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Effect of concentration of chitosan on the mechanical, morphological and optical properties of tapioca starch film

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Abstract

Recently, incorporation of natural filler such as chitosan into edible film to produce food packaging material is of particular interest due to the improvement in the properties of the film such as mechanical, morphological and optical properties of the edible film. In this work, tapioca starch-based edible film was prepared with different concentrations of chitosan; 0, 20, 40, 60, 80% w/w of dry starch solid weight. The effects of varying the chitosan concentration on the mechanical properties (tensile strength (TS), elongation at break (EAB), and Young modulus (YM)), morphological and optical properties (color and transparency) of the edible films were investigated. There was 492% increment in TS value of the edible films when chitosan was added up to 60% w/w (21.23 MPa) compared to neat starch film (3.58 MPa). The 60% w/w chitosan films exhibited smooth and even structure which indicated both chitosan and tapioca starch compounds were blended homogeneously. However, 60% w/w chitosan film exhibited very high YM value (2842.5 MPa) and low EAB value (2.46%) which might limit its application. Effects of chitosan concentration on optical properties could be considered negligible. The improvements in TS and morphological structure of the edible films will be beneficial for the potential application of film as food packaging material in which high TS is important to protect the structure of food and while morphological structure can be related to the aesthetical appearance of the food packaging materials.

Introduction

For many years ago, conventional plastic has been made up from the polymerization of petroleum which is a non-renewable source. Exploitation of petroleum source to produce packaging material has contributed to the pollution, toxicity and non-degradable rubbish on our land. Nowadays, potential biopolymers such as starch, cellulose, chitosan and gelatin (Othman, 2014) have attracted much interest among researchers due to their natural properties such as biodegradable, renewable and contain an active compound that can act as antibacterial agent, antioxidant and biosensor (Imran *et al*., 2010).

Among the biopolymers, starch is one of the most popular alternative materials used for food packaging application due to its easy availability, cheap, biodegradable and renewable (Jimenez *et al*., 2012; Bof *et al*., 2015). Tapioca starch is favorable compared to other starches because it is the cheapest raw material of starch (Bangyekan *et al*., 2006) and consistently available in the market (Santacruz *et al*., 2015). It can be easily casted into the edible film with the addition of glycerol. Tapioca starch film is more flexible and exhibits lower gas permeability than the films produced from other types of starches (Parra *et al*., 2004). However, starch-based edible film material exhibited poor mechanical properties, thus limiting its applications in food packaging (Bangyekan *et al*., 2006). The starch film can be blended with other natural biopolymers such as chitosan to improve these drawbacks. Chitosan has attracted interest from many researchers due to its special ability such as being a reinforcing agent to improve tensile strength, biocompatibility, biodegradable and antimicrobial properties (Mathew and Abraham, 2008; Dutta *et al*., 2009). Incorporation of chitosan into the edible film also can inhibit bacteria growth which ensures food safety and prolongs the shelf life of food.

Raw solid chitosan needs to be diluted into gel solution using aqueous acetic acid before blended into starch. Hydrogen ions (H^+) from acetic acid are attracted to the amino groups (NH_2) of chitosan thus easy to form the intermolecular hydrogen bonds with OH^-

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group in the starch molecules (Ashori and Bahrami, 2014). Intermolecular bonding is formed when two different molecules are attracted to each other. It is important to ensure that chitosan and starch are well mixed, so that their good affinity can form a rigid structure of the film (Salaberria *et al*., 2014). The combination of hydrogen bonding, opposite charge attraction between OH^- of starch molecules and NH_3^+ of chitosan provides strong adherence between starch and chitosan molecules, thus ensuring the stability of two different compounds in the film (Bangyekan *et al*., 2006).

