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Effect of Storage Temperature on the Antioxidant Properties of Active Bilayer Polyethylene/Soy Protein Isolate (PE/SPI) Film

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ABSTRACT

Polyethylene (PE) is considered to be one of the most widely used plastic in the world due to its vast array of applications depending on the particular type. It is well known that petroleum-based plastic is a good material for food packaging for its good mechanical properties. Meanwhile, biopolymer films have poor mechanical and water barrier properties compared to conventional plastics. Soy protein isolate (SPI) with mangosteen pericarps extract (MPE) was laminated on PE film to form a bilayer film with antioxidant property. The PE/SPI film was stored at two different temperatures; 25°C and accelerated temperature at 40°C for 9 weeks. The color and opacity of the film increased significantly ($P \leq 0.05$) after 9 weeks observation. There is no change in the morphology of the film after 9 weeks stored at both temperatures. The antioxidant properties of film were measured by total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay for each week interval. The antioxidant activities of films decreased significantly ($P \leq 0.05$) throughout the storage time at both temperatures. Higher temperature also reduced the antioxidant properties of bilayer film significantly ($P \leq 0.05$). Addition of mangosteen pericarps extract contributes to the enhanced antioxidant properties of bilayer film.

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1. Introduction

For decades, polyethylene (PE) has been one of the main materials used in food packaging industry. It is undeniably a good material for food packaging as it possesses good mechanical properties, flexible and has an outstanding environmental stress crack resistance that can protect the food from damages. Now, packaging does not only focusing on protecting its content from the interaction with the outer environment, it is improving into another level where the additive

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substances/compounds in the film packaging can interact directly with its content for better protection and one of the examples is active packaging materials. Active packaging is a type of packaging that incorporated with natural additives, giving an active function to the materials such as antioxidant agents, oxygen scavengers, moisture absorbers, antimicrobial agents or flavor releasing/absorbing systems [1]. This smart system encompasses the interactions between packaged food and packaging components where it involves different functions such as inhibition of lipid oxidation and microbial growth and generally leads to the prevention of food spoilage and deterioration [2].

The advancement of technology in packaging applications to improve the system has led to the development of films with the combination of several polymers with exploitation on each polymer's functional or aesthetic properties [3,4]. This includes the development of synthetic-biopolymer bilayer packaging films which benefits both types of polymeric films in reducing the limitations simultaneously [5]. In this study, bilayer film was developed by using soy protein isolate (SPI) as biopolymer layer with the addition of active compound from mangosteen pericarp and PE as the synthetic layer. Soy protein isolate (SPI) is a type of protein-based material that commonly used in food packaging development. SPI as a raw material has shown better properties among other sources due to its superior film-forming properties, relatively low cost and good barrier properties to oxygen, aromas and lipids under intermediate moisture conditions [6,7]. Furthermore, because of its structure, SPI also is a good carrier agent for antioxidant compounds [8-10].

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit that can be found in India, Myanmar, Malaysia, Philippines, Sri Lanka, and Thailand. Mangosteen has a milky white edible aril and its pericarp is dark red and it comprises about 75% of the whole fruit [11,12] which left as agricultural waste. Surprisingly, the pericarp fraction of mangosteen possesses higher antioxidant activity than the pulp fraction [12,13]. The pericarp contains various of phenolic compounds such as tannins, flavanoids, anthocyanins and xanthones [11,14-16]. These bioactive compounds give benefits as an antioxidant, antimicrobial and anti-cancer [19,20]. Hence, the usage of the pericarps for this film could be one way to reduce the generation of the agricultural waste.

The application of active packaging to package fatty foods could be one of the best strategies to control the oxidation of lipid compounds in the packaged food. However, storage time and temperature affected the properties of packaging film. For example, Ciannamea *et al*., [21] found that soybean protein concentrate (SPC) film stored at 25 and 65°C for 90 days promoted changes in molecular structure and film properties. Furthermore, the ageing of chitosan film up to 90 days resulted in significant variations in functional properties due to changes in structure related to Maillard reaction [22].

Moreover, phenolic compounds are easily sensitive to degradation by some factors such as light, oxygen, pH, storage temperature and time since they are unstable structures [23]. Therefore, the aim of this research was to investigate the effect of storage time and temperature on the antioxidant properties of mangosteen pericarp extract in PE/SPI bilayer film. In this research, mangosteen pericarp has been extracted and incorporated into SPI and laminated on PE film to provide bioactive compounds and enhance the antioxidant activity of the film with the intention to prevent oxidation process in foods. The effect of temperature was also investigated to observe the stability of antioxidant properties of the film.

2. Materials and Methods

2.1 Materials Preparation

For film preparation, commercial polyethylene (PE) film was purchased from the local plastic supplier in Taman Sri Serdang, Selangor, Malaysia with 80 µm thickness and soy protein isolate (SPI) was purchased from MP Biomedicals (Solon, Ohio). Glycerol, sodium carbonate and standard gallic acid were obtained from Sigma-Aldrich (St. Louis, USA). Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from TCI Development Co. Ltd (Shanghai, China). All the solvents and chemicals used for this research were analytically graded.

2.2 Mangosteen Pericarps Extract (MPE) Preparation

The pericarps of mangosteen at commercial maturation were obtained from markets around Serdang, Selangor, Malaysia. The mangosteen pericarps were peeled off, washed and cut into cubes (3 cm^3) before placed in a circulating oven (Universal Oven UF110, Memmert, DE) at 45°C for 48 h [24]. The dried pericarps were ground into fine powders using a grinder (Tefal, Rumilly, France). The powder of 100 g was macerated with 500 ml absolute ethanol (John Kollin Corporation, USA) for 48 h at room temperature. The solvent was removed under vacuum using rotary evaporator at 40°C. The dark yellow concentrated extract was kept in a freezer at -20°C until further use.

2.3 Film Preparation

For SPI layer, SPI was dispersed in distilled water at the concentration of 5% (w/v) to form the film-forming solutions (FFS) according to the method by Guerrero *et al*., [25]. Glycerol, as a plasticizer was added at 30% (v/w) of SPI concentration. The solution was stirred and heated to 80°C for 1 h. The FFS were cooled down to 40°C and then, 3% (w/w based on SPI) of mangosteen pericarps extract (MPE) was added into the FFS. Another FFS were prepared without the addition of MPE as a comparison. All FFS were cast onto PE film (10 cm^2) using a spreader with 50 µm thickness to form bilayer films. PE layer was cut into 10 cm^2 as control film. All the films were dried at an ambient temperature (25 ± 2°C) for 48 h. All films were conditioned at a temperature of 25°C with 50 ± 5% relative humidity (RH) for 24 h before stored at 25 and 40°C for 9 weeks for further analyses.

