Semirefined Carrageenan (SRC) Film Incorporated with α-Tocopherol and *Persicaria minor* for Meat Patties Application

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Abstract: *Semirefined carrageenan (SRC) plasticized with glycerol (G) and incorporated with antioxidants of 0.4% (v/v) of α-tocopherol and* Persicaria minor *(PM) extract was successfully developed. The objective of this study is to analyze the antioxidant effect of active packaging films from semirefined carrageenan incorporated with α-tocopherol and* Persicaria minor *on meat patties. Total phenolic content and antioxidant activity of α-tocopherol and PM extract were measured. The effects of α-tocopherol and PM extract incorporated with SRC-based films on meat patties were evaluated using thiobarbituric acid reactive substance (TBARS) assay, metmyoglobin assay, and pH value for 14 days of storage. The films with 0.4% (v/v) of α-tocopherol and PM extract exhibited lower lipid oxidation in meat patties compared to the control (SRC film only, $p < 0.05$). Also, brown color development of the meat patties of less than 50% was observed at the end of the 14-day storage. Meanwhile, the pH values for all samples decreased throughout the storage period with the SRC+G+α-tocopherol film showed the highest pH value. Hence, the formulation of SRC film with α-tocopherol or PM extract could be used as an alternative packaging for extending the shelf life of food product with high fat content.*

Keywords: *active packaging film; meat patties; α-tocopherol;* Persicaria minor *extract; lipid oxidation*

■ INTRODUCTION

The petroleum-based synthetic polymer is dominantly used in the production of food packaging film due to its durability, and water resistance properties [1]. The extensive use of synthetic packaging film which is practically non-biodegradable leads to an environmental concern and creates land pollution such as waste disposal problems. Alternatively, the development of degradable/edible packaging with similar characteristics to conventional plastic [2-3] is the current focus of research. Furthermore, the development of bioactive films containing biopolymer combined with antioxidant compound appears to be an interesting way not only provides the environmentally friendly packaging but also able to protect against oxidative reaction for longer shelf life [4-5].

A biopolymer, derived from either polysaccharides, proteins, or lipids, is the main component in the development of plastic/film due to its degradable behavior and abundance of resources. Carrageenan is one of the most abundant polysaccharide polymers extracted from a certain species of red seaweed that has good film-forming properties that provides efficient barriers against oxygen, oils, and lipids [6]. Carrageenan is one of the hydrocolloid products after starch and gelatin [7], and it is classified as a sulfated ester on 3,6-anhydro-d-galactose residues [6]. The production of carrageenan in the industry involves two processes which are refined carrageenan and semi-refined

carrageenan. Semirefined carrageenan (SRC) that is available at a reasonable cost has good gelling and binding properties to be used as film-forming material compared with refined carrageenan [1]. The addition of a plasticizer such as glycerol into biopolymer exhibits a good potential for the development of edible and/or biodegradable packaging films with enhanced mechanical and barrier properties [1].

Lipid oxidation is responsible for foods deterioration affecting the color, flavor, and odor of the foods and leads to the growth of microorganisms and vitamin losses [8]. Commonly, many food industries implement the use of synthetic antioxidants such as ter-butylhydroquinone, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) blended into food products to prevent oxidative deterioration [9-10], yet the consumption of synthetic antioxidants causes dubious health effect and toxic to human [11]. A natural antioxidant that contains phenolic compounds such as tocopherol, rosemary, oregano essential oil, and bearberry leaf extract not only reduces lipid oxidation in meat [12-14] but also extends the application of the film.

α-Tocopherol, also known as vitamin E, possesses many pharmacological benefits to human health including anticancer and anti-cardiovascular diseases due to its antioxidant capacity [15-16]. The potential use of α-tocopherol as an antioxidant coating in the film has been widely investigated, where the hydrophobic compounds of α-tocopherol exhibit antioxidant activity and improve the mechanical and barrier properties when incorporated into biodegradable films [17-18]. The formulation of an active film with the antioxidant compound is not limited to a single compound (e.g., α-tocopherol). Several studies showed that the formulation of natural antioxidant extract from bearberry leaf, *Caesalpinia decapetala*, and *Betula pendula* with biopolymer are able to inhibit the oxidative deterioration and retains the freshness in muscle foods [12,19-20]. *Persicaria minor* which is locally known as *daun kesum* is widely used as a condiment in cooking as well as a traditional medicine for digestion in South East Asia [21]. This edible plant extract contains many phenolic compounds such as rutin, catechin, flavonols, and hydroxycinnamic acids [22], which showed their antioxidant activity. Additionally, a pharmacological study conducted by Mackeen et al. [23] showed that *Persicaria minor* exhibited an antimicrobial and cytotoxic activity which could against human cervical carcinoma (HeLa). However, the ability of *Persicaria minor* extract in active film packaging has not been fully determined.

