Effect of dual-modification on some properties of rice starch based biodegradable films

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Abstract

The properties and characteristics of native rice starch and dual-modified rice starch in relation to dual-modified rice starch films were investigated. In this study, the rice starch was hydroxypropylated with 6–12% of propylene oxide followed by crosslinking with 2% sodium trimetaphosphate (STMP). Increasing of propylene oxide concentrations in the dual-modified rice starch yielded an increase in the molar substitution (MS) and degree of substitution (DS) of the modified rice starch. However, the pasting temperature, paste consistency, breakdown, setback and gel strength showed an inverse trend. Biodegradable films were prepared from the dual-modified rice starch. The results showed that the maximum tensile strength (TS) was presented when using the 8% propylene oxide. The Elongation at break (%E) and film solubility (FS) of the films were higher than native rice starch and these tended to increase when the concentration of propylene oxide increased. However, the water vapor permeability (WVP) of the films was lower than the native rice starch and these did not significantly change with an increase in the concentration of propylene oxide (p>0.05).

Keywords: rice starch, film, hydroxypropylation, crosslinking, dual-modification

1. Introduction

Synthetic plastic is an important material widely used in daily life because it is durable, safe, aesthetically pleasing and can be economically manufactured (Frost, 2010). The synthetic plastic can protect food from contamination and spoilage but may also be the source of substances migrating into the food (Risch, 1988). Most synthetic plastics are produced from petrochemicals and are not biodegradable. In addition, the synthetic plastics are difficult to recycle and quickly become dumped in landfills or incinerated; this leads to environmental

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pollution and attracts greater interest in biodegradable packaging from natural polymers. Among all biopolymers, starch is being investigated as a potential material for biodegradable films (Averous *et al*., 1999). Starch is quite cheap, abundant, biodegradable and edible. It consists of two types of polysaccharides, namely amylose and amylopectin (Alves *et al*., 2006). Amylose is a linear molecule with few branches, while the amylopectin molecule has many branches. Therefore, the amylose content contributes to film strength and the branched structure of amylopectin generally leads to film with low mechanical properties (Mali *et al.,* 2005). Muscat *et al*. (2012) found that, the films with high amylose content showed higher tensile strength and modulus of elasticity values but lower elongation values than those of low amylose starch films. There was an increase in the thermal and mechanical properties of the high amylose starch films. Based on this, the rice starch (Chiang rice) has a higher amylose content of 30.40% in its composition (Khunae *et al.,* 2007). However, the strength, flexibility and transparency of high amylose film are still poor and these undesirable properties limited its application in packaging.

Chemical modification is usually undertaken to improve film properties. Hydroxypropylation and crosslinking are methods widely used to produce modified starch. Starches that are etherified with propylene oxide (hydroxypropylated starches) exhibit lower gelatinization temperature, increased granule swelling, higher paste viscosity and greater paste clarity than native starches. Crosslinking reinforce the hydrogen bonds in the granules with chemical bonds that act as a bridge between the starch molecules (Neelam *et al.,* 2012). Crosslinking provides more stable pastes viscosity at high temperature and at low pH (Rutenberg and Solarek 1984). Reagents such as phosphorus oxychloride, sodium trimetaphosphate, and epichlorohydrin were reported to be generally crosslinking reagents (Wattanachant *et al.,* 2003).

In a previous study, the film from hydroxypropylation modified starches was formed and provided a higher elasticity and film transparency than native starch, while the tensile strength value was low. These results might have been due to the incorporation of a hydroxypropylated group which is capable of disrupting inter- and intra-molecular hydrogen bonds in the starch chains. It also reduced the formation of junction zones cause by the inhibition of amylose chain interactions, thus weakening the granular structure of the starch (Ramsden *et al.,* 1999; Lee and Yoo, 2011)

Kim and Lee (2002) reported that the mechanical properties of starch films prepared with crosslinked corn starch show higher values than native corn starch. Khan *et al.* (2006) showed that crosslinked sago starch/PVA blend films had mechanical properties higher than native sago starch/PVA blend films. In addition, Detduangchan *et al.* (2012) also reported that the properties of the rice starch films were improved by using crosslinking agents. Crosslinking reactions resulted in increasing the tensile strength of rice starch films. When starch is modified by both hydroxypropylation and crosslinking or dual modification, the functional benefits from each individual modification are realized. Currently there is no study on biodegradable film produced from hydroxypropylation and crosslinking or dual modification, and their mechanical properties and their possible applications. Hence the objective of this study was to investigate the properties and characteristics of dual-modified rice starch based biodegradable films.

2. Materials and methods

2.1 Raw material and chemical reagents

Rice starch grain (Chiang rice) was purchased from a local rice mill in Phattalung, Thailand. The rice grain was stored in an ambient temperature until used.

