

Semi-refined carrageenan film incorporated with α -tocopherol: Application in food model

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Abstract

Semi-refined carrageenan (SRC) film plasticized with glycerol and incorporated with α -tocopherol was prepared for food packaging application. Functional characterization, thermal stability, and microstructure of SRC-based films were analyzed. The effects of antioxidant α -tocopherol from SRC-based film were studied based on thio-barbituric acid-reactive substance assay, metmyoglobin assay, and pH value in food model (meat patties) for 12 days of storage. The development of lipid oxidation was delayed in the meat patties wrapped with antioxidant films during the storage with the final value of 0.68–0.37 mg malondialdehyde/kg sample. A less than 50% brown color development (metmyoglobin) of the meat patties wrapped with antioxidant film was observed in the first nine days of storage. Hence, the incorporation of α -tocopherol into the SRC-based film could be an alternative way to prolong the shelf life of food product, reducing the use of synthetic preservative directly into food product.

Practical applications

Recent strategy on the development of biodegradable film for food packaging is important as an alternative to the petrochemical-derived plastic that is harmful to the environment. SRC is one of the potential biopolymers that has the ability to form a strong gel and provides efficient barrier against gas, lipids, and oils, and addition of plasticizer glycerol into SRC enhanced mechanical and barrier properties of the films. The present study showed the potential of α -tocopherol within the SRC film plasticized with glycerol as active packaging on the film characteristics and increased the shelf life of meat patties. Hence, the active packaging film developed is not only contributes to reduce plastic waste discharged to environment but can be a potential substitute of synthetic preservative in food.

1 | INTRODUCTION

One of the major challenges in food preservation is to reduce the acceleration of deterioration rate of lipid which mainly occurs in food with a high content of unsaturated lipid. The oxidation of lipids leads to the growth of microorganisms and the formation of toxic aldehydes (Ganiari, Choulitoudi, & Oreopoulou, 2017) and affects the appearance, flavor, and nutritional quality of the product. Commonly,

several procedures have been applied to prevent the oxidative deterioration in foodstuffs such as the direct addition of antioxidant into the food product (Serrano-León et al., 2018) or the use of modified atmosphere packaging technology (Sánchez-Escalante, Torrescano, Djenane, Beltrán, & Roncalés, 2003). The current approach implemented in the food industries to prevent the oxidative deterioration in food is using synthetic antioxidant such as butylated hydroxyanisole, butylated hydroxytoluene, and ter-butylhydroquinone that

results in dubious health effects to consumers (Serrano-León et al., 2018; Trifković et al., 2014). In this sense, researchers currently investigate the development of active films from biopolymer incorporated with natural antioxidant to prolong the shelf life of food products and reduce waste disposal problems in the environment. Different authors demonstrated that the incorporation of natural antioxidants with phenolic compounds such as tocopherol (Liu, Dai, Zhu, & Li, 2010), rosemary, oregano essential oil (Vital et al., 2016), and acerola agroindustrial residue (Zegarra, Santos, Silva, & Melo, 2018) in biopolymer is able to inhibit the oxidation in the food, extending their shelf life by preserving color, odor, and texture.

Biopolymers derived from either polysaccharides, proteins, or lipids are the main component in the development of film that does not only account of their degradable behavior but also the advantage of renewability and abundance of resources (Shojaee-Aliabadi et al., 2013). Carrageenan is one of the most abundant polysaccharide polymers extracted from a certain species of red seaweed and a hydrocolloid product after starch and gelatin (Nur Fatin Nazurah & Nur Hanani, 2017). Carrageenan is classified according to the number and position of a sulfated ester on 3,6-anhydro-d-galactose residues and it has good film-forming properties that provide efficient barriers against gas, oils, and lipids (Shojaee-Aliabadi et al., 2013). The production of carrageenan in the industry involves two processes which are refined carrageenan and semi-refined carrageenan (SRC). SRC that is available at a reasonable cost has excellent gelling and binding properties to be used as film-forming material (Farhan & Hani, 2017). Furthermore, the addition of plasticizer such as glycerol into biopolymer exhibits a good potential for the production of edible/biodegradable packaging films with enhanced mechanical and barrier properties (Vieira, Da Silva, Dos Santos, & Beppu, 2011).

α -Tocopherol, also known as vitamin E, possesses many pharmacological benefits to human health including anticancer and ant cardiovascular diseases due to its antioxidant capacity (Azzi, 2017; Peh, Tan, Liao, & Wong, 2016). The potential use of α -tocopherol as antioxidant coating in film has been widely investigated, where the hydrophobic compounds of α -tocopherol exhibit antioxidant activity and improve the film properties when incorporated into biodegradable films (Martins, Cerqueira, & Vicente, 2012; Noronha, De Carvalho, Lino, & Barreto, 2014). The formulation of an active film with antioxidant compound is not limited to single compound (e.g., α -tocopherol). Several studies showed that the formulation of natural antioxidant extracts from mango peel (Kanatt & Chawla, 2018), propolis (Jafari, Kargozari, Ranjbar, Rostami, & Hamedi, 2018), and *Betula pendula* (Gallego, Gordon, Segovia, & Almajano Pablos, 2016) with biopolymer are able to inhibit the oxidative deterioration and retains the freshness in muscle foods.

Therefore, the present study aims to develop and characterize active packaging film from SRC plasticized with glycerol (G) and incorporated with different concentrations of α -tocopherol (0.1%, 0.2%, 0.3%, and 0.4% [v/v]). Nevertheless, studies on functional characteristic, thermal stability, and microstructure property of films have not been fully documented. The release effect of SRC-film

with antioxidant into meat patties was also determined by measuring the lipid oxidation rate, pH, and brown color development.

