

Mechanical Properties of Tapioca Starch-Based Film Incorporated With Bulk Chitosan and Chitosan Nanoparticle: A Comparative Study

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ABSTRACT

Tapioca starch-based film exhibits poor mechanical properties, thus limiting the application of the film as food packaging material. The present study aims to improve the mechanical properties of tapioca starch-based film by varying the concentration of glycerol (0, 5, 10, 15, 20, 25% w/w). Then, the starch-based films were incorporated with bulk chitosan (CH) and chitosan nanoparticle (CNP) to produce the starch/CH film and starch/CNP film, respectively. The mechanical properties of all the films which are tensile strength (TS), elongation at break (EAB), and Young's modulus (YM) were characterised using texture analyser. The morphological properties of both CH and CNP were observed under scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results revealed that 25% w/w of glycerol was sufficient to overcome the brittleness of film and improve the flexibility of the film. The addition of CH and CNP led to the increment in TS and EAB values of the films, thus confirming the role of the reinforcing agent of both CH and CNP in the films. A comparison study between CH and CNP demonstrated that CNP was more effective to improve the mechanical properties of the starch films compared to CH.

Keywords: Bulk chitosan, chitosan nanoparticles, food packaging, glycerol, ionic gelation, mechanical properties, tapioca starch.

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INTRODUCTION

Food packaging plays an important role in food industry to protect food products during processing, storage, and distribution,

thus maintaining the quality of the food products. The majority of food products use plastic as the packaging material due to its lightweight, low cost, convenient, and good barrier properties towards foreign contaminants. However, conventional plastic is usually made from petroleum-based sources which is non-degradable, leading to the dumping of municipal solid waste on the landfill (Othman et al., 2017). Biopolymer materials such as starch, cellulose, chitosan, and fish gelatin exhibit good film-forming properties and are easy to decompose, and thus are promising alternatives to replace conventional plastics (Rhim et al., 2015).

One of the most abundant biopolymers in tropical countries such as Malaysia, Thailand, Indonesia, Brazil, and some regions in Africa is tapioca starch or also known as cassava starch (*Manihot esculenta* Crantz) (Lagos et al., 2015; Versino & García, 2014). Tapioca starch is a promising biopolymer matrix film due to its good film-forming property, availability, and inexpensiveness (Jiménez, Fabra, Talens, & Chiralt, 2012; Kowalczyk et al., 2015). Another advantage of tapioca over other crops is its high tolerance to poor climate conditions, and therefore is a promising supply of starch for production of food packaging materials (Hanif et al., 2016; Piyachomkwan, 2011). In Malaysia, tapioca plant is cultivated for the starch production while other starches such as corn and rice are cultivated as the main food supplies for humans and animals (Hanif et al., 2016).

However, starch-based film usually exhibits poor mechanical properties which limit the application of the produced film for food packaging purposes. It is very important to produce a tough and flexible film so that the film is able to wrap the food properly. Addition of plasticiser such as glycerol, sorbitol, xylitol, and glucose into the biopolymer matrix can enhance the elasticity of the film (Vieira et al., 2011). Among various plasticisers mentioned, glycerol is widely used as a plasticiser in starch-based films due to its stability and compatibility with the structure of starch (Chillo et al., 2008b; Mali et al., 2005). However, high concentration of glycerol in the starch film reduces the strength of the film. This is due to the increase of free volume in starch matrix created by the interruption of glycerol molecules between the starch chain. Thus, it is very important to determine the optimum concentration of glycerol to produce efficient food packaging film.

Nevertheless, starch-based film can be blended with other biopolymers such as chitosan to improve the strength of the film (Chillo et al., 2008a; Shapi'i et al., 2016; Xu et al., 2005). Chitosan ($C_6H_{11}NO_4$) is a linear polysaccharide and the second most abundant polysaccharide found in nature after cellulose (Dutta et al., 2009). Chitosan is derived from chitin which is naturally found in the waste of crustacean shells such as crabs, shrimps, and lobsters (Goy et al., 2009). Chitosan is non-toxic, biodegradable, biofunctional, biocompatible, and exhibit good antimicrobial properties (Byun et al., 2013), and is a promising biopolymer to be incorporated in food packaging material. Several studies have revealed that the addition of chitosan into starch-based film increases the intermolecular

interaction between both biopolymers due to the different ionic charges (Shapi'i & Othman, 2016). The combination of hydrogen bonding and 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 the two different compounds in the film (Bangyekan et al., 2006).