Sodium chloride, sodium hydroxide, sodium sulphate, potassium phosphate, ammonium molybdate, ammonium metavanadate and ninhydrin were purchased from Univar (New South Wales, Australia). Sorbitol and propylene glycol were purchased from the Vidyasom (Bangkok, Thailand). Propylene oxide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium trimetaphosphate (STMP) was purchased from Xingfa (Hubei, China). Ethanol, sulfuric acid and hydrochloric acid were purchased from J.T. Baker (Center Valley, PA, USA).

2.2 Preparation of rice starch (RS)

Chiang rice starch was isolated by the alkaline method following the procedures of Sawai and Morita (1968), Sugimoto *et al.* (1986) and Ju *et al.* (2001). The rice grain was soaked in 4 times the volume of distilled water for 24 h, followed by wet grinding with a rice grinding machine. The slurry was isolated by centrifuging at 3,000xg for 30 min and was called rice flour. Then the flour was extracted with 4 times the volume of 5% NaCl for 2h (globulin extract) and centrifuged at 3000xg for 30 min. The flour was then extracted for the prolamin with 4 volumes of 70% ethanol for 2 h and followed by glutelin extraction with 4 times the volume of 0.35% NaOH and continuously stirred. After 16 h, the starch was adjusted to pH 7 with 1 M HCl and isolated by centrifuging (3,000xg, 30 min), and washed with 4 times the volume of distilled water for 3 times (with albumin extract and other chemical reagents). The starch was dried at 50°C overnight to ensure 10–12% moisture content.

2.3 Preparation of dual-modified rice starch

The rice starch was modified by its reacting with propylene oxide following modified procedures from Suwanliwong (1998), Wattanachant *et al.* (2003) and Hung and Morita (2005). Fifteen grammes of sodium sulphate (15% based on dry wt. of starch) were added to 300 ml of distilled water and stirred. When the salt was dissolved, 100 g of rice starch (dbs, equivalent to 30% starch solid in slurry) were added and stirred to make up a uniform slurry. Then a 5% sodium hydroxide solution was added with vigorous stirring to prevent starch gelatinization and to adjust the slurry to pH 11.5. Then 6–12% propylene oxide (vol. by weight of starch solid) was added and stirred at room temperature for 30 min. The slurry was then transferred to centrifugal bottles and placed in a 40°C water bath with continuous shaking for 24 h. The starch slurry was transferred into a mixing container at room temperature. Then it was cross-linked by using 2% STMP (w/w. of dry starch) based on the methods of Woo and Seib (1997), Suwanliwong (1998), Wattanachant *et al.* (2003) and Hung and Morita (2005). The slurry was held in a 40°C water bath for 3 h with continuous stirring. Then the slurry was adjusted to pH 5.5–6.0 with 10% hydrochloric acid solution to terminate the reaction. The starch slurry was washed with 1 time the volume of distilled water four times and isolated by centrifuging (3000xg, 5 min); thereafter the starch was dried at 50°C for 24 h.

2.4 Determination of hydroxypropyl group (HPG) and molar substitution (MS)

The hydroxypropyl group in the modified starch was determined according to the procedure described by the Joint FAO/WHO Expert Committee on Food Additives (2001). A sample (50–100 mg) was weighed into a 100-ml volumetric flask and 25 ml of 0.5 M sulfuric acid was added. The flasks were placed in a boiling water bath and heated until the solution became clear. The samples were cooled and diluted to 100 ml with distilled water. One milliliter of the solution was placed by pipette into 25 ml test tubes with glass stoppers. The test tubes were immersed in an ice bath and then 8 ml of concentrated sulfuric acid were dropped into each tube. The solution was mixed well and the tubes were placed in a boiling water bath for exactly 3 min. The tubes were immediately transferred to an ice bath until the solution was chilled. An aliquot (0.6 ml) of ninhydrin reagent was added, and the reagent was carefully allowed to run down the walls of the test tubes. The test tubes were immediately shaken well and placed in room temperature for 100 min. The volume in each tube was adjusted to 25 ml with the concentrated sulfuric acid and mixed by inverting the tubes several times. Portions of the solution were immediately transferred to 1-cm cells, and, after exactly 5 min, the absorbance was measured at 590 nm, using the starch blank as a reference. A calibration curve was prepared with an aliquot (1 ml) of standard aqueous solution, containing 10, 20, 30, 40 and 50 mg of propylene glycol per ml. The hydroxypropyl groups (by%) were calculated by the following equation:

$$\text{Hydroxypropyl groups } (\%) = \frac{\text{C x 0,7763 x 10 x F}}{\text{W}}$$

Where, C is amount of propylene glycol in the sample solution read from the calibration curve (mg/ml), F is the dilution factor and W is the weight of the sample (mg).

