Enhancement of the properties of biodegradable rice starch films by using chemical crosslinking agents

Detduangchan, N., Sridach, W. and *Wittaya, T.

Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand

Abstract

The effect of chemical crosslinking on the mechanical, thermal properties and water vapor permeability of rice starch-based films were investigated. Epichlorohydrin (EPI), Sodium trimetaphosphate (STMP) and the mixing of STMP/STPP (Sodium tripolyphosphate) were used as crosslinking agents. The viscosity of crosslinked rice starch decreased when the content of the crosslinking agent increased concomitant with the increasing of the pasting temperature. The results showed that the degree of crosslinking increased when the content of the cross-linking agents increased. The tensile strength of rice starch films increased concomitantly with decreases in elongation at break and water vapor permeability when chemical-crosslinking was applied. In addition, the crosslinking reaction decreased the crystalline peak structure and percentage of crystallinity of rice starch films. The rice starch films were characterized by Fourier Transform infrared spectroscopy (FT-IR) and this confirmed that crosslinking between starches molecules had occurred. The behavior of thermal properties of crosslinked rice starch films were investigated by means of dynamic mechanical thermal analysis (DMTA) and using a differential scanning calorimeter (DSC). It was found that the glass temperature (T_g) of crosslinked rice starch films shifted to a higher temperature than that of untreated rice starch films. In addition, the storage modulus (E') of crosslinked rice starch films decreased twice as much as untreated rice starch films, while the tan d curve of crosslinked rice starch film was higher than that of untreated rice starch films.

Introduction

Recently, environmentally friendly materials from natural and renewable resources have received much attention (Ma *et al.*, 2004; Nakamura *et al.*, 2005; Xiong *et al.*, 2008). The development of innovative materials as substitutes for synthetic polymers has become an important challenge. Among these materials are biopolymers from vegetable or animal proteins (Arvanitoyannis, 2006; Xiong *et al.*, 2008). Since 1970s there been much study of polar polymers or biopolymer such as polysaccharide and protein. Both of them are new alternative materials for use instead of synthetic polymers in plastic and plastic film manufacturing in the light of environmental problems (Garcia *et al.*, 2000).

Many researchers have found that the property of biopolymer such as polysaccharide, protein and fat can be formed into film and coating. Technically, biopolymer consists of naturally occurring polymers that are found in renewable resources. Using biopolymers will cause less harmful to our environment compared to the use of fossil fuel based commodity plastics. Basically, biopolymers can be degraded by the enzymatic action of living organisms.

These processes involve bacteria, yeasts, fungi and the ultimate end-products of the degradation process. These are CO_2, H_2O and biomass under aerobic conditions and hydrocarbons, methane and biomass under anaerobic conditions (Gerngross *et al.*, 2003).

Starches used in industrial applications are usually extracted from cereal seeds (corn, wheat, and rice), tubers (potato), and roots (tapioca) (Chiou *et al.*, 2005). Starch has been investigated widely for the potential manufacture of products such as water-soluble pouches for detergents and insecticides, flushable liners and bags, and medical delivery systems and devices. Starch granular organization as well as its amylose and amylopectin structure depends on the botanical source. It is an abundant raw material with low cost, and has been applied in the area of degradable plastics. Blend films containing starch are potential materials in the agriculture, medicine, and the packaging industries (Funke *et al.*, 1998; Hulleman *et al.*, 1998; Lu *et al.*, 2005; Xiong *et al.*, 2008).

Rice is the seed of the monocot plants *Oryza sativa* or *Oryza glaberrima.* It has many important roles in Thai society from food to work. Rice uses over half of the cultivable land area and labor force in

*Corresponding author.
Email: *thawean.b@psu.ac.th*
Tel: +66 74 286 359; Fax: +66 558866

Thailand. On the other hand, rice starch contains high amylose which is attractive raw materials for use as barriers in packaging materials. Rice starches have been used to produce biodegradable films to partially or entirely replace plastic polymers because of their low cost and renewability. They also possess good mechanical properties (Xu *et al.*, 2005; Bourtoom and Chinnan, 2008). However, the wide application of starch film is limited by their mechanical weaknesses and the fact that they are swollen by water depending on the relative humidity (RH) (Seow *et al.*, 1999).

Chemical modifications such as grafting or crosslinking are able to limit excessive water swelling and macromolecular motion. Basically, cross-linking means that the polymer molecules are interconnected by some sort of bonding. The bonding can be covalent, ionic, or it can result from intermolecular forces such as hydrogen bonding. The crosslink density in materials is necessary for the assessment of the quality of cross-linked polymers. In addition, the optimization of curing conditions and the tailored design of new products with improved mechanical and aging properties are important. The physical structure of cross-linked polymers, their network dynamics and molecular mobility, depend to a large extend on the density of crosslinks. Mechanical and oxidative aging as well as the different types and amounts of fillers affect the network dynamics, and these may lead to a change of crosslink density and/or to a degradation of the chemical structure by hydrocarbon chain scissions (Kuhn *et al.*, 2006). In addition, crosslinking tends to limit the interaction of starch with water and provides a structural integrity to starch based biodegradable materials during exposure to pressure and moisture (El-Tahlawy *et al.*, 2007).

Classical crosslinking leads to the modified starches used in food or paper additive applications in which crosslinking occurs in heterogeneous media using dry or semi-dry blending processes. For example, starch networks are usually achieved by treating granular starch with the following crosslinking agents in different media: sodium trimetaphosphate (STMP) (Woo and Seib, 1997); epichlorohydrin (EPI) (Kuniak and Marchessault, 1973); and a mixture of sodium trimetaphosphate (STMP) / Sodium tripolyphosphate (STPP). In this study the properties of rice starch films were improved by chemical treatment, using crosslinking agents such as STMP, EPI and a STMP/STPP mixture. In addition, the properties of crosslinked rice starch and rice starch films gained from chemical treatment were also investigated by comparing the viscosity, pasting temperature, degree of cross-linking,

mechanical property, thermal property and structural characteristics.

Materials and Methods

Materials

Rice starch was extracted from grains of Chiang Phatthalung rice (*Oryza sativa* L.) by using alkaline methods, and the rice grain was purchased from a local grocery. It contained amylose and moisture content of about 30.40% and 12.19% (determined in triplicate by vacuum drying at 70°C for 24 h using the EYELATM, Model VOS-300VD, Japan), respectively. Commercial grade sorbitol, used as a plasticizer, was purchased from the Vidyasom Co. Ltd. (Thailand). The crosslinking reagents used were Epichlorohydrin (EPI), which was purchased from MERCK Schuchardt OHG (Hohenbrunn, Germany), Sodium trimetaphosphate (STMP)m whichwas obtained from SciencePedia Co., Ltd. (Bangkae, Bangkok), and Sodium tripolyphosphate (STPP), which was purchased from SIGMA-ALDRICH Co., (St. Louis, MO, USA).

