CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Whey protein isolate BiPRO® (97.0% protein dry basis) was supplied by Davisco Foods International, Inc. (Le Sueur, Minnesota., USA). Konjac glucomannan, molecular weight ranges from 200,000 to 2,000,000, was purchased from Yunnan Gengyun Konjac Resources Developing Co., Ltd. (Kunming, the People's Republic of China). Glycerol QReC™ (99.5% purity), used as a plasticizer to improve film flexibility and Magnesium Nitrate Hexahydrate QReC™,used for saturated salt solution to equilibrate films at 50% relative humidity (RH) were purchased from QReC Chemical Co., Ltd. (Chonburi, Thailand).

3.2 Methods

3.2.1 Effect of incorporation of whey protein isolate (WPI) on properties of konjac glucomannan (KGM) based films

3.2.1.1 Film preparation and formation

One g KGM/ 100 g solution and 4.2 g WPI/100 g solution were prepared by adding KGM or WPI powder slowly into distilled water. Three mixture solutions were prepared by varying KGM at 0.8, 0.6 and 0.4 g and WPI at 3.4, 3.6 and 3.8 g per 100 g solution. The total biopolymer content of KGM and WPI in the mixture solution is 4.2 g biopolymer/100 g solution. Mixture solutions of KGM and WPI were prepared by slowly adding WPI and KGM powder, respectively, under constant stirring using a magnetic stirrer at room temperature for 75 min in order to totally dissolve both KGM and WPI, yielding homogeneous mixture. The casting solutions were then denatured in a water bath (Nealab EX10, Thermo fisher Scientific, Waltham, Massachusetts, USA) at 90 °C for 30 min and cooled down immediately to the room temperature in an ice bath. Glycerol was later added to the solutions as plasticizer at 1.5 or 1.8 g/ 100 g solution.

Casting solutions, containing 6 g of total solids, in order to maintain film thickness as constant as possible, were poured onto acrylic plates with casting dimension of 15 cm x 30 cm. Cast films were allowed to completely dry in a tray dryer (Contherm Thermotec 2000 oven, Contherm Scientific Ltd., Lower Hutt, New Zealand) at 50 °C for 15 h. Dried films were peeled off intact from the surface of the plates before conditioning at 50% RH and 25 °C for at least 48 h prior to tests.

3.2.1.2 Film properties measurements

Thickness

Thickness of the films was measured using a digital micrometer (Model ID-C112, Mitutoyo MFG Co. Ltd., Kanagawa, Japan) at 10 random positions of each test film. Mean values were used for the calculations of transparency, mechanical and barrier properties.

Color

The color of the films was measured by a Chroma meter (Model CR-400, Minolta Co., Ltd., Tokyo, Japan). Films were placed on a white standard plate and the Hunter color scale was used to measure color: L = 0 (black) to L = 100 (white), -a (greenness) to +a (redness), and –b (blueness) to +b (yellowness). Three measurements were made for each film replicate. The change of color was determined by total color differences between films. The total color difference (ΔE) was calculated using the following equation (1):

$$\Delta E = \sqrt{(L - L')^2 + (a - a')^2 + (b - b')^2} \qquad (1)$$

Where *L, a, b* value are Hunter color values of film samples and *L', a', and b'* value are Hunter color values of polypropylene (*L' =82.11, a' =-1.42, and b'=-0.4).*

Transparency

The transparency of the films was measured by a UV-visible spectrophotometer (Model Genesys 10, Rochester, New York, USA) according to ASTM D1746: the standard test method for transparency of plastic sheeting (ASTM, 1997b). The test films were cut into rectangular shape of 1 cm x 4 cm, then attached onto the surface of a cuvette. An empty cuvette was used as a reference. Five measurements were made for each film replicate. The transparency of the films was calculated by the following equation (2):

$$Transparency = \frac{\log \%T_{560}}{x} \qquad (2)$$

where T_{560} is the transmittance at 560 nm and x is the film thickness (mm).