The molar substitution (MS) of the modified starch was calculated in the normal fashion (Rutenburg *et al.,* 1998)

$$\text{MS (Hydroxypropylated starch)} = \frac{\text{moles of substituent}}{\text{mole of anhydro} - \text{glucose unit}}$$

$$\text{MS} = \frac{\text{\% HP x 162}}{\text{59.08 x (100} - \text{\%HP)}}$$

Where, %HP is the percentage of hydroxypropyl groups

2.5 Determination of phosphorus content (P) and degree of substitution (DS)

The content of the phosphorus in native and modified starches was determined according to a modified procedure as described by the A.O.A.C. (2000). A sample (2 g) was burned in a muffle furnace at 600°C for 4 h. This was then cooled to room temperature and 10 ml of 5 M hydrochloric acid was added in the ash. The contents were diluted to 100 ml with distilled water and 50 ml of the solution were then diluted again to 100 ml with distilled water. The vanadate molybdate (25 ml) was added to the solution and then the absorbance of the sample was measured at 470 nm. A calibration curve was prepared from 0, 2.5, 5, 10, 20, 30, 40 and 50 ml of potassium dihydrogen phosphate (KH_2PO_4), using the 0 ml of KH_2PO_4 as a reference. The phosphorous content was calculated from the absorbance curve which was obtained by using a standard phosphorous solution.

$$\text{Phosphorous } (\%) = \frac{\text{P x dilution volume in ml x 100}}{\text{(aliquot volume in ml x sample weight in g x 1000)}}$$

Where, P is the phosphorous content (mg/100 ml) from the calibration curve.

The degree of substitution (DS) of modified starch was calculated in the normal manner (Paschall, 1964; Rutenburg and Solarek 1984; Suwanliwong, 1998).

$$DS = \frac{162 \text{ x } \%P}{3100 - (124 \text{ x } \%P)}$$

Where, %P is the percentage of phosphorous

2.6 Functional properties of dual-modified rice starch

2.6.1 Pasting properties

The pasting properties of the starches were determined using a Rapid Visco Analyzer (RVA) model 3D (Newport Scientific, Warriewood, Australia). Distilled water (21.5 g) was added to the starch (3.5 g, db) in the RVA canister to obtain a total constant sample weight of 25 g (14% starch concentration). The slurry was then manually homogenized using a plastic paddle to avoid lump formation before the RVA run. A programmed heating and cooling cycle was set for 12 min. It was first held at 50°C for 1 min, heated to 95°C in 4 min, further held at 95°C for 2 min, cooled to 50°C within 4 min, and held at 50°C for 1 min.

2.6.2 Gel strength

The gel strength was measured using a Texture Analyzer (TA-XTplus, Stable Micro System, Ltd.). Starch pastes (15% db) were prepared by boiling in a water bath for 30 min and then the hot pastes were poured into cylindrical plastic tubes (20 mm diameter and 20 mm height). These were covered, cooled at room temperature for 1 h, and kept at 4°C f or 24 h. The starch gels were placed in room temperature for at least 1 h before measuring their gel strength. A cylindrical probe with a diameter of 35 mm was used to penetrate into the gel at a speed of 2 mm s^{-1} for distances of 20% strain. The maximum force of the penetration was recorded as the gel strength (g) and the measurements were repeated five times.

2.7 Film casting and drying

Native rice starch and dual-modified rice starch (3.5% db) were dissolved in distilled water and gelatinized by boiling in a water bath with continuous stirring (85°C for 15 min) and then cooled to 45°C. Sorbitol was added as 35% (w/w) of the total solid weight in solution. The mixtures were cast onto flat leveled, non-stick trays to set. Once set, the trays were held overnight at 50°C for 10 h undisturbed, and then cooled to room temperature before the films were peeled off the plates. The film samples were stored at 25°C and 55± 5 % RH until used for further testing. All the treatments were made in triplicate.

2.8 Properties of dual-modified rice starch films

2.8.1 Mechanical properties

Prior to the measurement of the mechanical properties, the films were conditioned for 72 h in a ventilated oven at 25°C and 55±5% RH. The tensile strength (TS) and elongation at break (%E) of the films were determined as described by Bourtoom and Chinan (2008) with a slight modification. This was done using a Universal Testing Machine (Lloyd Instruments, Hampshire, UK) equipped with tensile load cell of 100 N (ASTM, 1991). Ten samples (2.54 cm x 12 cm) with an initial grip length of 10 cm were used for testing. The samples were clamped and deformed under a tensile load with a cross-head speed of 50 mm/min until the samples were broken. The TS was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the %E was calculated as follows:

$$\%E = 100 \times (d_{after} - d_{before})/d_{before}$$

where, d was the distance between grips holding the specimen before or after the breaking of the specimen.