Advancement of nanotechnology reveals that bulk chitosan (CH) can be synthesised into nanoparticles, also known as chitosan nanoparticles (CNP), in which the size ranges from 1 to 100 nm (Othman, 2014). CNP can be produced via many methods such as ionic gelation (Calvo et al., 1997), reverse emulsion (Brunel et al., 2008), precipitation, and polyelectrolyte complexation (Nishimura et al., 2004). Among those methods, ionic gelation is the most popular method used to produce CNP because the process is relatively simple and non-toxic (Al-Qadi et al., 2012; Rampino et al., 2013). Addition of CNP into the matrix of biopolymer is more effective to improve the properties of the biopolymer compared to CH (Hannon et al., 2015). CNP acts as the reinforcing agent and tends to form good interfacial interaction with the biopolymer matrix, thus improving the structure and mechanical properties of the film compared to CH (Chang et al., 2010a; Hosseini et al., 2015; Moura et al., 2011; Shapi'i et al., 2017).

To the best of our knowledge, only Chang et al. (2010b) had investigated the effect of CNP in starch-based film, particularly potato starch. Moreover, no work has been published on the comparison of mechanical properties of starch/CH film and starch/CNP film. It is also crucial to find the suitable concentration of glycerol for tapioca starch-based film due to the difference in various sources and composition of tapioca starch used in this work compared to other previous works. Therefore, the objective of this study was to find the suitable concentration of glycerol to produce tapioca starch-based film and to compare the mechanical properties (TS, EAB, YM) of starch/CH film and starch/CNP film.

MATERIALS AND METHODS

Materials

Chitosan (low molecular weight) and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich, USA. Tapioca starch was obtained from Thye Huat Chan Sdn Bhd (Brand Kapal ABC, Thailand). Acetic acid and glycerol were purchased from R&M Marketing, UK.

Preparation of Starch Film-Forming Solution

An amount of 3 g tapioca starch was dispersed in 100 mL distilled water-glycerol solutions to obtain 3% w/v (weight of starch per volume of distilled water) of starch suspensions. The composition of glycerol added into the starch solution was varied from 0, 5, 10, 15, 20, and 25% w/w (weight of glycerol per weight of starch) as shown in Table 1. Then, the

solution was heated with continuous stirring until it completely gelatinised at 75°C. The starch solution was then subjected to film casting.

Table 1
Formulation of starch-film forming solutions

Film samples	Amount of tapioca starch (g)	Volume of distilled water (mL)	Concentration of glycerol (%)
1	3	100	0
2	3	100	5
3	3	100	10
4	3	100	15
5	3	100	20
6	3	100	25

Preparation of Starch/CH Film

CH solution (15% w/w of solid starch) was prepared by dispersing 0.45 g of chitosan flakes into 50 mL aqueous acetic acid solution (concentration of acetic acid: 1% v/v [volume of acetic acid per volume of distilled water]) for 30 min using a magnetic stirrer (FAVORIT HS0707V2, Indonesia). Then, the pH of the solution was adjusted to 4.6 using NaOH. The CH solution was mixed with gelatinised starch film-forming solution using a magnetic stirrer for 30 min and then subjected to film casting.

Preparation of Starch/CNP Film

The parameters to synthesise CNP were fixed based on the optimum parameters that produced the most stable and smallest size of CNP (3–12 nm) as reported by Gokce et al. (2014) with some modifications. First, CH solution (15% w/w of solid starch) was prepared by dispersing 0.45 g chitosan flakes into 50 mL aqueous acetic acid solution (1% v/v) for 30 min using a magnetic stirrer. Then, the pH of the solution was adjusted to 4.6 using NaOH. TPP solution was prepared according to the ratio of chitosan to TPP (5:1) by dissolving 0.09 g of TPP powder in 50 mL distilled water.