2.4 Film Thickness and Opacity

The thickness of film was measured using a hand-held digital micrometer screw gauge (Mitutoyo Corp., Kawasakishi, Japan) at 10 different film locations. Each film was cut into pieces (1 x 4 cm^2) and placed in a cuvette of UV-Vis spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Fisher Scientific, Wisconsin, USA). The opacity of the film was analyzed on every week for 9 weeks of storage time. The opacity of the films was determined by the following equation

$$\text{Opacity } (A_{600}/\text{mm}) = \text{Abs600}/y \quad (1)$$

where Abs600 is the value of absorbance at 600 nm and y is the thickness of the film (mm).

2.5 Film Color

The color of the film sample was determined using Minolta chromameter (Minolta CR 300 Series, Minolta Camera Co., Ltd., Osaka, Japan) using L, a, and b mode. The values range from L = 0 (black) to L = 100 (white), -a (greenness) to +a (redness), and –b (blueness) to +b (yellowness). The color of the film was analyzed on every week for 9 weeks of storage time.

2.6 Scanning Electron Microscope (SEM)

Morphology and cross-section of films on Week 0 and Week 9 were visualized using a scanning electron microscope (SEM) (JEOL JSM 6400, Tokyo, Japan). The films were glued on bronze stub using double-sided tape and were sputtered with gold using Sputter Coater SCD 005 (BAL-TEC AG, Balzers, Liechtenstein). The photographs were captured at an accelerating voltage of 10 kV with 1000x and 500x magnifications for surface and cross-section, respectively.

2.7 Total Phenolic Content (TPC)

TPC for each film was determined according to the method described by Hafsa *et al.* [26] from Week 0 till Week 9 with a one-week interval. Film extract was prepared by soaking 50 mg of sample in 3 mL of ethanol for 3 h. 300 µL of film extract was added to the test tubes followed by 2.5 mL Follin Ciocalteu's reagent (diluted 10 times with water) and 2 mL sodium carbonate (7.5% w/v). The mixture was stirred and incubated at 50°C for 5 min. The solution was measured using spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Fisher Scientific, Wisconsin, USA) with absorption at 765 nm and compared to a gallic acid calibration curve. The TPC was expressed as milligram gallic acid equivalent (mg GAE) per gram of films.

2.8 DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The antioxidant activity of the film from Week 0 till Week 9 with one-week interval was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according to the method described by Siripatrawan and Harte [27]. Three mL of film extract and 1 mL of 1 mM methanolic solution of DPPH was mixed and vortexes. Then, the mixture was incubated (30 min) in the dark at ambient temperature. The absorbance was measured at 517 nm using spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Fisher Scientific, Wisconsin, USA). The percentage of DPPH free radical quenching activity was calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(Abs_{DPPH} - Abs_{extract})/Abs_{DPPH}] \times 100\% \quad (2)$$

where Abs_{DPPH} is the absorbance for methanolic DPPH solution and $Abs_{extract}$ is the absorbance of sample extracts.

2.9 Statistical Analysis

All data for each analysis were statistically analyzed. Data is presented as the mean ± standard deviation of a triplicate of each measurement. A significant difference between means was evaluated using the analysis of variance (ANOVA). Tukey test was used for comparing mean values and differences between means were considered significant when $P \leq 0.05$.

3. Results and Discussion

3.1 Film Thickness and Opacity

Table 1 presents the data for film thickness and color at 25 and 40°C for 9 weeks. The thickness of control PE film was measured to be 80 µm while PE/SPI+MPE film was 130 µm. Changes in the film thickness may associate with the variations in network structures that have been changed throughout storage time [21]. However, the thicknesses of all films were found to be not statistically significant ($P > 0.05$) after 9 weeks of storage at both 25 and 40°C. As reported by Anker *et al*., [28] and Riquelme *et al*., [29], the loss of glycerol (plasticizer) and active compounds in the film matrix were too insignificant to affect the thickness of the film.

Table 1
Thickness of films stored at 25 and 40°C for 9 weeks

Storage time (Week)	PE (µm)		PE/SPI+MPE (µm)	
	25°C	40°C	25°C	40°C
0	80.7 ± 1.06	80.7 ± 1.06	130.6 ± 0.84	130.6 ± 0.84
1	80.7 ± 0.95	80.7 ± 0.48	130.6 ± 0.52	130.6 ± 0.70
2	80.7 ± 0.48	80.7 ± 0.48	130.6 ± 0.70	130.6 ± 0.70
3	80.7 ± 1.06	80.7 ± 0.48	130.6 ± 0.84	130.6 ± 0.52
4	80.7 ± 0.48	80.7 ± 0.68	130.6 ± 0.52	130.6 ± 0.70
5	80.7 ± 0.68	80.7 ± 0.68	130.6 ± 0.52	130.6 ± 0.70
6	80.7 ± 0.48	80.7 ± 0.68	130.6 ± 0.52	130.4 ± 0.52
7	80.7 ± 0.48	80.7 ± 0.68	130.4 ± 0.70	130.2 ± 0.63
8	80.7 ± 0.82	80.7 ± 0.63	130.4 ± 0.70	130.1 ± 0.57
9	80.7 ± 0.67	80.7 ± 0.63	130.4 ± 0.70	130.1 ± 0.74

*The data were presented as means ± standard deviation. There is no significant difference between films on the same day at both temperatures and no significant difference between same films at same temperature on every interval of storage time ($P \leq 0.05$).