Several studies have explored the effects of chitosan on starch film as well as their mechanical and water vapor permeability properties. Chillo *et al*. (2008) had studied the effect of chitosan on the mechanical and water vapor permeability properties of tapioca starch-based film. He reported that tensile strength value increased with the increase in chitosan concentration (0.1-1% w/v). The finding was consistent with the study of Xu *et al*. (2005), who studied the effect of chitosan addition on the mechanical properties and water vapor transmission rate of corn starch film by varying chitosan concentration from 30 to 100% w/w of corn starch. He reported that addition of chitosan into corn starch film with the ratio of up to 50% w/w had increased the tensile strength, elongation at break and water vapor permeability properties of the film. In general, the higher concentration ratio of chitosan to starch decreased their mechanical strength of starch film due to the phase separation between both chitosan and starch molecules (Xu *et al*., 2005; Bourtoom *et al*., 2008; Singh and Kamlesh, 2014). It should be noted that the behavior of tapioca starch film will be different compared to corn starch film due to their differences in physicochemical properties of the starch. For example, the flexibility of tapioca starch is higher than corn starch due to lower composition of amylose in tapioca starch than corn starch (Mali *et al*., 2006).

It is crucial to study the effects of chitosan concentration on the properties of the tapioca starch based film to find the optimum concentration of chitosan that can produce efficient food packaging film. Although there were several studies which explored the effect of chitosan concentration on the properties of tapioca starch film, the studies only focused on a few properties of the film such as physical, mechanical and water vapor permeability properties. The studies are clearly lacking discussion on the relationship between the properties mentioned. It is interesting to relate the mechanical properties of the film with morphological properties as well as optical properties to give a clearer explanation of the matrix-filler interaction in starch/chitosan film. In this study, the tapioca starch films incorporated with different concentration of chitosan varied from 20 to 80% w/w of dry starch were produced by solvent casting and characterized for mechanical, morphological, and optical properties.

Materials and Methods

Materials

Tapioca starch was obtained from Thye Huat Chan Sdn Bhd (Brand Kapal ABC). Food grade chitosan (70-97% deacetylation, white/yellow shapeless solid) was obtained from R&M Marketing, Essex, UK. Food grade glacial acetic acid and glycerol were purchased from R&M Marketing, Essex, UK.

Films preparation

3 g of tapioca starch was dispersed in 100mL water–glycerol solutions in order to obtain 3% w/w suspensions. The composition of glycerol added into starch solution was 25% w/w of the dry starch solid weight. All dispersions were heated on the hotplate with continuous stirring until gelatinized completely at 75ºC and then cooling to room temperature (30 ± 2 ºC).

Chitosan flakes at different weight (20 to 80% w/w of dry starch solid weight) were dispersed in 100 mL acetic acid (1% v/v) using magnetic stirrer in order to produce different concentration of starch/chitosan film. The dispersion was heated (80˚C) on a hotplate for 60 min under continuous stirring to completely dissolve the chitosan (Chillo *et al*., 2008).

The film forming solution was prepared by mixing different concentration of chitosan solution with tapioca starch solution (Xu *et al*., 2005; Chillo *et al*., 2008) in similar proportion (100 mL of starch and 100 mL of chitosan solution) for 20 minutes using magnetic stirrer. Then, the solution underwent sonication for 30 minutes (Chang *et al*., 2010) to homogenize the solution.

The blended solution was poured onto a square glass plate (30 cm x 20 cm) covered with Mylar plastic sheet. The solution was then casted using roller (wire bar coater; 32 cm film width) in order to control the uniformity of the film thickness, and the plate was placed on the level surface at room temperature and dried for 24 hours (De Moura *et al*., 2009). A neat starch film without the addition of chitosan was also prepared as the control. After drying, the film was peeled off from casting plate and conditioned (Relative Humidity: 50%, Temperature: 25ºC) in sealed plastic bag (Xu *et al*., 2005).

Thickness

The thickness of film was measured using a digital vernier caliper (Mannesmann, Germany) at five random positions around the film. The average values of the thickness were used to calculate the TS, EAB, YM, and transparency. Analysis of variance (ANOVA) was applied on the results using Minitab (Minitab Inc., State College, Pa., USA) to evaluate P-value.