CNP was spontaneously obtained upon the addition of 50 mL of TPP solution drop by drop to the 50 mL CH solution under vigorous magnetic stirring at room temperature (25°C) for 30 min. Then, an ultrasonic probe (QSonica Q500, USA) was used to disperse the CNP in the suspension for 15 min with a sequence of 1 min sonication and 10 s rest at an amplitude of 50%. The beaker containing CNP was placed in an ice bath during ultrasonication to ensure that the temperature of CNP suspension was in the favourable range (30 to 35°C). The starch/CNP film solution was prepared by mixing 100 mL CNP suspension with 100 mL gelatinised starch film-forming solution and stirred for 30 min using the magnetic stirrer.

Film Casting

An amount of 50 mL of the solution was poured into an acrylic petri dish (diameter: 14 cm) and left in an air-conditioned room (20°C) for 48 h on a flat table. Then, the petri dish containing starch/CNP film was dried at 40°C for 5 h in a ventilated oven to constant weight. A neat starch film without the addition of CNP was also prepared as control. After drying, the film was peeled off from the petri dish and conditioned in a desiccator containing saturated magnesium nitrate solution (R&M Marketing, UK) with a relative humidity of 51% and temperature of 30°C (Xu et al., 2005).

Mechanical Properties

Tensile strength, elongation at break, and Young's modulus were determined using a texture analyser (TA.XT2 Stable Micro Systems, UK) according to ASTM D882-09. Film strips (100 × 15 × 0.06 mm) were cut from each preconditioned sample and placed between the grips. Initial grip separation and test speed were set at 60 mm and 0.5 mm·s⁻¹, respectively. Force and distance were recorded during extension of the strips to break. TS, EAB, and YM were calculated according to Equations 1, 2, and 3, respectively. A minimum of five replications for each test sample were run.

TS was calculated according to the following equation:

$$TS = F/A \quad (1)$$

here F is the maximum force and A is the cross-sectional area of the film.

EAB was calculated according to the following equation:

$$EAB = \frac{l_f - l_o}{l_o} \times 100 \quad (2)$$

where l_f is the final length at specimen break and l_o is the initial length of the film.

YM was calculated according to the following equation:

$$YM = \frac{\text{Stress}}{\text{strain}} \quad (3)$$

Morphological Properties

Morphological properties of the CH and CNP were observed under SEM (LEO 1455 VP) and TEM (FE-TEM HF-3300). A drop of CH solution was deposited and left to dry on a specimen holder, and examined using SEM. For TEM analysis sample preparation, CNP was dispersed in deionised water using an ultrasonic bath for 10 min. The CNP solution was then dropped on a formvar grid and allowed to dry before being examined using the TEM.

RESULTS AND DISCUSSION

Effect of Glycerol Concentration on the Mechanical Properties of the Starch Film

The results for mechanical properties were obtained only for 10% to 25% w/w of glycerol. Mechanical properties of films containing 0% and 5% w/w of glycerol could not be measured because the films were very brittle, difficult to peel off from the petri dish, and easily broken into pieces as can be seen in Figure 1.



Figure 1. Neat tapioca starch film without the addition of glycerol

Figure 2(a) shows the effect of glycerol concentration (10% to 25% w/w) on the TS of the starch films. Increase in glycerol concentration from 10% to 25% has reduced the TS of the starch films from 7.12 to 1.12 MPa ($p < 0.05$). This finding is consistent with the study reported by Souza et al. (2012). The authors investigated the effect of glycerol concentration on tapioca starch film containing sucrose and inverted sugar. They revealed that the TS values of the films were around 6.06 to 2.72 MPa when glycerol concentrations were varied from 0% to 15% w/w. The trend of this finding is also consistent to the study done by Tang et al. (2008), who reported that addition of 20% w/w of glycerol into corn starch/montmorillonite films reduced the TS of the film from 20 to 6 MPa. This was due to the interruption of glycerol molecules that caused an increase of empty spaces within the starch matrix (Souza et al., 2012). Formation of empty spaces in the starch matrix reduced the intermolecular attraction force in amylose and amylopectin, thus producing starch films that were less dense and less compact. When the film was less dense and compact, it was easy to deform when stress was applied which was indicated by the low TS of the starch films.