2.8.2 Water vapor permeability (WVP)

The gravimetric modified cup method based on ASTME96- 92 (McHugh *et al.,* 1993) was used to determine the WVP of the films. The test cups were filled with 20 g of Silica gel (the desiccant) to produce a 0% RH below the film. A sample was placed between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hanau, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.5 cm between the film surface and the desiccant. The water vapor transmission rates (WVTR) of each film were measured at 55 ± 5% RH and 25 ± 2°C.

After taking the initial weight of the test cup, it was placed in a growth chamber with an air velocity rate of 135 m/min (Model KBF115, Contherm Scientific, Lower Hutt, New Zealand). The weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorious Corp.) every 3 h for 18 h. A plot of the weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit of time (g/h). The WVTR was expressed in gram units per square meter per day. The steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Six samples per treatment were tested. The WVP of the film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

2.8.3 Film solubility (FS)

A modified method from Jangchud and Chinnan (1999) was used to measure film solubility. Film pieces, 20 mm x 20 mm, were dried at 70°C in a vacuum oven for 24 h and then weighed to the nearest 0.0001 g for the initial dry mass. Films were immersed in 20 ml of distilled water in 50 ml screw cap tubes containing sodium benzoate (0.1% w/v) to prevent microbial growth. The tubes were capped and placed in a shaking water bath for 24 h at 25±2°C. A portion of the solution was removed and set aside for later use in protein solubility tests as described below. The remaining solution and film pieces were poured onto quality filter paper (Whatman #1). These were rinsed with 10 ml distilled water, and dried at 70°C in a vacuum oven for 24 h to determine the dry mass of the film. Five measurements were taken for each treatment. The total soluble matter was calculated from the initial gross mass and the final dry mass using the following equation:

$$\%\,\mathrm{FS(db)} = \frac{(\text{film mass before test} - \text{film mass after test})}{\text{film mass before test}} \times 100$$

2.9 Statistical analysis

All tests were performed at least in duplicate. Analysis of variance (ANOVA) was performed using the Duncan’s multiple-range test to compare treatment means (Steel and Torrie, 1960). Significance was defined at *p*<0.05.

3. Results and discussion

3.1 Effect of propylene oxide level on properties of dual-modified rice starch

3.1.1 Hydroxypropyl groups (HPG), molar substitution (MS), phosphorus content (P) and degree of substitution (DS)

The molar substitution (MS) of the hydroxypropyl group and the degree of substitution (DS) of the phosphate group into granular starches were used to measure the reaction efficiencies of hydroxypropylation and crosslinking. The MS and DS of dual-modified rice starch with 6–12% propylene oxide and using 2% STMP are shown in Table 1. The hydroxypropyl groups (HPG) and MS which presented the effect of hydroxypropylation were in the range 0.679–1.396% and 0.019–0.039 respectively. The phosphorus content (P content) and DS which presented the effect of crosslinking were in the range 0.0504–0.1189 % and 0.0026–0.0062 respectively. It was observed that all values were increased with the content of propylene oxide increase. The P content of all treatments is lower than the maximum level (0.4%) allowed by the U.S. regulations (Wattanachant *et al.,* 2002). The DS values of all treatments show a significance although they were crosslinked with 2% STMP.

Therefore, the different DS values obtained were due to the different amounts of propylene oxide. In addition, these results also show that the level of hydroxypropylation during the first stage of the dual-modification process enhanced the subsequent crosslinking. This was shown by the increase in the P content and DS values. Phosphate salts from the crosslinking reagent (STMP) enabled phosphate groups to produce the distarch phosphate derivative which has significant higher phosphorus content than the native rice starch (Wattanachant *et al.,* 2002). It is believed that the hydroxypropyl groups in the neighboring starch chains prevent interchain association and they facilitate water molecules to penetrate into the granules and thereby increase swelling and they had higher MS values (Gunaratne and Corke, 2007). Thus allowing the crosslinking reagent to react more with the starch chains results in higher DS values. Wattanachant *et al.* (2002) also reported that hydroxypropylation caused weaker granules by disrupting the hydrogen bonds between starch chains and results in the swelling of granules. In this way the crosslinking reagents reacted more easily with the starch molecules. In addition, Morikawa and Nishinari (2000) also found that the DS values of dual-modified potato starch increased when there was an increase in the amount of propylene oxide.

Table 1 Hydroxypropyl groups (HPG), molar substitution (MS), phosphorus content (P) and degree of substitution (DS) of native rice starch and dual-modified rice starches with various levels of propylene oxide.