2 | MATERIALS AND METHODS

2.1 | Materials

SRC was extracted from *Kappaphycus alvarezii* seaweed obtained in Sabah, Malaysia. Food grade G, α -tocopherol, phosphate-buffered saline tablet, thiobarbituric acid (TBA), 1,1,3,3-tetramethoxy propane, ethylenediaminetetra acetic acid, sodium chloride, potassium hydroxide (KOH), and hydrochloric acid were purchased from Sigma-Aldrich (Gillingham, United Kingdom). The minced meat was purchased from a local market in Kuantan, Pahang.

2.2 | Preparation of SRC from *Kappaphycus alvarezii* seaweed

SRC was prepared according to the established method by Mustapha, Chandar, Abidin, Saghravani, and Harun (2011). *Kappaphycus alvarezii* seaweed was cleaned under running tap water to remove debris and dried under sunlight for 8 hr. The extraction process was prepared by adding 150 g of dried seaweed into 1.0 M of KOH at 80°C for 2 hr. The extracted seaweed was neutralized by soaking it completely in water for 12 hr. Then, the neutralized seaweeds were dried at 50°C for 24 hr using a laboratory oven (Memmert, EQP004, Schwabach, Germany). Lastly, the samples were ground using a laboratory grinder (Retsch, ZM 200, Haan, Germany) with 0.5-mm mesh and stored in a desiccator for further analyses.

2.3 | Preparation of SRC-based films

The SRC-based films were prepared according to the method by Farhan and Hani (2017) with slight modifications. The SRC film solution was prepared by dissolving 2 g of SRC in distilled water (2% [w/v]) under continuous stirring and the solution was heated at 70°C for 10 min using a hot plate magnetic stirrer. After dissolution, plasticizer G was added at 0.9% (v/v) into the solution. The film-forming solution was heated to 80°C and maintained for about 10 min prior to the addition of antioxidant α -tocopherol (Tp) at different concentrations (0.1%, 0.2%, 0.3%, and 0.4% [v/v]) under continuous stirring. After the addition of Tp, the film-forming solution was heated to 90°C and maintained for approximately 5 min. The SRC film-forming solution was then cooled to 65°C and manually stirred using a glass rod to remove dissolved air bubbles produced during stirring. Then, 80 ml of the SRC film-forming solution was casted on a casting plate and dried using a laboratory oven at 50°C for 12 hr.

The SRC-control film was prepared without the addition of plasticizer and antioxidant, while the SRC + G film was prepared without the addition of antioxidant. The films were then cooled at room temperature before peeling off from casting plates and stored at 25°C and 50 \pm 5% relative humidity prior to further analyses.

2.4 | Thickness

Film thickness was measured by a micrometer (Mitutoyo Co, Tokyo, Japan) with a precision of 0.001 mm. Average of five random measurement positions were taken and the average value was used in the calculation of thickness and tensile strength (TS).

2.5 | Fourier transform infrared spectroscopy

The functional groups of the SRC-based films were determined using Fourier transform infrared [FTIR] spectrometer (Thermo Scientific Nicolet iS5 FTIR Spectrometer, Massachusetts, USA). The FTIR spectra were determined in a wavelength region from 400 to 4,000 cm^{-1} using OMNIC software.

2.6 | Thermal analysis

Thermogravimetric analysis (TGA) was performed to measure the thermal stability of SRC-based films. The analysis was performed using PerkinElmer, TGA7 at a heating rate of 10°C/min under nitrogen environment. The samples were heated from room temperature to 600°C.

2.7 | Scanning electron microscopy

The surface and cross-sectional microstructures of the SRC-based films were observed using scanning electron microscope (JEOL, model JSM-6460LV, Tokyo, Japan). The film samples were mounted on steel stubs using double-sided adhesive tape and coated with gold using a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). The image of film was captured under the microscope at 500 × -1,000 × magnification.

2.8 | Preparation of meat patties

The meat patties were purchased 7 days after slaughtering process to allow it to mature and kept at $-4 \pm 1^\circ\text{C}$ for further treatment. Approximately 20 g of meat was wrapped with different types of the SRC-based films: SRC-control, SRC + G, SRC + G + Tp0.1%, SRC + G + Tp0.2%, SRC + G + Tp0.3%, and SRC + G + Tp0.4%. The films sample were placed on the top and bottom to cover the whole meat with approximate size of 7 cm diameter. Each meat patty wrapped with treated films were prepared in triplicate. All the samples were placed in sterilized trays and stored in a chiller at $4 \pm 2^\circ\text{C}$ for 12 days of storage for analysis.

2.9 | Thiobarbituric acid reactive substance assay

Lipid oxidation that occurred in food model (meat patties) over the storage period was measured using thiobarbituric acid reactive substance (TBARS) method as described by Azman, Gallego, Julià, Fajari, and Almajano (2015). One gram of sample (meat patties) was mixed with 3 g/L of aqueous EDTA and immediately mixed with 5 ml of

0.375% (w/v) of TBA reagent using Ultra-Turrax (IKA, Germany) at 32,000 rpm until the mixture became homogeneous. The mixture then was incubated in hot water at $97 \pm 1^\circ\text{C}$ for 10 min under constant shaking. The liquid sample was recovered by filtration (Whatman, 0.45 μm), and the absorbance value was recorded at 532 nm using a UV-visible spectrophotometer (HITACHI, U-1800, Tokyo, Japan). The TBARS value was calculated from malondialdehyde (MDA) standard curve prepared with 1,1,3,3-tetraethoxypropane and analyzed using linear regression. All the results were reported in mg MDA per kg of sample (mg MDA/kg sample).