Figure 2(b) shows the effect of glycerol concentration on the EAB of the starch films. The addition of glycerol into starch film improved the EAB of the films. The EAB increased from 1.89% to 67% with the increase in glycerol concentration from 10% to 25% w/w, respectively, which was a 34-fold improvement. This finding revealed that the EAB of the starch films was highly dependent on the concentration of glycerol ($p < 0.05$). The result is similar to the work done by Alves et al. (2007), where they found that an increment in the concentration of glycerol from 20% to 45% w/w increased the EAB of tapioca starch films enriched with different amylose contents from 5.2% to 153.2%. In comparison, the trend of TS values (Figure 2(a)) was reciprocal to the trend of EAB values (Figure 2(b)). As the concentration of glycerol increased, the EAB increased but the TS decreased. This finding is consistent with the work reported by Tang et al. (2008), who studied the effect of glycerol concentration on the mechanical properties of corn starch film incorporated

with MMT. They found that an increment in glycerol concentration up to 20% w/w led to the increase in EAB but reduced the TS. The increase in the EAB of starch films when the glycerol concentration was increased was due to the formation of free volume created by glycerol molecules that interrupted the starch chain. The presence of empty spaces in the starch matrix facilitated the movement of starch chain when external stress was applied, thus improving the EAB of the films (Lim & Hoag, 2013). This phenomenon contributed to the increase in elasticity but reduced the strength of the film.

Figure 2(c) shows the effect of glycerol concentration on the YM of the starch films. It can be seen that the addition of glycerol into the starch film reduced the YM of the film. These findings revealed that the addition of glycerol reduced the rigidity of starch films, thus increasing the elasticity of the film. Starch films containing 10% w/w of glycerol exhibited the highest YM which was 378.09 MPa, followed by starch films containing 15% w/w of glycerol which was 178.87 MPa. Further increase in the concentration of glycerol to 25% w/w resulted in further reduction of YM to 5.96 MPa (62-fold reduction compared to 10% glycerol), which indicated that the film was flexible and less stiff. Suzana Mali et

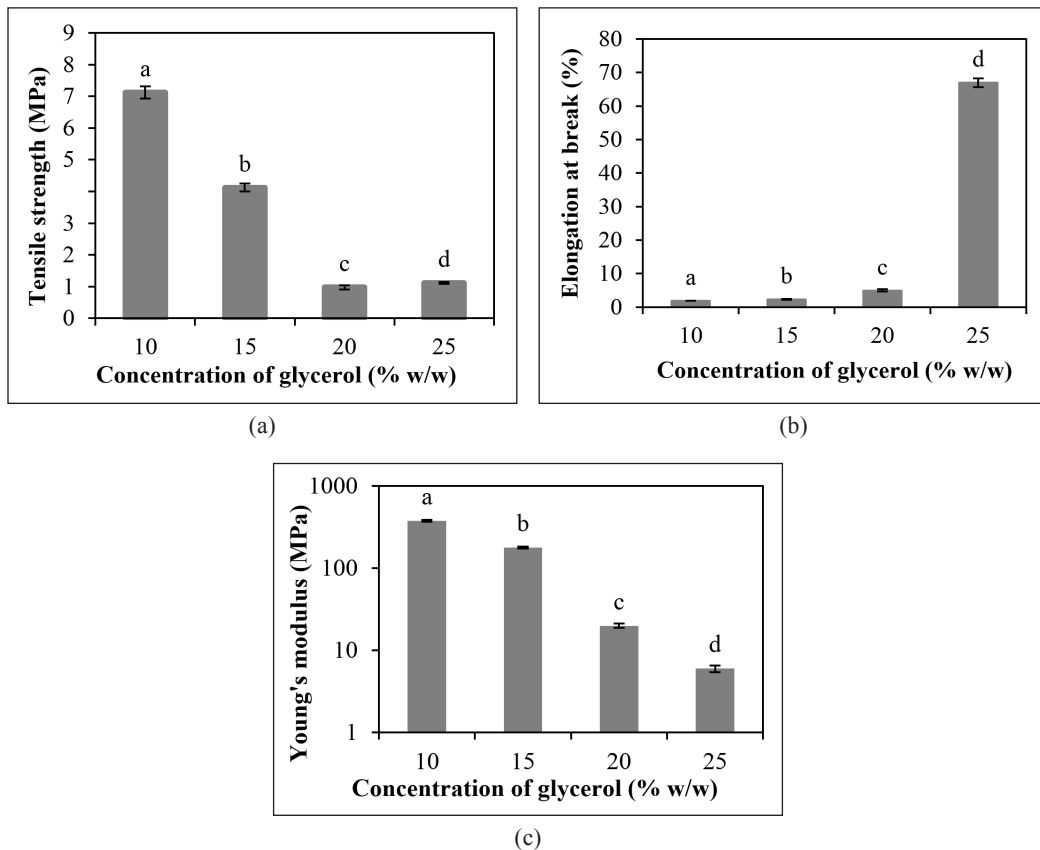


Figure 2. Effect of glycerol concentration on mechanical properties of tapioca starch film: (a) TS, (b) EAB, (c) YM