Starch	**HPG (%)**	**MS**	**P content (%)**	**DS**
Native	–	–	0.0037 ± 0.0000^{e}	–
HP6C	0.679 ± 0.021^{d}	0.019 ± 0.001^{d}	0.0504 ± 0.0005^{d}	0.0026 ± 0.0000^{d}
HP8C	0.922 ± 0.024^{c}	0.026 ± 0.001^{c}	0.0821 ± 0.0016^{c}	0.0043 ± 0.0001^{c}
HP10C	1.132 ± 0.043^{b}	0.031 ± 0.001^{b}	0.1021 ± 0.0006^{b}	0.0054 ± 0.0000^{b}
HP12C	1.396 ± 0.007^{a}	0.039 ± 0.000^{a}	0.1189 ± 0.0012^{a}	0.62 .0001^{a}

Note: Each value is mean of triplicate ± SD. Mean values in the same column with different letters are significantly different (p<0.05). HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

3.1.2 Pasting properties

Irreversible granule swelling follows gelatinization causes a rapid rise in viscosity to a peak value (Peak viscosity as well as peak time). The viscosity indicates the water-binding capacity of the starch. As the shear and heating continues, then cause granule rupture and lead to a “Breakdown” which dependent on the starch source and test conditions (Newport Scientific, 1998).

The pasting curve of native and dual-modified rice starch with different levels of propylene oxide determine by RVA is shown in Figure 1. The native rice starch started pasting at 82.43°C and showed quite stable viscosity with a slight breakdown during the holding period at 95°C. The native rice starch contained a high amylose content group. This resulted in strong internal bonding forces between the starch chain within granules due to low swelling, delayed pasting on setting, and a high pasting temperature. The dual-modified rice starch showed lower the pasting temperature, peak viscosity, breakdown viscosity and setback viscosity than native starch. However, the pasting profile of all dual-modified starches during the holding period at 95°C showed the similarity, as shown in Figure 1. The effects of dual-modification on reduction of pasting temperature and the viscosities (peak, breakdown and setback viscosity) are presented in the Table 2. It was observed that the pasting temperature and the viscosities of dual-modified rice starch tended to decrease when the amount of propylene oxide increased. The reduction of pasting temperature was probably due to the fact that the substitution occurred primarily in the amorphous region (Hung and Morita, 2005). Thus the substitution reflected swelling in that region and disrupted the crystalline phase, resulting in a lower temperature in melting, as compared with the native rice starch (Reddy and Seib, 2000).

These results reflected that thick (high viscosity) to thin (low viscosity) with increasing amount of propylene oxide. This is because the levels of propylene oxide induced high crosslinking in starch granules (Wattanachant *et al.,* 2002). In addition, Retenburg and Solarek, (1984) also reported that the breakdown viscosity can be reduced by crosslinking. The setbacks of all dual-modified rice starches were lower than the native rice starch and expressed a decreasing when the amount of propylene oxide increased. The hydroxypropylation resulted in weaker granules by disrupting hydrogen bonds between starch chains, as compared with the native rice starch (Gunaratne and Corke, 2007). Thus the addition of crosslinking reagent caused more reaction in the starch chain. The STMP was used to a crosslinking reagent which produced a distarch phosphate and it is reported to be a strongly effective crosslinking reagent (Woo and Seib, 1997)

This indicated that after dual-modification of rice starch, the starch granules were stronger and more difficult to breakdown. Thus the amylose chains inside the granules slightly leached out and they were then less likely to re-associate in an ordered structure, resulting in lower breakdown and setback. In addition, Yook *et al.* (1993) also reported that the setbacks in the dual-modified rice starch were decreased when the starches were dual-modified.

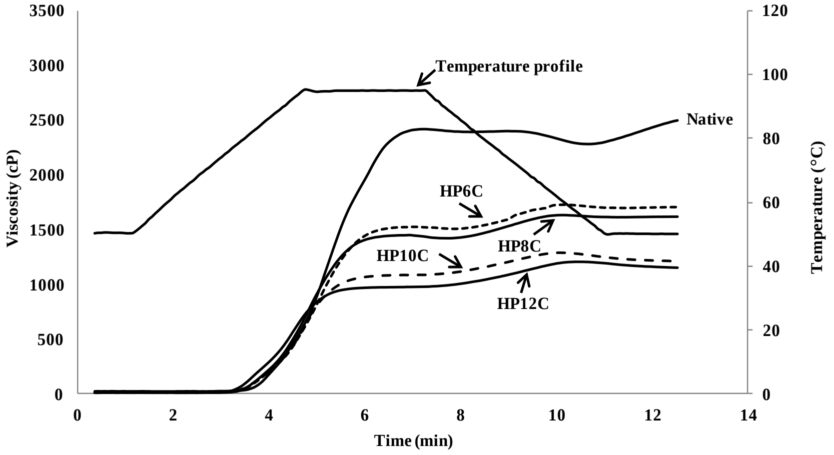

Figure 1 Pasting profiles of native rice starch and dual-modified rice starches with various levels of propylene oxide. HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

Table 2 Pasting properties of native rice starch and dual-modified rice starches with various levels of propylene oxide.