Therefore, this study aims to analyse the antioxidant effect of new active packaging films from semirefined carrageenan (SRC) that incorporated with α-tocopherol (0.4% [v/v]) and *Persicaria minor* (PM) (0.4% [v/v]) on meat patties, in addition, the changes in pH and brown color development in ground meat patties stored for 14 days at 4 °C were evaluated. Moreover, the total phenolic content (TPC) and antioxidant activity of α-tocopherol and *Persicaria minor* extract were evaluated.

■ EXPERIMENTAL SECTION

Materials

Semirefined carrageenan (SRC) was extracted from *Eucheuma cottonii* seaweed obtained in Sabah, Malaysia. *Persicaria minor* and meat patties were purchased from a local market in Kuantan, Pahang. Food grade glycerol, α-tocopherol, phosphate buffered saline tablet, thiobarbituric acid (TBA), 1,1,3,3-tetramethoxy propane, malonaldehyde, ethylenediamine tetra acetic acid, sodium chloride, and hydrochloric acid were purchased from Sigma Aldrich (Gillingham, England).

Procedure

Preparation of semi-refined carrageenan (SRC) from Eucheuma cottonii *seaweed*

SRC was prepared according to the established method by Mustapha et al. [24]. *Eucheuma cottonii* seaweed was cleaned under running tap water to remove debris and dried under sunlight for 8 h. The extraction process was prepared by adding 150 g of dried seaweed into 1.0 M of potassium hydroxide (KOH) solution at 80 °C for 2 h and subsequently soaked in water for 12 h. Then, the neutralized seaweeds were dried at 50 °C for 24 h using a laboratory oven (MEMMERT, EQP004,

Schwabach, Germany). Finally, the SRC samples were ground with 0.5 mm mesh using a laboratory grinder (Retsch, ZM 200, Haan, Germany) and stored in a desiccator (25 °C) for further analyses.

Extraction of Persicaria minor

The finely ground *Persicaria minor* (PM) (3 g) was mixed with 75:25 (v/v) ethanol:water in the ratio of 1:20 (w/v) on a dry weight basis of the ground PM. The extraction was performed using incubator shaker (Cole-Parmer Ltd, United States) at room temperature (25 °C) under constant stirring of 150 rpm for 24 h. The extract solution was centrifuged using a high-speed refrigerated centrifuge (Eppendorf, Centrifuge 5810 R, Hamburg, Germany) at 10000 g for 10 min at 4 °C and the solution was filtered using filter paper (Whatman, 0.45 μm). The supernatant sample was stored at -20 ± 1 °C prior to further analyses.

Fourier transform infrared (FTIR) spectroscopy

The functional groups of carrageenan, glycerol, and α-tocopherol or *Persicaria minor* extract in the SRC-based film were determined using Fourier transform infrared spectrometer (Thermo Scientific Nicolet iS5 FT-IR Spectrometer, Massachusetts, USA). The FTIR spectra were determined in a wavelength region from 400 to 4000 cm^{-1} using OMNIC software.

Determination of total phenolic content

Total phenolic content (TPC) of the extract and α-tocopherol were determined using the Folin-Ciocalteu method as reported by Azman et al. [25]. The calibration was performed using gallic acid as a standard. The sample (10 μL) was mixed with the solution containing 50 μL of 10% (v/v) Folin reagent and 150 μL of 7.5% (w/v) sodium carbonate. The assay was performed by diluted the solution mixture with MiliQ water (790 μL), and the absorbance at 765 nm was measured using a UV–visible spectrophotometer (HITACHI, U-1800, Tokyo, Japan) against water as a blank. The results were expressed as mg of gallic acid equivalents/g sample.