Table 2
Opacity of films stored at 25 and 40°C for 9 weeks

Storage time (Week)	PE (µm)		PE/SPI+MPE (µm)	
	25°C	40°C	25°C	40°C
0	$2.09\pm0.01^{a,A}$	$2.09\pm0.01^{a,A}$	$4.18\pm0.05^{a,J}$	$4.18\pm0.05^{a,J}$
1	$2.09\pm0.02^{a,A}$	$2.09\pm0.00^{a,A}$	$4.19\pm0.03^{b,I}$	$4.25\pm0.01^{a,I}$
2	$2.09\pm0.00^{a,A}$	$2.09\pm0.02^{a,A}$	$4.20\pm0.02^{b,H}$	$4.29\pm0.01^{a,H}$
3	$2.09\pm0.00^{a,A}$	$2.09\pm0.00^{a,A}$	$4.24\pm0.00^{b,G}$	$4.33\pm0.01^{a,G}$
4	$2.09\pm0.01^{a,A}$	$2.09\pm0.01^{a,A}$	$4.25\pm0.01^{b,F}$	$4.39\pm0.01^{a,F}$
5	$2.09\pm0.01^{a,A}$	$2.09\pm0.01^{a,A}$	$4.27\pm0.01^{b,E}$	$4.40\pm0.01^{a,E}$
6	$2.09\pm0.02^{a,A}$	$2.09\pm0.01^{a,A}$	$4.28\pm0.01^{b,D}$	$4.44\pm0.00^{a,D}$
7	$2.09\pm0.01^{a,A}$	$2.09\pm0.02^{a,A}$	$4.29\pm0.02^{b,C}$	$4.45\pm0.02^{a,C}$
8	$2.09\pm0.00^{a,A}$	$2.09\pm0.01^{a,A}$	$4.29\pm0.02^{b,B}$	$4.47\pm0.01^{a,B}$
9	$2.09\pm0.02^{a,A}$	$2.09\pm0.03^{a,A}$	$4.30\pm0.02^{b,A}$	$4.53\pm0.01^{a,A}$

*The data were presented as means ± standard deviation. Different letters (a-b) indicate significant differences between films on the same day with both temperatures and (A-J) indicate significant differences between each film at same temperature on every interval of storage time ($P \leq 0.05$).

Opacity is an essential property in food packaging, where it helps in controlling the light transmission on the inner product [30]. The opacity of film is correlated with the light transmission and transparency of film. Higher opacity indicates low transparency and good barrier towards light. The opacity of the films differed significantly ($P \leq 0.05$) for each film regardless of storage time and

temperatures. The result obtained in Table 2 showed that the films with the incorporation of MPE were less transparent than the control film. The additional layer of SPI and MPE on the PE film made the opacity of the film increased due to the higher amount of yellowish solid particles of SPI and MPE on the film after drying and thus, decreasing the transparency of the film. Also, storage time affected the opacity of active films in which the values increased significantly ($P \leq 0.05$), irrespective of temperature.

Moreover, at high temperature, the transparency of each film decreased in value and caused the film to be more opaque. The interference of glycerol with the protein-protein interactions by forming hydrogen bonds with protein molecule is reported to increase the transparency of the film matrix [31]. However, the loss of moisture and glycerol due to the high temperature during storage associated with the reduction in transparency of the film [21]. Upon storage, it is high probability for glycerol to diffuse to the surrounding since glycerol is fully soluble in water and also cause the residual moisture during the film-forming procedure to migrate too [21,32]. This is in agreement with the findings from Ciannamea *et al*., [21] and Jongjareonrak *et al*., [33] where the opacity of fish gelatin film and soybean protein film increased after prolonged storage time of 42 and 90 days, respectively. Among both films, PE/SPI+MPE film expressed the highest opacity value ($P \leq 0.05$). The incorporation of MPE into the film contributed to the darker color and hence increased the opacity of PE/SPI+MPE film, providing good light barrier to the product that sensitive to UV light.

3.2 Film Color

The color parameters (*L, a* and *b*) of PE and PE/SPI+MPE films are shown in Table 3, 4 and 5 respectively. Different types of film exhibited different *L, a* and *b* values ($P \leq 0.05$). From the data, it is proven that PE film has the lightest color compared to PE/SPI+MPE film which have the darkest color. The color of PE/SPI+MPE film changed significantly ($P \leq 0.05$) after 9 weeks of storage at both temperatures except for PE film which remain unchanged ($P > 0.05$). PE/SPI+MPE film was yellowish in color (greater value of *b*) due to the addition layer of SPI+MPE film on PE film. It is noted that the brown and yellowish color of SPI and MPE respectively, resulting in decreasing of *L* (lightness) and *a* (greenness) values. Meanwhile, the value of *b* (yellowness) increased significantly ($P \leq 0.05$).

Table 3
Color parameter of films (*L*) stored at 25 and 40°C for 9 weeks

Storage time	PE (µm)		PE/SPI+MPE (µm)	
(Week)	25°C	40°C	25°C	40°C
0	90.31±0.01^{a,A}	90.31±0.01^{a,A}	87.82±0.03^{a,A}	87.82±0.03^{a,A}
1	90.30±0.00^{a,A}	90.30±0.00^{a,A}	87.81±0.06^{a,A}	87.76±0.03^{a,A}
2	90.32±0.02^{a,A}	90.30±0.01^{a,A}	87.79±0.05^{a,AB}	87.59±0.05^{b,B}
3	90.30±0.01^{a,A}	90.26±0.01^{a,A}	87.72±0.08^{a,BC}	87.49±0.05^{b,B}
4	90.28±0.02^{a,A}	90.26±0.03^{a,A}	87.60±0.04^{a,CD}	87.38±0.04^{b,C}
5	90.30±0.01^{a,A}	90.31±0.01^{a,A}	87.54±0.05^{a,CDE}	87.32±0.02^{b,CD}
6	90.32±0.00^{a,A}	90.31±0.01^{a,A}	87.47±0.03^{a,DE}	87.25±0.03^{b,DE}
7	90.31±0.01^{a,A}	90.30±0.03^{a,A}	87.46±0.05^{a,E}	87.18±0.02^{b,E}
8	90.29±0.02^{a,A}	90.31±0.01^{a,A}	87.39±0.04^{a,E}	87.15±0.04^{b,E}
9	90.31±0.01^{a,A}	90.30±0.02^{a,A}	87.34±0.05^{a,E}	87.05±0.04^{b,F}

*The data were presented as means ± standard deviation. Different letters (a-b) indicate significant differences between films on the same day with both temperatures and (A-F) indicate significant differences between each film at same temperature on every interval of storage time ($P \leq 0.05$)

The storage temperature had affected the color of PE/SPI+MPE film significantly (P ≤ 0.05). At 40°C, the films became darker compared to films stored at 25°C. This could be due to the SPI polymer and MPE compounds had become concentrated when the solvent evaporated at high temperature, causing the color of the film becomes more intense [34]. The loss of glycerol and moisture may also affect the concentration of SPI and MPE, which were brown and yellow in color [21]. Furthermore, Maillard reaction (browning reaction) which is non-enzymatic browning reaction could be presented in the films due to the longer period of storage at 40°C, hence increased the yellowness (*b* value) of the films over 63 days [21,35].