2.10 | Metmyoglobin assay

The metmyoglobin assay was performed using the method developed by Xu et al. (2010). Five grams of meat patties were homogenized with 25 ml of ice-cold 0.04 M of phosphate buffer (pH 6.8) for 30 s using Ultra-Turrax at 18,000 rpm. The homogenized mixture was allowed to stand at 4°C for 1 hr and centrifuged using a high-speed refrigerated centrifuge at 12,000 rpm for 10 min. The absorbance of the filtered supernatant was read at 572, 565, 545, and 525 nm using a UV-visible spectrophotometer. The percentage of metmyoglobin (MetMb%) was determined using Equation 1.

$$\text{MetMb(\%)} = [-2.514(\text{Abs}_{572}/\text{Abs}_{525}) + 0.777(\text{Abs}_{572}/\text{Abs}_{525}) + 0.5(\text{Abs}_{545}/\text{Abs}_{525}) + 1.098] \times 100 \quad (1)$$

2.11 | pH measurement

The pH value of meat patties was measured periodically with a pH meter (Mettler-Toledo, model GLP 21). The pH of the samples was measured three times for each sample for a good standard variation.

2.12 | Statistical analysis

Statistical analysis was performed using one-way analysis of variance using SPSS statistical software version 17.0 (SPSS Inc., Chicago, IL, USA). Bonferroni's test was used to determine the significant differences at $p < 0.05$ level.

3 | RESULTS AND DISCUSSION

3.1 | Film appearance and thickness

The SRC + G film incorporating Tp with 0.1%, 0.2%, 0.3%, and 0.4% (v/v) displayed homogeneous blend of materials and the films were easy to handle and peeled from the casting plate. To identify the highest concentration of Tp that could be incorporated in SRC + G matrix, the increasing amount of Tp (0.5%, 0.6%, and 0.7% [v/v]) were tested in the film-forming solution. The film prepared with higher concentration of more than 0.4% (v/v) of Tp exhibited opaque and oily forms which might be attributed to the oily characteristic of Tp. The effect of active agent on the physical traits of the film generally depends on the type of compounds and their inherent hydrophilicity and hydrophobicity

indices (Shen & Kamdem, 2015). Furthermore, a study by Martins et al. (2012) also showed that higher percentage of Tp was discarded, as the surface of chitosan films was too greasy with more than 0.2% (w/w) of Tp in the chitosan-based films.

Previous study shows that the physical and mechanical properties of the SRC films were greatly affected by the addition of G and Tp (Hamid et al., 2018). The incorporation of Tp in the SRC film plasticized with G reduced the TS but the elongation and stretchability of the produced film increased significantly ($p < 0.05$) compared to that of the SRC-control. The solubility of the films was improved with the addition of Tp and the value was range between 49% and 59% (Hamid et al., 2018). They also demonstrated the opacity of SRC-based films reduced with the addition of G, where the incorporation of Tp into SRC plasticized with G films slightly increase the opacity value of the films.

The thickness of SRC + G with different concentrations of Tp is shown in Table 1. The films with 0.1%, 0.2%, 0.3%, and 0.4% (v/v) of Tp significantly ($p < 0.05$) affect the thickness of films compared with the SRC-control with values range between 0.0917 mm and 0.1443 mm. The addition of minimal amount of Tp (less than 0.2% [v/v]) demonstrated better blending of the SRC + G compounds into the matrices with a result of no significant changes of thickness compared with that of SRC + G. Our findings suggest that changes in Tp concentration led to the increment of thickness value that was relevant to the increasing of solid content in the film. Meanwhile, the film thickness was plausibly due to the protruding structures mediated by the interaction between the chemical compounds present in SRC, G, and Tp. The finding was supported by Jongjareonrak, Benjakul, Visessanguan, and Tanaka (2006) who explained that the cause of increased thickness in films was due to the dispersion of plasticizer and other components into interstitial spacing between polymer chains in film matrices. Peng, Wu, and Li (2013) demonstrated similar effect on the thickness of the chitosan films, where the thickness value increased with an increased amount of green tea extract added into the film solutions from 0.072 mm to 0.139 mm.

3.2 | Fourier transform infrared spectroscopy

The interaction between three components, namely carrageenan, G, and Tp in the SRC-based films was analyzed using FTIR spectroscopy

TABLE 1 The thickness of SRC-based films

Sample	Thickness (mm)
SRC-control	0.0653 ± 0.0005 ^a
SRC + G	0.0917 ± 0.0024 ^b
SRC + G + Tp0.1%	0.0973 ± 0.0021 ^c
SRC + G + Tp0.2%	0.1023 ± 0.0054 ^d
SRC + G + Tp0.3%	0.1247 ± 0.0086 ^e
SRC + G + Tp0.4%	0.1443 ± 0.0078 ^f

Note. Values are given as mean ± standard deviation. Different letters in the same column indicate significant difference ($p < 0.05$) when analyzed using Bonferroni's test.

in the wavenumber region of 400–4,000 cm^{-1} as shown in Figure 1. All films demonstrated a broad band ranging between 3,200 and 3,600 cm^{-1} that is attributed to the stretching of hydroxyl (-OH) groups of carrageenan and water (Nouri, Yaraki, Ghorbanpour, & Wang, 2018; Shankar, Wang, & Rhim, 2017). The peak at 1635 cm^{-1} corresponds to the stretching vibration of the -NH group, a typical amide I in all SRC-based films (Rhim & Wang, 2014; Shankar et al., 2017). The prominent peak observed at 1,221 cm^{-1} is assigned to sulfate ester (S = O) groups of carrageenan (Oun & Rhim, 2017; Rhim & Wang, 2014), while the peak observed at 1,035 cm^{-1} is attributed to glycosidic linkages of carrageenan (Farhan & Hani, 2017). In addition, all samples showed bands at 920 and 842 cm^{-1} which are attributed to 3,6-anhydro-d-galactose ring and d-galactose-4-sulfate which are associated with carrageenan functional groups (Distantina, Rochmadi, Fahrurrozi, & Wiratni, 2013; Rhim & Wang, 2014). Meanwhile, the incorporation of G and Tp into the film matrix shifted the band to a lower wavenumber. It can be observed in shift of the sulfate ester band from 1,221 to 1,217 and 1,218 cm^{-1} when G and Tp were added into the sample.