Determination of antioxidant activity

Antioxidant activity of the extract and α-tocopherol were determined using three different methods as followed: trolox equivalent antioxidant capacity (TEAC) assay as described by Azman et al. [26], 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) assay as described by Azman et al. [25], and ferric reducing antioxidant power (FRAP) assay as described by Gallego et al. [27].

TEAC assay. The mixture containing $ABTS^{\bullet-}$ radical cation (7 mM) and potassium sulfate (2.45 mM) was prepared and placed in the dark up to 16 h. The mixture then was adjusted by diluting with PBS (10 mM) incubated at room temperature (25 °C) for 30 min to an absorbance of 0.7 (± 0.02) at 734 nm. The assay then was performed by mixing the sample (PM extract or Tp) with the solution containing $ABTS^{\bullet-}$ radical and the absorbance was measured at 734 nm using UV-visible spectrophotometer for 20 min.

DPPH assay. A solution of 0.1 mM DPPH dissolved in ethanol appropriate dilutions was prepared. One aliquot (0.025 mL) of the sample (PM extract or Tp) was mixed with 0.975 mL of 0.1 mM DPPH. The solution was added to the cuvette, and the absorbance was measured at 585 nm using UV-visible spectrophotometer after 4 h.

FRAP assay. An acetate buffer (300 mM) solution was prepared by mixing acetic acid (279.7 mM) with sodium acetate (20.3 mM). FRAP reagent was prepared by mixing 300 mM acetate buffer with 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM ferric chloride hexahydrate with a ratio of 10:1:1. The sample (PM extract and Tp) was mixed with FRAP reagent incubated at 37 °C and the absorbance at 593 nm was measured using a UV-visible spectrophotometer. Each result was expressed as μmol of Trolox equivalent (TE) per gram sample.

Preparation of SRC films

The SRC-based films were prepared according to the method by Farhan et al. [1] with slight modifications. The SRC film was prepared by dissolving 2% (w/v) of SRC in distilled water under continuous stirring at 70 °C for 10 min using a hot plate magnetic stirrer. After dissolution, 0.9% (v/v) of plasticizer glycerol (G) was added and maintained heated at 70 °C for about 10 min. Then, 0.4% (v/v) of antioxidant α-tocopherol (Tp) or *Persicaria minor* extract were added before casting on the casting plates.

The films prepared were (i) SRC-control (no addition), (ii) SRC+G, (iii) SRC+G+Tp 0.4%, and (iv) SRC+G+PM 0.4%. The films were conditioned at 25 °C and 50 ± 5% relative humidity (RH) prior to further analyses.

Preparation of meat patties

The meat patties were purchased 7 days after slaughtering process to allow it to mature and kept at -20 ± 1 °C for further treatment. Approximately 20 g of meat was wrapped with different films as followed (i) SRC-control, (ii) SRC+G, (iii) SRC+G+Tp 0.4%, (iv) SRC+G+PM 0.4% and one sample prepared with no wrapping; meat sample only. All samples were placed in sterilized trays and stored at 4 ± 1 °C for 14 days for analysis. Each sample and the measurement was carried out in triplicates.

Thiobarbituric reactive substance (TBARS)

Lipid oxidation that occurred in food model over the storage period was measured using the TBARS method as described by Azman et al. [28]. One gram of sample (meat patties) was mixed with 3 g/L of aqueous EDTA and 5 mL of 0.375% (w/v) of thiobarbituric acid (TBA) reagent. The sample was homogenized using Ultra-Turrax (IKA, Germany) at 8,000 rpm for 5 min. The mixture then was incubated in hot water at 97 ± 1 °C for 10 min under constant shaking. The liquid sample was filtered 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). TBARS value was calculated from the malonaldehyde (MDA) standard curve. The results were expressed as mg malonaldehyde per kg of the sample (mg MDA/kg sample).

