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Research paper

Effect of montmorillonite in fish water soluble protein composite film as a prototype of biodegradable packaging materials

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ABSTRACT

Fish water soluble protein (FWSP) film from the surimi washing process was prepared by solvent casting. The results demonstrated that FWSP-oleic acid had the highest tensile strength (TS) and elongation at break (%E) at 12.11 ± 2.39 MPa and 7.14 ± 3.17% respectively. Water vapor permeability (WVP) of FWSP-oleic acid was higher than FWSP-palmitic acid at $5.27 \pm 0.50 \times 10^{-11}$ g m/m^2 s Pa. On the contrary, FWSP-oleic acid had the lowest oxygen permeability (OP) at 2.34 ± 0.14 cm^3 mm/m^2 d k Pa. FWSP-oleic acid with Mt content 5 g/100 g FWSP presented the lowest WVP. High amount of Mt loading resulted in an increase in the WVP and OP. The melting (T_m) temperature of FWSP-oleic acid was ~107 °C but FWSP-stearic acid and FWSP-palmitic acid had two T_m levels were ~73 °C ~ 122 °C and ~88 °C ~ 127 °C, respectively. FWSP-oleic acid with Mt content 5 g/100 g FWSP had the highest T_m level at ~113 °C. The thermal stability of the films increased from ~349 to ~357 °C with the addition of Mt content 20 g/100 g FWSP. The changes observed in the intensity of the bands corresponded to the amine group. It showed that FWSP interacts with Mt.

1. Introduction

Biopolymer protein-based films can be a potentially important material in biodegradable plastic packaging. They are an eco-friendly alternative material for use instead of non-degradable synthetic materials. Moreover, they help respond to the concern about the restricted availability of natural resources. Proteins have been widely utilized due to their film-forming ability and nutritional values (Weng et al., 2007). Many waste products from manufacturing can be turned into value-added inputs to the agricultural industry. Waste protein can be divided into two categories: plant protein and animal protein. One of the utilizable alternatives is biodegradable protein-based film. Many research studies have referred to plant protein-based film fabrication due to the availability of the raw material and the specific characteristics of the film such as uncomplicated fabrication, good appearance and compatible technology. For example, whey protein isolate (WPI) is a byproduct of the manufacture of cheese or casein which is derived from whey. WPI films are flexible, transparent, colorless and odorless films. The reason for the low oxygen permeability is the film's polar nature and the more linear structure of WPI leading to a higher cohesive energy density and a lower free volume (Oses et al., 2009; Kokoszka et al., 2010). Soy protein isolate (SPI) is the waste portion of soy oil manufacturing. SPI film is characterized by smoothness, flexibility, transparency, low solubility and good heat sealability (Denavi et al., 2009; Cho et al., 2010). However, there has been less research on animal protein-based films than for plant protein-based film. The complicated procedure is a barrier to development involving controlling the temperature of the raw material and the several steps in pure chitosan extraction. Nonetheless, animal protein-based films have more advantages than plant protein-based films; for example, chitosan prepared from shrimp processing waste (shells) is not only flexible, transparent, non-toxic and biodegradable but also has antimicrobial properties (Duan and Zhang, 2013). A large amount of FWSP is dissolved in fish mince washing water from the surimi process. This note principally refers to the mince produced when fish flesh is separated from skin and bone in a bone separator. The film is more water resistant than other protein-based films made from soy bean (Ghorpade et al., 1995), rice bran (Gnanasambandam et al., 1997), casein (Frinault et al., 1997) and myofibrillar protein (Cuq et al., 1995) due to the inclusion of the apolar amino acid element (Iwata et al., 2000; Shiku et al., 2004; Bourtoom et al., 2006; Chinabhark et al., 2007; Weng et al., 2007).

In the last decade, surimi products have added value to un-utilized fish or low-priced fish and their production has increased annually. Report of the End-of-project meeting of the working party on information collection of economically important species as Surimi raw materials in the Southeast Asian region by the Southeast Asian Fisheries Development Center (SEAFDEC) Training Department at Samut Prakarn, Thailand (SEAFDEC, 2009) demonstrated that the capacity of surimi processing plants in Thailand was approximately 170,200 million t per

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year. In particular, in 2008, Thailand exported approximately 65,000 million t of surimi products to Japan. In Japan in 2008, the estimated surimi consumption was 350,000 million t and the world market is expanding into the European Union and Southeast Asia with the major export countries being Thailand, India, Vietnam and Malaysia. Surimi processing involves a first washing and then another two washings, followed by the separation of the skin fat and soluble protein using cold water circulation in a larger tank. The cycling of washing is an important process to improve the gelling properties and produce colorless and odorless surimi. Consequently, the waste water from the surimi process contains a considerable amount of fish water-soluble protein (FWSP). Waste water treatment and the large amount of FWSP sludge could be concentrated using a nanofiltration membrane (Afonso and Borquez, 2002, Yeong et al., 2002; Wibowo et al., 2005; Velazquez et al., 2007). These abundant proteins are separated from the washing water from the surimi process and then mixed into fertilizers and animal feed at low cost. The conversion process of the surimi byproduct into a biodegradable film for food contact material application adds value to the food industry.