Mechanical properties

An Instron universal testing machine (Model 5565, Instron, Norwood, Massachusetts, USA) was used to measure tensile strength (TS), percent elongation (%E) and elastic modulus (EM) according to ASTM D882: the standard test method for tensile properties of thin plastic sheeting (ASTM, 1997a). The test films were cut into 30 mm wide and 120 mm long strips. The initial distance between the grips was 50 mm. The cross-head speed was 5.0 mm/sec and the load cell was 5 kg. Tensile strength was calculated by dividing the maximum load by the original cross-sectional area of the test film. The result was expressed in MPa. Elastic modulus was calculated from the slope of the initial linear portion of the stress-strain curve. The result was expressed in MPa. Percent elongation was calculated by dividing the elongation at the moment of rupture of the test film by the initial gauge length and multiplying by 100. The result was expressed in percent. A total of 5 measurements were made for each film replicate.

Solubility

The solubility of films was determined according to the modified method of Andreuccetti et al. (2001). A 2 cm x 2 cm test films were immersed in 25 ml of distilled water for 24 h with slow mechanical stirring at room temperature. The filtrated film residues were dried in a forced air oven (Memmert model 600, Memmert Gmbh & Co. KG, Schwabach, Germany) at 105 °C for 24 h to constant weight. The initial dry weight was determined from the sample moisture content. Percent total soluble matter was calculated from the initial and final dry weight of films as shown in equation (6). A total of 3 measurements were done for each film replication.

$$\%S = \frac{(Initial\ dry\ weight - Final\ dry\ weight)}{Initial\ dry\ weight} \times 100 \qquad (3)$$

Water vapor permeability

Water vapor permeability (WVP) was determined according to the WVP method as described by McHugh, Avena-Bustillos, and Krochta (1993), a modification of ASTM E96: the standard test method for water vapor transmission of materials (ASTM, 1989). Films were cut and mounted on test cups containing 6 ml of water. The cups were placed in desiccator cabinets containing fans and held at 0% RH using silica gel. Weight was taken after steady state was achieved. Changes in weight of the cell were plotted as a function of time. The water vapor transmission rate (WVTR) was calculated from the slope of the constant rate of weight loss divided by film area. A total of 5 measurements were done for each film replication. WVP was calculated from the following equation (7):

$$WVP = \frac{WVTR\ (gh^{-1}m^{-2})\ \times thickness\ (mm)}{p_1 - p_2\ (kPa)} \tag{7}$$

where p_1 is water vapor partial pressure at the film inner surface (kPa), and p_2 is water vapor partial pressure at the film outer surface (kPa).

Thermal transitions

Thermal transitions were determined using a differential scanning calorimeter (DSC) (Diamond, PerkinElmer Inc., Waltham, Massachusetts, USA) equipped with an Intracooler 2P (PerkinElmer Inc., Waltham, Massachusetts, USA) and nitrogen gas purge. The film was cut to the disc of approximately 0.5 cm diameter. Each disc weighed 5-10 mg. The disc samples were hermetically sealed in a large volume stainless steel pan (60 μL). The samples were heated from 30 °C to 200 °C at a rate of 10 °C / min. An empty stainless steel pan was used as a reference. The onset (T_o), peak temperature (T_p) and enthalpy (ΔH) of the endotherm were determined via Pyris^{TM} version 8 software (PerkinElmer Inc., Waltham, Massachusetts, USA).

Seal strength

Film samples were cut into strips of 7.62 x 2.54 cm. Two films strips were placed on top of one another, and an area of 2.54 x 1.5 cm (at the edge of the film) was heat-sealed by MULTIVAC Vacuum packer (Model C350, MULTIVAC SeppHaggenmüller GmbH & Co. KG, Wolfertschwenden, Germany) at 175 °C with heating time of 2.7 s and dwell time of 5 s allowing sealing area to cool down completely. All sealed film samples were conditioned at 50% RH, 48 h prior to determining seal strength. The seal strength was measured using an Instron universal testing machine according to ASTM F88: the standard test method for seal strength of flexible barrier material (ASTM, 2005). Each leg of the sealed film was clamped to the machine, with each end of the sealed film held perpendicularly to the direction of the pull. Seal strength (N/m) was calculated using the following equation (8):

$$Seal\ strength = \frac{Peak\ force}{Film\ width} \tag{8}$$

Trinocular phase contrast microscope

Coomassie Brilliant Blue solution was prepared by mixing the 10 ml of stock solution of Coomassie Brilliant Blue (dissolve 350 mg of Coomassie Brilliant Blue G-250 (Fluka™, Sigma-Aldrich Co. LLC., Buchs, Switzerland) in solvent containing 100 ml of 95% ethanol (QReC™, QReC Chemical Co., Ltd., Chonburi, Thailand) and 200 ml of 88% phosphoric acid (QReC™, QReC Chemical Co., Ltd., Chonburi, Thailand), respectively), 10 ml of 85% phosphoric acid, 5 ml of 95% ethanol and 150 ml of distilled water.

