Research Article

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Preparation and characterizations of antibacterial–antioxidant film from soy protein isolate incorporated with mangosteen peel extract

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Abstract: The mangosteen peel extract (MPE) was used to obtain soy protein isolate (SPI) films. The results show that MPE exhibited a high content of total phenolics and antioxidant activity. Moreover, the MPE can enhance the antibacterial–antioxidant properties, UV-visible light barrier properties, and water-resistant properties of the SPI films. The presence of MPE resulted in an increase in water vapor permeability and hydrophobicity. The extract addition also reduced the film's crystallinity along with a decrease in the mechanical property and lowering of the maximum degradation temperature. Attenuated total reflectance Fourier transform infrared spectroscopy revealed that the polyphenols in MPE could interact with SPI through hydrogen bonds and hydrophobic interactions, and the addition of MPE changed the secondary structure of SPI with a decrease in β -sheets and an increase in β -turns and random coils. Scanning electron microscopy showed that all the films exhibited smooth and homogenous morphology on the surface and on some layers through cross-sectional images. Our results suggested that the MPE would be a promising ingredient to make SPI films used as an active packaging material.

Keywords: SPI film, MPE, antibacterial–antioxidant

1 Introduction

Petroleum-based plastics with the advantages of lightness, barrier characteristics, processability, low cost, and good physical properties have been widely used in the consumer products packaging (1,2). But the problem is that these discarded plastics not only pollute soil and water, but also produce unwanted by-products (3). Concerns about the environmental problems caused by non-biodegradable plastics and the depletion of natural resources have prompted people to develop environmentally friendly and green composites using biodegradable and renewable materials. Biopolymer packaging, as an effective solution to reduce food waste, has additional functions such as biodegradability, improved food quality and shelf life (4). Antioxidant and antimicrobial functions are the main developmental direction in active bioplastic packaging (4,5). Using antimicrobials in food packaging have greater advantages than adding them directly to the food because antimicrobials added to the food surface via sprays or drops are not effective enough to inhibit microorganisms (6). This is due to the rapid spread of antimicrobials into foods and the denaturation of active compounds in food ingredients that reduce the activity of functional agents. Antimicrobials provide a slow and continuous migration of these agents from the packaging material to the food surface, which can maintain high concentrations of antimicrobial agents over a long period of time (7). Natural antimicrobial ingredients such as polyphenol-rich natural extracts are volatile and cannot be used alone. In order to improve the efficacy of these materials, active films are used (8).

As a natural substance, soy protein isolate (SPI) has higher protein content than other soybean protein products (9), and SPI film has good antioxidant and oil-proof performances at low relative humidity (RH) (10). However, due to the inherent hydrophilicity of natural proteins and

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strong molecular interactions, SPI films do not exhibit satisfactory mechanical properties or water vapor barrier properties, which become worse under highly humid conditions (11). In order to improve the mechanical properties of SPI films, various studies have reported that the blending of SPI and biodegradable polymers will be a promising method of structural modification, and the improvement in the basic properties of SPI films has been widely paid attention (12). Polyvinyl alcohol (PVA) (13) is a biodegradable and non-toxic synthetic polymer used to develop blend films. It imparts good tensile strength and biodegradability and hence has been widely used in biomaterial fields. Therefore, PVA is expected to improve the mechanical property of SPI films and dispersibility of antibacterial agent. In addition to serving as protective barriers, these films can also serve as carriers for bioactive compounds with antioxidant or antimicrobial properties. Waste from fruit processing can be considered as an economic source of antioxidants or antimicrobial agents, which may reduce the demand for synthetic preservatives.

Mangosteen (14,15) is a tropical fruit that is widely grown in Thailand, Malaysia, Indonesia, and South China. The skin of the mangosteen accounts for about two-thirds of its weight. The pericarp contains rich bioactive compounds, which can be used as therapeutic drugs (16) or functional food additives (17). Mangosteen peel extract (MPE) (18,19), a natural yellow-orange bioactive compound extracted from the mangosteen, is non-toxic and considered safe even at high concentrations and has a variety of functional properties, such as antioxidant, anti-inflammatory (20), antitumor, anticancer, and antibacterial activities (21). In recent years, antioxidant capacity and antibacterial activity of MPE have been frequently researched. However, no previous investigations have been conducted on MPE incorporation in SPI active packaging.

Therefore, this research was aimed to study the antioxidant properties and antibacterial activities of MPE, and antibacterial-antioxidant ability, mechanical properties, water vapor barrier capacity, water solubility, water contact angle, and optical properties of the SPI films incorporated with MPE. In addition, the films were also analyzed with attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, thermal gravimetric analyzer (TGA), scanning electron microscopy (SEM), and X-ray diffractometer (XRD).