FWSP compositions include protein, fat, ash and other components at 80.88, 2.94, 6.26 and 9.92 g/100 g, respectively (Bourtoom et al., 2006). The protein contains three amino acid elements: apolar-amino acids i.e. alanine, isoleucine, leucine, methionine, proline, tryptophan and valine; ionized polar amino acids i.e. arginine, asparagine, aspartic acid, glutamine, glutamic acid, histidine and lysine phenylalanine; and non-ionized polar amino acids i.e. cysteine, glycine, serine, threonine and tyrosine. Most of the fish amino acid is glutamic acid lysine acid, aspartic acid and leucine (Shiku et al., 2003; Gercia and Sobral, 2005, Rocha et al., 2013) which are all in the ionized polar amino acids category except for leucine which is in the apolar-amino acid category. These profiles are an important factor in determining the hydrophilic and hydrophobic properties of protein-based film.

FWSP film has poor tensile strength and elongation at break (%E) and high water vapor permeability (WVP). These poor characteristics are barriers to any food packaging material application. To overcome these disadvantages requires the influence of plasticizers and filler to increasing the capacity of film fabrication. The inclusion of glycerol, sorbital, sucrose and polyethylene glycol (PEG) decreases the brittleness of protein-based films which is demonstrated by cracking and chipping of the film during film forming and storage (Tanaka, Iwata et al. 2001). Fatty acid as a filler was incorporated not only to increase %E but also to decrease the WVP of the FWSP film (Tanaka, Ishizaki et al. 2001). Moreover, an increase in the Mt concentration resulted in a decrease in the WVP of soy protein isolate/montmorillonite-composite films decrease (Lee and Kim, 2010) and an increase in the glass transition temperature (T_g) of soy protein isolate (Kumar et al., 2010). From the literature review, research on the effect of the Mt concentration on the mechanical, barrier properties and thermal behavior to FWSP films properties is lacking and the FWSP samples were prepared in a scale-laboratory only.

The aim of this study was to analyze FWSP-based film made from surimi manufacturing composite to determine with two factors; suitable fatty acid plasticizer and the Mt content; in addition, the film characteristics were investigated. A successful outcome would produce multifunctional film for food packaging applications which would be stronger, more readily available and more biodegradable than currently available synthetic alternatives.

2. Materials and methods

2.1. Materials

FWSP derived from Threadfin Bream was recovered from the fish wash stage from the surimi plant of Pacific Marine Food Products Co., Ltd., Samutsakhon, Thailand. The wash water was transported to Thammasat University in a polypropylene container within 2 h and controlled temperature of 0–5 °C. Mt was purchased from Southern Clay Products (Austin, Texas, USA). Oleic acid and stearic acid were purchased from Panreac Qut́mica S.A. (Barcelona, Spain) and palmitic acid was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation of FWSP films

Fish wash water was precipitated at its isoelectric point. Freeze drying was used to preserve the FWSP drying quality and the FWSP was stored in a polyethylene bag at −20 °C until film-forming preparation. FWSP film-forming solutions were prepared according to the method described by Bourtoom et al. (2006) FWSP powder were dissolved in distilled water 3 g/100 mL using a homogenizer for 1 min. After adding the plasticizer; oleic palmitic and stearic acid at 50 g/100 g FWSP, the film-forming solutions were stirred with magnetic stirrers for 30 min before the pH level was adjusted to pH 10. The FWSP film-forming solutions were heated in a water bath at 70 °C for 15 min. Finally, the FWSP film-forming solutions were filtrated through a sifter sieve (mesh no. 140). Film-forming solution (4 mL) was cast onto a rimmed silicone resin plate (70 × 70 mm) and dried at room temperature for 24 h.

2.2.2. Preparation of FWSP/Mt bio-composite films

An appropriate plasticizer was used as plasticizer for FWSP/Mt bio-composite films. Mt content was mixed with FWSP powder at level of; 1, 3, 5, 10, 15, and 20 g/100 g FWSP and homogenized with distilled water. The method of FWSP/Mt bio-composite film followed the method of preparation of FWSP films.

2.3. Characterization

2.3.1. Mechanical properties

The FWSP and FWSP/Mt bio-composite films were determined for film thickness at 5 random positions using a thickness gauge micrometer to calculate the cross sectional area (m^2). The tensile strength (TS) and elongation at break (%E) were determined using a universal testing machine (Instron 5965, USA) and operated according to the American Society for Testing and Materials (ASTM) international standard method D 882-12 (D882-12). Two rectangular strips were cut with width 20 mm and length 45 mm using a razor-sharp cutter. The initial grip separation and cross-head speed were set at 30 mm and 0.5 mm/min, respectively. The tensile strength (MPa) was calculated at the maximum load (N). The percentage of elongation at break was calculated by dividing the film elongation at the moment of rupture by the initial grip length of samples multiplied by 100.

2.3.2. Morphological properties

Each film was cut into a square shape 2 × 2 mm, (length × width). The samples were coated with a thin layer of gold (to avoid electrostatic charging) for 15 min at a voltage of 13.0 kV. The surface and cross sectional morphologies were observed using a scanning electron microscope (model XL30&EDAX, Philips).