Preparation of cross-linked rice starch

Rice starch was modified by using 3 types of cross-linking agent. Crosslinked rice starch with Epichlorohydrin (EPI) was prepared by using the methodology of Wurtzburg (1960) and Wattanachant *et al.* (2003). Fifteen gram of sodium chloride was added to 250 ml of distilled water. After the salt was dissolved, 100 g of rice starch were added and stirred until the mixture was uniform. Then 5% of sodium hydroxide solution was added to adjust the slurry to pH 10.5 with vigorous stirring to prevent starch gelatinization. The crosslinking agent at 0.1, 0.3 and 0.5% of EPI was added into the mixture and stirred at room temperature for 5 h. After that the 1% of hydrochloric acid solution was added into slurry to terminate the reaction by adjusting the slurry to pH 5.5. Starch slurry was washed with water and neutralized with 5% NaOH. The slurry obtained was centrifuged at 8000 rpm for 15 min; thereafter the starch was dried at 50°C for 24 h.

Crosslinked starch with sodium trimetaphosphate (STMP) and mixtures of sodium trimetaphosphate/ Sodium tripolyphosphate (STMP/STPP: 99/1 w/w) was prepared according to the method of Woo and Seib (2002) and Koo *et al.* (2010). Native rice starch granules weighing 100 g were added to 250 ml of distilled water. Then, the cross-linking agents, STMP (1.0, 2.0 and 3.0%) and STMP/STPP (4.0, 8.0 and 12.0%), were added to the slurry. The mixtures were adjusted to pH 10.5 by 5% NaOH. The slurries were

stirred continuously and warmed up to 45ºC for 1 h. Later, 1 g/1000 ml of hydrochloric acid solution was added into slurry to terminate the reaction by adjusting the slurry to pH 5.5. Starch slurry was washed with water and neutralized with 5% NaOH. The slurry obtained was centrifuged at 8000 rpm for 15 min; thereafter the starch was dried at 50ºC for 24 h.

Viscosity, pasting temperature and degree of crosslinking of crosslinked rice starch analysis

The degree of cross-linking of modified starches was determined from the viscosity values, according to the modified methods of Kaur *et al.* (2006) and Koo *et al.* (2010). The viscosity and pasting properties of rice starch were measured by using a Rapid Visco Analyzer (RVA). A sample of 2.5 g (10 g/100 g dry basis) was suspended in 25 ml distilled water and heated from 50 to 95°C, held at 95°C for 3 min, then cooled from 95°C to 50°C and held at 50°C for 6 min. The pasting parameters were automatically computed and reported. The degree of cross-linking was calculated by using the equation below:

$$\text{Degree of cross-linking} = \frac{A-B}{A} \times 100 \quad (1)$$

Where A is the peak viscosity of the control sample (without crosslink), and B is the peak viscosity of the cross-linked rice starch.

Preparation of crosslinked rice starch films

Crosslinked rice starch films were prepared by using solution casting. A crosslinked rice starch solution with a concentration of 3 g/100 ml was prepared by dispersing crosslinked rice starch in distilled water and heating the mixtures and stirring it until gelatinized (85°C for 10 min) (Bourtoom and Chinnan, 2008). Then, the slurry was cooled to 50 ± 5ºC. Sorbitol (the plasticizer) was added to 50 g/100 g of starch and stirred for 2 min. The mixtures were cast onto flat, level, non-stick trays to set. Once set, the trays were held overnight at 55°C for 10 h undisturbed, and then cooled to an ambient temperature before peeling the films off the plates. The film samples were stored in plastic bags and held in desiccators at 55 ± 5 %RH for further testing. All treatments were made in triplicate.

Film testing

Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to a standard method, D618-61 (ASTM, 1993a).

The films were tested for water vapor permeability (WVP), tensile strength (TS) and elongation (E). This was done in conditions at 55 ± 5 %RH and 25 ± 2°C by placing them in desiccators over a saturated solution of $Mg(NO_3)_2 \cdot 6H_2O$ for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

Film thickness

The thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 (±5%) at five random locations on the film. The mean thickness values for each sample were calculated and used for water vapor permeability (WVP) and tensile strength (TS) calculations.

Tensile strength (TS) and elongation at break (E) testing

Tensile strength (TS) was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) in accord with the ASTM D882-91 Standard method (ASTM, 1993b). Ten samples, 1.5 cm x 12 cm, were cut from each film. The initial grip separation and cross-head speed were set at 70 mm and 30 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the present elongation at break (E) was calculated as in (2).

$$E = 100 \times (d_{after} - d_{before}) / d_{before} \quad (2)$$

Where, d was the distance between grips holding the specimen before and after the breaking of the specimen.

Water vapor permeability (WVP)

The gravimetric Modified Cup Method based on the ASTM E96-92 standard method (McHugh *et al.*, 1993) was used to determine the WVP of the films. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below that of the film. A sample was placed in between the cup and the ring cover of each cup coated with a silicone sealant (high vacuum grease, Lithelin, Hanau, Germany) and held with four screws around the cup's circumference. The air gap was approximately 1.5 cm between the film surface and desiccant. The rated water vapor transmissions (WVTR) of each film were measured at 55 ± 5% RH and 25 ± 2°C.

After measuring 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 Scient, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Satorious Corp.) every 3 h for 18 h. A plot of weight gained against the 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 time (g/h). The WVTR was expressed in gram units, per square meter, per day. 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.

Differential scanning calorimeter (DSC)

The thermal properties of rice starch films were analyzed with a Perkin-Elmer DSC-7 (Norwalk, Conn., U.S.A.) equipped with an intra-coolant Thermal Analysis Controller TAC7/DX (Perkin-Elmer). Samples (approx. 20 mg each,) were weighed in stainless steel pans (Perkin-Elmer) designed to withstand high pressures. Each stainless steel pan was sealed with an O-ring, and the moisture was allowed to reach equilibrium overnight. An empty DSC pan was used as a reference pan. The heating rate was programmed by holding the heat at -50°C for 1 min, followed by ramping the temperature range of -50°C to 180°C at a rate of 20°C/min, and holding it at 180°C for 1 min. The measurements were made at least in duplicate for each treatment.

Dynamic mechanical thermal analysis (DMTA)

The small deformation analysis of the rice starch films was performed in tension in a dynamic mechanical thermal analyzer (Rheometric Scientific, DMTA V). The tested strips of film were cut into small strips (20 x 5 mm) and clamped in the instrument with an initial grip separation of 5.5 mm. The films were subjected to a sinusoidal strain on top of static deformation. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.02% over a temperature range of 50 to 200°C, at a heating rate of 5°C/min. The measurements at each experimental point were done at least in triplicate. Dynamic mechanical spectroscopy was employed within the linear viscoelastic regime to determine T_g. The storage and loss modulus (E' and E'') and loss tangent (tan $= \Delta$E'/E'') were measured as a function of temperature at a constant frequency and a selected heating or cooling rate.