The absorption band at 2,937 cm^{-1} that is referred to alkane groups (C-H stretching vibrations) in the film matrix appeared when G was added into the film formulation. No band existed for the SRC-control sample which shows a good miscibility in the interaction between SRC and G in the film matrix (Nouri et al., 2018; Rhim & Wang, 2014). Moreover, the incorporation of G in the SRC-based films increased the peaks intensity compared with that of the

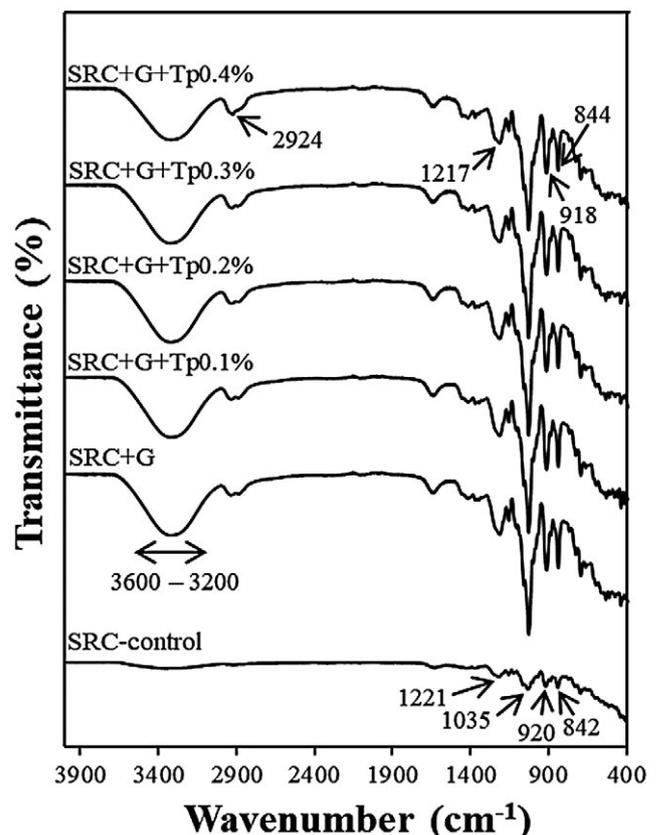


FIGURE 1 FTIR spectra of SRC-based films

SRC-based film without G addition (SRC-control). These reactions are in agreement with the findings reported by Cerqueira, Souza, Teixeira, and Vicente (2012) where the changes in the spectra peaks can be observed from the chemical interaction between the mixtures. The visibility of Tp spectrum was detected at higher peak intensity of $2,924\text{ cm}^{-1}$ in SRC + G + 0.4%Tp compared with that of the treated Tp sample and it is attributed to the asymmetric stretching of CH_2 vibration (Che Man, Ammawath, & Mirghani, 2005). Hence, the interaction between carrageenan, G, and Tp was observed in the changing of peaks intensity characteristic in the SRC-based films.

3.3 | Thermogravimetric analysis

TGA was carried out to determine the thermal stability and decomposition of the SRC-based films and how the interaction between carrageenan, G, and Tp influenced the stability. Figure 2a shows the thermal events and the changes in weight (% weight loss) of the SRC-based films. Mainly, the SRC-based films experienced three distinct steps of weight loss during the thermal degradation processes. The initial weight loss that occurred at around $60\text{--}100^\circ\text{C}$ is attributed to the evaporation of all moisture in the film sample (Kurt, Toker, & Tornuk, 2017). The second step of weight loss observed at $120\text{--}260^\circ\text{C}$ corresponds to the degradation of G component in all film samples except the SRC-control film (Colussi et al., 2017). Furthermore, the similar weight loss range in the second step may be related to the dehydration

of the saccharide rings and depolymerization step of the film sample (Basiak, Lenart, & Debeaufort, 2017). The third stage, ranging from $250\text{ to }360^\circ\text{C}$ is attributed to the thermal decomposition of polysaccharides backbone (Martins et al., 2012). These results illustrate that the SRC + G film and the SRC + G film that was incorporated with different concentrations of Tp exhibited no significant differences in the TGA profiles. The results also demonstrate that the thermal decomposition of the SRC-based films was affected by the incorporation of G and Tp into the film formulation when compared with that of the SRC-control film without the addition of G and Tp.

The maximum decomposition temperatures (T_{max}) of all films displayed by the highest peaks in the DTG curves (Figure 2b) were $229.5, 277, 267, 275, 266,$ and 267°C for SRC-control, SRC + G, SRC + G + Tp0.1%, SRC + G + Tp0.2%, SRC + G + Tp0.3%, and SRC + G + Tp0.4%, respectively. The DTG curves show that the addition of G and Tp could improve the thermostability of the SRC-based film. However, the incorporation of Tp slightly reduced the thermostable event in the SRC-based films, and it was reported that the incorporation of Tp enhances the thermal stability up to 240°C , thereafter Tp completely loses its mass about 95% at 450°C (Hwang et al., 2013). A similar result was found that indicates the incorporation of antioxidant did not influence the thermal stability of biodegradable films (Medina Jaramillo, Gutiérrez, Goyanes, Bernal, & Famá, 2016). Therefore, it appears that the incorporation of G and Tp could improve the thermostability of the SRC-based film.