2.3.3. Permeability properties

The water vapor permeability (WVP) was measured according to ASTM E96/E96M-10 (E96/E96M-10) with modification. The films were prepared by cutting square pieces sized 45 × 45 mm using a razor-sharp cutter. The film thickness was measured using a micrometer at 5 random positions for the WVP and OP tests. The FWSP films were sealed on a permeated aluminum cup containing silica beads (heated at 120 °C for 3 h and held in a desiccator before testing) and an O-ring to hold the film in place with paraffin. The cups were placed in a desiccator with distilled water at 30 °C. The cups were weighed at 1 h intervals over a 10 h period and the WVP (g m/m s Pa) was calculated using the following equation:

$$WVP = \frac{w \cdot x}{A \cdot t \cdot (P_2 - P_1)} \tag{1}$$

when w is the weight gain of the cup (g), X is the film thickness (mm), A is the area of the exposed film (m^2), t is the time of gain (s) and $P_2 - P_1$ is the vapor pressure differential across the film (Pa). Three replicates of each film were evaluated.

The oxygen permeability (OP) of the films was determined using an 8500 oxygen permeation analyzer (Illinois Instruments (Thailand) Co., ltd) according to the standard method ASTM D3985-05 (D3985-05). Each film was placed on an aluminum mask with an open circular area of radius 2.5 cm. The masks were placed into the test cell and exposed to 98% nitrogen on one side and to a pure oxygen flow on the other. The OP was calculated using the following equation:

$$OP = \frac{OTR \cdot X}{P_2 - P_1} \tag{2}$$

when OTR is oxygen transmission rate, X is the film thickness (m) and $P_2 - P_1$ is the oxygen pressure differential across the film (1 atm). Three replicates of each film were evaluated.

2.3.4. Thermal properties

Differential scanning calorimetry (model DSC1, Mettler Toledo) was used to determine the glass transition temperature (T_g) and melting temperature (T_m) according to Wang et al. (2013). A sealed empty aluminum pan was used as a reference. Film samples of approximately 20 ± 5 mg were weighed and sealed in the aluminum pan using sealer. The samples were cooled with liquid nitrogen as a cooling medium and scanned from −20 to 180 °C at a heating rate of 10 °C/min and a cooling rate of 10 °C/min. All DSC scans were performed three times for the same sample in an aluminum pan, namely first heating scan, cooling scan and second heating scan. The glass transition temperature was determined from the midpoint temperature of the step-wise change in the heat capacity and the melting temperature was investigated from the endothermic peak.

2.4. Statistical analyses

Analysis of variance (ANOVA) was performed using the SAS system. To determine significant differences, a comparison between the means of the control and treatment samples were performed using Duncan's test at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Mechanical and morphological properties

The stiffness brittleness and flexibility of protein based films depend on the raw material, plasticizer, heating time, heating temperature and drying conditions. The TS of the FWSP based-films were higher than for other protein-based films such as fish myofibrillar protein soy protein isolate (SPI) and whey protein isolate (WPI) films as in Table 1. In this study, the TS strength and %E of the FWSP-oleic acid films was the highest at 12.11 ± 2.39 MPa and 7.14 ± 3.67%, respectively.

The TS strength and %E of the FWSP-palmitic acid films were 7.42 ± 2.40 MPa and 2.02 ± 0.99%, respectively but Y was higher than for the FWSP-oleic acid films. The FWSP-oleic acid films had greater flexibility than the other films and it was easily peeled off from the silicone resin plate. The FWSP-stearic acid films ruptured after being manually peeled off. Film with added unsaturated fatty acid (oleic acid, C:18) had greater flexibility than with the saturated fatty acids (steric acid, C:18 and palmitic acid, C:16).

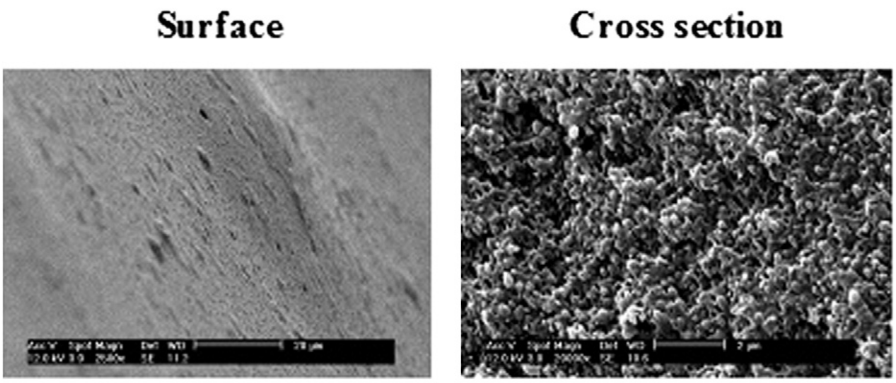
The increased chain length of the saturated fatty acids caused the flexibility of the FWSP films to decrease. These results were similar to those of Tanaka. (Tanaka, Iwata et al. 2001). SEM micrographs show the rough surface and fragile texture of the FWSP-stearic acid films (Fig. 1b) and FWSP-palmitic acid films (Fig. 1c). On the contrary, the surface morphology of the FWSP-oleic acid films (Fig. 1a) was smooth and the texture was continuous due to oleic acid being a liquid at room temperature and it is more compatible with the water than either stearic or palmitic acid during the stirring process (Limpisophon et al., 2010).

