



Original Article

Plasticizer effect on the properties of biodegradable blend film from rice starch-chitosan

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Abstract

The properties of biodegradable blend film from rice starch-chitosan with different plasticizers were determined. Three plasticizers comprising sorbitol (SOR), glycerol (GLY) and polyethylene glycol (PEG) were studied over a range of concentration from 20 to 60%. Increasing concentration of these plasticizers resulted in decreased tensile strength (TS) concomitant with an increase in elongation at break (E), water vapor permeability (WVP) and film solubility (FS). SOR plasticized films were the most brittle, with the highest tensile strength (TS, 26.06 MPa). However, its effect on WVP was low (5.45 g.mm/m².day.kPa). In contrast, GLY and PEG plasticized films had a flexible structure contradictory to a low TS (14.31MPa and 16.14MPa, respectively) providing a high WVP (14.52 g.mm/m².day.kPa and 14.69 g.mm/m².day.kPa, respectively). SOR plasticized films, demonstrated little higher FS compared to PEG and GLY plasticized films but not significant different ($p < 0.05$). The color of biodegradable blend film from rice starch-chitosan was more affected by the concentration of the plasticizer used than by its type. Nine moisture sorption models were applied to experimental data. Moisture content of the film increased at elevated water activity. The time to reach equilibrium moisture content (EMC) was about 20-24 days at lower humidity and 13-16 days at higher humidities. The EMC of glycerol and sorbitol rice starch-chitosan biodegradable blend films showed a logarithmic increase at above 0.59 a_w and reached the highest moisture content of 51.46% and 42.97 % at 0.95 a_w , whereas PEG rice starch-chitosan biodegradable blend films did not show much increase in moisture content.

Key words: Rice starch; chitosan; biodegradable blend film; plasticizer; moisture sorption

1. Introduction

The last 10 years have seen an explosion in the level of research devoted to the development of new biodegradable materials, essentially due to the desire to protect the environment. The growing demand for biodegradable polymers by emerging technologies including tissue engineering and regenerative medicine, gene therapy, novel drug delivery systems, implantable devices and nanotechnology has resulted in the development of a range of biodegradable polymers, mainly based on already known chemistry (Heller *et al.*, 2003). Polysaccharides such as starch and cellulose

have been studied in detail (Guilbert and Biquet 1996). These renewable biopolymers can also be used for the manufacture of materials, adhesives, coating or glazing agents and emulsifiers (Kolster *et al.*, 1997). Such films alone or combination are of interest since they have potential to improve food shelf life and quality and protect food from deterioration by microorganisms and physical damage (Sothornvit and Krochta, 2001).

Starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barrier in packaging materials. Starch often used in industrial foods. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, and it has good mechanical properties (Xu *et al.*, 2005).

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However, wide application of starch film is limited by its mechanical properties and efficient barrier against low polarity compounds (Azeredo *et al.*, 2000; Kester and Fennema, 1986). In order to overcome its shortcoming, Bourtoom and Chinnan (2007) blend rice starch with different chitosan to increase the tensile strength. A major component of biodegradable films is the plasticizer, as well as the film-forming polymer. The addition of a plasticizer agent to biodegradable blend films is required to overcome film brittleness, caused by high intermolecular forces. Plasticizers are generally small molecules such as polyols like sorbitol, glycerol and polyethylene glycol (PEG) that intersperse and intercalate among and between polymer chains, disrupting hydrogen bonding and spreading the chains apart, which not only increases flexibility, but also water vapor and gas permeabilities (Gontard *et al.*, 1993; Sorbal *et al.*, 2001; Baldwin and Banker, 2002). In previous work by the authors (Bourtoom, 2007), biodegradable blend film from rice starch-chitosan was determined. However, when no plasticizer was introduced in the film solution, the biodegradable blend film was relatively brittle. The brittleness of the films was most determined by the strength of polymer-polymer interaction, which was controlled by the polymer chemistry and addition of plasticizer. Information on the effects of sorbitol, glycerol and PEG on biodegradable blend film from rice starch- chitosan is poorly available at present. The aim of this investigation was to make a comparative study of different types of plasticizers and their concentrations incorporated into biodegradable blend film from rice starch-chitosan.