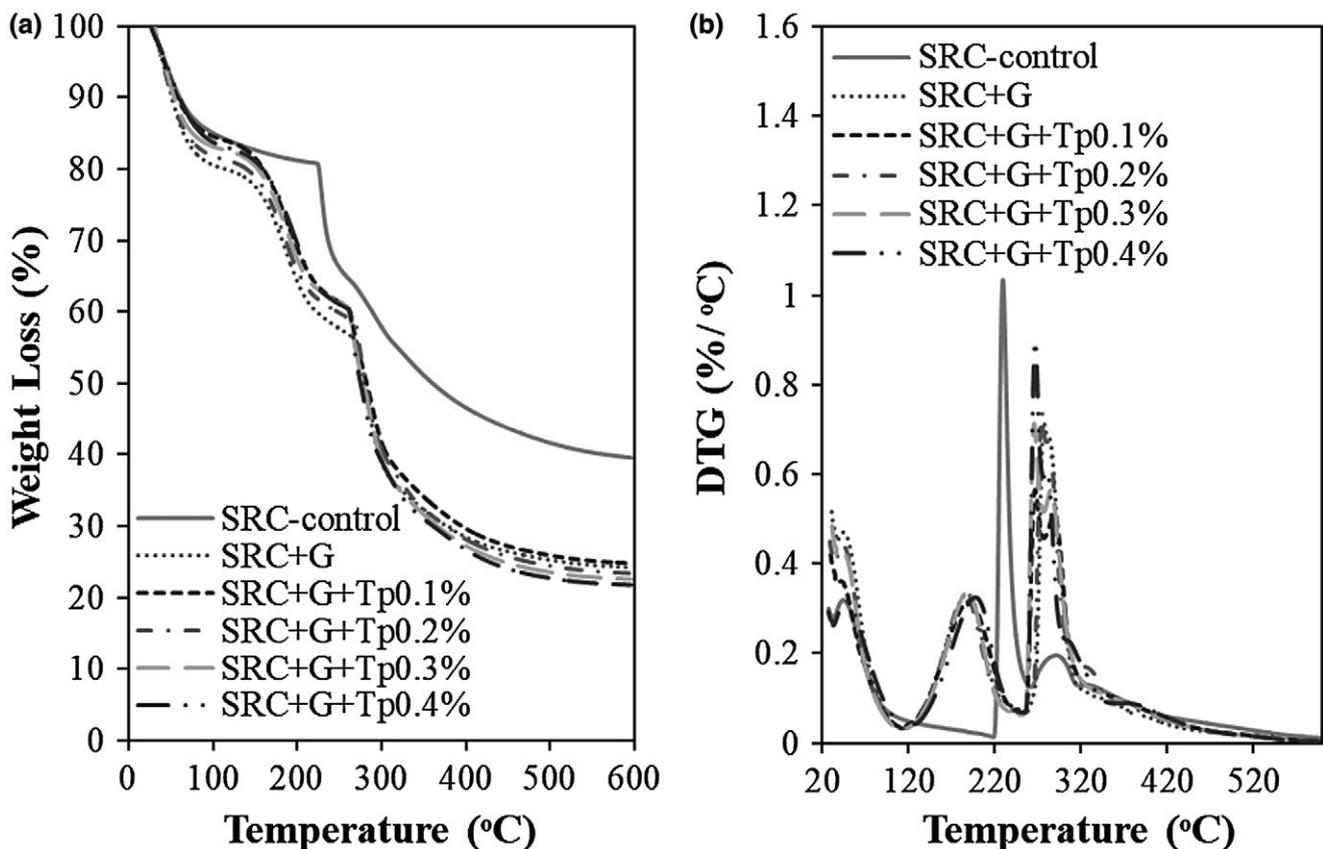


FIGURE 2 (a) Thermogravimetric analysis (TGA); (b) DTG curves of SRC-based films

3.4 | Film microstructure

Film-forming agents such as polymer, plasticizer, and antioxidant compound may be aligned in different ways depending on their hydrocolloid type, and the interaction between components during dispersion and drying processes of the films (Jiménez, Fabra, Talens, & Chiralt, 2012). The microstructure study on the film was performed to determine the influence of film-forming agents (SRC, G, and Tp) and its distribution in the film matrix. The structural surface and cross-sectional morphologies of SRC-control, SRC + G, and SRC + G incorporated with different concentrations of Tp (0.1%, 0.2%, 0.3%, and 0.4% [v/v]) are presented in Figure 3. The surface of the SRC-control film without the addition of plasticizer and antioxidant agent is smooth and the film shows a uniform and homogeneous cross section with no cracks. The addition of G unveiled the heterogeneous surface on the SRC-based film and formed cracks and pore cross-sectional area. However, the incorporation of 0.1% and 0.2% (v/v) of Tp demonstrated a slightly homogeneous surface of the SRC-based film compared with that of the SRC + G film. The miscibility of lower concentration of Tp in the SRC-based film may be finely distributed along the polymeric matrix and acted as the emulsifier between the components, while the surface of the SRC-based films with higher concentrations of Tp (0.3% and 0.4% [v/v]) resulted in an oil droplet form due to the presence of the lipid dispersed phase entrapped in the continuous SRC polymeric matrix (Hafsa et al., 2016; Jiménez et al., 2012). Nevertheless, these results show that the heterogeneity of film depended on the nature of lipid used in the film-forming solution where the size of oil droplets increased as the concentration of Tp was increased. The flocculation and coalescence of oil droplets in the film matrix was due to the forces interaction between the polymeric network during the evaporation process, where it can induce structural changes that increases the viscosity and concentration of the film dispersion and affects the stability of the polymeric network (Jiménez et al., 2012; Nouri et al., 2018). Besides that, the amount of lipid can increase the collision frequency between droplets and coalescence rate in the polymeric matrix (Hafsa et al., 2016). The results are in agreement with the findings reported by Nouri et al. (2018) where the addition of *Rosmarinus officinalis* L. extract formed a heterogeneous oil droplet surface in κ -carrageenan/nanoclay nanocomposite film. Hafsa et al. (2016) also demonstrated an oil droplet surface for *Eucalyptus globulus* (EG) essential oil incorporated with chitosan-based emulsifier film where the size of droplets increased as the concentration of EG oil was increased.