At various Mt contents, oleic acid was incorporated as a plasticizer in all films. The TS strength of the FWSP-oleic acid films with 5% Mt added was the highest at 12.62 ± 3.17 MPa. Mt particles were regularly distributed (Fig. 2a–c). The TS strength of the FWSP-oleic acid films decreased significantly ($P < 0.05$) with an added Mt concentration of >10%. The %E of the FWSP-oleic acid films without Mt was the highest at 7.14 ± 3.67%. The increased Mt concentration caused the %E to significantly ($P < 0.05$) decrease because Mt reduced the mobility of the biopolymer chain of the protein. The addition of >10% Mt (Mt overloading) resulted in brittleness due to Mt agglomeration. The SEM micrographs showed the Mt particles agglomerated as small flake on the surface of the films (Fig. 2d–f). This tendency was similar to the SPI/Mt plastics (Lee and Kim, 2010) and fish gelatin/Mt nanocomposite films (Bae et al., 2009). The addition of 5% Mt resulted in an increase in the TS strength of fish gelatin/Mt nanocomposite films increase from 30.01 to

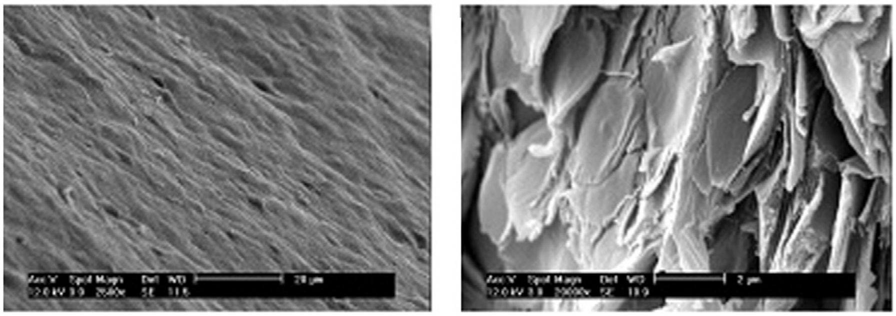
Table 1
Tensile strength and elongation at break of FWSP films at various plasticizers and Mt concentration comparing other components.

Film	TS (MPa)	E (%)	Y (MPa)	Ref.
FWSP	N/A	N/A	N/A	–
FWSP-50% oleic acid	$12.11 \pm 2.39^{A,a}$	$7.14 \pm 3.67^{A,a}$	$592.02 \pm 72.29^{A,a}$	–
FWSP-50% stearic acid	N/A	N/A	N/A	–
FWSP-50% palmitic acid	7.42 ± 2.40^{B}	2.02 ± 0.99^{B}	614.30 ± 60.96^{A}	–
FWSP-50% oleic acid −1% Mt	11.54 ± 3.13^{a}	4.34 ± 2.27^{b}	629.74 ± 27.23^{a}	–
FWSP-50% oleic acid −3% Mt	10.80 ± 3.11^{a}	5.69 ± 2.25^{b}	629.51 ± 64.19^{a}	–
FWSP-50% oleic acid −5% Mt	12.62 ± 3.17^{a}	5.38 ± 1.72^{c}	616.35 ± 93.03^{a}	–
FWSP-50% oleic acid −10% Mt	8.27 ± 3.20^{b}	3.51 ± 1.69^{c}	582.88 ± 58.46^{a}	–
FWSP-50% oleic acid −15% Mt	8.25 ± 3.35^{b}	3.39 ± 1.42^{c}	475.82 ± 41.20^{b}	–
FWSP-50% oleic acid −20% Mt	8.58 ± 3.54^{b}	2.86 ± 0.74^{c}	563.82 ± 64.03^{a}	–
FWSP-50% glycerol	3–5.5	40–70	–	1, 2, 3
FWSP-50% sorbital	1.70–3.02	8.50–14.72	–	1
FWSP-50% glycerol-10% oleic acid	5.8	205	–	4
Fish myofibrillar protein red tilapia (*Oreochromi niticus*) mince −50% glycerol	2.85	63.73	–	5
chitosan	9.75	1.36	–	6
SPI-2.5% glycerin	3.3	148.2	–	7
WPI-50% glycerol	2.4	104.8		8
WPI-50% sorbital	4.2	28.3		8

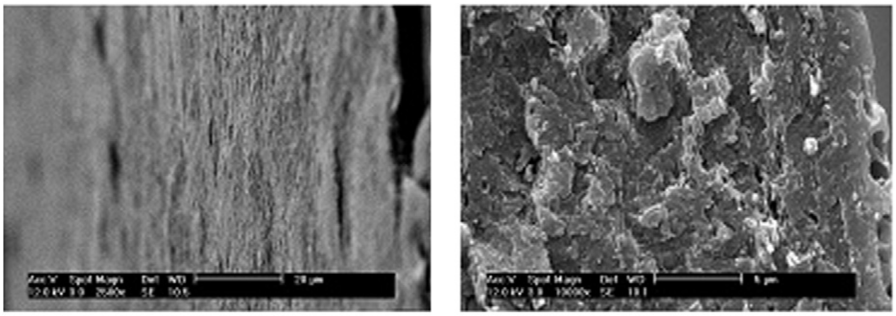
Note1 Mean values in a column followed by different letters are significantly different ($P < 0.05$).
Note2 (1) Bourtoom et al. (2006); (2) Tanaka et al. (2001); (3) Iwata et al. (2000); (4) Tanaka et al. (2001); (5) Tongnuanchan et al. (2013); (6) Oses et al., 2009.