X-ray diffraction (XRD)

The X-ray patterns of starch powders, starch nanocrystals, starch film, and starch film reinforced with starch nanocrystals were analyzed. This was done by using an X-ray diffractometer (Philips X, Pert MPD, Japan) with Cu Kα radiation at a voltage of 40 kV and 30 mA. The samples were scanned between 2θ = 3-40° with a scanning speed of 2°/min. Prior to testing the samples were dried and stored in desiccators.

Statistical analysis

A completely randomized experimental design was used to determine the character of the composite films. Analysis of variance (ANOVA) was used to compare mean differences of the samples. If differences in the means existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Viscosity and pasting temperature of crosslinked rice starch

The viscosity and pasting temperature of untreated rice starch (control) and crosslinked rice starch with EPI, STMP and STMP/STPP are demonstrated in Table 1 and Figure 1(A-C). The peak viscosity of the crosslinked rice starch (125.15-188.80 RVA) showed lower peak viscosity than did the untreated rice starch (338.60 RVU). The reduction in peak viscosity would result from the presence of the crosslinking agent which may reduce interactions of starch molecules with water molecules resulting in lower peak viscosity values. However, the results demonstrate that the initial pasting temperature of the crosslinked rice starch is higher than the untreated rice starch. These results could be explained by the crosslinking of molecular chains that makes the starch granules more ordered; consequently more energy would be required for swelling. Hirsch and Kokini (2002) reported that higher levels of crosslinking generally lead to reduced granule swelling solubility, paste clarity and paste peak viscosity (Wongsagonsup *et al.*, 2005; Kaur *et al.*, 2006).

The effects of the type and content of crosslinking agents on viscosity and pasting temperature of rice starch were compared. It was found that rice starch crosslinked with 8% STMP/STPP yielded a lower viscosity than with 0.5% EPI and 3% STMP. This would be because a higher density of crosslinking occurred. Moreover the viscosity peaks of rice starch crosslinked with EPI (Figure 1(A)) and STMP (Figure 1(B)) were decreased slightly whereas the viscosity peaks of STMP/STPP (Figure 1(C)) were dramatically decreased when the concentration of crosslinking agents increased. The application of STMP/STPP

Table 1. Peak viscosity and pasting temperature of crosslinked rice starch

Crosslinking agents	Viscosity			
	Peak Viscosity (RVU)	Viscosity@ 95ºC (RVU)	Viscosity@ 50ºC (RVU)	Pasting temperature (ºC)
Rice starch	338.60±0.42[a*]	316.80±3.25[a]	346.50±4.24[a]	84.03±0.03[f]
0.1%EPI	188.80±1.41[b]	176.55±0.77[b]	182.90±2.12[b]	92.30±0.42[e]
0.3%EPI	158.10±1.97[c]	149.10±1.83[c]	155.55±2.82[c]	93.10±0.60[bc]
0.5%EPI	156.50±1.41[cd]	148.05±0.63[d]	150.70±2.82[d]	93.83±0.71[b]
1.0%STMP	156.10±0.28[d]	148.60±042[d]	151.25±2.12[d]	94.30±0.03[b]
2.0%STMP	154.55±1.06[e]	146.00±1.83[e]	148.80±4.14[e]	94.38±0.00[b]
3.0%STMP	153.15±1.06[e]	142.65±0.63[g]	145.60±7.07[f]	94.70±0.64[b]
4.0%STMP/STPP	153.65±0.18[e]	144.05±0.64[f]	146.55±7.78[f]	92.75±0.07[d]
8.0%STMP/STPP	125.15±1.40[g]	118.35±1.27[i]	120.55±9.09[h]	95.08±0.03[a]
12.0%STMP/STPP	146.80±0.67[f]	135.40±0.78[h]	138.65±6.06[g]	94.30±0.00[b]

[*] Mean values with different letter are significantly different (p < 0.05).

Table 2. Degree of crosslinking of crosslinked rice starch

Crosslinking agent (g/100 g)	Degree of crosslinking (%)
rice starch	0.00
0.1EPI	44.24±1.14[i]
0.3EPI	53.31±1.97[h]
0.5EPI	53.78±1.44[g]
1.0STMP	53.90±0.28[f]
2.0STMP	54.36±1.06[e]
3.0STMP	54.77±1.06[c]
4.0STMP/STPP	54.62±1.41[d]
8.0STMP/STPP	63.04±0.42[a]
12.0STMP/STPP	56.65±1.12[b]

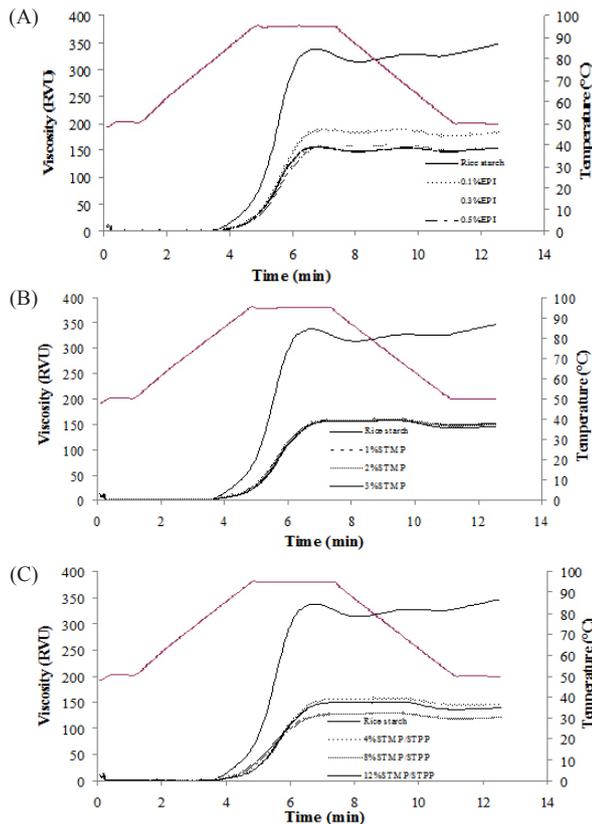[*] Mean values with different letter are significantly different (p < 0.05).

Figure 1. Viscosity profiles of untreated rice starch, EPI crosslinked rich starch (A), STMP crosslinked rice starch (B) and STMP/STPP crosslinked rice starch (C)

provided a greater reduction in viscosity. This might be because the high concentration of the crosslinking agent could provide the high crosslinking density. In addition, the results show that the crosslinked rice starch had a higher pasting temperature than the untreated rice starch.