2 Materials

SPI powder (more than 90% of dry protein) was provided by Henan Yuzhou Biotechnology Co., Ltd. PVA (1799,

$M_w = 9,000$) was purchased from Chengdu Kelong Chemical Co., Ltd. Glycerol was procured from Tianjin Aopusheng Chemical Co., Ltd. Mangosteen was bought from local market, and the waste pulp was removed, dried, and smashed for further use.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagents were obtained from Shanghai Macklin Biochemical Co., Ltd. All the other chemical reagents used in the experiment were of analytical grade. *Escherichia coli* ATCC 25922 (*E. coli*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*) were provided by Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.

2.1 Preparation of MPE

The mangosteen peel was washed three times, dried until no water existed, ground into powder, and screened by a 60-mesh sieve. Then, 20 g of the powder were added into 140 mL of 70% ethanol solution and stirred continuously at a temperature of 70°C for 120 min. The supernatant was obtained by centrifuging at 8,000 rpm for 10 min, filtered, and condensed under rotary evaporator. The MPE was obtained after freeze-drying to a constant weight. The dried MPE were stored under low temperature (4°C) and dark conditions for further use.

2.2 Film preparation

The SPI films were prepared by using solution-casting method (13). The SPI (5% w/w) solution was prepared in distilled water. The SPI solution mixing 30 wt% of glycerol was magnetically stirred at a temperature of 80°C for 30 min to denature the protein fraction. The PVA solution was prepared by adding 5% of PVA in 95°C water in a 100 mL capped beaker where it was maintained for 2 h, while being constantly shaken for good film-forming properties. The PVA solution at 5 wt% and MPE at concentrations of 0%, 1%, 5%, 10%, and 15% based on SPI were added to the SPI solution at room temperature. After stirring for 2 h, the large particles were removed by centrifugation at 6,000 rpm for 10 min, and the supernatant was collected in the glass bottle. Ultrasound technique (40 W for 10 min) was used to improve the solubility of mixture and to remove air bubbles. Twenty gram of the prepared solution were poured in petri dishes (9 cm diameter) and dried at a temperature of 45°C for 24 h at a RH of 45% to obtain the films. Before use, the film was

Table 1: Experimental formulations of the SPI films with and without MPE

Films	5% SPI (g) ^a	5% PVA (g) ^b	Glycerol (g) ^c	MPE (g) ^d
SPI control	25	1.25	0.375	0
SPI-1% MPE	25	1.25	0.375	0.0125
SPI-5% MPE	25	1.25	0.375	0.0625
SPI-10% MPE	25	1.25	0.375	0.125
SPI-15% MPE	25	1.25	0.375	0.1875

^a5% (w/w) solution; ^b5% (w/w) solution, 5 wt% based on SPI dry weight; ^c30 wt% based on SPI dry weight; ^d0%, 1%, 5%, 10%, and 15% based on SPI.

removed from the petri dish and stored at room temperature and 57% of RH for 72 h. The final formulations of the SPI and MPE-SPI films are shown in Table 1.

2.3 Determination of antioxidant activity

The total phenolic (TP) content and DPPH radical scavenging ability were determined to evaluate its antioxidant activity. 0.4 g MPE and 0.4 g SPI film samples containing different levels of MPE were solubilized in ethanol solution (10 mL, 10%) and placed in a thermostatic oscillator at a temperature of 30°C for 16 h, followed by centrifugation at 6,000 rpm for 10 min. The final volume was diluted to 100 mL. The supernatants were employed by Folin–Ciocalteu method (22). Briefly, the test solution (1 mL) was mixed with Folin phenol reagent (1 mL) and reacted at room temperature for 4 min. After the incorporation of 2 mL of 10% sodium carbonate solution, the mixture was reacted for 2 h at room temperature. The absorbance was measured at 760 nm. Distilled water was blank and TP content was expressed as gallic acid equivalents (GAE) using a standard curve of the gallic acid and was calculated using Eq. 1:

$$\text{TP (mg GAE/g)} = \frac{a \times V}{W} \quad (1)$$

where a (mg/mL) is the concentration of the polyphenols in the test solution, V (mL) is the volume of the test solution, and W (mg) is the mass of the extract or the dried film.

The free radical scavenging activities of the extract and the films were estimated using the DPPH method (23). Different concentrations of test solution (1 mL) were mixed with 4 mL of DPPH ethanol solution (25 mg/L). The absorbance was measured at 517 nm after reaction for 30 min at

room temperature. Meanwhile, the absorbance of 1 mL ethanol solution mixed with DPPH ethanol solution was determined for comparison. The formula used for calculation is as follows (Eq. 2):

$$\text{Scavenging rate (\%)} = \frac{A_1 - A_2}{A_1} \times 100\% \quad (2)$$

where A_1 is the absorbance of the blank DPPH solution and A_2 is the absorbance of the sample extract or the test film.