Mechanical properties

Tensile tests were performed using Instron Universal Testing Machine Model 5566 to determine the TS, EAB, and YM. The films stretched at a crosshead speed of 10 mm/min. The procedure was in accordance to ASTM D882-02 (2002). Analysis of variance (ANOVA) was applied on the results using Minitab (Minitab Inc., State College, Pa., USA) to evaluate P-value.

Morphological properties

Morphological properties of the films were observed using scanning electron microscope (SEM). All the films coated with gold before being observed under SEM (600x magnification).

Optical properties

Color and transparency of the films were measured using a color spectrophotometer (Ultra Scan Pro). The percentage of transmittance at visible light, 660 nm was determined to indicate the transparency of the films (Shankar *et al*., 2015). Color parameters (L*, a*, b*) obtained via the computerized system using Hunter Lab. All results calculated based on a mean of at least three replicates. Analysis of variance (ANOVA) was applied on the results using Minitab (Minitab Inc., State College, Pa., USA) to evaluate P-value.

Results and Discussion

Thickness

The thickness of films produced was found to be between 0.04 to 0.05 mm as tabulated in Table 1. There was no significant increase in the starch/chitosan film thickness when compared with the neat starch film ($p>0.05$). Consistency value of film thickness indicates the reliability of mechanical and optical properties data as thickness has been proven to affect the properties of the film as well as mechanical and optical properties (Shahrul *et al*., 2016).

Mechanical properties

The mechanical properties which include TS, EAB, and YM of starch film incorporated with chitosan as reinforcing agent are presented in Figure 1. Figure 1(a) shows the effect of concentration of chitosan on TS and EAB of the film. TS value is the maximum tensile stress that the film sustain when forces are being exerted on the film continuously (Lee *et al*., 2008). EAB is the parameter that can determine the film's flexibility and elongation capacity before the film can break (Lopez *et al*., 2014). TS and EAB for the neat starch film were found to be 3.58 MPa and 3.92% respectively. It shows that the neat starch film itself has very low tensile strength and flexibility. When chitosan was blended with starch film, the tensile strength of film significantly improved. The tensile strength of film reached a maximum value of 21.23 MPa when 60% w/w of chitosan added into the film's composition. There was 492% improvement in tensile strength compared to the neat starch film without the addition of chitosan. A good molecular interaction between starch and chitosan has led to the formation of the intermolecular bond. When strong intermolecular bonding between molecules formed, the film exhibited strong affinity and high tensile strength (Xu *et al*., 2005).

Although the ability of chitosan to improve the tensile strength of the starch film was proven, the flexibility of film was not improved. As can be seen in Figure 1(a), EAB values of films decreased with the increase of chitosan in starch films. There was around 36% decrement in EAB value of 60% w/w chitosan film (2.52%) compared to neat starch film without chitosan (3.92%). A mathematical modeling study by Chillo *et al*. (2008) had proven that improvement of EAB only influenced by the amount of plasticizer particularly glycerol concentration. The brittle characteristic of chitosan may lead to the

Table 1 Film thickness, transmittance and apparent color

Concentration of chitosan (w/w)	Thickness (mm)	T_{660} (%)	Parameter L* (luminosity)	Parameter a* (green-red)	Parameter b* (blue-yellow)
0%	0.04 ± 0.002	84.78 ± 0.04	93.84 ± 0.07	-0.90 ± 0.03	3.22 ± 0.17
20%	0.04 ± 0.002	85.34 ± 0.20	94.03 ± 0.02	-0.95 ± 0.04	3.21 ± 0.12
40%	0.05 ± 0.001	85.29 ± 0.22	93.95 ± 0.08	-0.97 ± 0.02	3.42 ± 0.16
60%	0.05 ± 0.002	85.21 ± 0.27	93.76 ± 0.06	-1.03 ± 0.04	4.46 ± 0.11
80%	0.05 ± 0.003	85.80 ± 0.29	94.05 ± 0.07	-1.03 ± 0.03	3.86 ± 0.15

increment of TS and decrement of EAB (Bangyekan *et al*., 2006). The neat starch film itself has very low EAB value although 25% w/w of glycerol added into film formulation. Thus, a further study which varies the concentration of plasticizer is recommended to improve EAB as well as the flexibility of films.