(a) FWSP-oleic acid



(b) FWSP-steric acid



(c) FWSP-palmitic acid

Fig. 1. SEM micrographs of FWSP/Mt bio-composite films, bottom surface (left) and cross section surface (right): (a) oleic acid (b) stearic acid (c) palmitic acid.

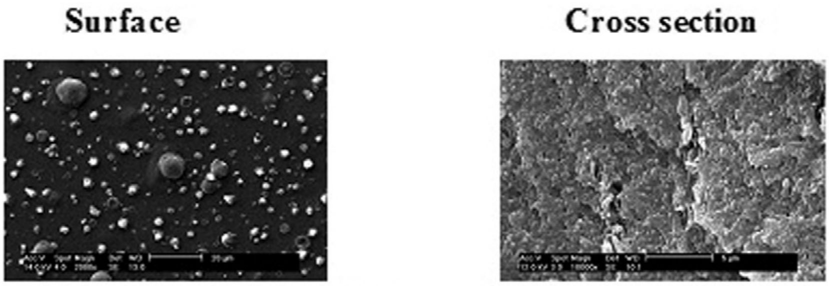
40.71 MPa but the %E dropped from 48.24 to 20.16%. The FWSP-fatty acid had a low %E (Table 1) because it had a high melting temperature. Generally, glycerol is used as plasticizer for protein based films, as the low melting temperature of glycerol results in better solubility with the water solvent but induces a higher water solubility.

3.2. Barrier properties

In this study, the barrier properties of the FWSP/Mt bio-composite films were investigated. Before testing, all films were conditioned to 50% relative humidity at 25 ± 5 °C over 48 h in order to decrease any moisture effect (Perz-Gago and Krochta, 2001, Avena-Bustillos et al., 2011). The effect of different plasticizer types of saturated fatty acid (stearic and palmitic acid) and unsaturated fatty acid (oleic acid) and effect of Mt content on the WVP and OP are shown in Fig. 3a and b, respectively. Additional explanation ware described in Sections 3.2.1 and 3.2.2 for water vapor permeability and oxygen permeability, respectively.

3.2.1. Water vapor permeability

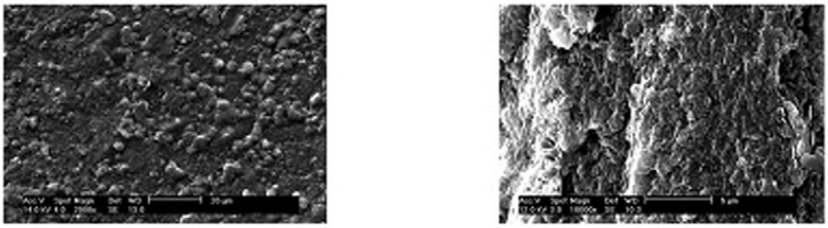
The WVP of the FWSP-oleic acid films was higher than in the FWSP-palmitic acid films at $5.27 \times 10^{-11} \pm 5.01 \times 10^{-12}\ \mathrm{g \cdot m \cdot m^{-2} \cdot s^{-1} \cdot pa^{-1}}$ ($4.56\ \mathrm{g \cdot mm \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}}$) whereas WVP of FWSP-palmitic acid films was $1.29 \times 10^{-11} \pm 1.39 \times 10^{-12}\ \mathrm{g \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}}$ ($1.11\ \mathrm{g \cdot mm \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}}$). The results suggest that the low melting temperature of oleic acid results in a high solubility coefficient therefore the oleic film had a higher WVP. These results for the WVP are lower than for other protein-based film such as FWSP-sorbital films, FWSP-glycerol films, blue shark (*P. gluca*) gelatin-fatty acid emulsion films and bigeye snapper (*Priacanthus tayenus*) surimi films. The WVP was close to that of red tilapia (*Oreochromis niloticus*) at 3.49–$5.07 \times 10^{-11}\ \mathrm{g \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}}$ (Tongnuanchan et al., 2013). The different oleic and palmitic acid structures caused the reorganization of the protein network and increased the free volume in the protein matrix (Cuq et al., 1997a, 1997b). On the other hand, nonpolar component of protein was a main factor in its resistance to water permeability although lipid material was incorporated into the fish protein film.



(a) FWSP-oleic acid/1%Mt

(b) FWSP-oleic acid/3%Mt

(c) FWSP-oleic acid/5%Mt



(d) FWSP-oleic acid/10%Mt

(e) FWSP-oleic acid/15%Mt

(f) FWSP-oleic acid/20%Mt

Fig. 2. SEM micrographs of FWSP-oleic acid film with (a) 1% Mt added, (b) 3% Mt added, (c) 5% Mt added, (d) 10% Mt added, (e) 15% Mt added and (f) 20% Mt added.