2.4 Determination of antimicrobial activity

The antimicrobial activity of MPE on *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) was tested by using the agar diffusion method (Oxford cup method) (24,25). All the strains were incubated in 50 mL of Luria–Bertani broth at a temperature of 37°C until microbial tests. After 20 h of incubation, 100 μ L of the diluted culture broth prepared through ten-fold serial dilutions were coated onto MH agar media and then left to dry for 2 min. Sterile stainless steel drill was used to make wells (diameter of 6 mm) in the medium. Each well was filled with 100 μ L diluted extract at concentrations of 0, 0.75, 1.5, 3, 6, and 12 mg/mL. The plates were left in a refrigerator for overnight to allow diffusion of active components in the media. 0 mg/mL MPE was used as negative control under the same conditions. The plates were incubated at a temperature of 37°C for 12–48 h until there was significant microbial growth on the control plate. The inhibition zone around the well was measured in mm (including well diameter). The antimicrobial activity was indicated by the diameter of the inhibition zones produced by the extract on the test microorganism. All the experiments were repeated 3 times.

The macrodilution method was used to evaluate the biocide property of the SPI films (26). All film samples were stored in a biosafety cabinet and sterilized by UV irradiation overnight. Same weight of each film specimen was added to the test tubes containing *E. coli* and *S. aureus* culture. The bacterial suspensions were incubated at a temperature of 37°C for 1 h. Then, 100 μ L of the sample were taken and spread on a MH agar plate, which was incubated at a temperature of 37°C for 24 h. The number of colonies was counted with a colony counter. The inhibition of bacterial growth was calculated using Eq. 3:

$$\text{Reduction (\%)} = \frac{a - b}{a} \times 100\% \quad (3)$$

where a and b are the number of bacterial colonies of the control and test films, respectively.

2.5 Mechanical properties

The thickness of each sample was measured randomly at different positions with an Electronic digital micrometer (EVERTE, awt-chy01, Zhejiang) having an accuracy of 1 μm and the average value was reported (27). Three repetitions were used for each sample.

Mechanical properties of the films were determined according to ASTM D882 (28) using an Instron universal testing machine (Instron 8801, Britain). The film samples were placed in a desiccator at room temperature and 57% of RH for at least 2 days prior to measurement. All performance measurements were done immediately after the film samples were removed from the laboratory to minimize humidity differences. Each sample was repeated five times. To determine the tensile strength (TS, MPa) and elongation at break (EB, %), the films were cut into strips of 60 mm $\times$ 10 mm and installed between the tensile clamps. The initial separation of the tool head was 50 mm, and the crosshead speed was 0.8 mm/s. Three repetitions were used for each sample.

2.6 Water solubility (WS)

The WS of the samples was measured according to a method described by Rambabu et al. (29). In short, the film samples were dried in an oven at a temperature of 103°C for 24 h, cut into a size of 40 mm $\times$ 10 mm, and weighed to determine the initial solid content (W_i). The pre-weighed film samples were immersed in 50 mL of distilled water for 24 h at a temperature of 30°C under constant stirring. The remaining films were then filtered and dried in a dry oven at a temperature of 103°C for 24 h. The WS of each film was calculated using Eq. 4:

$$\text{WS (\%)} = \frac{W_i - W_f}{W_i} \times 100\% \quad (4)$$

where W_i is the initial weight of the dried film (g) and W_f is the final weight of the dried film (g).

Five repetitions were used for each film, and the arithmetic mean was reported as solubility percentage.

2.7 Water vapor permeability (WVP)

The WVP of the films was determined by the modified method of Qin et al. (30). Anhydrous calcium chloride (CaCl_2) and weighing bottle were dried at a temperature of 105°C for 24 h. Then 10 g of CaCl_2 were poured into a

weighing bottle and sealed with a film. The weighing bottles were then placed at room temperature with a RH of 90%. The bottles were weighed every 1 h for 12 h. WVP is calculated using Eq. 5 as follows:

$$\text{WVP} = \frac{\Delta W \times d}{t \times A \times \Delta P} \quad (5)$$

where WVP (g/cm s MPa) is the water vapor permeability, ΔW (g) is the mass of water passing through the films, d (m) is the film thickness, t (s) is the time interval, A (m^2) is the effective film area, and ΔP (MPa) is the partial pressure of water vapor over the film samples. All experiments were performed in triplicate.

2.8 Water contact angle (WCA)

The contact angle of water on the surface of the films were measured to estimate the hydrophobicity. The films were cut into rectangular pieces (3 cm $\times$ 10 cm) and placed on a horizontal movable platform (black Teflon coated steel, 7 cm $\times$ 11 cm) equipped with a WCA analyzer (Fangrui, JCY-1, Shanghai). Then, a micro-syringe was used to drop 2 μL of water on the surface of the films. The WCA on both sides of the water drop was measured and the average value was taken. The liquid used was distilled water and the experiments were performed at room temperature and $57 \pm 2\%$ of RH.

2.9 Color measurement

A colorimeter was used to determine the color of the film and the white board was used as a reference. Each treatment was measured at least 3 times, and the values of L^* , a^* , b^* , and ΔE were recorded. Three replicate film samples were tested.