2. Materials and Methods

2.1 Materials

Native rice starch (Thai Flower brand) used as obtained from Bangkok Starch Industrial Co. Ltd. The general specifications are as follows: 14.0% moisture content, 0.5% ash, 0.2% fat, 0.35% protein, pH value of 4.5-7.0 and peak viscosity (8% solid) of 500 BU (Brabender unit). Commercial grade- chitosan flake (approx. 85% degree of deacetylation) with average molecular weight of about 75KDa was purchased from Bona Fides Marketing Co. Ltd. (Thailand). Commercial grade glycerol, polyethylene glycol (PEG- 400) and sorbitol were obtained from Vidyasom Co. Ltd. (Thailand).

2.2 Film preparation

Starch solution with concentration of 2% (w/v) were prepared by dispersing rice starch, in distilled water and heating the mixtures on hot plates with stirring until they gelatinized (85°C for 5 min), and then cooling to 27±2°C. The chitosan solutions (2%, w/v) were prepared by dispersing 20 g of chitosan (deacetylated degree of 85%; Sigma-Aldrich Chemie, Damstadt, Germany) in 1000 ml of acetic

acid (1%, v/v; Sigma-Aldrich Chemie, Damstadt, Germany). After the chitosan dissolved completely, the solution was filtered through a polyester screen (mesh no. 140 with mesh opening of 106 µm) by vacuum aspiration to remove any small lumps in the solution. While pulling a vacuum with an aspirator, the vacuum flask containing the chitosan solutions were sealed with a rubber stopper and held for a few hours to de-gas the solution to prevent the formation of air bubbles in the films when the casting solvent evaporated. A starch/chitosan blend film was prepared by mixing 100 ml of 2% starch solutions with 100 ml of 2% chitosan solution. Sorbitol, glycerol or polyethylene glycol was added in the giving concentration (20, 40 and 60% of the total solid weight). 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 ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60% RH for further testing. All treatments were made in triplicate.

2.3 Film Testing

Conditioning. All films were conditioned prior to subjecting them to permeability and mechanical tests according to Standard method, D618-61 (ASTM, 1993a). Films used for testing water vapor permeability (WVP), tensile strength (TS) and elongation (E) were conditioned at 60% RH and 27±2°C by placing them in a desiccators over a saturated solution of Mg (NO₃)₂ ·6H₂O for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

Film thickness. 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. Mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

Film Solubility. The method taken 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 into 20 ml of distilled water in 50 ml screw cap tubes containing 0.01g/100 g sodium benzoate. 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 use later in protein solubility tests as described later. The remaining solution and film piece were poured onto (Whatman #1) qualitative filter paper, rinsed with 10 ml distilled water, and dried at 70°C in a vacuum oven for 24 h to determine the dry mass of film. Five measurements were taken for each treatment triplicate. Total soluble matter was calculated from the initial gross mass and final dry mass using the following equation:

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

Water Vapor Permeability (WVP). The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh *et al.*, 1993) was used to determine the WVP of films. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the film. A sample was placed in between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hannau, 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 desiccant. The water vapor transmission rates (WVTR) of each film was measured at 60 ± 2 % RH and 25 ± 2 °C. After taking initial weight of the test cup, it was placed into a growth chamber with an air velocity rate of 450 ft/min (Model KBF115, Contherm Scientific, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001g with an electronic scale (Sartorius Corp.) every 3 h for 18 h. A plot of 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 time (g/h). 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 film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

Tensile Strength and Elongation at Break (TS and E). Tensile strength was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) as per ASTM D882-91 Standard Method (ASTM, 1993b). Ten samples, 2.54 cm x 12 cm, were cut from each film. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by initial specimen cross-sectional area, and percent elongation at break was calculated as follows:

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

Where, d was the distance between grips holding the specimen before or after the break of the specimen.

Color. A CIE colorimeter (Hunter Associates Laboratory, Inc., VA, USA) was used to determine the film L*, a* and b* color value (L* = 0 (black) to 100 (white); a* = -60 (green) to +60 (red); and b* = -60 (blue) to +60 (yellow)). Color (means of five measurements at different locations on each specimen) was measured on 10 cm X 10 cm segment of film.

Prior to taking color measurements, film specimens were pre conditioned at 60% RH and 27 ± 2 °C for 72 h.

2.4 Moisture sorption studies

Water sorption isotherms were determined by placing rice starch- chitosan biodegradable blend films into a

controlled humidity environment at a constant temperature until equilibrium. After drying at 75°C and < 1 mmHg pressure for 24 h, EYELA™, Model VOS-300VD, Japan), the films were placed into environments of various relative humidities above salt solutions in desiccators. The relative humidities were 11% RH (lithium chloride), 23% RH (potassium acetate), 33% RH (magnesium nitrate), 43% RH (potassium carbonate), 52% RH (magnesium nitrate), 59% RH (sodium bromide), 75% RH (sodium chloride), 85% RH (potassium chloride), and 95% RH (disodium hydrogen phosphate). The sorption experiments were carried out by keeping approximately 1,000 mg of blend films (2 cm x 2 cm) in desiccators, removing at frequent intervals and weighing until they reach constant weight (within ± 5 %). All chemicals were of analytical grade (AR).