Percentage metmyoglobin

Brown color development of meat patties over the storage period was analyzed using metmyoglobin method developed by Xu et al. [29]. Phosphate buffer solution (0.04 M) at pH 6.8 was prepared. Five grams of meat patties were mixed with 25 mL of phosphate buffer solution and homogenized for 30 s using Ultra-Turrax at 8,000 rpm. The homogenized mixture was located for 1 h at 4 °C and centrifuged at 12,000 g for 10 min at 4 °C using a high-speed refrigerated centrifuge. The mixture was filtered using filter paper and the absorbance was read at 572, 565, 545, and 525 nm using a UV–visible spectrophotometer (HITACHI, U-1800, Tokyo, Japan). The percentage of metmyoglobin (MetMb%) was calculated using the Eq. (1).

$$\begin{aligned}\text{MetMb}(\%) = [&-2514(\text{Abs}_{572}/\text{Abs}_{525}) + 0.777(\text{Abs}_{572}/\text{Abs}_{525}) \\ &+ 0.8(\text{Abs}_{545}/\text{Abs}_{525}) + 1.098] \times 100\end{aligned} \quad (1)$$

pH measurement

pH measurement method was adapted from Jin et al. [30], with the ratio of meat patties to the distilled water is 1:10 (w/v). The sample was homogenized using Ultra-Turrax at 8,000 rpm, and the readings were taken at three different points for each treatment. The change of pH value of meat patties over the storage period was measured periodically with a pH meter (Mettler-Toledo, model GLP 21).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) 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. Each sample was measured in triplicates, and the average standard deviation for each sample was less than 5%.

■ RESULTS AND DISCUSSION

Fourier Transform Infrared (FTIR) Spectroscopy of SRC

Fig. 1(a-d) shows the possible interaction of the functional groups between carrageenan, glycerol, *Persicaria minor* (PM) extract and α-tocopherol (Tp) in the wavenumber region of 4000–400 cm^{-1}. The broadband between 3200 and 3600 cm^{-1} in all films represents the O–H stretching that was formed by the hydroxyl group of carrageenan and water [1]. The peaks observed at 1221 cm^{-1} correspond to sulfate ester (S=O) groups of carrageenan and this band is in the range with the observation of other literature [31]. This band shifted to a lower wavenumber of 1217–1218 cm^{-1} when glycerol, PM extract, and Tp were incorporated into the SRC-based films (Fig. 1(b-d)). The absorption band at 1035 cm^{-1} was attributed to glycosidic linkages of

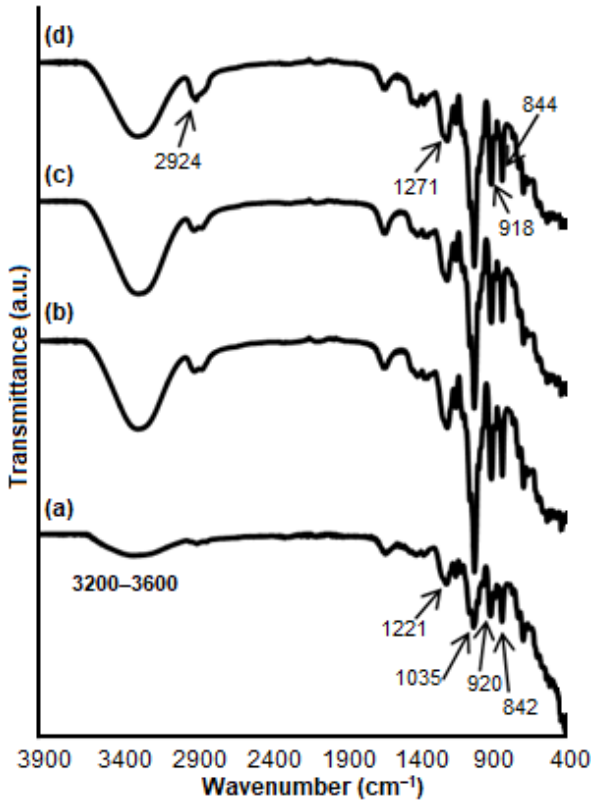

Fig 1. Fourier transform infrared (FTIR) spectrum of (a) SRC-control, (b) SRC+G, (c) SRC+G+PM 0.4%, and (d) SRC+G+Tp 0.4% film