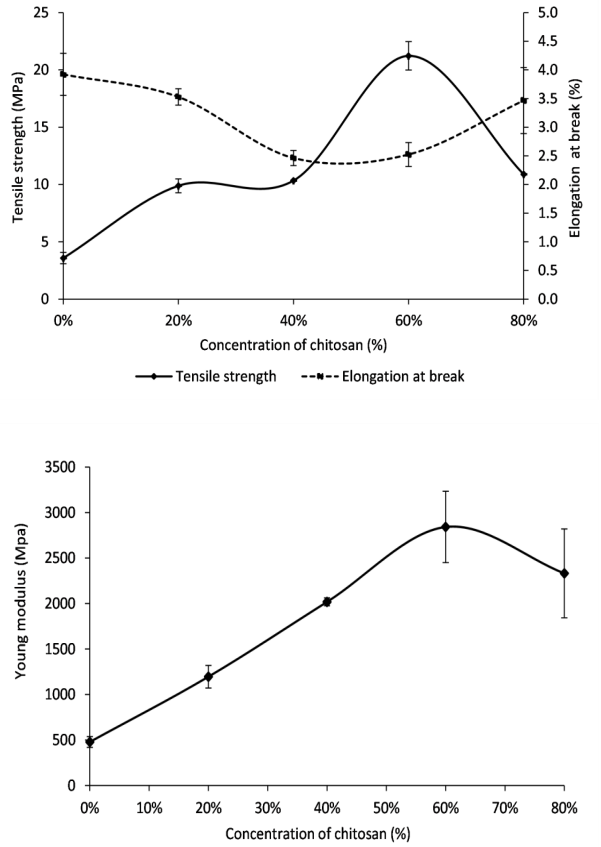

Figure 1. Effect of chitosan concentrations on tapioca starch film (a) tensile strength and elongation at break, (b) young modulus

It can also be seen from Figure 1 that further addition of chitosan (>60% w/w) into the starch film decreased the TS value. TS value drops dramatically from 21.23 MPa to 10.89 MPa which was 49% decrement, when 80% w/w of chitosan was added into the starch matrix. The tensile strength decreased due to the phase separation between two components which were chitosan and starch (Xu *et al*., 2005). When chitosan molecules were too concentrated compared to starch molecules, intermolecular bond was difficult to be formed. Amino group cation (NH_3^+) in chitosan could not form hydrogen bonding with the hydroxide ion (OH^-) in starch easily, thus resulting in more intramolecular bond formed compared to intermolecular bond. The intramolecular force led to the phase separation between two different components, thus producing non-homogenous matrix-filler interaction (Xu *et al*., 2005; Chang *et al*., 2010). Interestingly, the EAB value for 80% w/w of chitosan film (3.47%) increased by 37% compared to 60% w/w of chitosan film. Phase separation of both chitosan and starch compounds led to less affinity between the molecules. Therefore, the molecules arrangements were less compact and became more flexible compared to 60% w/w of chitosan film.

YM is a useful parameter that indicates stiffness characteristic of a film by measuring the tensile forces needed to stretch the film, which is low YM value indicates the film has high elasticity (Lee *et al*., 2008). Figure 1(b) shows the effect of chitosan on the YM of the film. The addition of 20% w/w chitosan significantly increased the YM of the film (150% increment) compared to YM of the neat starch film. The addition of chitosan up to 60% w/w further increased the YM value but, further addition of chitosan to 80% w/w slightly decreased the YM value (18% decrement). The decrement in YM value for 80% w/w chitosan film was due to the low TS as explained earlier. Therefore, YM values were comparable with the trend of TS and EAB results. Both YM and TS exhibited optimum value when the concentration of chitosan was 60% w/w. However, too high YM value will limit the application of the film because the film can be more brittle and less elastic. Statistical analysis shows that the effect of chitosan concentration on mechanical properties including tensile strength, elongation at break and Young modulus of tapioca starch film was significant which ($P<0.05$).