2.5 Statistical Analysis

A completely randomized experimental design was used to study the plasticizer effect on the properties of biodegradable rice starch-chitosan blend films. Analysis of variance (ANOVA) was used to determine the effect of plasticizer. If differences in mean existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

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

Previous work demonstrated that the unplasticized biodegradable blend films from rice starch- chitosan were relatively brittle and needed to be handled very carefully. Hence, biodegradable blend films from rice starch-chitosan were prepared by using three types of plasticizers (sorbitol, glycerol and polyethylene glycol) at different concentrations. The mechanical properties of films plasticized by sorbitol, glycerol or polyethylene glycol were assessed by measuring their tensile strength (TS) and elongation at break (E) for three plasticizer concentrations. The results showed that an increase in the concentration of these plasticizers yielded a decrease in TS. TS decreased from 26.60 to 10.66 MPa, 14.31 to 2.52 MPa and 16.14 to 8.28 MPa when sorbitol, glycerol and polyethylene glycol increased from 20% w/v to 60% w/v, while E increased from 7.36 to 67.22%, 11.00 to 93.60% and 14.95 to 46.41%, respectively (Figure 1). The changes in mechanical properties of characterized by the plasticizers weakening the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant (Sorbal *et al.*, 2001). Thus the increase in the plasticizer concentration causes a reduction of the TS due to the decrease in the intermolecular interactions. Besides, the increase in the plasticizer concentration increases the moisture content of the film because of its high hygroscopic character, which also contributes to the reduction of the forces

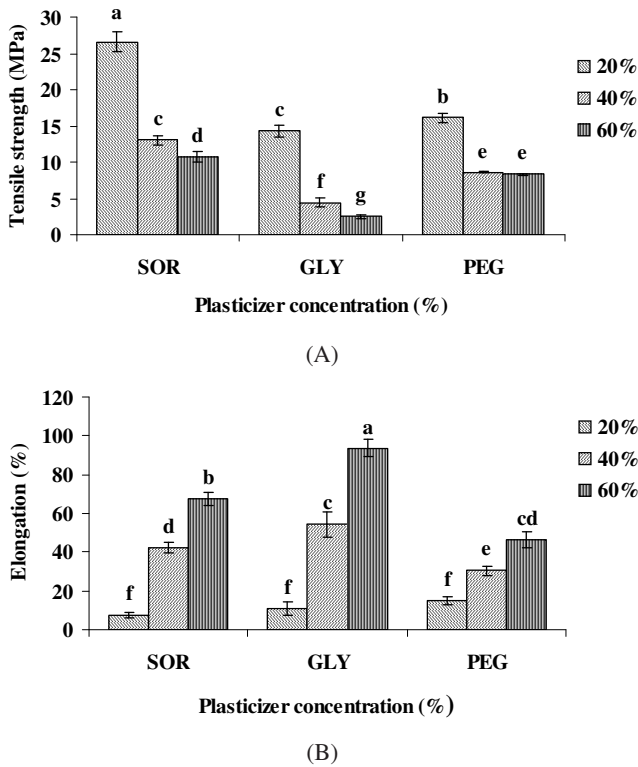


Figure 1. Effect of plasticizer type and concentration on (A) the tensile strength and (B) elongation at break of biodegradable blend films from rice starch-chitosan. Mean values with different letters are significantly different ($p < 0.05$).

between the adjacent macromolecules (Sorbal *et al.*, 2001). The effect of plasticizer on reduction of the mechanical properties is well known and its explanation is reported by some researchers (Cuq *et al.*, 1997; Gontard *et al.*, 1993 and McHugh and Krochta, 1994). Gontard *et al.* (1993) observed a linear reduction of the puncture force in gluten films, from 1.9 N to 0.3 N between 19 and 49 % glycerin. Cuq *et al.* (1997) also observed a linear reduction of the puncture force of edible films based on myofibrillar proteins of Atlantic sardine from 5.1 to 2.6N, between 0 and 40 g of glycerol/100 g of protein. The mechanical properties of sorbitol, glycerol and PEG plasticized films at an equal concentration were compared (Figure 1). The sorbitol plasticized films had significantly higher TS and lower E than glycerol and polyethylene glycol plasticized films. These results might be explained by the ring structure of sorbitol, which lead to difficult to interact between efficiently rice starch-chitosan molecules resulting in less effectiveness in disrupting the rice starch- chitosan interruptions (Yang and Paulson, 2000). Furthermore, the low water-attracting ability of sorbitol limits its ability to reduce rice starch- chitosan- chain hydrogen bonding compared to the straight-chain polyethylene glycol and glycerol of similar molecular weight. The glycerol plasticized films were less mechanically resistant but had higher stretchability than the polyethylene glycol plasticized films (Figure 1). This suggests that glycerol could be more