carrageenan [1]. The prominent peaks at 920 and 842 cm^{-1} are assigned to 3,6-anhydro-d-galactose ring [32] and d-galactose-4-sulfate of carrageenan [1,31,33]. Similar peaks of carrageenan groups were observed in all the SRC-based films. However, the interaction between the carrageenan polymer with glycerol, PM extract, and α-tocopherol can be observed from the shifted band to a lower wavenumber as shown in Fig. 1(b-d). These results are in agreement with those reported by Zainuddin and Samsudin [34] where the changes of peak intensity and band shift were due to the interaction between the polymer and dopant. In Fig. 1(d), the α-tocopherol spectrum appeared in the SRC+G+Tp0.4% film at 2924 cm^{-1} for asymmetric stretching (CH_2) vibration [35]. However, there was no significant change in functional groups observed in the SRC+G (Fig. 1(b)) and SRC+G+PM 0.4% (Fig. 1(c)), which may be due to the formation of a homogeneous film-forming solution. In general, the peak intensity increased when the glycerol, PM extract, and α-tocopherol were blended into the SRC-based films compared to that of the SRC-control film. Similar results of FTIR were observed for carrageenan-based films that were combined with nanofillers silver nanoparticles (AgNPs) and clay mineral where there was no change in the intensity of peaks when AgNPs and clay mineral were included in the carrageenan-based films [31].

Total Phenolic Content (TPC) and Antioxidant Activity of PM Extract and α-Tocopherol

Persicaria minor (PM) was extracted using ethanol as the extraction solvent, a generally recognized as safe (GRAS) solvent, where it is safe to be consumed in the food industry application [36]. Table 1 illustrates the total phenolic content (TPC) and antioxidant activity of α-tocopherol (Tp) and PM extract. Tp showed a higher phenolic content, and antioxidant activity than that of the PM extract ($p < 0.05$) and the value of total phenolic content of Tp and PM extract correlated with the antioxidant activity value determined by TEAC, DPPH, and FRAP assays. The measurement of polyphenol content in PM extract allows the estimation of all polyphenol compounds such as rutin, catechin, and quercetin present in the sample as reported by Maizura et al. [37]. In the present study, Tp showed significantly increased ($p < 0.05$) in phenolic content with a value of 82.81 mg GAE/g sample compared to PM extract (37.14 mg GAE/g sample). Qader et al. [38] demonstrated that the PM extract exhibited the highest phenolic content (55.5 mg/g) compared to five Malaysia herbs using ethanol aqueous as solvent extraction. On the other hand, Tp is one of polyphenol compound that naturally obtained from oils, vegetables and nuts possess the strong antioxidant capacity and pharmacological benefits towards human health [39]. Many studies on pharmacology showed potent compound of vitamin E used as anticancer, antitumor and antioxidant agent [15-16,39].

Nevertheless, Table 1 shows the antioxidant activity of TEAC assay gives higher antioxidant values compared to that of the FRAP and DPPH assay in Tp and PM extract. TEAC assay showed the ability of antioxidant compound in Tp and PM extract to scavenge

Table 1. Total phenolic content and antioxidant activity of Tp and PM extract

Assay	Tp	PM
TPC (mg GAE/g sample)	82.81 ± 1.53	37.14 ± 0.06
TEAC (μmol TE/g sample)	552.64 ± 1.16	307.75 ± 0.04
DPPH (μmol TE/g sample)	409.15 ± 0.96	299.01 ± 1.05
FRAP (μmol TE/g sample)	216.52 ± 1.83	110.04 ± 0.28

Values are the mean ± standard deviation; GAE: gallic acid equivalent; TE: trolox equivalent.

ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) long-life radical cation, whereas, the DPPH assay shows the scavenging ability of Tp and PM extract as antioxidant properties towards the DPPH radical [26]. The antioxidant activity of Tp and PM extract using FRAP assay displayed the ability of the compounds to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) [40]. While this antioxidant activity context, polyphenol compound entails its hydroxyl group on the aromatic ring compound structure as hydrogen or electron donators to capture the free radicals in the oxidation process [37]. Similar observation found by Qader et al. [38] that showed the ethanol aqueous extract of PM has the highest antioxidant activity value of FRAP assay with comparison to water extract. They also demonstrated a positive correlation between phenolic content and antioxidant activities in the DPPH assay ($R^2 = 0.7611$) and FRAP assays ($R^2 = 0.9697$) for PM which may contribute to many polyphenols content such as rutin, catechin, and quercetin in the extract [38]. Meanwhile, α-tocopherol experienced the high value of antioxidant activities in all antioxidant assays including FRAP, DPPH, and TEAC, and often used as a standard for all tocochromanols comparisons due to its promising activities in biological and antioxidant assays [41]. Therefore, the results show that the phenolic content in Tp and PM extract may contribute to the antioxidant activity by TEAC, FRAP, and DPPH assay.