These results are in agreement with the study of Xiao *et al.* (2011) who reported that the pasting properties of untreated potato starch and crosslinked starch with epichlorohydrin (EPI) decreased the peak, setback and final viscosities of potato starch. Lim and Seib (1993) investigated the properties of starch phosphates. They found that a distarch phosphate (crosslinked starch) prepared from a mixture of phosphate salts (STMP) and (STPP) provided better results than using STMP. This is because the formation of crosslinks between starch

molecules strengthen the swollen granules against breakage under conditions of high temperature and shear. In addition, after starch granule rupture, the covalent crosslinks may provide sufficient granule integrity to keep the swollen granules intact and minimize or prevent loss in viscosity (Lim and Seib, 1993; Jyothi *et al.*, 2006). The amylose does not leach out from the granule because the phosphate groups have strengthened the granule and prevented it from swelling (Muhammad *et al.*, 2000).

Degree of crosslinking

The degree of crosslinking was calculated from the maximum viscosity values, as described by Kaur *et al.* (2006); Koo *et al.* (2010) are shown in Eq. (1). The degree of cross linking is therefore directly proportional to the number of covalent bonds in the formation of cross inking which are produced during reaction with a crosslinking agent. The degree of crosslinking of crosslinked rice starch is depicted in Table 2. Higher levels of degree of crosslinking generally lead to reduced granule swelling (Hirsch and Kokini, 2002), solubility, paste clarity and paste peak viscosity (Kaur *et al.*, 2006; Wongsagonsup *et al.*, 2005). In the results may be observed that the degree of crosslinking increased with the increasing of the concentration of the crosslinking agents (EPI, STMP and STMP/STPP). The highest degree of crosslinking was found when 0.5% EPI, 3.0% STMP and 8.0% STMP/STPP were used. However, using 12.0% STMP/STPP yielded a decreasing degree of crosslinking from 63.04 ± 0.42 (8% STMP/STPP) to 56.65 ± 1.12 (12.0% STMP/STPP). This was because it was an excessive amount as described above. Hirsch and Kokini (2002) also noticed that the degree of crosslinked waxy maize starch increased when the concentration of phosphorous oxychloride (POCl$_3$), sodium trimetaphosphate (STMP) and epichlorohydrin (EPI) increased.

Bajpai and Bhanu (2007) studied the impact of crosslink density on the percentage of dissolution of EPI crosslinked starch. They found that the extent of dissolution gradually decreased with increased concentrations of the crosslinking agents. This may be
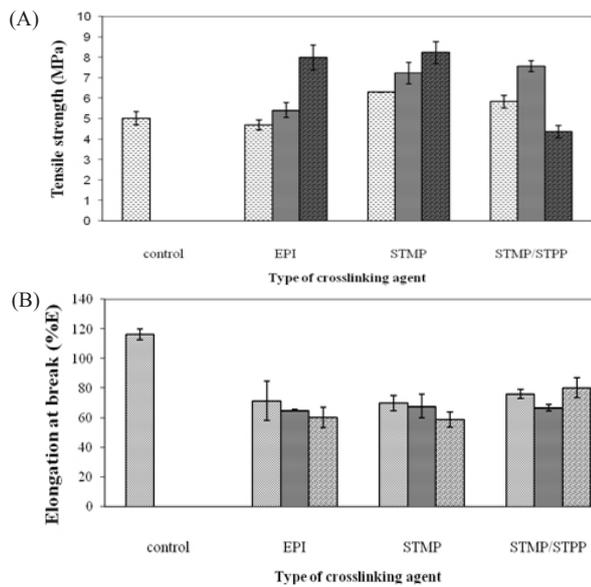
(A)

(B)

Figure 2. Effect of crosslinking agents on tensile strength
(A) and elongation at break (B) of rice starch films.

explained by the fact that increasing the crosslinking agent yielded the promotion of the crosslink density of starch, resulting in greater compactness and resistance to dissolution. Ayoub and Rizvi (2007) also reported that an increase in the EPI concentration from 0 to 0.5% was accompanied by an increase in the degree of crosslinking of extruded wheat starch due to increasing the EPI concentration. The relative self-diffusion coefficient (D) decreased rapidly, reaching a negligible value at an EPI concentration higher than 0.5% in untreated starch. This confirmed good crosslinking.

Effect of crosslinking agents on the properties of crosslinked rice starch films

Tensile strength (TS) and Elongation at break (E)

Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile at yield or at break, respectively (ASTM, 1995). The effect of crosslinking agents effect on tensile strength (TS) and elongation at break (E) of rice starch films are shown in Figure 2(A-B). The results demonstrate that the impact of crosslinking agents affected the TS of rice starch films. The TS of crosslinked rice starch films was higher than that of untreated (native) rice starch films. The TS of untreated rice starch film was 5.01 MPa, whereas that of rice starch films crosslinked with 0.1, 0.3 and 0.5% EPI were, 4.70, 5.42, 7.99 MPa, respectively. The TS of rice starch films crosslinked with 1.0, 2.0 and 3.0% STMP were 6.30, 7.23, 8.23 MPa, respectively. The TS of rice starch films crosslinked with 4.0, 8.0 and

12.0% STMP/STPP were 5.84, 7.57 and 4.36 MPa, respectively.

The increase in TS is due to the increase of crosslinking density in starch films that is caused by the crosslinking reaction between hydroxyl groups and crosslinking agent resulting in crosslinked starches. Similar results were reported by Kim and Lee (2002) who reported that the TS of the films prepared with crosslinked corn starch was higher than that of untreated corn starch. In addition, Khan *et al.* (2006) reported that the TS of crosslinked sago starch/PVA blend films was higher than that of untreated sago starch/PVA blend films. The results point out that rice starch films crosslinked with 0.1% EPI provide relatively little improvement in TS, and hat of E shows an inverse trend. In addition, the TS was also slightly increased when the concentrations of STMP/STPP agents were increased. However, the TS of the films showed an inverse trend when 12.0% STMP/STPP was applied. This could be explained by the bulky molecules of STMP/STPP which coagulated and limited the crosslinking reaction in starch granules and the free volume was increased resulting in the decreasing of the TS value of the film. Figure 2(A) shows that using STMP and a mixture of STMP/STPP had a better action than others. Hence, the TS of the resulting films were increased, while EPI provided the least impact. Technically, the optimum concentration of a crosslinking agent is an important factor for improving the mechanical properties of crosslinked starch films. At low concentrations of the crosslinking agent there is not enough crosslinking between the starch molecules to improve the TS of the films. Whereas, at higher concentrations, there is excess crosslinking that limits the mobility of the starch molecules, leading to a lower TS (Yang *et al.*, 1996; Yang *et al.*, 1997).