Morphological properties

SEM images of starch films produced with and without the addition of chitosan are shown in Figure 2. Figure 2a shows that the microstructure of the neat starch film surface was very rough and withered large granules of starch. Whereas, the addition of 60% w/w of chitosan into the starch film matrix (Figure 2b) reduced the size of granules and improved the surface structure. As can be seen from Figure 2b, the film with 60% w/w chitosan exhibited homogeneous and smooth matrix structure, and a few chitosan particles also appeared on the film surface. The film with 60% w/w chitosan also shows smoother and more even structure compared to the neat starch film structure. This finding is consistent with the finding of Ashori and Bahrami (2014), whereby their SEM micrograph of starch/chitosan film also shows a smooth and homogeneous structure with small particles of chitosan sparsely distributed on the film surface. Similarly, Singh and Kamlesh (2014) also found that SEM micrograph of neat corn starch film has rough and large pebbles structure. They also reported that the large size of pebbles structure in starch film disintegrated to the smaller size when the

concentration of chitosan was increased.

However, when the concentration of chitosan was further increased to 80% w/w, the chitosan was not fully blended with the starch solution and many chitosan microparticles formed on the film surface as can be seen in Figure 2c. The surface structure from Figure 2c was smoother than Figure 2b, but more chitosan particles were appeared on the surface of the film compared to that with 60% w/w of chitosan. High concentration of chitosan in the starch film may not facilitate the anion and cation to attract each other. The cation from chitosan may form intramolecular bonding in their molecules itself rather than intermolecular bonding with starch due to the lack of anion in starch molecular structure. Intramolecular bonding led to the phase separation between chitosan and starch, thus reducing the rigidity of the films and decreased their tensile strength (Xu *et al*., 2005).

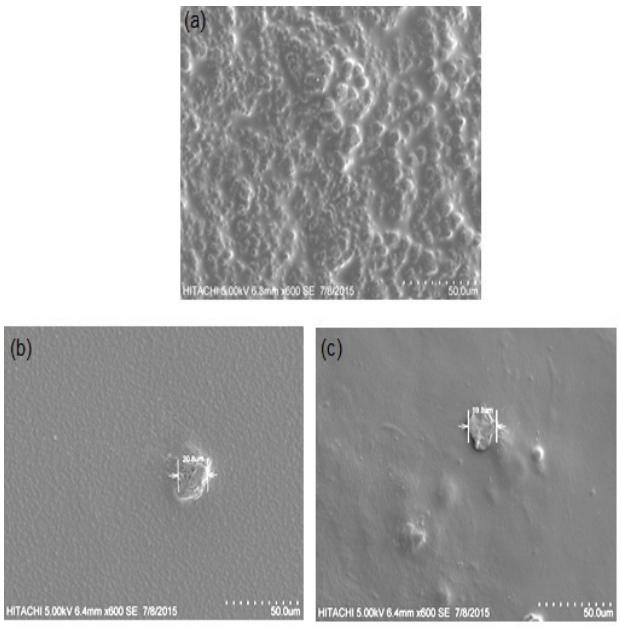

Figure 2. SEM images of films: (a) neat starch, (b) 60% w/w chitosan in starch matrix, (c) 80% w/w chitosan in starch matrix

Optical properties

The optical property of food packaging material is one of the important aesthetical factors that influence food product appearance as well as consumer acceptance (Hosseini *et al*., 2013). **Figure 3** compared the edible films produced with three different compositions of starch and chitosan. The glossiness of films was compared by observing the images in **Figure 3**. All films produced were transparent as the alphabet "P" can be seen clearly through the films. The addition of 60% w/w of chitosan into the starch film (Figure 3b) produce more glossy and transparent film compared to neat starch film (Figure 3a). Based on the morphological structure, the molecules were distributed homogeneously inside the film, thus producing clearer and even film. Bangyekan *et al*. (2006) also study the effect of chitosan on tapioca starch coating material. He reported that 1% w/w of chitosan had significantly increased the glossiness of tapioca starch film. He also reported that the highly glossy film was due to the smooth surface of the film. However, further addition of 80% w/w of chitosan slightly reduced the glossiness and transparency of the film as can be compared in Figures 3b and 3c, due to the poor blending of chitosan and starch that explained in Section 3.2.