At various Mt contents, the WVP of FWSP-oleic acid with 5% Mt added was the lowest at $2.65 \times 10^{-11} \pm 1.84 \times 10^{-12}$ g·m·m^{-2}·s^{-1}·Pa^{-1} (2.29 ± 0.15 g·mm·m^{-2}·d^{-1}·kPa^{-1}) followed by the addition of 10–20% Mt resulting in an increase in the WVP. The trend of this result was similar to that reported for corn zein/Mt film, where zein film reinforced with 3% Mt had the lowest WVP which was higher than for films of FWSP-oleic acid with 3% Mt added. Moreover, the WVP of them increased with an increase in the reinforcement content (Luecha et al., 2010). Conversely, the trend in fish gelatin/Mt nanocomposite films and soy protein isolatate/Mt composites showed a decrease in the WVP with added reinforcement up to 10% Mt and 15% Mt In this study, non-modified Mt was difficult to have the reaction with molecular structure of protein. It may subsequently caused the poor distribution among Mt particle. Agglomerated Mt flakes were observed on the SEM micrographs (Fig. 2d–f).

3.2.2. Oxygen permeability

The oxygen resistance of the FWSP-oleic acid films was better than for the FWSP-palmitic acid films. The OP of FWSP-oleic acid films was 3.19 ± 0.04 g·μm/day·kPa while the OP of FWSP-palmitic acid films was 3.34 ± 0.20 g·μm/day·kPa. The liquid state of oleic acid at room temperature led to greater chain mobility and thus increased the resistance to of OP. Furthermore, the unsaturated fatty acid formed micropores in the protein based film matrix (Bertan et al., 2005). These resulted in an increase in the gas diffusion of the films.

The FWSP-oleic acid films without any added Mt had an OP of 3.19 ± 0.04 g·μm/day·kPa. The OP of FWSP-oleic acid films containing 1, 3, and 5% added Mt was similar to the control film without any added Mt with values of 3.27 ± 0.17, 3.66 ± 0.22 and 3.36 ± 0.09 g·μm/day·kPa, respectively. On the contrary, the OP of the film increased when the Mt concentration increased to 15%. Generally, exfoliated Mt layers acted as impermeable layers and lengthened the diffusion length which resulted in reduced OP [40]. Bae et al. (2009) reported that the oxygen barrier properties of fish gelatin/nanoclay composite films decreased as the Mt content increased to 9% from 402.8×10^{-6} cm^3·m/m^{-2}.day.atm to 114.48×10^{-6} cm^3·m/m^2·day·atm. In the current study, the prepared film solution was casted onto a BYTAC ® (Norton Performance Plastics Corporation, Wayne, NJ, USA) coated 8″ × 10″ glass plate which was formed utilizing a custom designed film applicator at pH 10. More Mt was incorporated resulting in an poor distribution. The FWSP/Mt bio-composite films containing 10–20% Mt had increased oxygen permeability due to the small flake agglomeration on the surface of films which was observed using SEM (Fig. 2e and f). Although the nanocomposites prepared using solution blending showed greater dispersion than the nanocomposites using the melt blending process, the excessive Mt content effected the agglomeration and the reduction of the mechanical properties and diffusion pathways (Bharadwaj, 2001, Kelnar et al., 2013). Similar results occurred with the SPI/Mt composite films, with those containing 3% Mt showing reduced oxygen permeability. On the contrary, films containing > 12% Mt showed increased oxygen permeability. Polar groups of the SPI structure and the ionic clay clusters influenced the gas barrier more than the degree of crystallinity of the polymer.

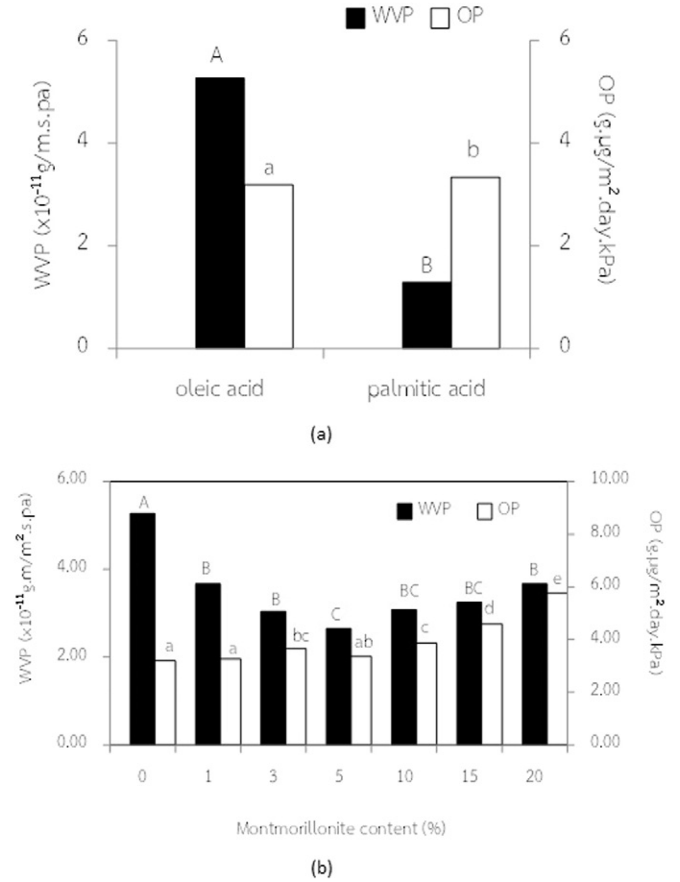


Fig. 3. WVP and OP of FWSP/Mt bio-composite films (a) using oleic and palmitic acids as plasticizers and (b) at different added levels of Mt.