Elongation at break (E) is an indication of the films flexibility and stretching ability (extensibility), which is determined by the point when the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen. Figure 2(B) shows the effect of type and concentration of crosslinking agents on the E of rice starch films. It was found that the E of untreated rice starch film was higher than all crosslinked rice starch films. In addition, increasing the crosslinking agents resulted in a decreasing of E from 116% to about 58%; E was dramatically decreased when using 0.5% EPI, 3.0% STMP and 8.0% STMP/STPP. Furthermore, the lowest E occurred when 0.5% EPI was applied. This was because the crosslinking reaction limited chain movement and strengthened the starch chain. The crosslinked rice starch films have greater rigidity than
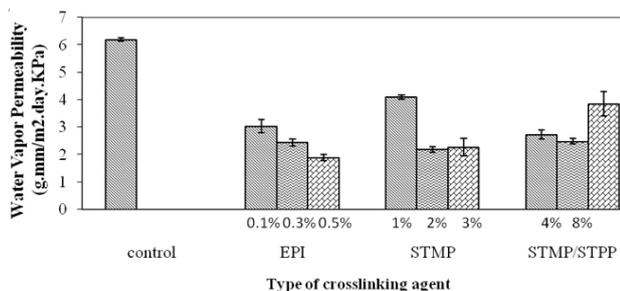
Figure 3. Effect of crosslinking agents on water vapor permeability (WVP) of rice starch films.
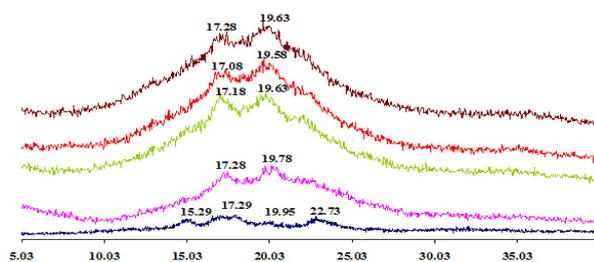

Figure 4. XRD patterns of rice starch powder, untreated rice starch films and crosslinked rice starch films (0.5 EPI, 3.0 STMP and 8.0 g/100 g STMP/STPP)

uncrosslinked films. In addition, Yin *et al.* (2005) also reported that starch–polyethylene films crosslinked with boric acid (0.5–2.5%) had a TS ranging from 8.9 to 10.1 MPa, whereas E decreased from 60% to 8% after crosslinking. A comparison between types of crosslinking agents on the E of rice starch films revealed that the type of crosslinking agent did not significant affect the E of the resulting films.

Water vapor permeability (WVP)

Starch is a biopolymer that is sensitive to moisture, which affects the mechanical properties of thermoplastic starch materials. Thus any improvement in decreasing moisture sensitivity and enhancing water resistance of thermoplastic starch material is valuable (Zhou *et al.*, 2008). Water vapor permeability (WVP) results can be useful to understand possible mass transfer mechanisms and solute and polymer interactions in biodegradable films. According to the thermodynamics of irreversible processes, the difference in water chemical potential is the driving force of water transfer though films. When the process occurs at a constant temperature and pressure the results of the water chemical potential difference are proportional to the water vapor concentration difference between the two faces (Morillon *et al.*, 2000; Bertuzzi *et al.*, 2007).

The WVP values of untreated rice starch and crosslinked rice starch films are shown in Figure 3. The crosslinked rice starch films showed superior water barrier properties compared with untreated rice starch films. The WVP of untreated rice starch films was 6.19 g.mm/m^2.day.KPa, whereas rice starch films crosslinked with 0.1, 0.3 and 0.5% EPI were 3.02, 2.44 and 1.89 g.mm/m^2.day.KPa, respectively. However, the rice starch films crosslinked with 1.0, 2.0 and 3.0% STMP were 4.08, 2.19 and 2.28 g.mm/m^2.day.KPa, respectively. In addition, rice starch films crosslinked with 4.0, 8.0 and 12.0% STMP/STPP were 2.72, 2.48 and 3.84 g.mm/m^2.day.KPa, respectively. These results could be because the decreasing of the WVP value by a hydrophilic group (OH$^-$) of rice starch decreased when crosslinking

reaction occurred.

The interaction between rice starch and the molecules of crosslinking agents limits water absorption by restricting the mobility of starch chains in the amorphous region (Gunaratne and Corke, 2007). In addition, Muñoz *et al.* (2003) reported that the WVP of glutenin-rich film values decreased by around 30% when formaldehyde (FA), glutaraldehyde (GTA) or glyoxal (GLY) were used as crosslinking agents. Figure 3 shows that the WVP of rice starch films crosslinked with EPI and STMP were slightly decreased when the concentrations of the crosslinking agents were increased. This is because the EPI and STMP are slow acting crosslinking agents and gradually penetrated into the starch granules and slowly induced the crosslinking reaction within these starch granules. The results demonstrate that the WVP of rice starch films crosslinked with the mixture of STMP/STPP dramatically decreased when the concentration of STMP/STPP increased from 4.0 and 8.0% STMP/STPP. However, the WVP value of the resulting films showed increases when 12.0% STMP/STPP was applied. This was because an excess concentration of STMP/STPP provided an increase in the bulky molecules and the free volume in the films resulted in an increase in the hydrophilic content of starch film. Chung *et al.* (2004) reported that corn starches crosslinked with STMP/STPP highly concentrated when measured at 10% concentration of the mixture of STMP/STPP.

XRD analysis

The XRD graphs of the individual components (rice starch powder, rice starch films and crosslinked rice starch films (0.5% EPI, 3.0% STMP and 8.0% STMP/STPP) were selected for XRD analysis. The results show that the gelatinized untreated rice, rice starch films and crosslinked rice films had different structures (Figure 4). The characteristic diffraction peaks of untreated rice starch powder were observed at 15.29º, 17.29º and 22.73º, and weak diffraction peaks at 19.95º. Rice starch films and crosslinked rice starch films were observed to be about 17.08º and

Table 3. Relative crystallinity of crosslinked rice starch films

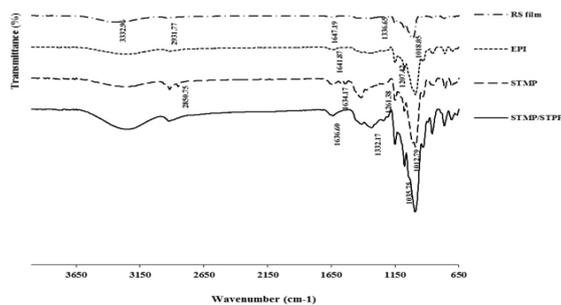| Type of starch	%Crystallinity (Area(peak)/Area(total)x100)
rice starch powder	23.19± 0.56
rice starch films	16.10± 0.70
0.5% EPI crosslinked rice starch films	15.67± 0.23
3.0% STMP crosslinked rice starch films	15.13± 0.15
8.0% STMP/STPP crosslinked rice starch films	14.79± 0.24

Figure 5. FTIR spectrum of untreated rice starch films (control), EPI, STMP and STMP/STPP crosslinked rice starch films.