al. (2006) also reported that tapioca starch film containing 40% w/w glycerol exhibited a low YM of 9 MPa. They explained that addition of glycerol increased the hydrophilic behaviour of starch films, thus increasing the tendency of films to hold water molecules from the surrounding. As the moisture content of starch films increased, the structure of the film became less stiff, compact, and rigid, thus increasing the flexibility of the films.

It was found that 25% w/w glycerol was sufficient to be used for producing starch film. The EAB of starch films produced using 25% w/w glycerol was 67%, which was comparable with the EAB of commercial LDPE film (69%) (Cirillo et al., 2015). Although further increment of glycerol concentration could further increase the EAB of the films, the TS of starch film is expected to further decrease with further increase in glycerol concentration. Starch films containing 25% w/w glycerol exhibited low TS which was not comparable with the TS of commercial LDPE film (16 MPa) (Cirillo et al., 2015). Too high concentration of glycerol in the starch films weakened the attraction forces in the starch chains and created a higher number of glycerol–glycerol bonds than glycerol–starch bonds (Müller, Yamashita, & Laurindo, 2008). Formation of the glycerol–glycerol bonds in starch matrix led to the phase separation of glycerol zones and starch–glycerol zones, thus producing non-homogenous film which was sticky, soggy, and wrinkly (Kim et al., 2015). Therefore, CNP was utilised as the reinforcing agent to further improve the mechanical properties of the starch films.

Effect of CH and CNP on the Mechanical Properties of Starch Film

Previous studies have proven the ability of CH to improve the properties of starch films, especially mechanical properties (Chillo et al., 2008b; Shapi'i & Othman, 2016; Xu et al., 2005). However, there is a study done to compare the mechanical properties of films incorporated with CH and CNP. A comparison study between CH and CNP is very important to find out the efficiency of CNP in improving the properties of films compared to CH. To the best of our knowledge, only one study has been done to compare the effect of CH and CNP on tara gum (TG) films in terms of thermomechanical, physicochemical, morphological, and barrier properties (Antoniou et al., 2015). Therefore, a comparative study was done to determine the efficiency of CNP as a filler compared to CH. The concentrations of both CH and CNP in starch films were fixed to 15% w/w. All the films produced in this part of work were added with 25% w/w of glycerol. Table 2 shows the mechanical properties of neat starch films with the addition of CH and CNP.

From Table 2, neat starch film exhibited poor mechanical properties where the values of TS, EAB, and YM were 1.12 MPa, 67%, and 5.96 MPa, respectively. The addition of CH and CNP into neat starch films increased the TS and EAB of the films, thus confirming the potential of chitosan to improve the mechanical properties of the neat starch film. This result is consistent with the studies by Shapi'i and Othman, (2016), and Xu et al., (2005),

Table 2
Comparison of CH and CNP as filler

Film	TS (MPa)	EAB (%)	YM (MPa)
Neat starch	1.12 ± 0.03 ^a	67.00 ± 1.32 ^a	5.96 ± 0.58 ^c
Starch/CH	3.16 ± 0.07 ^b	76.90 ± 1.23 ^b	4.43 ± 0.16 ^a
Starch/CNP	4.95 ± 0.01 ^c	90.77 ± 1.40 ^c	5.70 ± 0.15 ^b

The data are reported as mean ± SD with n=3 and p<0.05

who reported that incorporation of suitable amount of chitosan into tapioca starch films improved the mechanical properties of the films.