Film samples were cut into small pieces and dipped in Coomassie Brilliant Blue solution. After that, placed it on slide with cover slip. Trinocular phase contrast microscope using 1000X magnification (10X eyepiece lens and 100X objective lens) (Olympus BX51, Olympus Optical Co., Ltd., Tokyo, Japan) under oil immersion was used to visualize the slides.

Statistical analysis

All property tests were replicated three times. Completely randomized design (*CRD*) was used in the experiment. Analysis of variance (ANOVA) was performed. Mean comparison test was done using Duncan's New Multiple Range Test (DNMRT) at a confidence level of 95%.

3.2.1.3 Criteria for selecting the composite film for the next step

The ratio of blend film which gave the highest transparency, lower water solubility and could be heat sealed was selected to use in the next step of experiment.

3.2.2 Effect of drying rate on mechanical and physical properties of konjac glucomannan-whey protein isolate blend films

3.2.2.1 Film preparation and formation

Casting solution was prepared by adding WPI and KGM powders at their optimized concentrations, 3.8% and 0.4% (wt/wt), respectively (selected from section 3.2.1) under constant stirring using a magnetic stirrer at room temperature for 75 min. The casting solutions were then denatured in a water bath (Nealab EX10, Thermo fisher Scientific, Waltham, Massachusetts, USA) at 90 °C for 30 min and cooled down immediately to the room temperature in an ice bath. Glycerol was later added to the solutions as plasticizer at 1.5 g/ 100 g solution.

Then, casting solution was poured onto acrylic plates and allowed to completely dry by either fast or slow drying rates utilizing tray dryers (Contherm Thermotec 2000 oven, Contherm Scientific Ltd., Lower Hutt, New Zealand and UM-Oven 950L, Umac Scientific Co., Ltd., Khonkaen, Thailand, respectively) until the film weight reach constant weight. In the case of fast drying rate using inlet air temperature 50 $^{\circ}$C, air velocity 3.04 ± 0.67 m/s and 19% RH, films were completely dried and able to be peeled intact off acrylic plates within 3 h. For slow drying rate using inlet air temperature 50 $^{\circ}$C, air velocity 0.92 ± 0.15 m/s and 18% RH, the drying took 15 h. Dried films were peeled off from the surface of the plates, before conditioning at 50% RH and 25 °C for at least 48 h prior to tests.

3.2.2.2 Film properties measurements

The film samples were measured for thickness, color (for ΔE calculation, L', a', and b' value in equation (1) are Hunter color values of the film dried by slow drying rate), transparency, mechanical properties, solubility, water vapor permeability, thermal transitions and trinocular phase contrast microscope as in section 3.2.1. All property tests were replicated three times. One-way ANOVA was performed. Mean comparison test was done using DMNRT test at a confidence level of 95%.

3.2.3 Effect of storage temperatures on properties of konjac glucomannan-whey protein isolate blend films

3.2.3.1 Film preparation and formation

Casting solution was prepared with the same formulation and method as listed in section 3.2.2.1. Then, casting solution was poured onto acrylic plates and allowed to completely dry in a tray dryer (UM-Oven 950L, Umac Scientific Co., Ltd., Khonkaen, Thailand) at 50 °C for 3 h. Dried films were peeled off intact from the surface of the plates and stored at 50% RH in three different temperatures (4, 25 and 35 °C) for 24 days.

3.2.3.2 Film properties measurements

The film samples were measured for color (for ΔE calculation, L', a', and b' value in equation (1) are Hunter color values of the film stored for 3 days), transparency, mechanical properties, solubility, water vapor permeability and trinocular phase contrast microscope as in section 3.2.1. All property tests were replicated three times. One-way ANOVA was performed. Mean comparison test was done using DMNRT test at a confidence level of 95%.