



Original Research Article

Effect of rice bran protein and cassava starch ratio on physical, mechanical and structural properties of rice bran protein-cassava starch composite film

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ABSTRACT

The objective of the present work was to investigate the effect of rice bran protein (RBP) extracted from cold pressed defatted RB and a commercial cassava starch (CS) ratio on physical, mechanical, and structural properties of the composite film. The RBP-CS films were prepared by casting of 5% (w/v) film forming solution with various RBP:CS weight ratios (0:100, 10:90, 20:80 and 30:70) and glycerol (60% w/w of total solid) was used as plasticizer. Results showed that increasing the RBP:CS ratio significantly increased yellowness, redness, opacity, water activity (a_w), thickness (T), and elongation at break (% E) of the films. The increased RBP content resulted in an insignificant decrease of TS ($P>0.05$) but markedly improved the % E of the RBP-CS composite film (from 6.03 to 39.64%). ATR-FTIR spectra of RBP-CS composite films showed the main interactions between rice bran protein and cassava starch were hydrogen bonds that occurred by protein chain and hydroxyl groups of starch. Thus, a molecular miscibility between these two components was evidenced. The RBP:CS ratio at 20:80 was chosen due to its greatest elongation and desired tensile strength, transparency, and color value.

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INTRODUCTION

In the recent years, the development of packages used in the food commercials and industries is rapidly progressing. The food packaging that has been widely used and well-known is plastic films. Most of all made from petroleum-based polymeric materials synthesized, due to their numerous advantages, including large scale availability, relatively low production cost, lightweight, versatile and good mechanical and barrier properties (Tharanathan 2003). However, these materials synthesized from a non-renewable source are not biodegradable and accumulation of residues can cause environmental problems. Alternative materials from agricultural sources such as proteins and polysaccharides have been used to produce biodegradable and edible films to substitute synthetic plastic films (Cuq *et al.*, 1998). Edible films based on starch and protein have received much attention as potential packaging materials due to their good film-formers with excellent oxygen, aroma, and lipid barriers at low relative humidity (Krochta 1997).

Improvement of biopolymeric film properties can be achieved by blending of biopolymers. A large number of starch-protein composites have been developed to produce effective by several researches i.e. Rhim *et al.* (1998), Wongsasulak *et al.* (2006) and Sun *et al.* (2013).

Thailand is one of major leading exporters of rice (*Oryza sativa L.*) and cassava (*Manihotesculenta Crantz*) products to the world market. Rice bran, a by-product which is obtained from the rice milling process, which is further extracted for oil. The defatted rice bran is considered as a byproduct of rice bran oil manufacture. However, defatted rice bran still contains important nutrients such as protein about 15.4% (Hamada 2000). Rice bran protein (RBP) is a high-quality, hypoallergenic food ingredient with unique nutritional value and nutraceutical properties (Saunders 1990; Helm and Burks 1996). Few information on RBP film-forming characteristics and physicochemical properties has been reported (Adebiji *et al.*, 2008; Shin *et al.*, 2011; Shin *et al.*, 2012). Cassava starch has been extensively used to produce edible films as it exhibits appropriate physical characteristics. They are odorless, tasteless, colorless and impermeable to oxygen. However, films show brittleness with inadequate mechanical properties (Chillo *et al.*, 2008).

The aim of this study was to fabricate composite films by combining of RBP and CS to develop new edible films with their advantages and reduce their disadvantages, we investigated physical, mechanical and structural properties of RBP-CS composite films with variation of RBP-CS weight ratios.

MATERIALS AND METHODS

Materials

Cold pressed defatted rice bran (CDRB) was obtained from the CEO Agrifood Co.,Ltd. (Bangkok, Thailand). A commercially available cassava starch (CS) (food grade, Thai Wah Food Products Public Co.,Ltd., Thailand) was purchased from a supermarket in Khon Kaen, Thailand. Sodium hydroxide, hydrochloric acid and glycerol (Qrec Chemical, New Zealand) were analytical grade.

Preparation of rice bran protein concentrate (RBP)

The cold pressed defatted rice bran (CDRB) was dispersed in distilled

water (rice bran:distilled water 1:5 w/v) then pH of the mixture was adjusted to 9.5 with 2 N NaOH.

The dispersion was stirred for 45 min at room temperature and centrifuged at 5,000 x g for 15 min at 4 °C. The supernatant was filtered through cheesecloth. The protein in supernatant was then precipitated by adjusting pH to an isoelectric pH of 4.5 with 1 N HCl and centrifuged at 10,000 x g for 15 min at 4 °C. The precipitated proteins were washed twice with distilled water, and then were lyophilized. The lyophilized RBP were packed under vacuum condition, and stored at -30 °C until use.