Table 4
Color parameter (*a*) of films stored at 25 and 40°C for 63 days

Storage time	PE film		PE/SPI+MPE film	
(Week)	25°C	40°C	25°C	40°C
0	-1.09±0.01^{a,A}	-1.09±0.01^{a,A}	-1.91±0.02^{a,A}	-1.91±0.02^{a,A}
1	-1.09±0.01^{a,A}	-1.10±0.00^{a,A}	-1.92±0.02^{a,A}	-1.91±0.01^{a,A}
2	-1.10±0.03^{a,A}	-1.09±0.01^{a,A}	-1.91±0.01^{a,A}	-1.92±0.00^{a,A}
3	-1.08±0.02^{a,A}	-1.09±0.00^{a,A}	-1.92±0.00^{a,A}	-1.95±0.02^{b,A}
4	-1.09±0.02^{a,A}	-1.09±0.01^{a,A}	-1.91±0.01^{a,A}	-1.97±0.02^{b,A}
5	-1.09±0.01^{a,A}	-1.10±0.03^{a,A}	-1.91±0.01^{a,A}	-2.04±0.05^{b,B}
6	-1.10±0.00^{a,A}	-1.09±0.00^{a,A}	-1.92±0.01^{a,A}	-2.11±0.02^{b,C}
7	-1.09±0.01^{a,A}	-1.09±0.01^{a,A}	-1.93±0.02^{a,AB}	-2.19±0.02^{b,D}
8	-1.09±0.01^{a,A}	-1.08±0.02^{a,A}	-1.94±0.03^{a,AB}	-2.24±0.02^{b,D}
9	-1.09±0.03^{a,A}	-1.09±0.01^{a,A}	-1.96±0.02^{a,B}	-2.25±0.02^{b,D}

*The data were presented as means ± standard deviation. Different letters (a-b) indicate significant differences between films on the same day with both temperatures and (A-D) indicate significant differences between each film at same temperature on every interval of storage time (P ≤ 0.05)

Table 5
Color parameter (*b*) of films stored at 25 and 40°C for 63 days

Storage time	PE (μm)		PE/SPI+MPE (μm)	
(Day)	25°C	40°C	25°C	40°C
0	2.21±0.01^{a,A}	2.21±0.01^{a,A}	10.77±0.02^{a,F}	10.77±0.02^{a,G}
7	2.21±0.01^{a,A}	2.21±0.01^{a,A}	10.90±0.08^{a,EF}	11.00±0.09^{a,G}
14	2.22±0.01^{a,A}	2.22±0.01^{a,A}	10.95±0.03^{b,EF}	11.61±0.08^{a,F}
21	2.21±0.02^{a,A}	2.22±0.01^{a,A}	11.30±0.03^{b,DE}	12.20±0.01^{a,E}
28	2.21±0.01^{a,A}	2.22±0.02^{a,A}	11.60±0.13^{b,CD}	12.70±0.05^{a,D}
35	2.20±0.01^{a,A}	2.21±0.02^{a,A}	11.63±0.20^{b,BCD}	13.39±0.06^{a,C}
42	2.21±0.00^{a,A}	2.22±0.02^{a,A}	11.80±0.09^{b,BC}	13.70±0.15^{a,C}
49	2.22±0.01^{a,A}	2.23±0.02^{a,A}	12.03±0.03^{b,AB}	14.30±0.21^{a,B}
56	2.21±0.01^{a,A}	2.22±0.02^{a,A}	12.28±0.10^{b,A}	14.82±0.16^{a,A}
63	2.22±0.02^{a,A}	2.24±0.02^{a,A}	12.43±0.08^{b,A}	15.02±0.05^{a,A}

*The data were presented as means ± standard deviation. Different letters (a-b) indicate significant differences between films on the same day with both temperatures and (A-G) indicate significant differences between each film at same temperature on every interval of storage time (P ≤ 0.05)

3.3 Scanning Electron Microscopy (SEM)

The morphology of the films was determined by using scanning electron microscopy (SEM) that analyzed the surface and cross section of the films as seen in Figure 1 and 2, respectively. Obviously, the structure of PE film was smoother compared to SPI/PE and PE/SPI+MPE films without crack and irregularity. The films with SPI layer showed rough and irregular structures with the presence of

granules. As indicated by Kaewprachu and Rawdkuen [36], the globulin components of soy protein made the SPI formed a less organized matrix that may cause the unevenness to the structures. Between PE/SPI film and PE/SPI+MPE film, the latter was observed to have the roughest and uneven structure. The incorporation of MPE into the film interfered the ordered structure of the film matrix and hence produced the irregularity on the surface with granules [37]. However, there is no crack present on the structures of each film.