Starch	Pasting Temperature (°C)	Viscosity (mPa.s)		
		Peak	Breakdown	Setback
Native	82.43±0.03^{a}	454.67±28.22^{a}	329.67±8.08^{a}	507.67±14.01^{a}
HP6C	81.62±0.08^{b}	1472.33±9.07^{b}	50.67±13.65^{b}	203.67±5.03^{b}
HP8C	81.55±0.05^{b}	445.67±11.15^{b}	45.33±5.86^{b}	212.67±5.51^{b}
HP10C	81.67±0.06^{b}	1086.00±6.24^{c}	18.67±6.35^{c}	139.00±4.58^{d}
HP12C	79.43±0.40^{c}	972.00±10.58^{d}	7.33±1.53^{c}	176.33±2.08^{c}

Note: Each value is mean of triplicate ± SD. Mean values in the same column with different letters are significantly different (p<0.05). HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

3.2.2 Gel strength

The gel property of native rice starch and dual-modified rice starch are shown in Figure 2. The gel strengths of all dual-modified rice starches were lower than those of native rice starch. At higher levels of propylene oxide induced high crosslinking in the starch granule.

The STMP was used to crosslinking reagent which gave distarch phosphate. Distarch phosphates have the phosphate esterified with two hydroxyl groups, often from two neighboring starch molecules. This leads to the formation of a covalent bridge or crosslinking in the starch granule (Sitohy *et al.,* 2000; Neelam *et al.,* 2012). It is believed that the hydroxypropyl groups in the neighboring starch chains prevent amylose association and results in weaker granules. Thus, allowing the crosslinking reagent to react more with the starch chains, results in stronger granule compared with native starch granules (Gunaratne and Corke, 2007).

This indicated that after dual- modification of rice starch, the starch granules were stronger and more difficult to breakdown. Thus after gelatinization, the amylose chains inside the granules slightly leached out and they were then less likely to re-associate in an ordered structure, resulting in lower breakdown and decreased the amylose leaching (Wattanachant *et al.,* 2002; Gunaratne and Corke, 2007). A reduction of amylose leaching may decrease the amylose concentration in the continuous network. Furthermore, it would also reduced the deformability of gelatinized swollen granules in the gel matrix and introduce more junction zones (Gunaratne and Corke, 2007). Hence, all dual-modified rice starches showed a decrease in the gel strength. As was seen, the gel strength of the dual-modified rice starch was reduced when the amount of propylene oxide increased. This indicates that the propylene oxide levels induced high crosslinking in starch granules. This result may occur from weakening in the starch granule through hydroxypropylation, as the crosslinking reagents react more with the starch molecules (Wattanachant *et al.,* 2003). This caused a substantial lessening of the breakdown in the starch granule and decreased the amylose leaching (Gunaratne and Corke, 2007).

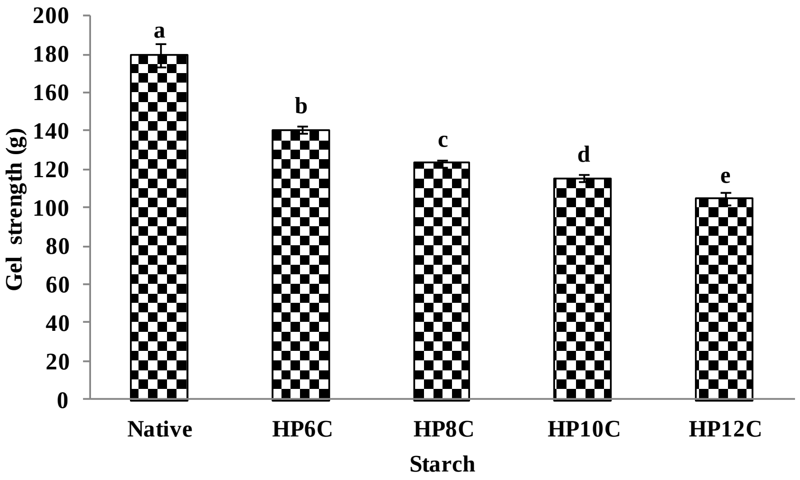

Figure 2 Gel strength of native rice starch and dual-modified rice starches with various levels of propylene oxide. Mean values in the same column with different letters are significantly different (p<0.05). HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

3.3 Effect of propylene oxide level on properties of dual-modified rice starch film

3.3.1 Mechanical property

Tensile strength (TS) and Elongation at break (%E) of native rice starch film and dual-modified rice starch films are shown in Figure 3. The TS values and %E values of native rice starch film and dual-modified rice starch films were in the range 6.18–8.07 MPa and 68.70–121.85% respectively. The native rice starch film was the lowest of both TS value and %E value of all dual-modified rice starches films. This result reflects that the dual-modified rice starch films were stronger and more flexible. This might due to the hydroxypropylation caused a weakening of the hydrogen bonding between starch chains and this led to more crosslinking reagents reacting with the starch molecules (Wattanachant *et al*., 2003; Gunaratne and Corke, 2007). Das *et al.* (2010) reported that the increase of crosslinking density induced the increase in TS values. The crosslinking reagent reacts with the -OH groups present in starch and makes ether linkages with the available hydroxyl groups. This helps to increase the TS values. Kim and Lee (2002) also reported that the TS values of starch films prepared with crosslinked corn starch show higher values than those from native corn starch.