19.58º. The XRD pattern described by Zobel (1964) and Kim and Lee (2002) showed that the untreated rice starch and the crosslinked rice starches belong to the A type. This result was in agreement with Xiao et al. (2011) who reported that A-type crystallinity is characterized by a well-defined peak at about 16.9-17.5 º (2θ).

The crystallinity values of rice starch films and crosslinked rice starch films are displayed in Table 3. A reduction of the % crystallinity of rice starch and crosslinked rice starch films can be observed. This was because the starch was gelatinized for forming the film and after gelatinization the crystallinity of the starch granules was destroyed (Zhang *et al.*, 2007) as well as their participation in crosslinking reactions (Das *et al.*, 2010). The results suggested that the crosslinking reaction decreased the crystallinity of rice starch films. This resulted from an increase in the density fluctuation as an increase of lattice defects introduced by crosslinking within the crystalline phase, or to the change in local density in the vicinity of the crosslinks in the amorphous phase (Chen and Yeh, 1991).

FT-IR analysis

FT-IR spectroscopy was used to verify the change in the chemical structure of starch molecules resulting from crosslinking reaction with EPI, STMP and STMP/STPP. FT-IR spectra of all films are shown in Figure 5. The broad band of rice starch films at 3332.96 cm^{-1} was the OH stretching. The peak at 2931.77 cm^{-1} corresponded to the C-H stretching vibration. Another characteristic absorption band occurred at 1018.05

Table 4. Glass transition temperatures and specific heat change values of untreated rice starch films (control) and EPI, STMP and TMP/STPP crosslinked rice starch films

Film samples	Transition temperature (ºC)			ΔCp (J/g* ºC)
	T_g onset	T_g midpoint	T_g endpoint	
rice starch films	23.33	25.60	27.00	0.001195
EPI crosslinked rice starch films	23.00	35.59	47.66	0.098895
STMP crosslinked rice starch films	21.04	29.45	37.33	0.074048
STMP/STPP crosslinked rice starch films	24.004	31.67	35.67	0.081237

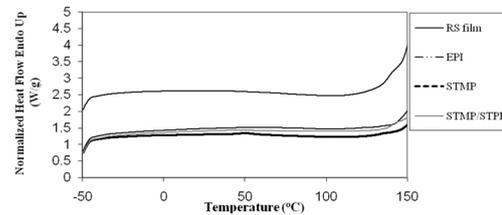Figure 6. DSC curves of untreated rice starch films (control) and EPI, STMP and TMP/STPP crosslinked rice starch films.

cm^{-1} and at about 990 cm^{-1} in crosslinked films, which was attributed to C–O stretching. One characteristic absorption band occurred at 1647.19 cm^{-1} which presumable originated from the tightly bound water present in the starch molecules. Rice starch films crosslinked with EPI demonstrated a weak intensity peak that should correspond to the ether stretch band at 1207.42 cm^{-1}. In STMP crosslinked rice starch films the peak was shown at 2850.75 and 1634.17 cm^{-1} which corresponds to the CH stretch attached to O and OH bending band and the peak at 1012.79 and 1261.38 cm^{-1} that corresponds to phosphate stretching (P-O-C) and vibration (P=O) (Singh and Nath, 2012), respectively. A peak at 1035.75 corresponded to phosphate stretching (P-O-C) was observed on in STMP/STPP crosslinked rice starch films.

Differential scanning calorimeter (DSC)

Differential scanning calorimeter (DSC) measurements were performed for untreated rice starch films (the control) and crosslinked rice starch films. All the samples were heated from -50 to 180ºC with a heating rate of 20ºC/min. The DSC traces are shown in Figure 6. The chemical crosslinking impacted on thermal properties of the resulting films. The results show that the glass transition temperature (T_g) of untreated rice starch film was 25.60ºC, while those of the EPI, STMP and STMP/STPP crosslinked rice starch films were 35.59ºC, 29.45ºC and 31.67ºC, respectively (Table 4). The increasing of the T_g was due to the crosslinking that occurred which resulted in decreased chain mobility in the film matrix. Ackar *et al.* (2010) reported that the T_g of wheat starch film was increased by modification in 0.1 and 0.3% EPI. It was noted that STMP/STPP and EPI crosslonked rice starch films had higher a T_g than STMP crosslinked rice starch films. These results are possibly because
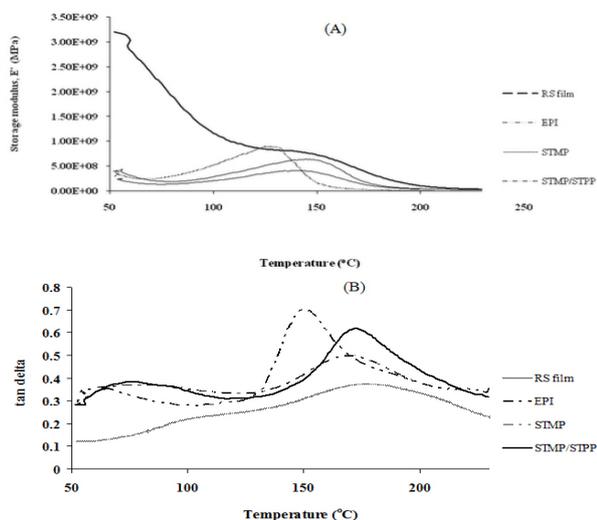
Figure 7. The dynamic mechanical behaviors of storage modulus (E') (A) and loss factor (tan d) (B) as a function of temperature for untreated rice starch films and crosslinked rice starch films.

the introduction of phosphate groups (STMP/STPP) and glycerol bonds (EPI) into starch tightened the molecular organization in the starch molecules, thus gelatinization is carried out at a higher temperature. Figure 6 depicts the decreasing of the endothermic heat flow of crosslinked rice starch films compared with untreated rice starch films. These results are in accordance with the research of Carmona-Garcia *et al.* (2009) and Jyothi *et al.* (2006). In addition, Woo and Seib (2002) and Deetae *et al.* (2008) also reported that crosslinked starches showed higher gelatinizing temperatures and lower gelatinizing enthalpies than their untreated counterparts. The decrease in the heat flow of crosslinked rice starch films may due to increases in the free volume in the starch chain in the presence of bulky groups of crosslinking agents. The increasing of T_g and the heat capacity change (ΔCp) (Table 4) suggests that the crosslinked rice starch was resistant to a higher temperature than the untreated rice starch films.