effective plasticizer in biodegradable blend film from rice starch-chitosan than polyethylene glycol. The effectiveness of glycerol in biodegradable blend films from rice starch-chitosan is most likely due to its small size which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the mechanical properties than the larger polyethylene glycol molecule. Donhowe and Fennema (1993) found that plasticizer with low mole-cular weights such as glycerol and polyethylene glycol 400 was more effective than those with high molecular weights in methylcellulose-based films. Similarly, McHugh and Krochta (1994) suggested that, due to its small size, glycerol was more effective than sorbitol in plasticizing whey protein films. In addition, at an equal percentage concentration, the total number of glycerol molecules in the film solution is greater than that of the higher molecular weight polyethylene glycol and therefore glycerol has more functional groups (-OH) than polyethylene glycol which should promote the plasticizer-polymers interactions in the films (Donhowe and Fennema, 1993; McHugh and Krochta, 1994). Yang and Paulson (2000) reported that the molecular size, configuration and total number of functional hydroxide groups of the plasticizer as well as its compatibility with the polymer could affect the interactions between the plasticizer and the polymer.

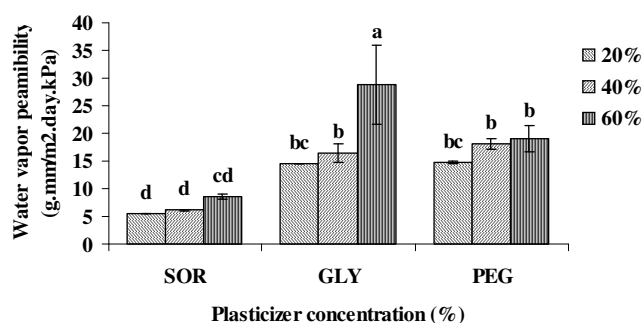
3.2 Water vapor permeability (WVP)

Water vapor permeability of biodegradable blend films with different type and concentrations of plasticizer were examined at a vapor pressure difference of 0/60%RH across the film. Figure 2 shows that the film water vapor permeability increased with increasing plasticizer in the films. The water vapor permeability of biodegradable blend film from rice starch-chitosan increased from 5.45 g.mm/m².day.kPa to 8.68 g.mm/m².day.kPa, 14.53 g.mm/m².day.kPa to 28.73 g.mm/m².day.kPa and 14.70 g.mm/m².day.kPa to 19.10 g.mm/m².day.kPa when sorbitol, glycerol and polyethylene glycol concentration increased from 20% to 60%, respectively. This tendency could be explained by structural modifications of the polymer network. The network may be come less dense because of an increase in the mobility of the polymeric chains and in the free volume of the film. These consequences of the plasticizing action are favorable to the adsorption or desorption of water molecules (Gontard *et al.*, 1993). Furthermore, the increase of water vapor permeability might be related to the hydrophilicity of all tested plasticizers. It is well known that the presence of plasticizers increases the concentration of polar residues in hydrocolloid based film and, thus consequently the solubility of water (Gontard *et al.*, 1993). Since permeability is defined as the product of the diffusion constant and the solubility coefficient, the water vapor permeability is substantially increased. Glycerol plasticized films provided the films with higher water vapor permeability than polyethylene glycol and sorbitol at each plasticizer concentrations (Figure 2)