The TS values of the dual-modified rice starch tended to increase with adding the 6-8% propylene oxide and it’s gradually decrease with adding the 10% propylene oxide. However, there are not significances the TS values with adding the 10–12% propylene oxide. This might be because a reduction of amylose concentration after dual-modification. The 10–12% propylene oxide induced the crosslinking reagent more reaction in the starch chains and this results in stronger granule and less to breakdown than 6–8% propylene oxide, as seen the P content in Table 1. In addition, a reduction of TS values when using 10–12% propylene oxide might be due to the high hydroxypropyl groups in starch structure (Table 1) provided an increasing the bulky molecules.

However, It can be observed that the gel strength of dual–modified rice starches were lower than native rice starch and tended to decrease with an increase the propylene oxide content while the TS values of dual-modified rice starch films were higher than native rice starch and tended to increase with 6-8% propylene oxide. This might be due to the different structure of starch gel and starch film. Basically, a starch gel is a solid–liquid system having a solid, continuous network in which the liquid phase is entrapped and has a characteristic structural shape resistant to flow (Eliasson, 1985; Tester and Morrison, 1990). In addition, Gunaratne and Corke, 2007 reported that the dual-modification caused to stronger and more rigid granules and it resulted in a decrease the amylose leaching. A reduction of the amylose leaching would reduce the deformability of gelatinized swollen granules in the gel matrix and this affected to decrease the gel strength. While the starch film are normally prepared by casting gelatinized starch dispersion (Zobel, 1984). The amylose chains after gelatinization formed the films through aggregation and compaction of swollen starch granule, probably driven by water evaporation and introduced a two-dimensional network during drying (Rindlav-Westling and Gatenholm, 2003). Liu and Han (2005) reported that the water evaporation leads to the concentration of the solution and accelerates the amylose re-associations. When all possible chain associations have been completed, further evaporation of water reduces the bulk of the gel until the majority of the free water is removed. Often, the surface upon which the solution is cast serves as a sufficient restraint to prevent the film from shrinking (Protzman *et al*., 1967).

The % E values of all dual-modified rice starches were higher than those of the native rice starch and tended to increase when adding the propylene oxide. This might be due to the bulky hydroxypropyl groups in the dual-modified rice starch inhibiting the re-association of the amylose chains. In addition, the propylene oxide may act like a plasticizer in the films and this induces the formation of starch-plasticizer interactions resulting in higher %E.

In this results, the effect of crosslinking reagents are expressed in higher both TS and %E values. These results show that the dual-modification of rice starch was more efficient than crosslinking alone. However, Detduangchan and Wittaya (2011) reported that only photo-crosslinked rice starch showed an increase in the tensile strength but there was a decrease in the elongation at break.

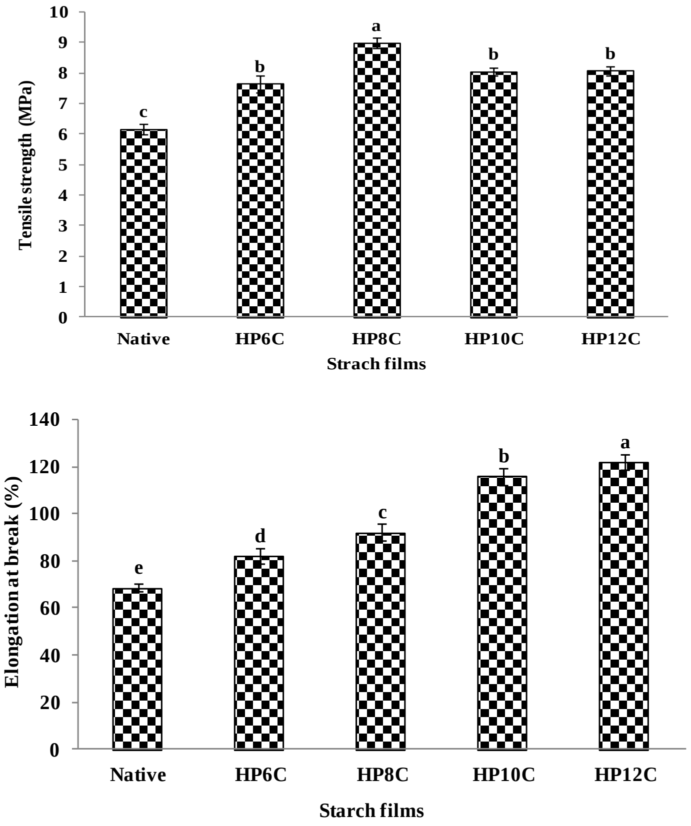

Figure 3 Tensile strength and elongation at break of native rice starch and hydroxypropylated rice starch films containing various concentrations of propylene oxide. Mean values in the same column with different letters are significantly different ($p<0.05$). HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