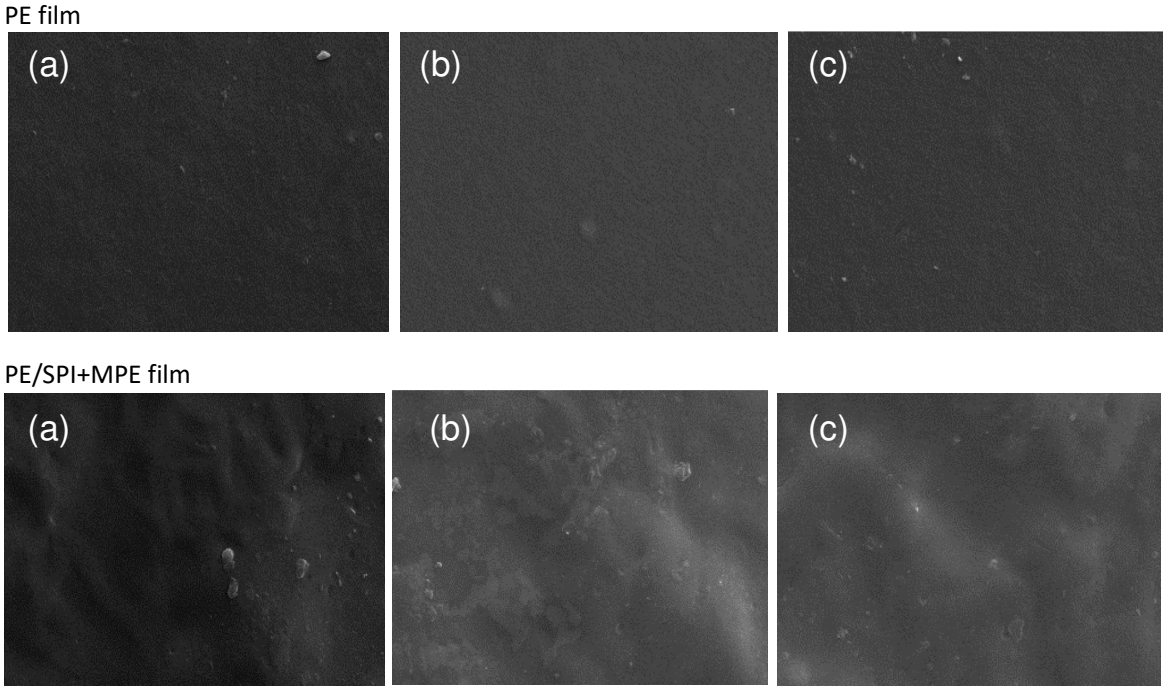

Fig. 1. SEM – surface images of PE and PE/SPI+MPE films in (a) week 0 at 25 and 40°C, (b), week 9 at 25°C and (c) week 9 at 40°C at magnification of 1000x

The cross section images for all films were presented in Figure 2. It can be seen that cross section of PE film was the smoothest compared to other two bilayer films. Both bilayer films (PE/SPI film and SPI+MPE/PE) showed the images of two layers being adhered together. Compared to PE layer, SPI layer was more compact and had a fibrous structure. The contribution of those images was discussed above with due regard to SPI and MPE compounds. In addition, it is possible to affirm that the morphology of PE film, PE/SPI and SPI+MPE/PE films were not affected by the different storage temperature since the morphology of films remained unchanged after 9 weeks at both temperatures.

3.4 Antioxidant Activity of Film

Lipid peroxidation had caused a great loss in food industry. Hence, the incorporation of active compounds (antioxidant) in the packaging film is believed to help in slowing down the occurrence of autoxidation and rancidity in fatty foods. The release of active compounds into the packaged food can be triggered by chemical, biochemical or biological changes [38]. Antioxidant activities of all films were determined using Folin-Ciocalteu method and DPPH scavenging assay for every week in order to measure the loss of antioxidant in the films at both storage temperatures. Folin-Ciocalteu method

was used in determining the content of crude estimate of phenolic compound in the films as seen in Figure 3 while Figure 4 displays the values of DPPH scavenging activity for all films.

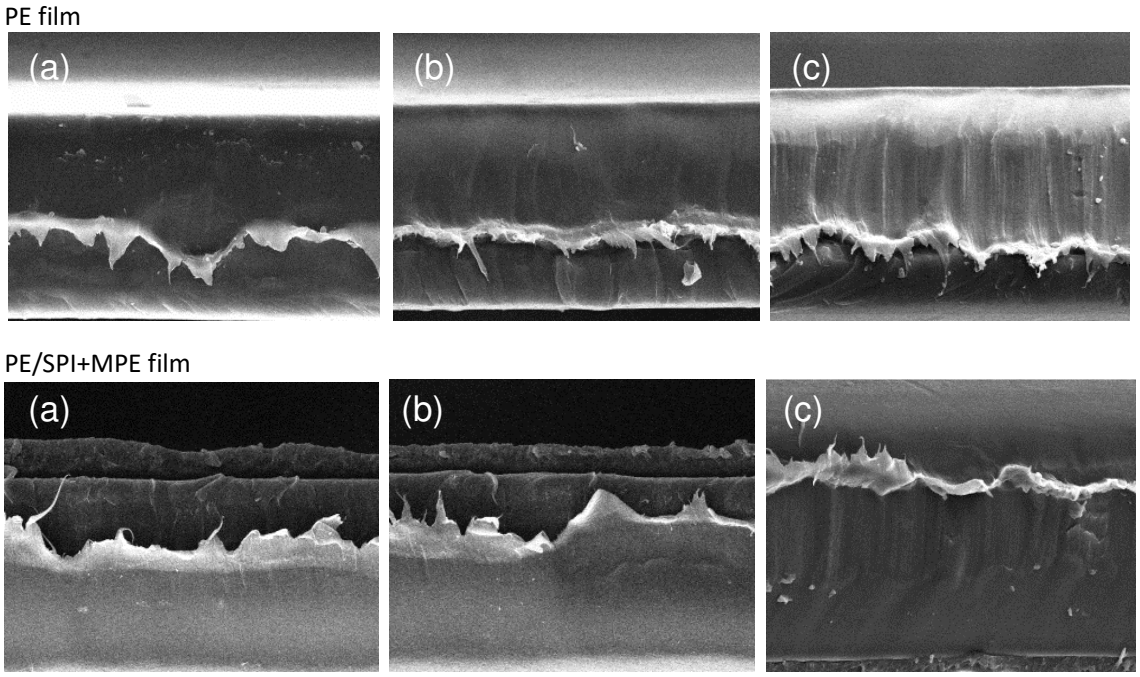

Fig. 2. SEM – cross section images of PE and PE/SPI+MPE films in (a) week 0 at 25 and 40°C, (b), week 9 at 25°C and (c) week 9 at 40°C at magnification of 500x