Preparation of films

The RBP-CS films were prepared by casting of 5% (w/v) film forming solution with various RBP:CS weight ratios (%) 0:100, 10:90, 20:80 and 30:70. The mixture of RBP and CS was prepared by the following steps. (1) RBP solution was prepared by dissolving specific amount of RBP powder in distilled water, then pH was adjusted to 8.0 with 1 N NaOH solution with continuous stirring at 150 rpm at 80 °C for 30 min. (2) 6% (w/v) CS solution was prepared by adding CS powder into distilled water under constant stirring at 80 °C for 30 min. Glycerol as plasticizer, was added to solution at the concentration of 60% w/w of total solid. (3) The RBP and CS solutions were mixed under constant stirring at 80 °C for 40 min. (4) The solution was vacuum filtered to remove air and undissolved impurities. The mixtures were casted onto acrylic plates with 20 cm in width x 30 mm in length x 1 mm in height and dried at 45 °C for 4 h, then the casted film were cooled to ambient temperature before peeling the films off the plates. The dried films were conditioned in an environmental chamber at 25 °C and 50% relative humidity (RH) using saturated $Mg(NO_3)_2 \cdot 6H_2O$ for 48 h prior to testing.

Film thickness

Thickness was determined as the average of 10 measurements for five samples of each film with a digital micrometer (Insize, model 3109-25, Australia).

Mechanical properties

The tensile strength (TS) and percent elongation at break (%E) of films were determined using Texture Analyser TA.XT plus (Stable Micro Systems, Surrey, England) according to the ASTM Standard Test Method D882-12 (ASTM 2012). The dried films were conditioned in the controlled chamber at 25 °C and 50% relative humidity (RH) for 48 h before testing. Ten samples (25x150 mm) of each film were tested. The initial grip distance was 100 mm and crosshead speed was 0.5 mm s⁻¹. The TS and %E values were reported as averages of at least five measurements performed for each type of film. The TS was calculated according to Equation (1)

$$TS = \frac{F}{A} \quad (1)$$

where TS is the tensile strength in MPa; F is the force maximum at rupture of the film in N; A is the initial cross-section area of the film in mm².

The %E was calculated according to Equation (2)

$$\%E = \frac{L-L_0}{L_0} \times 100 \quad (2)$$

where %E is percent elongation at break; L₀ is initial gage length; L is the final length

Color

The color of films was measured with a colorimeter (UltraScan XE, Hunter Lab system, USA) . The films were cut into 5x5 cm pieces and Average CIE scale L*, a* and b* color values were determined to investigate the change in lightness, redness-greenness and yellowness-blueness of film, respectively.

Opacity

Opacity was measured accordingly to the HunterLab method (Teixeira *et al.*, 2014) using the same equipment of color measurement. The opacity (%) of films was calculated with reflectance measurements of each film (n=5) with standard black and white backing plates, accordingly to the following equation (3)

$$\text{Opacity} = \frac{Y_{\text{blackbacking}}}{Y_{\text{whitebacking}}} \times 100 \quad (3)$$

where Y is the CIE tristimulus value of the film with the black ($Y_{\text{blackbacking}}$) or white ($Y_{\text{whitebacking}}$) backing plates.

Water activity (a_w)

The water activity (a_w) of films was measured at 25° C using Water activity meter (AquaLab, Series 3TE, USA).

ATR-FTIR analysis

The chemical nature and the structural interactions of cassava starch films incorporated with RBP were analyzed by Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance mode (ATR-FTIR). FTIR spectra of films were obtained using a Bruker FTIR Tensor 27 spectrometer with Bruker Opus7 software. Three samples were analyzed to confirm the homogeneity of each film. Scanning was carried out in the range between 4000 and 600 cm^{-1} with 4 cm^{-1} resolution and 128 scans, using air as background.

Statistical analysis

SPSS version 19.0 was used for all of the statistical analyses. The experimental data were subjected to analysis of variance (ANOVA). The statistical significance of differences between mean values was established at $p \leq 0.05$ and the Duncan’s multiple range test (DMRT) was performed to determine significance.

RESULTS AND DISCUSSION

Thickness

The thicknesses of films are presented in Table 1. It ranged from 0.042 to 0.09 mm, the results indicated that the thickness of films was significantly increased ($p \leq 0.05$) as the RBP weight ratio increased.

Table 1. Thickness, tensile strength (TS) and percent elongation at break (%E) of the CS and RBP-CS films with various ratios of BP:CS.