From Table 2, the starch/CNP film exhibited higher improvement of TS (4-fold increment) compared to starch/CH film (2.9-fold increment), where the TS of both films were 4.95 and 3.16 MPa, respectively. The EAB of starch/CNP film (1.36-fold increment) also exhibited a slightly higher improvement than the EAB of starch/CH film (1.15-fold increment), where the EAB of both films were 90.77% and 76.90%, respectively. Addition of both CNP and CH into starch films slightly reduced the YM of the film where the decrement were 0.04-fold and 0.26-fold, respectively. These findings revealed that CNP was more efficient to increase the strength, elasticity, and flexibility of the film compared to CH. The difference in the performance of CH and CNP to improve the mechanical properties of starch films was due to the difference in the morphological properties of CH and CNP. Figure 3 shows the morphology of CH and CNP viewed under SEM and TEM, respectively. As shown in Figure 3(a), CH is in a continuous phase and not in a regular shape compared to CNP. From Figure 3(b), CNP has a regular spherical shape and is tiny in size due to the crosslinking of chitosan and TPP during ionic gelation process.

Different morphological properties of both CH and CNP resulted in the difference in matrix–filler interaction mechanism between chitosan and starch. In order to produce

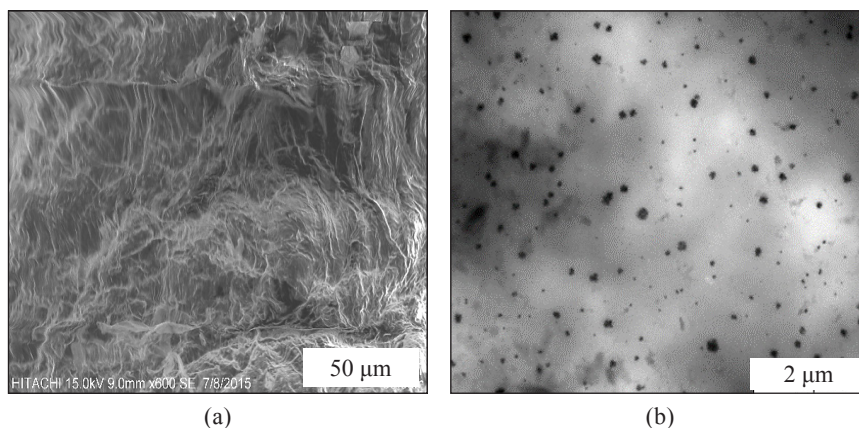


Figure 3. Morphology of (a) bulk chitosan and (b) chitosan nanoparticle

starch/CH film, starch and CH were blended together immediately when CH was added into the starch solution due to the similar continuous phases (Silva-Weiss et al., 2013). The blending of starch and CH changed the physical and rheological properties of the film-forming solution. This was different when CNP was incorporated into the starch matrix as a filler where CNP did not change the physical and rheological properties of the film-forming solution. The tiny size and regular shape of CNP facilitated the CNP to fill in the empty spaces between the matrix of the starch film (Lorevice et al., 2015).

Furthermore, the tiny size of CNP provided a large surface area of CNP to be exposed to the starch, thus increasing the intermolecular interaction between the starch and CNP. Good molecular interaction that occurred between CNP and starch chain increased the intermolecular attraction forces which brought the distances of adjacent starch chain closer (Ma et al., 2009). This phenomenon increased the density of the starch film to become more compact, strong, and higher resistant towards physical stress. Meanwhile, improvement of film elasticity was also attributed to the good intermolecular interaction between both CH and CNP to the starch matrix (Ghani et al., 2016). Good interaction between starch and chitosan led to the strong intermolecular forces that were able to resist the applied tensile stress. When the intermolecular forces in the starch/CNP films were sufficient to resist the applied forces, the films were difficult to deform and break and thus increased the film elasticity and flexibility.

CONCLUSION

The optimum concentration of glycerol in the starch film was successfully determined, where 25% w/w glycerol was sufficient to overcome the brittle property of tapioca starch films. Incorporation of CH and CNP into the starch films improved the mechanical properties of the films, where the TS and EAB of the films increased significantly, thus proving the role of both CH and CNP as reinforcing agents in the films. The comparison study of CH and CNP revealed that CNP was more effective to improve the mechanical properties of starch film compared to CH due to the stronger intermolecular interaction between CNP and starch compared to CH and starch. This finding indicates that it is worth to explore the potential of CNP as a filler in the biopolymer materials to improve the properties of the material. Other properties of the starch/CH film and the starch/CNP film such as thermal, water vapor permeability, and oxygen permeability properties should be characterised to better understand the application of the film.

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