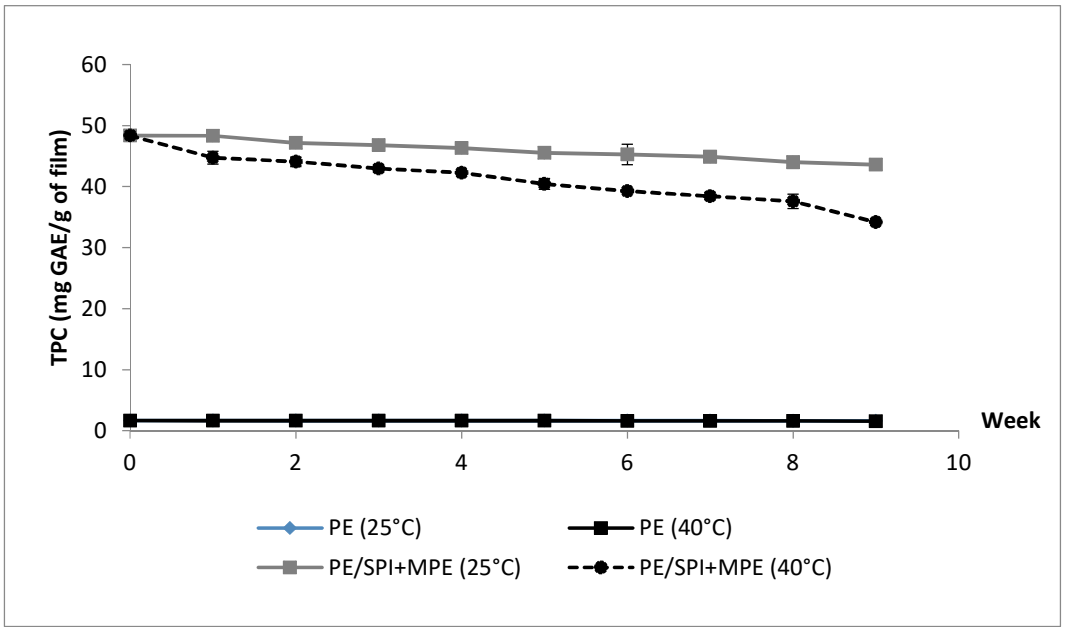

Fig. 3. Total phenolic content of films for 9 weeks stored at 25 and 40°C

TPC of films is correlated with the DPPH scavenging activity of the films. No antioxidant activity was found in control film after 9 weeks of storage at 25 and 40°C as this was served for comparison purpose. PE/SPI+MPE films recorded the presence of antioxidant activities with high value of antioxidant activity. SPI as a protein polymer is a good carrier agent for antioxidant molecules with such amino acids that possessed more binding sites for phenolic compounds [39,40].

The antioxidant activity of films is largely associated with the phenolic content in the mangosteen pericarp [16,41]. It has been declared that mangosteen pericarps are rich in phenolic compounds such as tannins [15], xanthones and anthocyanins [42] that can act as natural antioxidants. Undeniably, the incorporation of 3% MPE into the film layer played a vital role in increasing the TPC and DPPH scavenging activity of the film. Consequently, phenolic compounds in MPE helped in influencing the antioxidant activity in PE/SPI+MPE where it showed about 70% increased of antioxidant activity compared to control film on week 0.

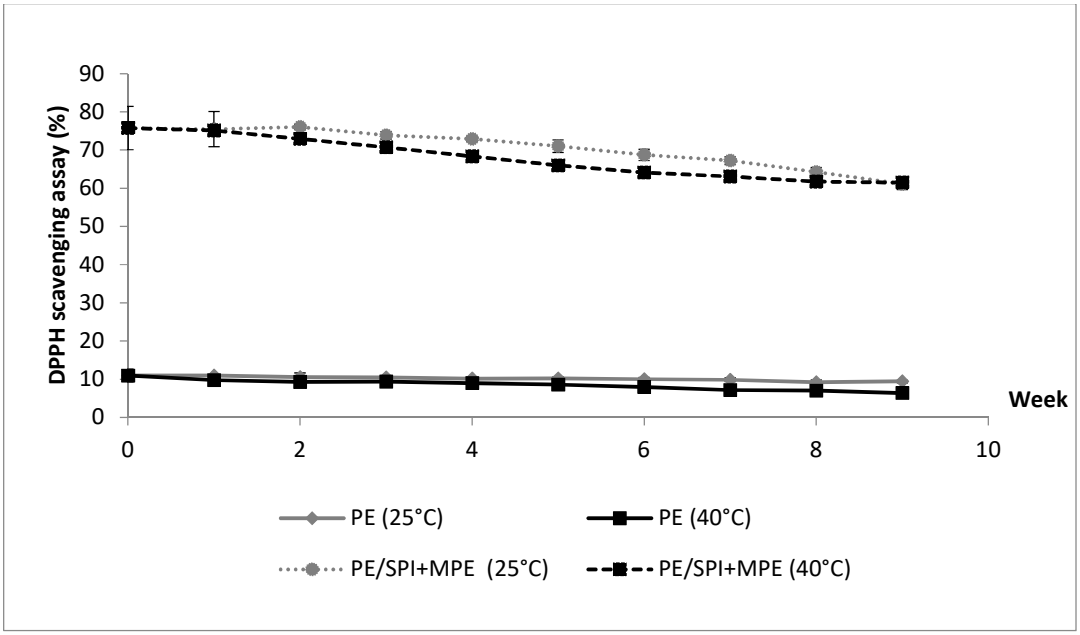

Fig.4. DPPH scavenging assay of films for 9 weeks stored at 25 and 40°C

The TPC and the DPPH scavenging activity of PE/SPI+MPE film decreased significantly ($P \leq 0.05$) over 9 weeks for both storage temperatures. Furthermore, at 40°C, both TPC and DPPH values of all films started to decrease prominently ($P \leq 0.05$) after week 0 compared to films stored at 25°C. These results showed that the presence of phenolic compounds was influenced by accelerated storage temperature. Temperature is one of the important factors affecting antioxidant activity. The expose of phenolic compounds of MPE at high temperature for a long time (9 weeks) could lead to three possible mechanisms; the thermal degradation of the phenolic compounds, the release of bound phenolic compounds and the release of phenolic acid derivatives to the surrounding [43].

Besides, since phenolic compounds are unstable structures, they are easily sensitive to degradation by some factors such as light, oxygen, pH, storage temperature and time [23]. The loss of antioxidant activity in the film may attributed to the deterioration of phenolic compounds in MPE due to the heat sensitivity of the bioactive compound and the increased of temperature [44,45]. Hence, as the storage temperature increase, the degradation of the phenolics and antioxidant activity will occur faster.