2.10 Films opacity

The opacity of SPI films was measured with the absorbance of 600 nm per mm thickness by a Shimadzu UV-2450 UV-Visible (UV-Vis) spectrophotometer. Each sample was cut into rectangular pieces and placed in the UV spectrophotometer test cell, and an empty test cell as a reference for measurement. The following formula was used to calculate the optical properties of the films (Eq. 6):

$$\text{Opacity (\%)} = \frac{\text{Abs } 600}{L} \quad (6)$$

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

2.11 Films transmittance

The transmittance spectra of the SPI films were obtained using -Vis spectrophotometer. The spectra were recorded at room temperature in steps of 1 nm, in the range of 200–800 nm. For this, a rectangular piece of film (4.5 cm × 1 cm) cut from each film sample was previously conditioned in a dry desiccator at room temperature for 48 h, and three measurements were performed.

2.12 Characterizations

The thermal properties of the films were measured by TGA (NETZSCH, STA 449 F3, Germany). Approximately 5 mg samples were added to a standard aluminum pan and heated from 30°C to 600°C at a rate of 10°C/min. Nitrogen was used as the purge gas at a flow rate of 20 mL/min.

The ATR-FTIR spectra of SPI films were analyzed by ATR-FTIR spectrometer (Shimadzu, IRTracer-100, Japan). The samples were placed on the X-ray exposure table and the scanning frequency was 4,000–650/cm with a spectral resolution of 4/cm. The measurements were carried out at room temperature. All the data treatments were performed with Peakfit software version 4.12 (SYSTAT Software, Richmond, CA, USA).

The surface and cross section of SPI films at 10,000× and 1,500× were observed by SEM (Phenom Pro, 800-07334, Netherlands) at 15 kV voltage, respectively. Before scanning, the film sample was cut into 5 mm × 1 mm pieces and installed on the bronze stub with double-sided carbon tape. Before visualization, the films were gold-plated with a sputter coater.

XRD (Rigaku, D/max, 2,500 V, Japan) was used to study the crystal structure of the films at 40 kV and 40 mA in $2\theta = 5\text{--}40^\circ$. The scanning speed was 2°/min.

2.13 Statistical analysis

The SPSS statistical software was used for analysis of ANOVA and Duncan triplicate range test for significance analysis at $P < 0.05$ level (SPSS Inc. Chicago, IL, USA).

The results are expressed as mean values $\pm$ standard deviation (SD). If $P < 0.05$, the significance level was defined.

3 Results

3.1 TP content and antioxidant activity

The Folin–Ciocalteu method is used to evaluate the antioxidant activity of MPE both individually and when incorporated into the SPI matrix. Since the order of addition of extracts directly reflects the availability of phenolic hydroxyl groups, it can be used as an effective method to evaluate the effect of the order of addition of extracts on the antioxidant capacity of final materials.

The results obtained by the Folin–Ciocalteu method showed that the MPE presented the TP content of 81.34 mg GAE/g, which were lower than that in other similar studies (31). Emam-Djomeh et al. (32) reported that the total amount of phenolic compounds in pomegranate fruit peel extract was 186 mg GAE/g.

These differences may be due to that TP content in different extracts is not only related to the variety, species, and seasonality of the fruit, but also related to the extraction method, the type of solvent used, the temperature, or stirring (33).

The incorporation of MPE had a significant effect on the TP contents of the formulated SPI film as shown in Figure 1 ($P < 0.05$). The TP content increased from

Figure 1: TP content and DPPH radical scavenging activity of the SPI films with and without MPE. All data are expressed as mean values $\pm$ standard deviation (SD). Different letters indicated significant differences ($P < 0.05$).

1.5 mg GAE/g for the peel free film to 3.0 mg GAE/g for the film sample that contained 15% of MPE. The low level of TP observed in the control film may be due to the presence of amino acid residues such as tyrosine and histidine in SPI, which can react with Folin-Ciocalteu reagent. The TP detected in the film was due to the compounds present in MPE. The phenolic compounds are active hydrogen donors and are good antioxidants, which may be responsible for the oxidative activity of mangosteen pericarp containing phenol (34), xanthone (35), tannin, anthocyanin (36), and carotenoids (37).

Antioxidant ability of hydrogen atom to absorb DPPH free radical was determined by the DPPH radical scavenging activity (38). When the DPPH solution is quenched by antioxidants, its color changes from dark purple to light yellow, and its absorbance decreases at 514 nm (3). DPPH assay is a rapid and simple detection method, which is an effective method to evaluate the antioxidant free radical scavenging activity. Figure 2 indicates that MPE has antioxidant and health-promoting potential due to its content of phenolic compounds. Compared with vitamin C, MPE had similar DPPH radicals scavenging ability, that is, 0.2 mg/mL. Therefore, MPE could be used effectively as an antioxidant.