TBARS Analysis in Meat Patties

Thiobarbituric acid reacting substances (TBARS) method is used to determine the malondialdehyde (MDA) compound that is produced from the secondary oxidation in lipids such as meat [28]. MDA compound is responsible for the off-odor and flavor, and undesirable taste in food products [42]. The development of active packaging film consisting of plasticized SRC incorporated with α-tocopherol (Tp) or *Persicaria minor* (PM) could be an effective approach to inhibit the oxidation in meat throughout the 14-day refrigerated storage condition (Fig. 2). All samples demonstrated increment of TBARS values throughout the storage period, and the control sample (meat patties without wrapping -no wrap) had the highest value of MDA ($p < 0.05$). This result demonstrates that the unwrapped meat was highly susceptible to oxidation in muscle food due to high exposure to atmospheric oxygen [28]. The SRC-control and SRC+G samples showed no significant difference in TBARS values because the incorporation of glycerol plasticizer in SRC film did not have any antioxidant effect on the meat ($p > 0.05$). Therefore, the addition of plasticizer glycerol into

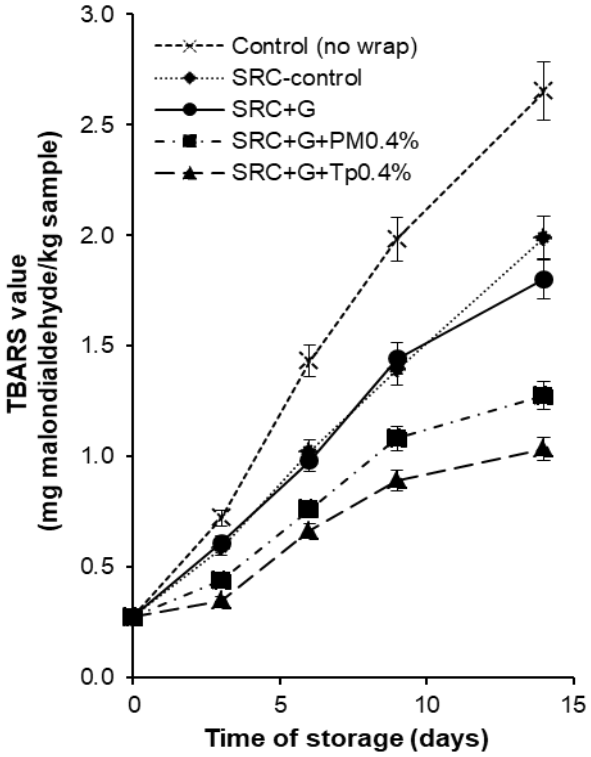

Fig 2. TBARS values (mg malondialdehyde/kg sample) of meat patties wrapped with SRC-based films for 14-days storage at 4 ± 1 °C

the SRC-based films did not significantly influence the inhibition of lipid degradation in meat patties throughout the storage and there was no study reported the antioxidant activity of glycerol towards the inhibition of lipid degradation. The addition of glycerol into the SRC-based films as a plasticizer is to improve the mechanical and barrier properties of the films as demonstrated by Farhan et al. [1].

The samples wrapped with antioxidant-treated films (SRC+G+Tp 0.4% and SRC+G+PM 0.4%) experienced lower oxidation rates at the end of the storage period ($p < 0.05$) with the TBARS values of 1.03 and 1.27 mg MDA/kg sample, respectively. These results indicate that the interaction between polymer (SRC), plasticizer (glycerol), and chemical composition of active compounds in Tp and PM extract may influence the inhibition of lipid oxidation in the meat patties.