4. Conclusion

The physical properties of PE/SPI+MPE film such opacity and color were affected by the storage temperatures. The opacity of PE/SPI+MPE film increased ($P \leq 0.05$) after 9 weeks of storage from 4.18 A_{600}/mm to 4.30 A_{600}/mm at 25°C and 4.53 A_{600}/mm at 40°C. However, SEM images for all films showed no significant different on the surface morphology of films. Incorporation of MPE possessed higher antioxidant activity compared to control PE film. MPE added into the bilayer PE/SPI+MPE film increased the total phenolic content (TPC) of the film up to 48.36 mg GAE/g compared to PE film with only 1.66 mg GAE/g. Moreover, the antioxidant activity (DPPH scavenging assay) of active PE/SPI+MPE film still exhibits higher value after 9 weeks of storage (more than 50%) at 25 and 40°C. This is due to the stability of bioactive compounds of MPE in the film. Therefore, this active PE/SPI+MPE film could be a potential alternative as an active bilayer film that can be used to enhance the quality of food products by slowing down the lipid oxidation in foods.

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References

[1] Realini, Carolina E., and Begonya Marcos. "Active and intelligent packaging systems for a modern society." *Meat science* 98, no. 3 (2014): 404-419.
[2] Rešček, Ana, Ljerka Kratofil Krehula, Zvonimir Katančić, and Zlata Hrnjak-Murgić. "Active bilayer PE/PCL films for food packaging modified with zinc oxide and casein." *Croatica Chemica Acta* 88, no. 4 (2015): 461-473.
[3] Ganiari, Sophie, Evanthia Choulitoudi, and Vassiliki Oreopoulou. "Edible and active films and coatings as carriers of natural antioxidants for lipid food." *Trends in Food Science & Technology* 68 (2017): 70-82.
[4] Marsh, Kenneth, and Betty Bugusu. "Food packaging—roles, materials, and environmental issues." *Journal of food science*72, no. 3 (2007): R39-R55.
[5] Rahmani, Bahareh, Hedayat Hosseini, Mohammadreza Khani, Mehdi Farhoodi, Zohreh Honarvar, Ehsan Feizollahi, Babak Shokri, and Saeedeh Shojaee-Aliabadi. "Development and characterisation of chitosan or alginate-coated low density polyethylene films containing Satureja hortensis extract." *International journal of biological macromolecules* 105 (2017): 121-130.
[6] Friesen, Kara, Chang Chang, and Michael Nickerson. "Incorporation of phenolic compounds, rutin and epicatechin, into soy protein isolate films: Mechanical, barrier and cross-linking properties." *Food chemistry* 172 (2015): 18-23.
[7] Kokoszka, Sabina, Frederic Debeaufort, Andrzej Lenart, and Andree Voilley. "Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films." *International Dairy Journal* 20, no. 1 (2010): 53-60.
[8] Alves, Maria M., M. P. Gonçalves, and Cristina MR Rocha. "Effect of ferulic acid on the performance of soy protein isolate-based edible coatings applied to fresh-cut apples." *LWT-Food Science and Technology* 80 (2017): 409-415.
[9] Kang, Hye-Jin, Sung-Jin Kim, Young-Sun You, Monique Lacroix, and Jaejoon Han. "Inhibitory effect of soy protein coating formulations on walnut (Juglans regia L.) kernels against lipid oxidation." *LWT-Food science and technology* 51, no. 1 (2013): 393-396.
[10] Wang, Yufang, and Xiaoyong Wang. "Binding, stability, and antioxidant activity of quercetin with soy protein isolate particles." *Food chemistry* 188 (2015): 24-29.
[11] Zarena, A. S., and K. Udaya Sankar. "Isolation and identification of pelargonidin 3-glucoside in mangosteen pericarp." *Food chemistry* 130, no. 3 (2012): 665-670.
[12] Fu, Caili, Alvin Eng Kiat Loo, Fiona Ping Ping Chia, and Dejian Huang. "Oligomeric proanthocyanidins from mangosteen pericarps." *J. of Agricultural and Food Chemistry* 55, no. 19 (2007): 7689-7694.
[13] Naczk, M., M. Towsend, R. Zadernowski, and F. Shahidi. "Protein-binding and antioxidant potential of phenolics of mangosteen fruit (Garcinia mangostana)." *Food chemistry*128, no. 2 (2011): 292-298.
[14] Nguyen, Lien-Hoa D., Ganpathi Venkatraman, Keng-Yeow Sim, and Leslie J. Harrison. "Xanthones and benzophenones from Garcinia griffithii and Garcinia mangostana." *phytochemistry* 66, no. 14 (2005): 1718-1723.