DPPH scavenging assay was used to indicate antioxidant activity of the film. The antioxidant activity of the film was determined by DPPH scavenging assay. As shown in Figure 2, all the films tested showed DPPH radical scavenging activity in a dose-dependent manner. Due to the lack of a hydrogen atom donor, SPI films showed the lowest antioxidant capacity. With the increase in MPE content, the DPPH radical scavenging ability of SPI

Figure 2: DPPH radical scavenging activity of MPE and vitamin C.

Table 2: Antimicrobial effect of MPE on the tested organisms

Mangosteen extracts dilutions (mg/mL)	Diameter of inhibition zone (mm)	
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923
12	16.3	17.6
6	14.9	16.5
3	13.6	15.2
1.5	12.6	13.9
0.75	12.2	13.6
0	0	0

films was significantly enhanced ($P < 0.05$). The enhanced antioxidant capacity of SPI film was mainly due to the presence of polyphenols in MPE, which could capture free radicals by providing phenol hydrogen. The DPPH radical scavenging activity was slightly lower than that of similar studies reported previously. The DPPH activity of khorasan wheat starch film containing 1% of moringa leaf extract has been reported to be 37.9% (33). The DPPH activity of the SPI film with 1% of MPE was 32% (Figure 2). These differences may be attributed to different key ingredients and film matrix.

3.2 Determination of antimicrobial activity of SPI films

The antimicrobial activity of MPE against *S. aureus* and *E. coli* was determined using agar well diffusion method (Table 2 and Figure 3). Table 2 presents diameters of inhibition zones exerted by the different concentrations of MPE toward tested microorganisms. The results showed that the MPE had antibacterial activity against both the strains. 0 mg/mL MPE had no activity on the tested

Figure 3: Photos of the inhibitory zones of the extracts. (1) 12 mg/mL, (2) 6 mg/mL, (3) 3 mg/mL, (4) 1.5 mg/mL, (5) 0.75 mg/mL, and (6) 0 mg/mL concentration extracts against (a) *E. coli* and (b) *S. aureus*.

microorganisms, which indicated that the obvious inhibitory effect was only related to the extract. The difference in the antibacterial activity of MPE could be partly explained by the change in the phenol content in the extracts. The toxic mechanism of phenols in microorganisms was related to the reaction of protein sulfhydryl groups and the inability of the microorganisms to utilize substrates (11). MPE interfered with the bacterial protein secretions (15). Liang and Wang (39) showed that there was no inhibition zone against *E. coli* on the cortex phellodendron extract but showed clear inhibition zones against *S. aureus*. The differences in the activities of the peel extracts between the studies could be explained in part as due to the changes in the phenol content of the extracts, the strain sensitivity, and the antibacterial procedures used in the tests.

Table 3 shows the antibacterial activities of the different SPI films against *E. coli* and *S. aureus*. The SPI film containing 15% of MPE showed the highest antimicrobial potency towards both *E. coli* (66.87%) and *S. aureus* (80.43%). The antibacterial effect increased with the increasing amount of MPE. Phenols react with oxidative compounds such as sulfhydryl groups or form non-specific complexes with proteins, thereby causing enzyme inhibition. In short, all these compounds prevent oxidative phosphorylation by inhibiting the enzymatic mechanism of microorganisms, thereby contributing to the rupture of microbial cell walls (26). As the antibacterial activity test was performed on both gram-negative bacteria and gram-positive bacteria, the results showed that the SPI film combined with MPE has activity against a broad spectrum of microorganisms. Natural analogies in plant extracts with different bioactivity and potency are highly likely to be retained for bioactivity.

Table 3: Antibacterial properties of SPI films with and without MPE

Films	Bacteria	Viable colony numbers (CFU/mL)	Antibacterial potency (%)
SPI control	<i>E. coli</i>	492.0	0
	<i>S. aureus</i>	92.0	0
SPI-	<i>E. coli</i>	474.0	3.7
1%MPE	<i>S. aureus</i>	74.0	19.6
SPI-	<i>E. coli</i>	327.0	33.5
5% MPE	<i>S. aureus</i>	41.0	55.4
SPI-	<i>E. coli</i>	280.0	43.1
10% MPE	<i>S. aureus</i>	24.0	73.9
SPI-	<i>E. coli</i>	163.0	66.9
15% MPE	<i>S. aureus</i>	18.0	80.4

3.3 Mechanical properties

Table 4 shows that the thickness of the SPI films containing 0%, 1%, 5%, 10%, and 15% MPE were 0.103, 0.108, 0.110, 0.123, and 0.129 mm, respectively. Obviously, as the concentration of MPE increased, the thickness of the film increased.

The mechanical properties of biopolymer-based films, particularly TS and EB, have a great influence on packaging materials (40). All the samples were preconditioned at medium constant RH of $45 \pm 5\%$ because the plasticizing effect of water produce positive and negative changes in film elasticity and mechanical stress, respectively. In general, both TS and EB of the SPI films increased with the concentration of MPE, and the film containing the highest MPE concentration (15 g/100 g SPI) reached the comparable levels in the TS and EB when compared with those from the control film (Table 4). The result was due to the phenol–protein interaction, which is mainly stabilized by the hydrogen bonds and hydrophobic interactions. However, a small amount of MPE may cause lower formation of hydrogen bonding in the SPI films, hinder the interaction between molecules, and thereby reduce TS and EB. The strength of SPI films were improved than that in the study by Wang et al. (41) because of cross-linking between SPI and PVA.