On the other hand, cross-sectional image of SRC-based film showed that the SRC-control film has homogeneous and compact microstructure, while SRC film that incorporated with G and Tp displayed multiple layer arrangement. These results in agreement with the finding of Ili Balqis, Nor Khaizura, Russly, and Nur Hanani (2017) that showed compact cross-sectional image on the control film without the addition of plasticizer due to the strong cohesion forces during slow-drying of aqueous-based materials. The author also pronounced that the formation of layer arrangement could increase the flexibility of the film due to the weak cohesive force that

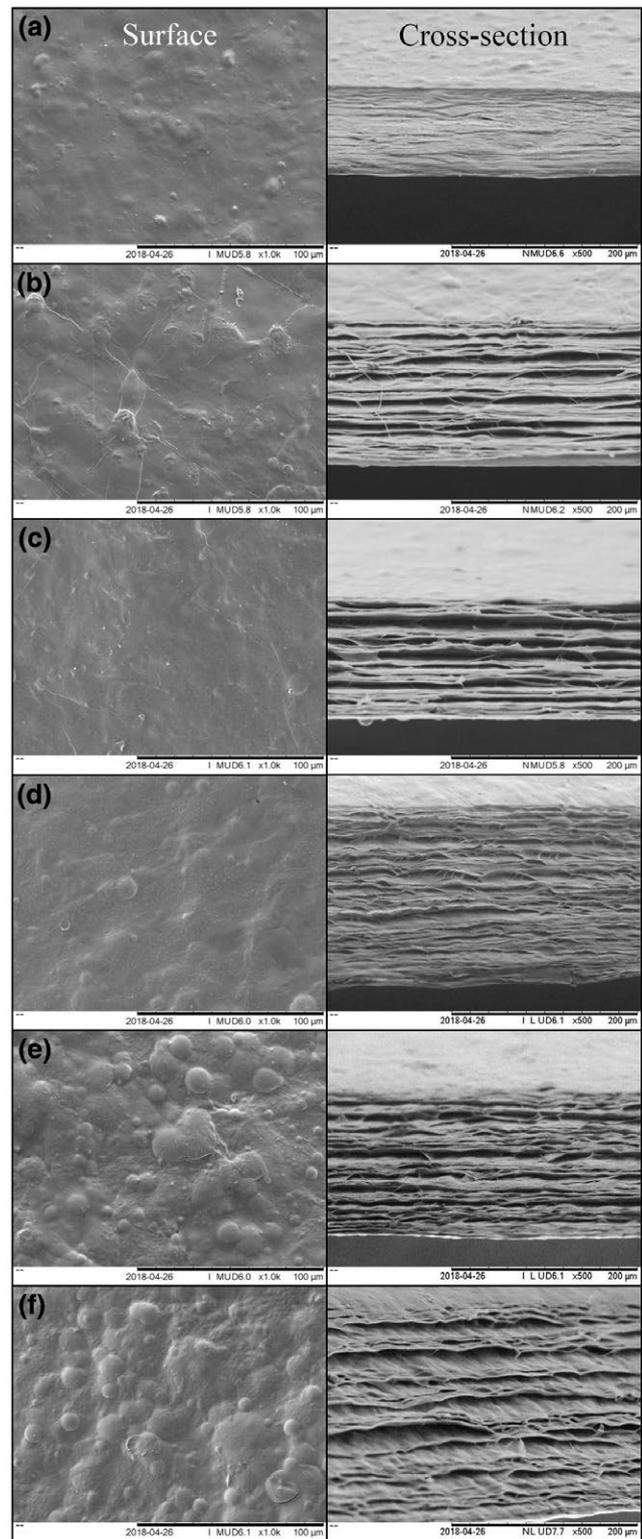


FIGURE 3 Surface and cross-sectional images of (a) SRC-control, (b) SRC + G, (c) SRC + G + Tp0.1%, (d) SRC + G + Tp0.2%, (e) SRC + G + Tp0.3%, and (f) SRC + G + Tp0.4% films

may increase the polymer mobility. Therefore, this study shows that the aggregation of oil droplets is more acceptable compared with the films that used emulsifier and shows similar effects on the film microstructure.

3.5 | TBARS analysis in meat patties

MDA compound formation during secondary lipid oxidation in lipid product is responsible for the alteration of flavor, undesirable taste, and rancid odor of the food. Figure 4 shows the formation of MDA in meat patties (mg MDA/kg sample) of all samples in 12 days of storage. The meat patties wrapped with the SRC-control film exhibited the highest value of MDA compound ($p < 0.05$). However, there is no significant difference in the TBARS values ($p < 0.05$) of the meat patties wrapped with the SRC-control and SRC + G films and the final value of 0.89 and 0.82 g MDA/kg sample, respectively. The plasticizer is widely used to improve the functional properties of the film as a packaging material and it displayed no significant effect on the inhibition of lipid oxidation in the meat patties.

The samples wrapped with antioxidant treated SRC-based films gave lower TBARS values where the meat patties wrapped with SRC + G + Tp0.4% film exhibited the lowest oxidation rate at the end of the storage period with a value of 0.37 mg MDA/kg sample ($p < 0.05$). These results indicate that lipid oxidation in meat patties could be minimized using the SRC-based film incorporated with antioxidant Tp compared with those by the use of the SRC-control and SRC + G films. It was noted that the TBARS value of 1.5 mg MDA/kg and above showed an unacceptable off-odor for meat product

