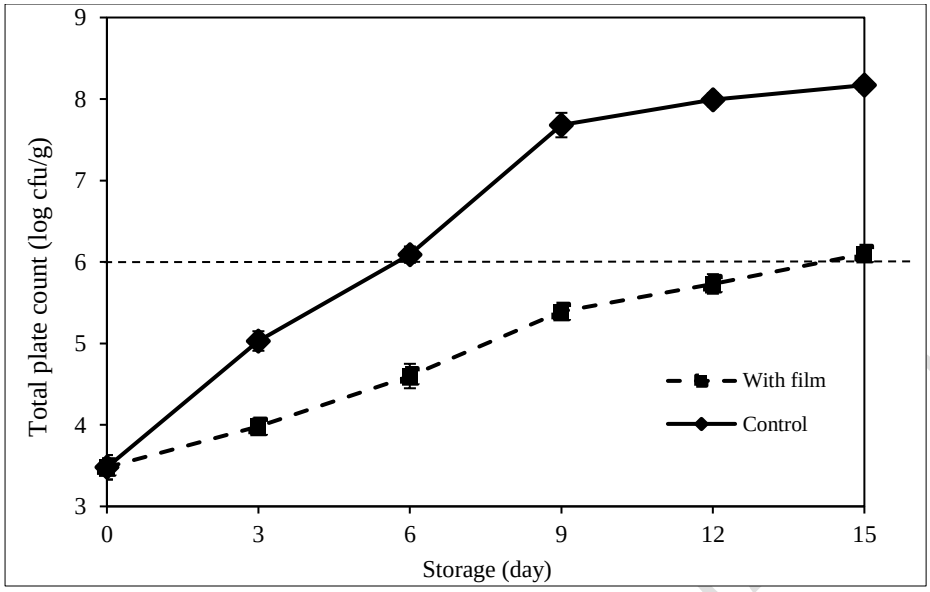

Figure 6. Changes in total plate count in log (CFU/mL) throughout the storage period

Conclusion

This study has demonstrated the incorporation of 1.5% (v/v) glycerol and 10 mM (w/v) NaCl into chitosan film incorporated with *Garcinia atroviridis* extract had the highest elongation at break and tensile strength that fulfilled the conventional standard. Therefore, glycerol and NaCl improved the mechanical properties of film. Storage tests showed that that the chicken meat wrapped with the film had a shelf life less than 14 days whereas the controls had a shelf life of 5 days. Additionally, the results from the storage test indicated that applying the film onto meat products such as chicken meat can extend its shelf life. Hence, edible films with better mechanical properties are able to extend shelf life of food products and were developed in this study.

Acknowledgement

This work was supported by UCSI University Pioneer Scientist Incentive Fund (Proj-In-FAS-056) from UCSI University and UCSI Fundamental Research Grant Scheme (FRGS/1/2020/STG01/UCSI/02/3) from the Ministry of Education Malaysia.

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