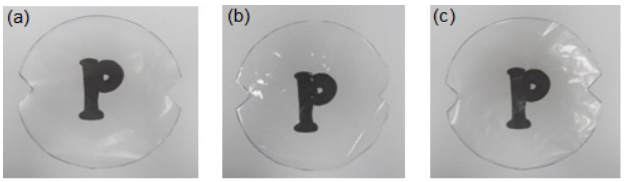

Figure 3. Appearance of films on alphabet of 'P'; (a) starch film (control), (b) 60%w/w chitosan-starch film, (c) 80%w/w chitosan-starch film

The transparency values and color attribute parameters (L*, a*, b*) of the edible films are shown in Table 1. The percentage of transmittance at visible light wavelength provides the basis of transparency measurement. According to Lee *et al*. (2008), transparency of films can be affected by the homogeneity and amount of inert filler such as chitosan. From Table 1, the neat starch film exhibited 84.78% of transmittance whereas 20% w/w of chitosan exhibited 85.34% w/w of transmittance. In general, there was only a slight improvement of film transparency with the addition of chitosan to starch film whereby the transparency of film with 80% w/w of chitosan was 85.80% w/w. Transparency values of films produced with the addition of chitosan did not differ markedly from neat starch control film (Table 1). Statistical analysis also shows that the effect of chitosan on tapioca starch film transparency was not significant ($P>0.05$).

Parameter L* indicate the value of lightness (Molinaro *et al*., 2013), brightness and luminosity (Lopez *et al*., 2014). As tabulated in Table 1, all films show the high value of L* indicating that those films exhibit colorless appearance. For parameter a*, all samples have very low negative a* values indicating that the films have a tinge or almost negligible of green color. Statistical analysis also shows that the effect of chitosan concentration on a* value of tapioca starch film was not significant ($P>0.05$).

For parameter b*, all samples have the small positive value indicating that the films have a tinge of yellow color. Statistical analysis shows that the effect of chitosan concentration on b* value of tapioca starch film was significant ($P<0.05$). The

addition of chitosan (40-60% w/w) into edible films increased the b* values due to Maillard reaction between amino and hydroxyl groups of chitosan (Bof *et al*., 2015). This reaction has produced insoluble melanoidin compound, thus exhibiting slight yellow color of edible film. Overall, the addition of chitosan only slightly affected the optical properties included transparency, L*, a*, and b* value of starch films.

Conclusion

Edible films were made with tapioca starch incorporated with varying concentration of chitosan. Increasing the concentration of chitosan up to 60% w/w resulted in the increase of TS and YM values but decrease in EAB values, thus the toughness of films was improved. Further addition of chitosan up to 80% w/w decreased the TS and YM, and slightly increased the EAB value. The morphological study shows that both chitosan and starch compounds were blended homogenously, thus improved the surface structure of starch film. Nonetheless, the effect of chitosan on the optical properties of edible films could be considered as almost negligible. Findings of this study suggest that the addition 60% w/w chitosan into tapioca starch film exhibited optimum improvement of food packaging material properties. The film produced exhibited highest tensile strength with improved glossiness and transparency that enhance their desired properties in food packaging application. The SEM image also presented smooth and homogeneous structure which revealed that there is a good interaction between starch and chitosan. Addition of chitosan into tapioca starch films may increase their commercial values as food packaging material because the films exhibit improved mechanical properties and aesthetical value as well.

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