Sample	Thickness (mm)	TS (MPa)	%E
RBP-CS 0:100	0.042 ± 0.007 ^a	11.44 ± 0.50 ^b	0.87 ± 0.15 ^a
RBP-CS 10:90	0.071 ± 0.006 ^b	1.22 ± 0.11 ^a	6.03 ± 0.64 ^b
RBP-CS 20:80	0.082 ± 0.007 ^{bc}	0.43 ± 0.04 ^a	39.64 ± 2.01 ^c
RBP-CS 30:70	0.090 ± 0.005 ^c	0.38 ± 0.01 ^a	37.09 ± 1.43 ^c

Any two means in the same column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan’s multiple range test.

Mechanical properties

The mechanical properties of the RBP-CS films with various RBP-CS ratios are shown in Table 1. The tensile strength (TS) values of RBP-CS composite films are in the range from 0.38 to 1.22 MPa. An increase in the RBP proportion from 10 to 30 % resulted in a significant decrease of TS compared to the CS (RBP:CS 0:100) film (11.44 MPa) ($p \leq 0.05$). However, the increased RBP ratio in the CS film from 10 to 30% resulted in a slight decrease in TS values ($p > 0.05$). Thus, the presence of RBP in CS film yielded a decrease in TS, which was inversely proportional to the increase of RBP proportion.

The incorporation with RBP in CS film increased the extensibility or elongation at break (%E, Table 1) up to 45 times compared to the CS film without RBP. The %E values increased from 0.87 to 39.64% with increasing RBP ratio (0-30%). Changes in %E of the composited films suggests plasticizing effect by rice bran protein. The results of TS and %E of the RBP-CS films also suggest that RBP would act as a plasticizer of the CS network. A similar effect was also observed for other biopolymeric films as described by Cian *et al.* (2014). There are two main types of plasticizers; (1) molecules capable of forming hydrogen bonds, thus interacting with polymers by interrupting polymer-polymer bonding and maintaining the further distance between polymers chains, (2) molecules capable of interacting with large amounts of water to retain more water molecules, thus resulting in a higher moisture content and larger hydrodynamic radius. Sun *et al.* (2013) found that addition of peanut protein isolate decreased the tensile strength and increased elongation of pea starch film. These might be attributed by the interaction between the swollen pea starch granules and peanut protein isolate, which led to the formation of a flexible network. In this case, the RBP may also act as both types of plasticizer, since RBP contains lots of amino acids residues that have reactive group such as -OH, -COOH, -NH₃⁺ or amine groups which can form hydrogen bond and interact with water yielding an increase flexibility, a_w and a reduced brittleness of RBP-CS film (Salgado *et al.*, 2011; Valenzuela *et al.*, 2013; and Cian *et al.*, 2014). Al-Hassan and Norziah (2012) reported that the tensile strength of sago film decreased with increasing amount of fish gelatin in sago film due to the interaction between hydroxyl groups between starch and protein. The interaction between starch chains and gelatin seemed to act as a plasticizer which enhanced film flexibility and reduced brittleness.

The mechanical properties of film were characterized by tensile strength (TS) and elongation (E) value. High TS and E values represent good mechanical properties. The results in this study suggests that the addition of suitable RBP content could be applied to greatly improve the flexibility with small decreases of strength of a composite films. Thus, RBP:CS ratio at 20:80 was selected due to its good mechanical properties.

Film color and opacity

The effect of RBP proportion on CS film color and opacity is shown in Table 2. The presence of RBP in CS films significantly affected ($p \leq 0.05$) color value of the films. The CS film without RBP (RBP:CS 0:100) showed the highest L* values. Films with RBP were darker (lower L* value) when increasing its proportion. The L* values decreased from 91.52 to 86.68, but a* and b* values increased from 0.41 to 1.78 and from 9.11 to 12.42, respectively, when the RBP proportion was increased from 0 to 30%.

The opacity values of RBP-CS composite films range between 60.33 and 87.07 % (Table 2). The CS film without RBP was more transparent (lower %opacity) than those CS films incorporated with RBP. The results showed that increasing the RBP content significantly increased opacity ($p \leq 0.05$). An increase in film opacity as a result of the addition of protein has also been reported in pea starch and peanut protein blend films (Sun *et al.*, 2013).

Water activity (a_w)

The a_w values of the RBP-CS composite films (0.75-0.81) were higher than the CS film (0.49) as shown in Table 2. It was found that increasing the RBP ratio in films resulted in an increase in a_w values, indicating that free water in composite films is higher than in the CS film. This may relate to the hydrophilic character of protein molecules and their capable of interacting with large amounts of water to retain more water molecules corresponding with described by Cian *et al.*, 2014.

Table 2 Color values, Opacity and Water activity (a_w) of CS and RBP-CS films in different ratios.