3.3. Thermal properties

The DSC thermogram of the films using different varying plasticizers and added levels of Mt are shown in Fig. 4a and b. The function of the plasticizer was not only to assist lubrication but also to increase the free volume in the polymer system and thus result in greater mobility of the polymer chains and crystallinity. Many studies have reported that dry fish had only a second-order phase transition, glass transition temperature (T_g) (Cuq et al., 1997a, 1997b, Hashimoto et al., 2004). For example, the sarcoplasmic protein and myofibrillar proteins of freeze dried bonito (*Katsuwonus pelamis*) muscle had a T_g value of 100 and 107 °C, respectively, and the same two proteins in freeze dried cod (*Gadus macrocephalus*) muscle had T_g values of 93 and 107 °C, respectively. The thermogram of sarcoplasmic protein of the native striped catfish presented as both a major exothermic peak and as an endothermic peak at around 67–85 °C (Tadpitchayangkoon et al., 2010). Native Tilapia had two endothermic peaks on thermograms at 51 and 69 °C due to myosin and actin denaturation, respectively, whereas films with added plasticizer had both T_g and melting temperatures (T_m) that were dependent on the raw material, the plasticizer type and the reaction between the polymer and the plasticizer (Ghanbarzadeh and Oromiehi, 2009) while sometimes, the thermogram of the plasticized film had a T_g value similar to that of a gelatin-like film, with no endothermic peak because it had totally denatured.

The FWSP-oleic acid film as a control film had a T_g value of 55 °C and one endothermic peak as the T_m at 107 °C with enthalpy of 2.44 J·g^{-1}. On the contrary, the T_g of the FWSP-steric acid films and the FWSP-palmitic acid films was not found but there were two T_m values for FWSP-stearic acid at 73 and 122 °C, respectively, with enthalpy of 2.42 and 12.54 J·g^{-1}, respectively. The T_m values of the FWSP-palmitic acid films were at 71 and 127 °C with enthalpy at 3.83 and 5.40 J·g^{-1} respectively. The absence of a T_g caused by the solid state at room temperature of both stearic and palmitic acid restricted the mobility of the FWSP polymer chain (de la Caba et al., 2012). The results was similar for soy protein isolate film with added stearic and palmitic acid mixing in when the film-forming solution was adjusted at pH 7. Two T_m values were observed at 57 and 70 °C but they shifted to 117 and 131 °C when the film-forming solution was adjusted to pH 10.

The first T_m value was close to the T_m of stearic acid and palmitic acid. From this result, it can be hypothesized that the first T_m did not react to the fatty acid change from a solid phase to a liquid phase and the endothermic peak was observed in the second heating scan. The second T_m can be hypothesized by the formation of a helical structure

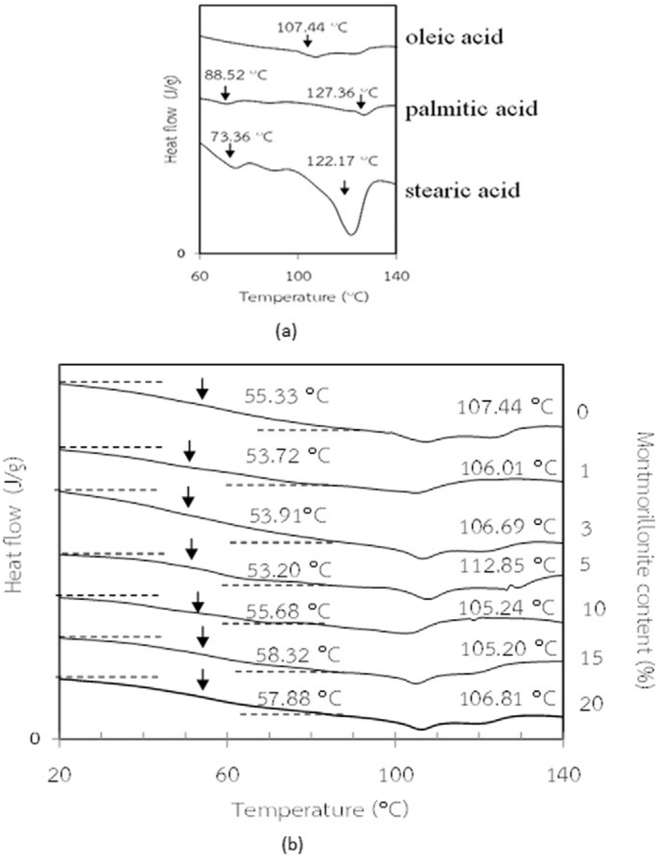


Fig. 4. DSC thermograms of FWSP/Mt bio-composite films (a) with plasticizers of oleic, stearic and palmitic acid and (b) at various Mt content.