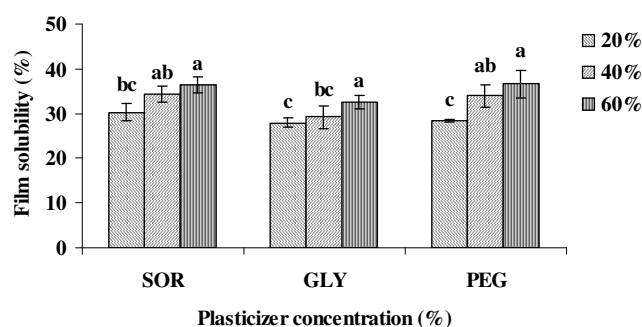
which should be the result of the high hydrophilicity of the glycerol molecule, which is favorable to the adsorption of water molecules and could also contribute to the increase in the film water vapor permeability (Gennadios *et al.*, 1993). Additionally, at high glycerol concentration, glycerol could cluster with itself to open the polymer structure, enhancing the permeability of the film to moisture (Lieberman and Gilbert, 1973). An increase in inter chain spacing due to inclusion of glycerol molecules between the polymer chain may promote water vapor diffusivity through the film and hence accelerate the water vapor transmission (Yang and Paulson, 2000).

3.3 Film solubility (FS)

From visual observations and irrespective of plasticizer type and concentration, the biodegradable blend films from rice starch-chitosan were clearly not dispersed without visual loss of integrity after a 24 h immersion in water. Irrespective of plasticizer type, an increase in its concentration provided an increase in film solubility (Figure 2). Film solubility increased from 30.19% to 36.46%, 27.93% to 32.54% and 28.45% to 36.54% when sorbitol, glycerol and polyethylene glycol increased from 20% to 60%, respectively. The solubility values obtained in this work were



(A)



(B)

Figure 2. Effect of plasticizer type and concentration on (A) the water vapor permeability and (B) film solubility of biodegradable blend films from rice starch-chitosan. Mean values with different letters are significantly different ($p < 0.05$).

relativity lower than those obtained with films based on cellulose (55-84%) or carrageen (41%) (Rhim *et al.*, 1997) but higher than those obtained with fish myofibrillar proteins (Monterrey and Sorbal, 2000). An increase of film solubility with increasing plasticizer concentration could be briefly explained by hydrophilic plasticizers enhancing film solubility in water. The dry matter solubilized in water is likely to be constituted mainly by the plasticizer. Plasticizer solubilization in water was already observed for film based on wheat gluten or treated soy proteins or produced by transglutaminase catalytic cross-linking of whey protein (Gontard *et al.*, 1992; Stuchell and Krochta, 1994). Stuchell and Krochta (1994) have pointed out an increase in the content of protein solubilized in water when the hydrophilic content increased for treated whey protein- and soy protein-based films. A decrease in the polymer network interaction density due to the plasticizer presence was thus associated with this increase in solubility properties. On the other hand, Marquet *et al.* (1995) have displayed a large decrease in the content of dry matter solubilized in water for cottonseed protein-based reticulated films when glycerol content increased. It was observed that sorbitol plasticized films showed little higher film solubility than polyethylene glycol and glycerol but not significantly different ($p > 0.05$) (Figure. 2). It might be explained that sorbitol had a ring molecular conformation which may sterically hinder insertion between the chain of polymers resulting in an easy escape into the solution, while polyethylene glycol and glycerol have straight chains, which are inserted and positioned within the tri-dimensional polymer network (Yang and Paulson, 2000).

3.4 Film Color

The results of the measurements performed on the films were expressed in accordance with the Hunter system, and the rectangular coordinates (L^* , a^* and b^*) were defined. The color of biodegradable blend film from rice starch-chitosan was more affected by the concentration of the plasticizer used than by its type. L^* and a^* values of biodegradable blend film from rice starch-chitosan plasticized by sorbitol, glycerol and polyethylene glycol were not significantly different ($p < 0.05$, Figure 3). However, increased yellowness (high $+ b^*$) occurred when higher all these plasticizers were used (Figure 3). This was somewhat expected since color change is attributed to the concentration of plasticizer.

3.5 Moisture sorption properties

The relationship between a_w and moisture content (at constant temperature) is described by moisture isotherm. Moisture content of the film increases at elevated water activity (a_w). The time to reach equilibrium moisture content (EMC) was about 20-24 days at lower humidity and 13-16 days at higher humidities. The sorption isotherm curves for EMC (db) obtained from different type of rich starch-chito-