[15] Pothitirat, Werayut, Mullika Traidej Chomnawang, Roongtawan Supabphol, and Wandee Gritsanapan. "Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity." *Fitoterapia* 80, no. 7 (2009): 442-447.
[16] Zadernowski, Ryszard, Sylwester Czaplicki, and Marian Naczk. "Phenolic acid profiles of mangosteen fruits (Garcinia mangostana)." *Food Chemistry* 112, no. 3 (2009): 685-689.
[17] Chong, Yee Mun, Sui Kiat Chang, Winne Chiaw Mei Sia, and Hip Seng Yim. "Antioxidant efficacy of mangosteen (Garcinia mangostana Linn.) peel extracts in sunflower oil during accelerated storage." *Food Bioscience* 12 (2015): 18-25.
[18] Tjahjani, Susy, Wahyu Widowati, Khie Khiong, Adrian Suhendra, and Rita Tjokropranoto. "Antioxidant properties of Garcinia mangostana L (mangosteen) rind." *Procedia Chemistry* 13 (2014): 198-203.
[19] Suksamrarn, Sunit, Narisara Suwannapoch, Piniti Ratananukul, Nantana Aroonlerk, and Apichart Suksamrarn. "Xanthones from the Green Fruit Hulls of Garcinia m angostana." *Journal of Natural Products* 65, no. 5 (2002): 761-763.
[20] Wang, Jing J., Qing H. Shi, Wei Zhang, and Barbara JS Sanderson. "Anti-skin cancer properties of phenolic-rich extract from the pericarp of mangosteen (Garcinia mangostana Linn.)." *Food and chemical toxicology* 50, no. 9 (2012): 3004-3013.
[21] Ciannamea, Emiliano Manuel, Pablo Marcelo Stefani, and Roxana Alejandra Ruseckaite. "Storage-induced changes in functional properties of glycerol plasticized–Soybean protein concentrate films produced by casting." *Food Hydrocolloids* 45 (2015): 247-255.
[22] Leceta, I., M. Peñalba, P. Arana, P. Guerrero, and K. De La Caba. "Ageing of chitosan films: Effect of storage time on structure and optical, barrier and mechanical properties." *European Polymer Journal* 66 (2015): 170-179.
[23] Kim, Ah-Na, Hyun-Jin Kim, Jiyeon Chun, Ho Jin Heo, William L. Kerr, and Sung-Gil Choi. "Degradation kinetics of phenolic content and antioxidant activity of hardy kiwifruit (Actinidia arguta) puree at different storage temperatures." *LWT* 89 (2018): 535-541.
[24] Khonkarn, Ruttiros, Siriporn Okonogi, Chadarat Ampasavate, and Songyot Anuchapreeda. "Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines." *Food and Chemical Toxicology* 48, no. 8-9 (2010): 2122-2129.
[25] Guerrero, P., ZA Nur Hanani, J. P. Kerry, and K. De La Caba. "Characterization of soy protein-based films prepared with acids and oils by compression." *Journal of Food Engineering*107, no. 1 (2011): 41-49.
[26] Hafsa, Jawhar, Me ali Smach, Med R. B. Khedher, Bassem Charfeddine, Khalifa Limem, Hatem Majdoub, and Sonia Rouatbi, "Physical, antioxidant and antimicrobial properties of chitosan films containing *Eucalyptus globules* essential oil." *LWT – Food Science and Technology* 68 (2016): 356-364.
[27] Siripatrawan, Ubonrat, and Bruce R. Harte. "Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract." *Food Hydrocolloids* 24, no. 8 (2010): 770-775.
[28] Anker, M., M. Stading, and A. M. Hermansson. "Aging of whey protein films and the effect on mechanical and barrier properties." *Journal of Agricultural and Food Chemistry* 49, no. 2 (2001): 989-995.
[29] Riquelme, Natalia, Maria L. Herrera, and Silvia Matiacevich. "Active films based on alginate containing lemongrass essential oil encapsulated: Effect of process and storage conditions." *Food and Bioproducts Processing* 104 (2017): 94-103.
[30] Carpiné, Danielle, Joao L. A. Dagostin, Eriel F. de Andrade, Larissa C. Bertan, and Marcos R. Mafra. "Effect of natural surfactant *Yucca schidigera* extract on the properties of biodegradable emulsified films produced from soy protein isolate and coconut oil." *Industrial Crops and Products* 83 (2016): 364-371.
[31] Sothornvit, R., and J. M. Krochta. 2005. "Plasticizers in edible films and coatings." In *Innovations in food packaging,* edited by H. H. Jung, 403-433. London: Academic Press.
[32] Olabarrieta, Idoia, Sung-Woo Cho, Mikael Gällstedt, Jose-Ramon Sarasua, Eva Johansson, and Mikael S. Hedenqvist. "Aging properties of films of plasticized vital wheat gluten cast from acidic and basic solutions." *Biomacromolecules* 7, no. 5 (2006): 1657-1664.
[33] Jongjareonrak, Akkasit, Soottawat Benjakul, Wonnop Visessanguan, and Munehiko Tanaka. "Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α-tocopherol." *Food Hydrocolloids* 22 (2008): 449-458.
[34] Janjarasskul, Theeranun, Kanitha Tananuwong, Manusawee Leuangsukrerk, Thunyaluck Phupoksakul, and Chaleeda Borompichaichartkul. "Effects of hasten drying and storage conditions on properties and microstructure of Konjac Glucomannan-whey protein isolate blend films." *Food Biophysics* 13, no. 1 (2017): 49-59.
[35] Martucci, J. F., and R. A. Ruseckaite. "Biodegradation of three-layer laminate films based on gelatin under indoor soil conditions." *Polymer Degradation and Stability* 94 (2009): 1307-1313.
[36] Kaewprachu, P., and S. Rawdkuen. "Mechanical and physico – chemical properties of biodegradable protein-based films: A comparative study." *Food and Applied Bioscience Journal* 2, no. 1 (2014): 15-30.

[37] Ma, Q., D. Hu, H. Wang, and L. Wang. "Tara gum edible film incorporated with oleic acid." *Food Hydrocolloids* 56 (2016): 127-133.
[38] Khaneghah, A. Mousavi, Seyed M. B. Hashemi, and Sara Limbo. "Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions." *Food and Bioproducts Processing* 111 (2018): 1-19.
[39] Denavi, Gabriela A., Miriam Pérez-Mateos,Maria C. Añón, Pilar Monterro, Adriana N. Mauri, and M. Carmen Gómez-Guillén. "Structural and functional properties of soy protein isolate and cod gelatin blend films." *Food Hydrocolloids* 23, no. 8 (2009): 2094-2101.
[40] Wang, Haixia, Dongying Hu, Qianyun Ma, and Lijuan Wang. "Physical and antioxidant properties of flexible soy protein isolate films by incorporating chestnut (*Castanea mollissima*) bur extracts." *LWT - Food Science and Technology* 71 (2016): 33-39.
[41] Chaovanalikit, A., A. Mingmuang, T. Kitbunleuwit, N. Choldumrongkool, J. Sondee, and S. Chupratum. "Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products." *International Food Research Journal* 19, no. 3 (2012): 1047-1053.
[42] Suttirak, Weerayuth, and Supranee Manurakchinakorn. "In vitro antioxidant properties of mangosteen peel extract." *Journal of Food Science and Technology* 51, no. 12 (2014): 3546-3558.
[43] Maillard, Marie-Noeelle, and Claudette Berset. "Evolution of antioxidant activity during kinling: role of insoluble bound and phenolic acids of barley and malt." *Journal of Agricultural and Food Chemistry* 43 (1995): 1789-1793.
[44] Buchner, Nadja, Angelika B. Krumbein, S. Rohn, and Lothar W. Kroh. "Effect of thermal processing on the flavonols rutin and quercetin." *Rapid Communications in Mass Spectrometry* 20 (2006): 3229-3235.
[45] Kim, D. O., and O. I Padilla-Zakour. "Jam processing effect on phenolics and anti- oxidant capacity in anthocyanin-rich fruits: Cherry, plum, and raspberry." *Journal of Food Science* 69, no. 9 (2004): 395-400.