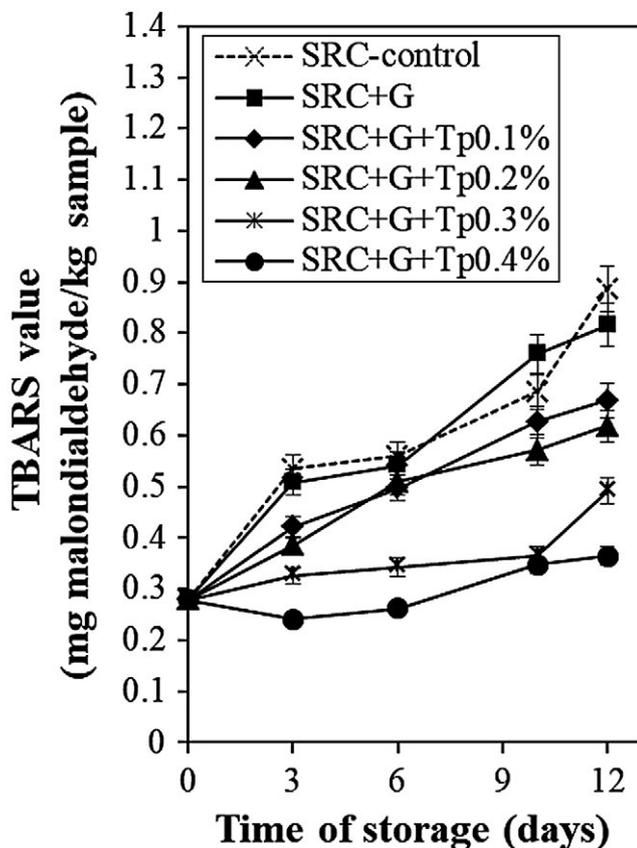


FIGURE 4 TBARS analysis of meat patties wrapped with SRC-control, SRC + G, SRC + G + Tp0.1%, SRC + G + Tp0.2%, SRC + G + Tp0.3%, and SRC + G + Tp0.4% films for 12-day storage at 4°C

(Gallego et al., 2016). The TBARS value in Figure 4 suggest that the antioxidant Tp released in the meat patties until 12 days was influenced by the SRC polymer and G blended in the film. Natural plasticizer such as G that is classified as generally recognized as safe blended into the SRC polymer may be an alternative mass transport for active agents such as Tp with no specific migration limits due to their low toxicity and migration (Vieira et al., 2011). Barbosa-Pereira, Aurrekoetxea, Angulo, Paseiro-Losada, and Cruz (2014) displayed similar results when low-density polyethylene with natural plasticizer incorporated with different concentrations of Nutrabiol-T90 that contained Tp compound inhibited the lipid oxidation on salmon product compared with the use of the control film without antioxidant agent. Furthermore, the incorporation of natural antioxidant that contained phenolic compound from plant extract such as *Caesalpinia spinosa* and white tea extracts into gelatin films could reduce the lipid oxidation in beef patties throughout the storage as reported by Gallego et al. (2016).

In the past, Tp was considered as the most active form of vitamin E for human consumption and it was reported to exhibit the highest biological activity to protect our bodies against cancer and cardiovascular diseases (Rizvi et al., 2014; Sabliov et al., 2009). On the contrary, the active film containing antioxidant Tp allowed the diffusion of antioxidant compounds to the meat patties that could block further degradation caused by oxidation to prolong the shelf life of the food sample. Krinsky (2001) reported that the antioxidants Tp and phenolic compounds are capable to scavenge free radicals and inhibit the chain initiation or break the chain propagation during the oxidation stress because they quench singlet oxygen. Therefore, the SRC-based film incorporated with Tp may increase the shelf life of meat products throughout the storage by delaying the occurrence of lipid oxidation.

3.6 | Metmyoglobin in meat patties

Metmyoglobin is a pigment formed when meat is oxidized and it is responsible for the deterioration of product and gives the brown color to the meat. Azman, Segovia, Martínez-Farré, Gil, and Almajano (2014) reported that the formation of metmyoglobin is proportional to the oxidation rate measured by TBARS assay. Figure 5 shows the percentage of metmyoglobin in all samples increased throughout the storage time under refrigerated condition ($p < 0.05$). Similar to TBARS analysis, the SRC-control and SRC + G films showed no significant difference ($p > 0.05$) in metmyoglobin percentage with the final value of 72% and 67%, respectively. A slower formation of metmyoglobin in all samples was observed in the first three days and increased rapidly onward ($p < 0.05$). The antioxidant film delayed the formation of brown color during the storage period with the metmyoglobin percentage in the average of 20%–50%. Gallego et al. (2016) reported that the metmyoglobin percentage of greater than 40% in fresh meat will affect the red color of the product that correlates with its freshness and this phenomenon is not accepted in the aspect of consumer panel. Therefore, the acceptable value of

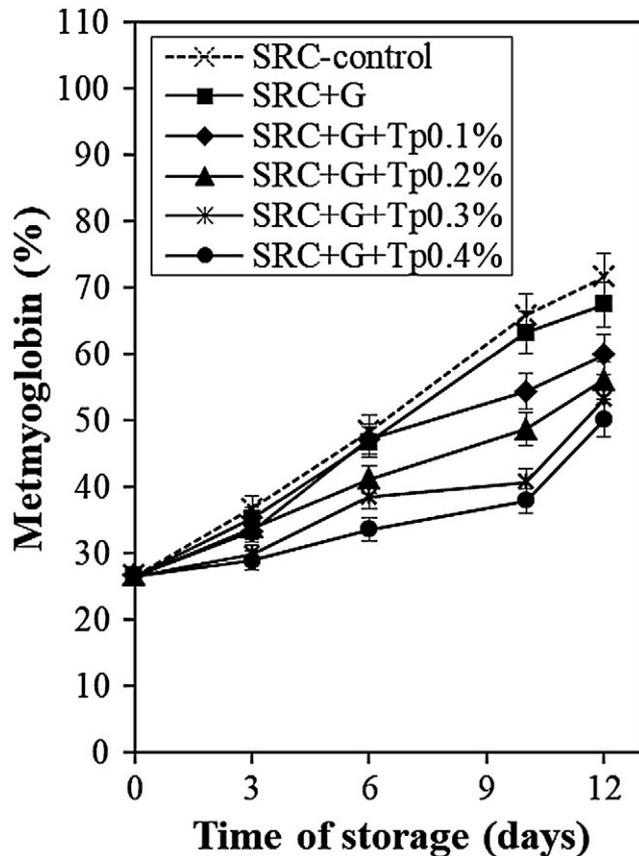


FIGURE 5 Metmyoglobin percentage in meat patties wrapped with SRC-control, SRC + G, SRC + G + Tp0.1%, SRC + G + Tp0.2%, SRC + G + Tp0.3%, and SRC + G + Tp0.4% films for 12-day storage at 4°C

metmyoglobin in the samples treated with antioxidant films was satisfactory until day 9, while the samples wrapped with the SRC-control and SRC + G films were rejected before the samples reached day 6 in the refrigerated storage. These results display the role of natural antioxidant Tp to inhibit the formation of metmyoglobin throughout the storage. The formation of metmyoglobin in wrapped meats significantly increased in the following order: SRC + G + Tp0.4% < SRC + G + Tp0.3% < SRC + G + Tp0.2% < SRC + G + Tp0.1% < SRC + G < SRC-control.