These results suggest that the use of SRC-based film containing α-tocopherol or *Persicaria minor* extract could minimize lipid deterioration in meat patties due to their strong antioxidant capacities released from the film to the food products. Active packaging film based on SRC with Tp and PM extract not only promotes the application of degradable packaging but also acts as a replacement of synthetic antioxidant applied directly into the food where it can retain the freshness of the meat throughout the storage. These results are in agreement to those reported by Vital et al. [13] that found lower TBARS values in the meat samples coated with rosemary and oregano essential oil (EO) where the EO coating decreased lipid oxidation throughout the 14-day storage. Furthermore, the incorporation of *Caesalpinia spinosa* and white tea extracts into gelatine-based film reduced the inhibitory effect of lipid oxidation in beef patties as reported by Gallego et al. [20]. Thus, the SRC-based film incorporated with α-tocopherol or PM extract may prolong the shelf life of meat products by minimizing lipid oxidation.

The antioxidant property of α-tocopherol and phenolic compounds in PM extract is capable to scavenge free radicals and inhibits the chain initiation or breaks the chain propagation during oxidation stress because they quench singlet oxygen [43]. The engagement of Tp in films is not new as Martins et al. [18] demonstrated the positive effect of Tp in physicochemical properties of chitosan-based films. However, this is the first study reported the potential of the SRC-based film incorporated with α-tocopherol and PM extract to prevent lipid degradation in meat patties.

Percentage Metmyoglobin in Meat Patties

The redness in meat is a common visual trait indicating the freshness of the meat patties. Previous research by Azman et al. [25] found that the color changes in meat are proportional to the oxidation rate measured via TBARS. Furthermore, the deterioration of meat is due to the changes of metmyoglobin, known as brown color formation in muscle foods [44]. Fig. 3 presents the effect of the SRC-based films with or without the incorporation of antioxidants (α-tocopherol and *Persicaria minor* extract) on relative metmyoglobin percentages in meat patties. Generally, the percentage of metmyoglobin increased throughout the storage, and the control sample (no wrap) showed the highest metmyoglobin formation ($p < 0.05$). The SRC-control and

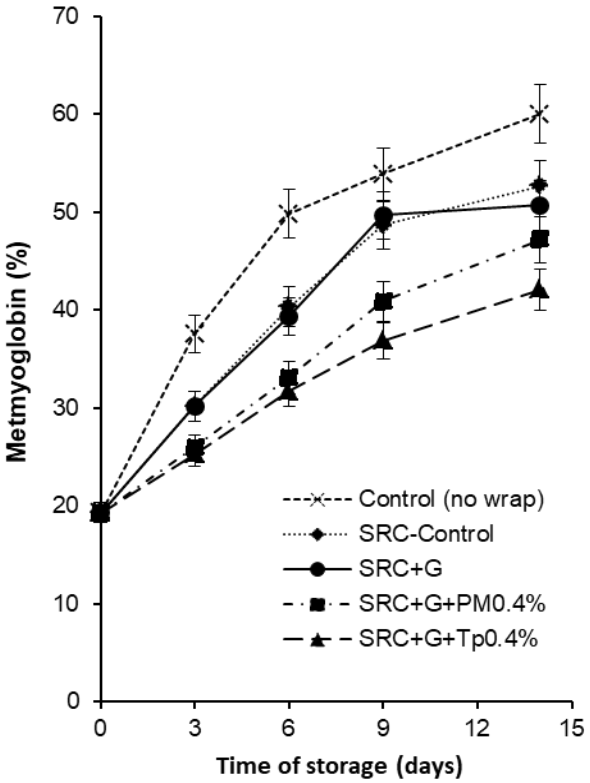

Fig 3. Metmyoglobin percentages in meat patties wrapped with SRC-based films for 14-days storage at 4 ± 1 °C

SRC+G samples showed no significant difference during storage with the final values of 52.7 and 50.7%, respectively ($p < 0.05$). The samples treated with α-tocopherol and *Persicaria minor* extract had slower development of metmyoglobin with less than 50% at the end of the storage period. This finding is expected due to the role of α-tocopherol as a natural antioxidant and phenolic compounds in the PM extract that reduces the formation of metmyoglobin, so the color of the meat retains. Gallego et al. [20] reported that a consumer panel would not accept the metmyoglobin percentage of greater than 40% in fresh meat due to the poor visual red color that collerates with the food freshness. Hence, the acceptable brown color formation in the meat wrapped with antioxidants films in this study was satisfactory until day 9.