3.4 WS

The WS of a film in water may also provide insight into the behavior of the films in an aqueous environment, which is a measure of its water resistance (42). Since the weakness of SPI-based films is their higher susceptibility to moisture, many studies are targeted to improve hydrophilicity, which is highly desirable for food

Table 4: Thickness and mechanical properties of SPI films as affected by MPE incorporation

Films	Thickness (mm)	TS (MPa)	EB (%)
SPI control	0.10 ± 0.03^b	4.1 ± 0.3^a	186.1 ± 10.5^a
SPI-1% MPE	0.11 ± 0.08^b	3.1 ± 0.1^b	85.8 ± 15.1^b
SPI-5% MPE	0.11 ± 0.04^b	3.7 ± 0.1^a	143.6 ± 39.8^a
SPI-10% MPE	0.12 ± 0.02^a	3.8 ± 0.1^a	159.2 ± 14.8^a
SPI-15% MPE	0.13 ± 0.06^a	4.1 ± 0.3^a	187.5 ± 23.7^a

All data are shown as mean values $\pm$ standard deviation (SD). Different superscript letters in the column indicate significant differences ($P < 0.05$).

Table 5: WVP, WS, and WCA of SPI films as affected by MPE incorporation

Films	WVP $\times 10^{-14}$ (g/cm s MPa)	WS (%)	WCA ($^{\circ}$)
SPI control	6.92 ± 0.02^c	22.38 ± 0.49^{ab}	58.8 ± 0.7^a
SPI-1% MPE	7.14 ± 0.06^c	23.58 ± 0.12^a	55.8 ± 1.8^{ab}
SPI-5% MPE	7.7 ± 0.02^b	21.74 ± 0.69^b	54.4 ± 2.3^b
SPI-10% MPE	8.34 ± 0.01^a	22.16 ± 0.62^{ab}	52.7 ± 2.6^{bc}
SPI-15% MPE	8.6 ± 0.01^a	22.34 ± 0.58^{ab}	50.1 ± 1.8^c

All data are shown as mean values $\pm$ standard deviation (SD). Different superscript letters in the column indicate significant differences ($P < 0.05$).

packaging application (10). The solubility of the film remains the same, with only a small difference ($P < 0.05$) (Table 5). Similar results were obtained by other authors (43). Based on our findings, SPI films were much more water-resistant, the probable reason behind considerable differences in WS of the films could be the difference in the methods applied. The resistance of the film to water, which is determined by the solubility of the film in water, is critical to the potential application of the film. Originally, the WS of polymers is determined by their molecular structures. Therefore, thermal stability information can be obtained by dissolution test.

3.5 Water vapor permeability

The WVP values for the samples are listed in Table 5. WVP is a parameter that is affected by multiple factors, such as roughness, hydrophilic tightness, and the distribution and orientation of particles in the film, so it is difficult to account for all the changes observed in this parameter for the samples under study (44). Compared to others (30), the WVP of SPI film was reduced because the new hydrogen bond between SPI and PVA would reduce the free hydrogen group in SPI films. Compared to the control films, incorporation of MPE increased the WVP of the SPI films significantly ($P < 0.05$). It was possible that the introduction of MPE led to the recombination of

the polymer layers, which in turn increased the free volume and inter-chain distance. Therefore, more chain mobility and free movement space could promote the transfer of water vapor.

3.6 WCA

The surface hydrophobicity of the SPI film was evaluated by measuring. Generally, the higher the WCA, the more the hydrophobicity of the film surface (45). The addition of MPE had a profound effect on the hydrophobicity of the resulting films ($P < 0.05$), which may be due to the hydrophilicity of MPE (Table 5). Compared with the composite films, the control film has a higher WCA ($58.78 \pm 0.38^{\circ}$). When an increasing concentration of MPE was added to the SPI films, a decrease in WCA was observed ($P < 0.05$).

3.7 Color measurement

It is usually found that the addition of various plant extracts will change the original color of the biopolymer-based film to some extent, depending on the source and concentration of the plant extracts (46). L (0 = black and 100 = white), a (-60 = greenness to $+60$ = redness), b (-60 = blueness to $+60$ = yellowness), and total color difference (ΔE) of these films are presented in Table 6. The addition