The development of free radicals from lipid oxidation is capable to commence the reaction of oxidizing oxymyoglobin (red pigment) to metmyoglobin (brown pigment) which results in the discoloration of meat during storage (Azman et al., 2015). Several findings observed that the fresh meat packaged with antioxidant film could inhibit metmyoglobin formation throughout the storage (Gallego et al., 2016; Lorenzo, Batlle, & Gómez, 2014; Nerin, Tovar, & Salafraanca, 2008; Xu et al., 2010). Similar trends were demonstrated by Liu, Xu, Dai, and Ni (2015) where the direct incorporation of vitamin E in raw beef patties delayed the formation of metmyoglobin during storage. Therefore, the present study displays that the incorporation of Tp in the SRC-based film could delay the development of metmyoglobin in the meat samples during storage.

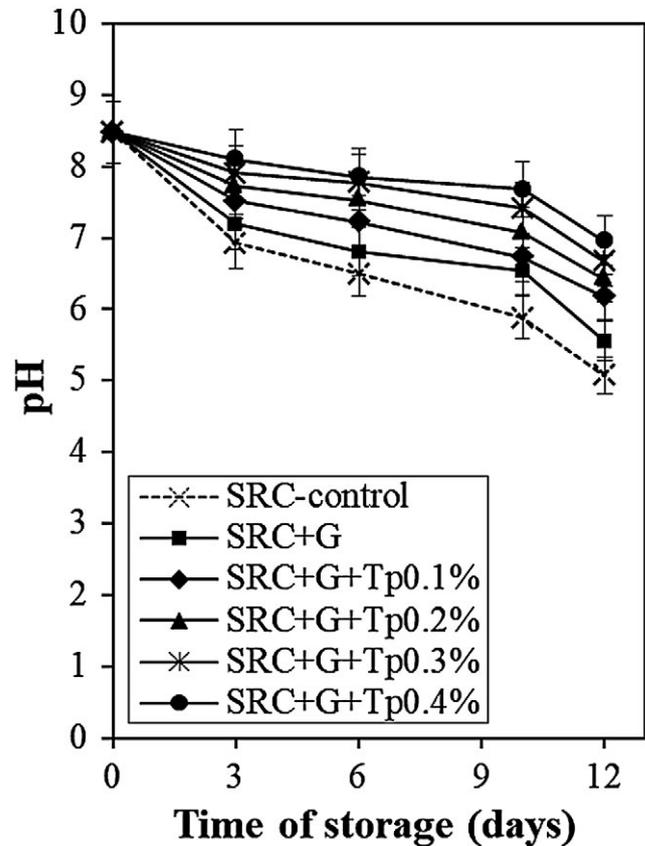


FIGURE 6 pH values of meat patties wrapped with SRC-control, SRC + G, SRC + G + Tp0.1%, SRC + G + Tp0.2%, SRC + G + Tp0.3%, and SRC + G + Tp0.4% films for 12-day storage at 4°C

3.7 | pH measurement

Figure 6 illustrates the pH value profile of meat patties wrapped with the SRC-based films during the 12-day storage. A decreasing trend was observed where the sample that wrapped with the SRC-control and SRC + G films rapidly became acidic throughout the storage with the final value in the range of 5.0–5.5. On the contrary, the pH values of meat patties that treated with antioxidant films steadily decreased and the results between the treated and untreated samples are significantly different ($p < 0.05$). The formation of highly unstable hydroperoxides that results in the development of ketones, epoxides, or organic acids produces an acidic condition and changes the pH of the food materials (Skowrya, Falguera, Azman, Segovia, & Almajano, 2014). Besides that, the decreasing pH value in the meat patties during storage reported by Kılıç, Şimşek, Claus, Karaca, and Bilecen (2018) occurred due to the growth of microorganisms in the samples. Jones (2004) noted that the pH value of the meat wrapped using a vacuum-packing technique decreased due to the development of microbial flora of lactic acid bacteria. However, Skowrya et al. (2014) demonstrated a positive effect of pH on the oxidation rate in food model that was influenced by the incorporation of natural antioxidant into the biopolymer film. At the end of the storage, the pH values of

the meat samples significantly decreased in the following order: SRC-control > SRC + G > SRC+G + Tp0.1% > SRC + G+Tp0.2% > SRC + G+Tp0.3% > SRC + G + Tp0.4%. Thus, the incorporation of antioxidant Tp into the SRC-based film could retain the pH of the meat during storage.

4 | CONCLUSION

The interaction between SRC, G, and Tp was demonstrated via FTIR spectroscopy analysis. The thermal stability of the SRC-based film improved when Tp and G were incorporated into the film matrix. The antioxidant effect of Tp in the SRC-based films was tested using meat patties and the highest antioxidant effect was shown by the incorporation of the highest concentration of Tp in the SRC-based film. The antioxidant film delayed the development of lipid oxidation and brown color formation in the meat patties during storage. The pH value of the meat patties treated with the antioxidant film showed a positive effect where the value decreased steadily throughout the storage. In conclusion, the formulation of SRC incorporated with Tp may be an alternative not only to prolong the shelf life but also to avoid the direct contact of synthetic preservative with foods.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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