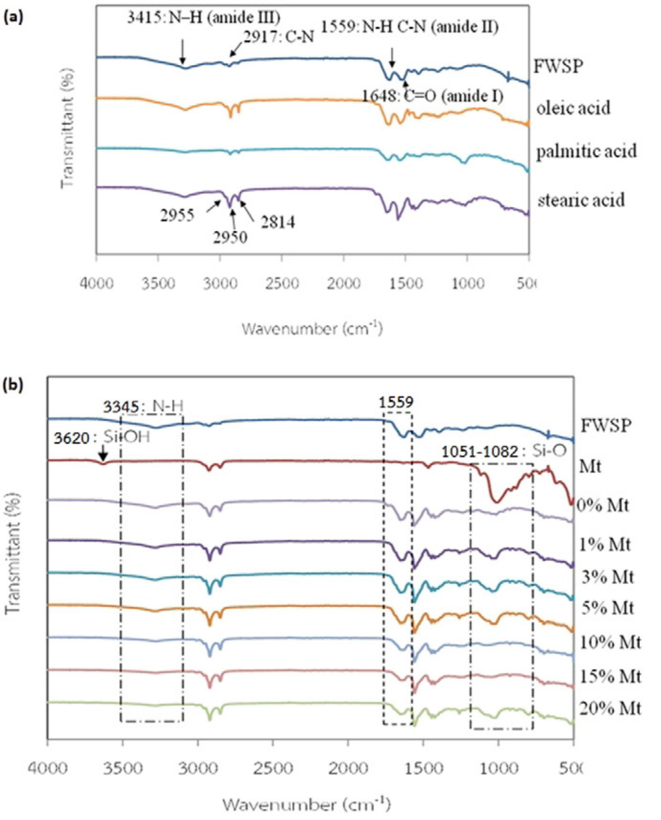


Fig. 5. ATR-FTIR spectra of FWSP films (a) at various plasticizers and (b) at various Mt content.

during the drying process at room temperature which was locked inside the film. The protein and fatty acid interaction led to a crystalline structure after the cooling process. Then, with the temperature increase, the protein structure unfolded and partial denaturation occurred (Guerrero et al., 2010). The moisture content affected the phase transition of the T_g shift and the endothermic peak as the T_m dropped.

The FWSP-oleic acid films with 1, 3, 5, 10, 15 and 20% Mt added had T_g values at 53–58 °C, each with one T_m at 106 °C. The addition of Mt caused only a very small shift of the thermal transition. Most of the films showed T_g and T_m values close to the control film at 55 and 106 °C, respectively. In contrast, T_g increased with the addition of 10–20% Mt because the Mt blocked the polymer chain rearrangement and broke down the bond interactions. There was an endothermic peak (T_m) shift with the addition of 5% Mt. The Tm value indicated that the crystalline arrangement of the helical structure of the protein had been broken. This result was different from the result with the SPI/Mt nanocomposite films, where the T_g value increased from 142 to 200 °C with the increased addition of Mt from 5 to 15%.

3.4. Chemical properties

The ATR-FTIR spectra of the FWSP films with various plasticizers are shown in Fig. 5a as a fingerprint pattern. The sharp peak near 1559 cm^{-1} of FWSP was the bending vibration of the N—H group and the stretching vibration of the C—N group (*Ami*de II). The band near 1648 cm^{-1} was the stretching vibration of the C═O carbonyl group (Amide I). Furthermore, the bands near 2917 and 3415 cm^{-1} were the stretching vibration of C—H and the stretching vibration of N—H (Amide III), respectively. The spectral pattern of the FWSP was similar to that of bigeye snapper (*Priacanthus tayenus*) (Limpan et al., 2010). The bands near 2955, 2950 and 2814 cm^{-1} were the vibration of methylene symmetric and asymmetric of methylene. Because of these group conjugated with carbon chain of fatty acid resulting in intensity around 2800–3000 cm^{-1} increase.

The ATR-FTIR spectra of the FWSP films with various Mt content are shown in Fig. 5b. In the spectral pattern of Mt, the band near 1051–1082 cm^{-1} was Si—O stretching. The amide II spectral intensity of the FWSP/Mt composite films near 1559 cm^{-1} decreased. The absence of both a band near 3620 cm^{-1} of structural OH vibration of MT and 3415 cm^{-1} of OH stretch of absorbed water was detected. The characteristic peak of 3345 cm^{-1} was due to the stretching vibration of N—H (amide III) interpreted that Mt existed the protein matrix. However, it was important to note that with the high amount of Mt on molecular structure, the featureless peak was evident. It was difficult to detect to N—H bond when Mt was existed. The region of wavenumber was in the range of 3000–3500 cm^{-1}.

4. Conclusions

FWSP, by-product from surimi process was utilized to form bio-composite films. Oleic acid resulted in the highest TS and %E at 12.11 ± 2.39 MPa and 7.14 ± 3.67%. Incorporation of Mt at 5% (w/w FWSP) induced upper tensile strength of bio-composite films but lower elongation at break, compare with bio-composite films without Mt. Moreover, oleic acid led to WVP of films higher than palmitic acid at $5.27 \times 10^{-11} \pm 5.01 \times 10^{-12}$ $g \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$ because the low melting temperature of it had an effect on a high solubility coefficient. WVP of bio-composite films increase when Mt content higher than 10% due to initial crack from phase of Mt agglomeration. The oxygen resistance of the FWSP-oleic acid films was better than for the FWSP-palmitic acid films at 3.19 ± 0.04 g·μm/day·kPa but did not affect OP of FWSP-oleic acid with Mt bio-composite films. Thermal behavior of FWSP-oleic acid was presented both of T_g and T_m but stearic and palmitic acid were shown only T_m because solid state of them restricted the mobility of the FWSP polymer chain. Mt content induced T_m increase from 107.44–112.85 °C at 5% of Mt content. ATR-FTIR confirmed Mt reinforcing the FWSP matrix.

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