Table 6: Color and opacity of SPI films as affected by MPE incorporation

Films	L	a	b	ΔE	Opacity
SPI control	90.94 ± 0.22^a	2.52 ± 0.47^d	7.69 ± 0.23^e	3.46 ± 0.07^a	1.75 ± 0.02^d
SPI-1%MPE	85.31 ± 0.49^b	4.88 ± 0.36^c	15.09 ± 0.51^d	6.85 ± 0.54^b	1.78 ± 0.08^d
SPI-5% MPE	84.01 ± 0.90^c	5.94 ± 0.33^b	17.7 ± 2.16^c	9.79 ± 1.88^c	2.21 ± 0.02^c
SPI-10% MPE	80.85 ± 1.22^d	8.31 ± 1.58^a	22.25 ± 1.93	16.78 ± 2.93^d	2.52 ± 0.08^b
SPI-15% MPE	77.20 ± 1.85^e	10.87 ± 1.30^a	23.66 ± 2.23^b	19.09 ± 2.87^e	2.93 ± 0.01^a

All data are shown as mean values $\pm$ standard deviation (SD). Different superscript letters in the column indicate significant differences ($P < 0.05$).

Figure 4: Appearance of SPI films: (a) SPI control, (b) SPI-1% MPE, (c) SPI-5% MPE, (d) SPI-10% MPE, and (e) SPI-15% MPE.

of MPE to SPI films significantly reduced their whiteness and increased their reds and yellows ($P < 0.05$). The changes in ΔE of SPI films were increased by the addition of MPE which could be confirmed visually in Figure 4. In general, the phenolic pigments in plant extracts may be contributors to the various colors observed in these biopolymer films (47).

3.8 Film opacity

The opacity at different concentrations of MPE in SPI films are shown in Table 6. The lowest opacity was

observed in the control. The films with higher concentration of MPE showed substantial ($P < 0.05$) higher opacity. This indicated that the opacity of the SPI film was caused by the polyphenol compound added in the SPI film which caused light scattering and refraction, resulting in darkening of the film (29). Although food packaging materials are mostly transparent and colorless, colored films may also be an advantage in protecting against exposure to UV-Vis light, which can affect food quality (48).

3.9 Film transmittance

Figure 5 shows the UV-Vis light transmission patterns of all samples in the range of 200–800 nm. The spectrum of all films showed transmittance values of 0–42% in the UV range (200–400 nm). Therefore, the SPI film effectively prevents the transmission of UV light at these wavelengths, independent of the addition of MPE. The visible light transmittance (400–800 nm) of all films was between 0% and 70%. By adding different contents of MPE, a significant change in the light transmittance of the obtained film could be observed in the visible light range. The transmittance value was greatly reduced in SPI films containing MPE. This also showed that MPE-SPI films had higher UV-Vis light barrier performance than SPI control film. In addition, the light barrier performance of the SPI film increased with the increase in the MPE content. Therefore, it could be concluded that the UV-Vis light barrier properties of the SPI film might

Figure 5: UV-Vis spectra of SPI films with and without MPE.

Table 7: Thermal degradation temperature (T_d , °C) and weight loss (Δw , %) of films incorporated without and with MPE

Films	Δ_1		Δ_2		Δ_3	
	$T_{d1,onset}$	Δw_1	$T_{d2,onset}$	Δw_2	$T_{d3,onset}$	Δw_3
SPI control	56	0.44	222	1.10	319	2.70
SPI-1% MPE	78	0.37	228	1.21	320	3.16
SPI-5% MPE	758	0.30	243	1.04	313	2.75
SPI-10% MPE	65	0.39	231	1.16	316	2.77
SPI-15% MPE	75	0.35	230	1.13	316	2.55

be due to the UV-absorbing ability of the polyphenolic compounds in MPE as researchers reported earlier (49).

3.10 TGA

TGA was used to measure the thermal properties of the SPI films, and the results are shown in Table 7 and Figure 6. As shown in the TGA thermograms, all the films showed three main stages of weight loss. The first thermal degradation (30–150°C) was mainly attributed to the evaporation of water remaining in SPI films. The second (150–280°C) and third (280–600°C) stages of degradation could be due to the degradation of residual glycerol and the thermal decomposition of SPI matrix and MPE, respectively. It is worth noting that the addition of MPE did not significantly change the TGA curve and derivative thermogravimetric (DTG) curve of the film (50). As shown in Table 6, the weight loss and onset thermal degradation temperature of the film containing MPE was almost the

Figure 7: ATR-FTIR spectra of SPI films with and without MPE.

same as the control film. This result shows that the incorporation of MPE cannot improve the thermal stability of the SPI film.

3.11 ATR-FTIR

The ATR-FTIR spectra of SPI films showed hydrogen interaction between components (Figure 7). For SPI control film, the absorption bands are associated with C=O stretching of $1,634\text{ cm}^{-1}$ (amide I) and N–H bending of $1,539\text{ cm}^{-1}$ (amide II). The wide absorption band (amide – A) observed at $3,275\text{ cm}^{-1}$ is attributable to the stretching

Figure 6: TGA (a) and DTG (b) patterns of SPI films with and without MPE.

