Research Article

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Mechanical, thermal, and barrier properties of starch films incorporated with chitosan nanoparticles

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Abstract: The mechanical, thermal, and barrier properties of tapioca starch films produced using a solvent casting method with varying concentrations of chitosan nanoparticles (CNPs; 5, 10, 15, 20, 25, 30, 35% w/w of solid starch) were investigated. The addition of 15% w/w CNP (size range: 20–50 nm) to the starch films (starch/CNP films) enhanced the tensile strength from 1.12 to 10.03 MPa (7.96-fold increment) and elongation at break from 67.00 to 90.77% (0.35-fold increment). However, the thermal stability of the starch films reduced slightly whereby the maximum degradation temperature decreased from 316.32 to 289.11°C (0.09-fold decrement) with the addition of CNP. The opacity of 15% w/w starch/CNP films increased from 8.07 to 14.67 due to the presence of CNP within the starch matrix that hinders the light transmission pass through the film. Furthermore, reductions in the water vapor permeability from $1.1 \times 10^{-7}$ to $0.63 \times 10^{-11}$ g/Pa h m (4-fold increment) and oxygen permeability from $7.38 \times 10^{-3}$ to $3.59 \times 10^{-3}$ cm$^3$/m day Pa (0.51-fold increment) of the films were observed. Starch/CNP films fabricated in this work exhibit enhanced the mechanical and barrier properties, thus proving the promising potential to be employed as food packaging materials.

Keywords: biopolymer, chitosan nanoparticles, mechanical, nanocomposite, starch, thermal

1 Introduction

Most of the conventional food packaging materials are made up of fossil-based polymers such as polyethylene, polypropylene, and polystyrene. They are nondegradable and can cause solid waste accumulation in the landfill and ocean [1]. Nonetheless, biopolymer materials are promising to replace conventional food packaging materials due to their biodegradability, nonpollutant, nontoxicity, and availability [2]. Biopolymers can be classified as synthetic biopolymers such as polylactic acid, polycaprolactone, and polyethylene succinate and natural biopolymers such as starch, cellulose, chitosan, and gelatin. The advantages of materials made from synthetic biopolymers are high transparency, durability, and flexible properties that are almost similar to conventional plastic. However, synthetic biopolymers are expensive compared to natural biopolymers, which are more natural, cheaper, and nontoxic, thus promising for food packaging applications [3].

In this work, a natural biopolymer particularly tapioca starch was chosen as the matrix phase of the biopolymer films because it can be abundantly found in tropical countries, involves low processing cost, and is a sustainable resource. Tapioca starch is obtained from the Cassava plant tuber (Manihot esculenta), which is easy to be grown in tropical climate countries such as Malaysia, Indonesia, and Thailand, thus ensuring a steady supply of high-quality starch sources in these countries. Thin films prepared from tapioca starch are usually colorless, odorless, tasteless, nontoxic, and biodegradable, and thus their application is...
suitable for food packaging materials [4]. Although starch is promising for the large-scale production of biopolymer films, the produced films usually exhibit poor physical, mechanical, thermal, and barrier properties including exhibit brittle structure, poor stability, and low heat distortion temperature [5]. These poor properties lead to the limitation of their application as food packaging materials. Plasticizers such as glycerol, sorbitol, and glucose may be added to the biopolymer matrix to enhance the elasticity of the films, thus improving the brittleness of the films [6]. However, the addition of plasticizer into starch films increases the sensitivity of starch films toward moisture, whereby the films become soggy and the structure of the films becomes weak [7]. This will restrict their use in a variety of applications, especially for food packaging.

Incorporation of the reinforcing agent such as nanosized filler (1–100 nm) into the starch-based polymer, producing bionanocomposites, can overcome the lack of properties of starch-based films [8]. This nano-reinforcement method is a promising technique to improve the biopolymer properties while also introducing new functionalities to starch-based materials. Chitosan nanoparticles (CNPs) have a tremendous attraction in material sciences compared to bulk chitosan because of the different sizes, shapes, and chemical structures, thus involving distinctive mechanisms to strengthen the biopolymer films [9]. The tiny size and the spherical shape of CNP provide a substantial surface area of CNP, therefore increasing the ability of CNP to interact with the matrix starch [7,10]. Besides their intrinsic properties, CNP is also environmentally friendly as the synthesis process is mild, consumes less energy, and does not produce a toxic residue. The incorporation of CNP at optimum concentration into biopolymer particularly starch films may result in the improvement of the mechanical, thermal, optical, and barrier properties of the films. Due to the promising properties of CNP, previous works of literature have reported the incorporation of CNP into various biopolymers including potato starch [11], tara gum [12], fish gelatin [13], pectin [14], hydroxypropyl methylcellulose [15], and carboxymethyl cellulose [16].