DMTA

The thermo-mechanical behavior of untreated rice starch films and crosslinked rice starch films was studied using a dynamic mechanical thermal analyzer (DMTA). Figure 7(A) shows the storage modulus (E') as a function of the temperature of rice starch films. It was found that the storage modulus of crosslinked rice starch films was lower than that of untreated rice starch films. For example, the storage modulus of untreated rice starch films and crosslinked rice starch films with 0.5% EPI, 3.0% STMP and 8.0% STMP/STPP, was about 3.02×10^9 MPa, 2.21×10^8 MPa, 3.01×10^8 MPa and 4.00×10^8 MPa, respectively. The storage modulus of chemical crosslinking was

decreased to twice that of untreated rice starch films. The decrease of the storage modulus was due to decreasing of structural symmetry and H-bonding. In addition, some covalent bonds were formed between the starch molecules (Ray *et al.*, 2009).

Figure 7(B) shows the tan d curve of untreated rice starch film and crosslinked rice starch films as a function of temperature. Usually the position of the tan d peak is used as the definition of transition temperature. The results show that the transition temperature of crosslinked rice starch film was slightly increased. It was observed that 8.0% STMP/STPP crosslinked rice starch films had the highest transition temperature. The increase in the transition temperature indicates that there were structural changes in the amorphous phase in starch. Normally, an increase in crosslinking density reduces the chain mobility and consequently increases the T_g. This effect can be understood in terms of decreasing free volume (Montserrat, 1995; Chung *et al.*, 2004). However, 3.0% STMP crosslinked rice starch films showed a decrease in the transition temperature. These results could be because the bulky molecules of crosslinking agent that remained in the starch molecules caused the increased free volume.

Conclusions

The properties of the rice starch films were improved by using crosslinking agents (EPI, STMP and STMP/STPP). The viscosity of crosslinked rice starch and the percentage of crystallinity decreased when the contents of the crosslinking agent increased concomitant with an increase in the pasting temperature and degree of crosslinking. Crosslink reactions resulted in increasing the TS, and the E and WVP of rice starch films. It was found that the glass temperature (T_g) of crosslinked rice starch films shifted to higher temperatures than in the untreated rice starch films. In addition, the storage modulus (E') of crosslinked rice starch films decreased two-fold compared with untreated rice starch films, and the tan d curve of crosslinked rice starch film was higher than in untreated rice starch films. These results suggest that treating rice starch films with chemical crosslinking can improve the properties of rice starch films.

Acknowledgments

References

Ackar, D., Babic, L., Subaric, D., Kopjar, M. and Milicevic, B. 2010. Isolation of starch from two wheat varieties and their modification with epichlohydrin. Journal of Carbohydrate Polymer 81: 76-82.

Arvanitoyannis, I. S. 2006. Biodegradable edible films and coatings based on protein resources: physical properties and applications in food quality management. In Mallapragada, S.K. and Narasim, B. (Eds). Handbook of Biodegradable Polymeric Materials and their Applications, p. 151-182. New York: American Scientific Publishers.

ASTM. 1993a. Standard practice for conditioning plastics and electrical insulating materials for testing: D618-61 (Reproved 1990). In: ASTM. Annual book of American Standard Testing Methods (Vol. 8.01, pp. 146-148). Philadelphia, PA.

ASTM. 1993b. Standard test method for water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor: D1249-90. In: ASTM. Annual book of American Standard Testing Methods (Vol. 15.09, pp. 1168-1172). Philadelphia, PA.

ASTM. 1995. Standard test methods for tensile properties of thin plastics sheeting D882-91. In: ASTM. Annual Book of American Standard Testing Methods. Vol.8.01. pp. 182-190. West Conshohochem. PA.

Ayoub, A. and Rizvi, S. S. 2007. Properties of supercritical fluid extrusion-based crosslinked starch extrudes. Journal of Applied Polymer Science 107: 3663-3671.

Bajpai, A. K. and Bhanu, S. 2007. Dynamics of controlled release of heparin from swellable crosslinked starch microspheres. Journal of Materials Science: Materials in Medicine 18: 1613-1621.

Bertuzzi, M. A., E. F. Vidaurre, C., Armada M. and Gottifredi, J. C. 2007. Water vapor permeability of edible starch based films. Food Engineering 80(3): 972-978.

Bourtoom, T. and Chinnan, M. S. 2008. Preparation and properties of rice starch-chitosan blend biodegradable film. LWT-Food Science and Technology 41: 1633-1641.

Carmona-Garcia, R., Sanchez-Rivera, M. M., Mendez-Montealvo, G., Garza-Montoya, B. and Bello-Perez, L. 2009. Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisiacal*). Journal of Carbohydrate Polymer 76: 117-122.

Chen, C. J. and Yeh, G. S. Y. 1991. Radiation-induced crosslinking: III. Effect on the crystalline and amorphous density fluctuations of polyethylene. Colloid and Polymer Science 269(4): 353-363.

Chiou, Bor-S., Glenn, G. M., Imam, S. H., Inglesby, M. K.,

Wood, D. F. and Orts, W. J. 2005. Starch Polymers: Chemistry, Engineering, and Novel Products. In Mohanty, A. K., Misra, M. and Drzal., L. T. (Eds). Natural Fibers, Biopolymers, and Biocomposites: p. 35-68. New York: Taylor & Francis.

Chung, H. J., Woo, K. S. and Lim, S. T. 2004. Glass transition and enthalpy relaxation of cross-linked corn starches. Carbohydrate Polymers 55: 9–15.

Das, K., Ray, D., Bandyopahyay, N. R., Gupta, A., Sengupta, S., Sahoo, S., Mohanty, A. and Misra, M. 2010. Preparation and Characterization of Cross-Linked Starch/Poly(vinylalcohol) Green Films with Low Moisture Absorption. Journal of Industrial Engineering Chemistry Research 49: 2176-2185.

Deetae, P., Shobsngobm S., Varanyanond, W., Chinachoti, P, Naivikul, O. and Varavinit, S. 2008. Preparation, pasting properties and freeze-thaw stability of dual modified crosslink-phosphorylated rice starch. Journal of Carbohydrate Polymer 73: 351-358.

El-Tahlawy, K., Venditti, R. A. and Pawlak, J. J. 2007. Aspects of the preparation of starch microcellular foam particles crosslinked with glutaraldehyde using a solvent exchange technique. Carbohydrate Polymers 67(13): 319-331.

Funke. U., Bergthaller, W. and Lindhauer, M. G. 1998. Processing and characterization of biodegradable products based on starch. Polymer Degradation and Stability 59: 293-296.

Garcia, M. A., Martino, M. N. and Zaritzky, N. E. 2000. Microstructural characterization of plasticized starch-based films. Starch/Starke 52: 118–124.

Gerngross, T., Slater, S. and Gross, R. A. 2003. Biopolymers and the Environment. Science Magazine 7: 822-825.

Gunaratne, A. and Corke, H. 2007. Functional properties of hydroxypropylated cross linked, and hydroxypropylated cross linked tuber and root starches. Cereal Chemistry 84: 30-37.