Table 8: Percentage of secondary structures of SPI films with and without MPE

Films	α -helix (%)	β -sheet (%)	β -turn (%)	Random coil (%)
SPI control	24.2	45.8	18.3	11.6
SPI-1% MPE	27.3	40.8	15.5	16.4
SPI-5% MPE	30.5	37.4	18.0	14.1
SPI-10% MPE	25.1	43.3	18.3	13.4
SPI-15% MPE	26.6	42.4	16.9	14.1

vibrations of the free and bonded O–H and N–H groups (9,51,52).

The amide – A shifted slightly to higher wavenumbers ($3,275\text{--}3,281\text{ cm}^{-1}$) in SPI films incorporated with 1% and 5% of MPE. This result indicated that the unfolding of soy protein may lead to new hydrophobic interactions and weak hydrogen bonds. Generally, the intensity of amide – A in SPI film added with MPE was weaker than the film control. This observation may be attributed to the hydrogen bonding between SPI and MPE. Therefore, hydrogen bond interactions between polyphenols and proteins may be enhanced at higher MPE concentrations. The amide I band is composed of several bands corresponding to different secondary structures of the protein, e.g., α -helix ($1,646\text{--}1,664/\text{cm}$), β -sheet ($1,615\text{--}1,637$ and $1,682\text{--}1,700/\text{cm}$), β -turn ($1,664\text{--}1,681/\text{cm}$), and random coil ($1,637\text{--}1,645/\text{cm}$) (53). Table 8 shows the percentage of different secondary

structures of protein in the SPI films containing MPE. For the control film, the main structures resulted from α -helix, β -sheet, β -turn, and random coil corresponded to the percentage areas of around 24.21%, 45.81%, 18.34%, and 11.64%, respectively. The addition of MPE as an antioxidant to the SPI film did not significantly affect the β -turn and caused the β -sheet to transform into random coils and α -helix, in which the areas of random coils and α -helix increased. In general, the content of β -sheet contributed to the strength of SPI films, while the flexibility of SPI films was related to the content of α -helix and β -turn (54). In the SPI film, the addition of MPE resulted in the conversion of β -sheets to α -helix, and the percentage area of α -helix increased. Meanwhile, it was believed that the heating of the MPE-SPI solution may destroy the internal hydrogen bonds of the peptide groups, resulting in random coils. Random coils could provide complexing sites where the carbonyl group of the polypeptide

Figure 8: SEM images of surface and cross section of the SPI films with and without MPE. (a) SPI control, (b) SPI-1% MPE, (c) SPI-5% MPE, (d) SPI-10% MPE, and (e) SPI-15% MPE.

Figure 9: XRD diffraction patterns of SPI films with and without MPE.

could interact with the polyphenols in MPE. Overall, it could be included that the interaction between SPI and MPE attributed to hydrogen bonds and hydrophobic interactions lowered the mechanical properties of SPI films.

3.12 SEM

The characterization of biopolymer microstructure is an important factor to understand its behavior. Figure 8 shows SEM micrographs of cross section of all the films prepared in this study. All films have similar morphological images, and the effect of MPE addition was not detected by analysis. However, some layers were detected for all the samples in cross section which is an indication of the phase separation between the component. Sun *et al.* (55) reported similar findings. The micrographs of the fractured cross sections showed slight differences between the samples, evidencing small changes in the mechanical properties and WVP.

3.13 XRD

In order to explore the crystal structure of the control SPI film and reveal the effect of incorporation of MPE, XRD analysis was performed. It is evident from the spectrum in Figure 9 that SPI peaks about 9° and 19° at 2θ reflect the α -helix and β -sheet structures of the secondary conformation of SPI, respectively. Similar results have been reported by Liang and Wang (39). After MPE was added to

the SPI films, peak around 19° became sharper as the concentration of MPE increased to 15%. Moreover, when the ratio of MPE was 5%, one peak around 22° appeared. When MPE was added to SPI film, the peak intensity was much lower than that of the control film. This indicated that the crystalline structure of SPI films had collapsed after the addition of MPE. Compared with the control film, the XRD peak of SPI film was gentler when MPE was added. It showed that the crystallinity of SPI film was decreased by MPE. The results showed that MPE inserted the macromolecular structure of the SPI film and destroyed the crystallization of the SPI film. These findings are in compliance with previous reports where “*Pequi*” peels addition reduced the crystallinity of chitosan film (56).

4 Conclusion

MPE contains a lot of total polyphenols which causes the significant antioxidant and antibacterial properties. When they were added into the SPI film, the bio-activities of the film increased as extract level increased in film. Compared with the control film, the UV-Vis barrier and hydrophobicity of the film were improved with the addition of MPE. And the TS and EB properties of the SPI films containing MPE were changed. SEM and TGA analyses indicated that the addition of MPE did not change the microstructure and thermal stability of the films. XRD results showed that the crystalline structure of SPI films was reduced by MPE. ATR-FTIR analysis showed that the reaction between polyphenol and protein changed the secondary structure through hydrogen bonds and hydrophobic interactions. These results indicate that the MPE would be a promising ingredient to use the SPI films as an active packaging material.

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