3.3.2 Water vapor permeability (WVP) and film solubility (FS)

WVP is proportionality constant and assumed to be independent of the water vapor pressure gradient applied across films. The WVP of native rice starch and dual-modified rice starch films are shown in Table 3. These results show that the WVP of dual-modified rice starch films were in the range 1.23–1.32 g.mm/day.m^2.kPa. It was observed that the WVP of all dual-modified rice starch films were lower than the native rice starch film (2.98 g.mm/day.m^2.kPa). These results suggest that the dual-modified rice starch films showed superior water barrier properties than the native rice starch films. A reduction of WVP values by dual-modification would due to the hydroxyl groups (-OH groups) of rice starch were decreased when crosslinking reaction occurred between starch chain and crosslinking reagent. This crosslinking reagent would limit the water absorption by restricting the mobility of starch chains in the amorphous region and also decrease the free volume in the film structure (Gunaratne and Corke, 2007). Thus, the crosslinking expresses to limit the interaction of starch chains with water and provides a structural integrity of starch-based film during exposure to pressure and moisture (El-Tahlawy *et al.,* 2007). Furthermore, the crosslinking reagents react with the -OH groups present in starch and make ester linkages with the available hydroxyl groups. This helps to increase the mechanical properties of the films as well as to reduce the water absorption behavior (Das *et al.,* 2010). However, there are not differences in WVP value with various levels of propylene oxide. This reflected that the higher amount of crosslinking reagent, as seen in P content (Table 1) did not affect to WVP values. This might due to the high P content when using 8% propylene oxide induced an excess concentration of crosslinking reagent in the starch structure and this affected to no change in water absorption behavior. In addition, Chung *et al.* (2004) reported that the crosslinked corn starches that crosslinked with STMP/STPP had an excess concentrate measured at 10% concentration of the mixture of STMP/STPP.

FS is an important property for application in food protection where water activity is high, or when the film must be in contact with water during the processing of the coated food. The FS of dual-modified rice starch films were in the range 6.53–7.09%. It was observed that they were higher than those of native rice starch film and tended to increase with adding the propylene oxide, as shown in Table 3. This might due to the hydroxypropyl groups after hydroxypropylation induced the increase the hydrophilicity in the nature of the starch, thus giving higher MS values, as seen in Table 1 (Chun and Yoo, 2007). In addition, the crosslinking reagents, STMP produced starch phosphates which presented the negative charge in the phosphate groups apparently reducing the inter-chain associations and gave increased levels of hydrated molecules (Lim and Seib, 1993). Thus, the solubility of the native rice starch film and dual-modified rice starch films most likely reflected the leaching of plasticizer, starch chains and a hydrophilic modification reagent from the film matrix after 24 h immersion and shaking in water.

Table 3 WVP and FS of native rice starch and dual-modified rice starches with various levels of propylene oxide.

Film	Propylene oxide concentration (%w/w)	WVP g.mm/day.m^2.kPa	FS (%)
Native	-	2.98 ± 0.14^b	4.45 ± 0.14^a
HP6C	6	1.23 ± 0.04^a	6.53 ± 0.33^b
HP8C	8	1.26 ± 0.03^a	6.83 ± 0.31^{bc}
HP10C	10	1.32 ± 0.15^a	6.79 ± 0.26^{bc}
HP12C	12	1.29 ± 0.11^a	7.09 ± 0.19^c

Note: Each value is mean of triplicate ± SD. Mean values in the same column with different letters are significantly different ($p<0.05$). HP6C-HP12C = dual-modified rice starches at various levels of propylene oxide (6–12%).

4. Conclusion

The properties of dual-modified rice starch were significantly different to those of the native rice starch and were dependent on the concentration of propylene oxide. An increase the amount of propylene oxide in the dual-modified rice starch resulted in increasing the MS and DS. However, the pasting temperature, paste consistency, breakdown, setback and gel strength tended to decrease with addition the propylene oxide. The properties of rice starch based biodegradable films presented that the %E and FS values in the film structures tended to increase when the propylene oxide increased while the highest TS value was presented when using 8% propylene oxide. However, the water vapor permeability of the films did not significantly change with an increase the amount of the propylene oxide ($p>0.05$).

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