Lipid deterioration in meat sample is capable to initiate the formation of oxidizing red pigment (oxymyoglobin) to brown pigment (metmyoglobin) which affects the discoloration of meat during storage [28]. Several findings observed that the fresh meat packaged with antioxidant film from plant extracts could inhibit metmyoglobin formation [20,45]. Similar finding on the brown color development was demonstrated by Liu et al. [14] where the direct incorporation of vitamin E in raw beef patties could delay the formation metmyoglobin during the 8-day storage with the percentage metmyoglobin value less than 50%.

pH Measurement

Fig. 4 illustrates that the pH values of the meat patties wrapped with the SRC-based films dropped over time and these values were inversely proportional to the metmyoglobin percentage and TBARS values. The control sample showed the lowest pH value followed by the sample that wrapped with the SRC-control film. These results indicate the acidic condition in the untreated meat patties where the pH value dropped continuously throughout the storage. The formation of organic acids, ketones, or epoxides which is highly unstable and acidic could reduce the pH value of the food material [46]. Generally, the pH value showed decreasing value for all samples throughout the 14-day storage. According to

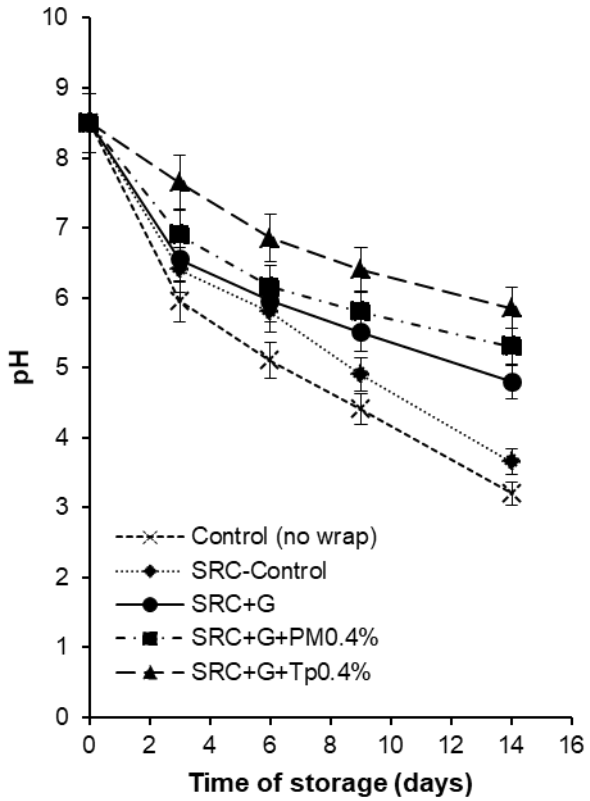

Fig 4. pH values of meat patties wrapped with SRC-based films for 14-days storage at 4 ± 1 °C

Kılıç et al. [47], the decreasing pH value during storage may be due to the growth of microorganisms in the samples. Jones [48] reported a pH decrease in vacuum-packed meat due to the development of microbial flora of lactic acid bacteria. However, Skowyra et al. [46] showed that the natural antioxidants from *Perilla frutescens* could minimize the reduction of pH value in food emulsion during storage. Thus, the incorporation of antioxidant α-tocopherol or *Persicaria minor* extract into the SRC-based film could minimize the change of pH in meat during 14-day storage.

■ CONCLUSION

α-Tocopherol and *Persicaria minor* (PM) extract exhibited different levels of phenolic content and antioxidant activity measured by TEAC, FRAP, and DPPH assays. The properties of bioactive the SRC-based films were greatly affected by α-tocopherol and PM extract incorporation. The addition of α-tocopherol and PM extract into the SRC-based films delayed the lipid

oxidation and metmyoglobin formation in the meat patties throughout the 14-day refrigerated storage. In conclusion, the formulation of SRC incorporated with α-tocopherol or *Persicaria minor* extract 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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