Hirsch, J. B. and Kokini, J. L. 2002. Understanding the mechanism of cross linking agents (POCl3, STMP and EPI through swelling behavior and pasting properties of crosslinked waxy maize starches. Cereal Chemistry 79: 102-107.

Hulleman, S. H. D., Janssen, F. H. P. and Feil, H. 1998. The role of water during plasticization of native starches. Polymer 39(10): 2043-2048.

Jyothi, A. N., Moorthy, S. N. and Rajasekharan, K. N. 2006. Effect of cross-linking with epichlohydrin on the properties of cassava (*Manihot esculenta* Crantz) starch, Starch/Starke 58: 292-299.

Kaur, L., Singh, J. and Singh, N. 2006. Effects of cross-linking on some properties of potato starches. Journal of Science Food Agricultural 86: 1945-1954.

Khan, M. A., Bhattacharia, S. K., Kader, M. A. and Bahari, K. 2006. Preparation and characterization of ultra violet (UV) radiation cured bio-degradable films of sago starch/PVA blend. Journal of Carbohydrate Polymer 63: 500-506.

Kim, M. and Lee, S. J. 2002. Characteristics of crosslinked potato starch and starch-filled linear low density polyethylene films. Journal of Carbohydrate Polymer

50: 331-337.

Koo, S. H., Lee, K. Y. and Lee, H. G. 2010. Effect of cross-linking on the physicochemical and physiological properties of corn starch. Food Hydrocolloids 24: 619-625.

Kuhn, W., Peregi, E., Fei, Z., Giese, U., Schuster, R. and Zhao, S. 2006. Network Dynamics of Crosslinked Polymers Crosslinking, Filler and Aging Characterized by NMR Parameters. World Polymer Congress 41: 1-4.

Kuniak, L. and Marchessault, R. H. 1973. Study of the crosslinking reaction between epichlorohydrin and starch. Die stärke 24: 110-116.

Lim, S. and Seib, P. A. 1993. Location of phosphate ester in a wheat starch pjhosphate by 31-P NMR spectroscopy. Cereal Chemistry 70(2): 146-152.

Lu, Y., Weng, L. and Cao, X. 2005. Biocomposites from plasticized starch and cellulose crystallites from cotton seed linter. Macromolecular Bioscience 68; 251-260.

Ma, X. F. and Yu, J. G. 2004. The plasticizers containing amide groups for thermoplastic starch. Carbohydrate Polymers 57: 197–203.

McHugh, T. H., Avena-Bustillos, R. and Krochta, J. M. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. Journal of Food Science 5: 899-903.

Montserrat, S. 1995. Effect of crosslinking density on ΔCp (Tg) in an epoxy network. Polymer 36: 435–436.

Morillon, V., Debeaufort, F., Blond, G. and Voilley, A. 2000. Temperature influence on moisture transfer through synthetic films. Membrane Science 168: 223–231.

Muhammad, K., Hussin, F., Man, Y. C., Ghazali, H. M. and Kennedy, J. F. 2000. Effect of pH on phosphorylation of sago starch. Carbohydrate polymer 42: 85-89.

Muñoz, P. H., Villalobos, R. and Chiralt, A. 2003. Effect of crosslinking using aldehydes on properties of glutenin-rich films. Food Hydrocolloids 18: 403-411.

Nakamura, E. M., Cordi, L., Almeida, G. S. G., Duran, N. and Mei, L. H. I. 2005. Study and development of LDPE/starch partially biodegradable compounds. Materials Processing Technology 162–163: 236–241.

Ray, D., Roy, P., Sengupta, S., Sengupta, S. P., Mohanty, A. K. and Misra, M. 2009. A Study of Dynamic Mechanical and Thermal Behavior of Starch/Poly(vinylalcohol) Based Films. Polymer Environment 17: 49–55.

Seow, C. C., Cheah, P. B. and Chang, Y. P. 1999. Antiplasticization by water in reduced-moisture food systems. Food Science 64: 576-581.

Singh, A.V. and Nath, L. K. 2012. Synthesis and evaluation of physic-chemical properties of cross-linked sago starch. Biological Macromolecule 50: 14-18.

Wattanachant, S., Muhammad, K., Hashim, D. M. and Rahman, R. A. 2003. Effect of crosslinking reagents and hydroxylpropylation levels on dual-modified sago starch properties. Food Chemistry 80: 463–471.

Wongsagonsup, R., Shobsngobm S., Oonkhanond, B. and Varavinit, S. 2005. Zeta potential and pasting properties of phosphorylated or crosslinked rice starches. Starch/Starke 57: 819-825.

Woo, K. S. and Seib,P. A. 2002. Cross-linked resistant starch: preparation and properties. Cereal Chemistry 79: 819-825.

Wurzburg, O. B. 1986. Cross-linked starches. In: Wurzburg, O.B., editor. Modified starches: Properties and uses. p.41-53. Florida: CRCPress.

Xiao, H., Lin, Q., Liu, G-Q., Wu, Y., Tian, W., Wu, W. and Fu, X. 2011. Effect of green tea polyphenol on the gelatinization and retrogradation of rice starches with different amylase contents. Journal of Medicinal Plants Research 5(17): 4298-4303.

Xiong, H. G., Tang, S. W., Tang, H. L. and Zou, P. 2008. The structure and properties of a starch-based biodegradable film. Carbohydrate Polymers 71: 263–268.

Xu, X. Y., Kim, K. M., Hanna, M. A. and Nag, D. 2005. Chitosan-starch composite film: preparation and characterization. Industrial Crops and Products 21: 185-192.

Yang, C. Q., Wang, X. and Kang, I. 1997. Ester cross-linking of cotton fabric by polymeric carboylic acids and citric acid. Journal of Textile Research 67(5): 334-342.

Yang, Y., Wang, L. and Li, S.J. 1996. Formaldehyde- free zein fiber-preparation and investigation. Journal of Applied Polymer Science 59: 433-441.

Yin, Y., Li, J., Liu, Y. and Li, Z. 2005. Starch cross-linked with poly(vinyl alcohol) by boric acid. Journal of Applied Polymer Science 96: 1349-1397.

Zhang, Q., Yu, Z., Xie, X., Naito, K. and Kagawa, Y. 2007. Preparation and crystalline morphology of biodegradable starch/clay nanocomposites. Polymer 48: 7193-7200.

Zhou, J., Zhang, J., Ma, Y. and Tong, J. 2008. Surface photo-crosslinking of corn starch sheets. Carbohydrate Polymer 74: 405–410.

Zobel, H. F. 1964. X-ray analysis of starch granule starches. In Whistler, R. L. (Ed). Methods in Carbohydrate Chemistry, p. 109-113. New York: Academic Press.