Sample	L^*	a^*	b^*	%Opacity	a_w
RBP-CS 0:100	91.52 ± 1.58 ^b	0.41 ± 0.17 ^a	9.11 ± 0.29 ^a	60.33 ± 0.84 ^a	0.49 ± 0.01 ^a
RBP-CS 10:90	90.42 ± 0.69 ^b	0.81 ± 0.57 ^b	10.06 ± 0.43 ^a	63.11 ± 0.03 ^a	0.75 ± 0.012 ^b
RBP-CS 20:80	87.13 ± 1.19 ^a	1.25 ± 0.092 ^c	11.73 ± 0.11 ^b	72.84 ± 1.22 ^b	0.80 ± 0.018 ^c
RBP-CS 30:70	86.68 ± 1.82 ^a	1.78 ± 0.057 ^d	12.42 ± 0.22 ^b	87.07 ± 0.78 ^c	0.81 ± 0.017 ^c

Any two means in the same column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test.

ATR-FTIR analysis

Both polymers in this blend systems containing the proton acceptor and proton donor usually undergo some interaction such as hydrogen bonding (Khurma *et al.*, 2005; Lewandowska, 2011). The miscibility of both polymers can be monitored using the Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) technique.

The interaction between rice bran protein and cassava starch was investigated by FTIR in ATR mode. Figure 1 shows the FTIR spectra of RBP film, CS film, and RBP-CS composite films.

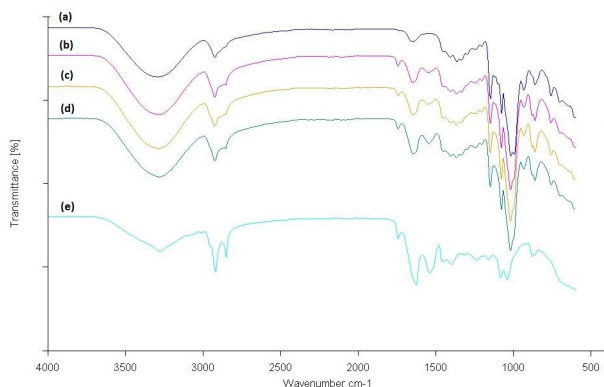


Figure 1 ATR-FTIR spectra of cassava starch film (a), RBP-CS (10:90) film (b), RBP-CS (20:80) film (c), RBP-CS (30:70) film (d), and FTIR spectra of rice bran protein film (e).

The characteristic absorption peaks observed for the RBP film were: the main absorption peaks at 3279 cm^{-1} (broad band) are indicative of the O-H stretching which are able to form hydrogen bonding with carboxyl group of the peptide linkage (Gonzalez *et al.*, 2010); 2922 cm^{-1} (CH_2 asymmetrical stretching); 1629 cm^{-1} (C=O stretching, amide I); 1541 cm^{-1} (N-H bending, amide II); and 1236 cm^{-1} (C-N stretching, amide III).

In the CS spectra, the following peaks were observed: a broad band at the 3297 cm^{-1} (O-H stretching of CS hydroxyl groups); 2926 cm^{-1} (C-H stretching vibration); 1649 cm^{-1} (C=O stretching); strong peak at 1149 cm^{-1} (O-H bending); and 1077 cm^{-1} (C-O-C bending).

The RBP-CS composite films spectra with various weight ratios suggested that when RBP was added to the CS film, they presented a shift of the broad absorption band due to a shift of O-H vibration from 3297 cm^{-1} to 3285-3281 cm^{-1} indicating that the hydrogen bonding had occurred between protein chain and hydroxyl groups of starch.

Also, the RBP-CS composite film showed a shift of absorption band from 1629 to 1650 cm^{-1} for amide I band and from 1541 to 1547 cm^{-1} for amide II band, compared with the RBP film. Moreover, these bands gradually increased while RBP content was increased. These results point out that the main interaction between RBP and CS in composite film would be through the formation of hydrogen bonds, as has been suggested by Abugoch *et al.* (2011).

CONCLUSION

The RBP-CS composite films were successfully prepared by casting solution of rice bran protein and cassava starch. Including of RBP to form the RBP-CS composite film could improve mechanical properties of the film compared to the CS film. The addition of RBP resulted in an increase in thickness, redness, yellowness, opacity, and a_w of the RBP-CS films but a decrease in lightness and tensile strength. The elongation at break of the CS film was improved markedly by incorporating RBP into the CS film due to the plasticizing characteristics of the rice bran protein. Moreover, the structural properties of RBP-CS composite film determined by ATR-FTIR analyses showed good compatibility between both polymers. The main interaction was hydrogen bonds that occurred by protein chains and hydroxyl groups of starch. The RBP:CS ratio at 20:80 was selected for further study due to its greatest elongation and desired tensile strength, transparency, and color value.

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