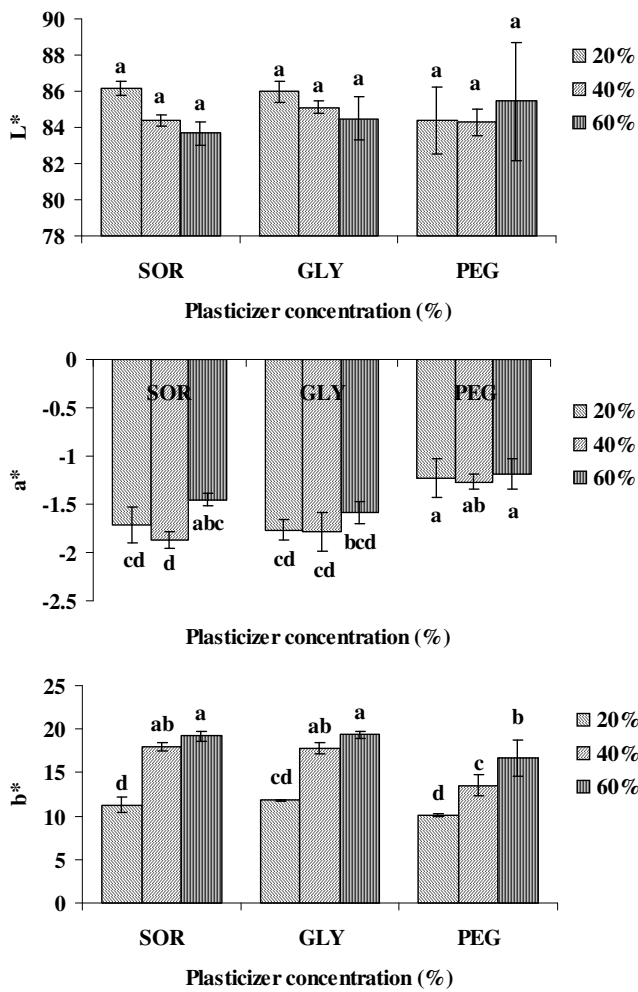


Figure 3. Effect of plasticizer type and concentration on (A) the L* value, (B) a* value and (C) value of biodegradable blend films from rice starch-chitosan. Mean values with different letters are significantly different (p<0.05).

san plasticizer blends are shown in Figure 4. At lower a_w the slope of the curve was less; with increase in a_w the slope increased rapidly. Considering the individual rice starch-chitosan biodegradable blend films, some mould growth was observed by visual inspection at edges and surfaces at the end of the storage period in plasticizer biodegradable blend films, but in native rich starch-chitosan less number of colonies were observed, may be due to less moisture holding capacity of such films. Experimental data for moisture adsorption at $27 \pm 2^\circ\text{C}$ revealed sigmoid shape curves for all. The EMC of glycerol and sorbitol blend films showed logarithmic increase at above 0.59 a_w and reached to highest moisture content of 51.46% and 42.97% at 0.95 a_w , whereas PEG rice starch-chitosan biodegradable blend films did not show much increase in moisture content, at 0.59 a_w , the uptake of moisture was more compared to other films, and this showed a linear increasing trend up to 0.75 a_w , but later no exponential increase was observed.

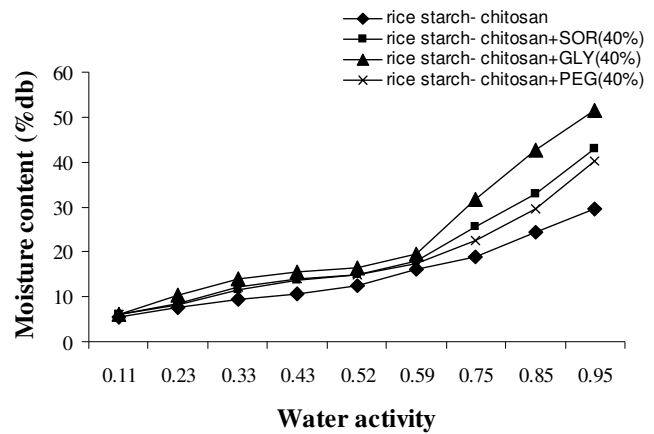


Figure 4. Sorption isotherm of rice starch-chitosan biodegradable blend films

4. Conclusions

The results of this study demonstrate that sorbitol plasticized films provided the films with highest mechanical resistance, but the poorest film flexibility. In contrast, glycerol and polyethylene glycol plasticized films exhibited flexible structure; however, the mechanical resistance was low, while inversely affecting the water vapor permeability. Increasing the plasticizer concentration decreased TS with concomitant with an increase E, WVP and FS. Type and concentration of plasticizers affected the film solubility. Increasing the plasticizer concentration resulted in higher solubility. Sorbitol plasticized films demonstrated little higher FS compared to polyethylene glycol and glycerol plasticized films. The color of biodegradable blend film from rice starch-chitosan was more affected by the concentration of the plasticizer used than by its type.

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