To the best of our knowledge, there is only one study conducted to investigate the effects of CNP concentrations on the properties of starch films, whereby Chang et al. [11] produced and examined the mechanical, barrier, and thermal properties of potato starch films incorporated with different CNP concentrations (0–8% w/w of solid starch). No study has investigated the effects of CNP concentrations on the properties of tapioca starch films. It is worth noting that different types of starch used in film processing may result in different properties of the films due to the different compositions of amylose and amylopectin. Furthermore, limited studies have been performed to incorporate CNP in a suspension form into the biopolymer films. Many of the previous studies incorporated CNP in the form of powder, which was more challenging to handle and prone to agglomeration [17] including the work by Chang et al. [11]. The use of freshly prepared CNP in the form of suspension is a better approach to improve the performance of CNP as the filler. Therefore, the current work is directed on developing tapioca starch-based films added with varying concentrations (0, 5, 10, 15, 20, 25, 30, and 35% w/w of solid starch) of CNP in the suspension form that exhibits improved properties. It is important to determine the suitable concentration of CNP in the starch/CNP films since concentration is a critical parameter that affects the properties of the films, which will influence the final applications of the films.

2 Materials and methods

2.1 Materials

Chitosan (low molecular weight: 50 kDa, viscosity: 20–300 cP in 1% w/v of acetic acid, deacetylation: 75–85%) and sodium tripolyphosphate (TPP) were obtained from Sigma-Aldrich, USA. Tapioca starch (amylose: 19%, amylopectin: 81%) was procured from Thye Huat Chan Sdn. Bhd. (Brand Kapal ABC, Thailand). Acetic acid, glycerol, sodium hydroxide (NaOH), magnesium nitrate (Mg(NO3)2), anhydrous calcium chloride (CaCl2), paraffin wax, and bee wax were acquired from R&M Marketing, UK.

2.2 Preparation of CNP

CNP suspension was synthesized via the ionic gelation method. All parameters were controlled based on the work reported by Shapi et al. [10]. Table 1 presents the concentrations of CNP in dry starch solid weight and the respective amount of chitosan and TPP with chitosan to TPP ratio of 5:1 to synthesize the CNP.

Different amounts of chitosan as tabulated in Table 1 were dissolved in 50 mL of aqueous acetic acid solution (1% v/v) for 30 min using a magnetic hot plate stirrer (FAVORIT, Indonesia) to prepare different concentrations of chitosan solution. Then, 10% v/v NaOH was used to adjust the pH of the solution to pH 4.6. At the same time, different amounts of TPP powder as tabulated in Table 1
Table 1: The concentration of CNP in dry starch solid weight and the respective amount of chitosan and TPP based on chitosan to TPP ratio of 5:1

| Concentration of CNP in dry starch solid weight (% w/w) | Amount of chitosan (g/50 mL distilled water) | Amount of TPP (g/50 mL distilled water) |
|--------------------------------------------------------|---------------------------------------------|----------------------------------------|
| 5                                                      | 0.15                                        | 0.03                                   |
| 10                                                     | 0.30                                        | 0.06                                   |
| 15                                                     | 0.45                                        | 0.09                                   |
| 20                                                     | 0.60                                        | 0.12                                   |
| 25                                                     | 0.75                                        | 0.15                                   |
| 30                                                     | 0.90                                        | 0.18                                   |
| 35                                                     | 1.05                                        | 0.21                                   |

were dissolved in 50 mL distilled water to produce different concentrations of the TPP solution according to the optimum ratio of chitosan to TPP (5:1).

An amount of 50 mL of the TPP solution was added drop by drop to the 50 mL of chitosan solution with vigorous magnetic stirring for 30 min. The addition of TPP in the chitosan solution formed CNP spontaneously. CNP in the suspension was then dispersed using an ultrasonic probe (QSonica Q500, USA) at an amplitude of 50% for 15 min with a sequence of 1 min of sonication and 10 s of rest at an amplitude of 50%. During sonication, the beaker containing CNP was put in an ice bath to keep the temperature of the CNP suspension in the desired range (25–30°C).

2.3 Preparation of starch-based films

A 3% w/w solution of tapioca starch was obtained by dispersing 3 g of tapioca starch in 100 mL distilled water–glycerol solution, whereby the concentration of glycerol was set at 25% w/w of the dry starch solid weight [7]. The solution was then heated until fully gelatinized at 75°C with continuous stirring using a magnetic hot plate stirrer. Then, the starch solution was cooled until 40 ± 2°C before being mixed with the CNP suspension.

Next, 100 mL of CNP suspension was mixed with 100 mL gelatinized starch solution and stirred for 30 min to produce starch/CNP film solution. The starch/CNP solution was subjected to sonication for 5 min using the ultrasonic probe to obtain a homogenous solution. A 50 mL of the solution was then poured into an acrylic petri dish (diameter: 14 cm) and placed on the flat table in an air-conditioned room (20°C) for 48 h. The Petri dishes holding the films were dried at 40°C in a ventilated oven (Memmert Universal Oven UN110, Germany) for 5 h. Neat starch films without CNP were also produced as the control.

The films were peeled off from the Petri dishes and conditioned in a desiccator having saturated magnesium nitrate (MgNO₃) solution (relative humidity (RH): 51%, temperature: 30°C) (Xu et al. [23]). The thickness of the films was measured using a digital micrometer (Mitutoyo, Japan) at five random positions around the film. The average value of the film thickness (0.07 ± 0.002 mm) was used to determine the tensile strength (TS), elongation at break (EAB), and Young’s modulus (YM) of the films.

2.4 Molecular interaction of CNP and starch

Fourier-transform infrared (FTIR; Nicolet, UK) analysis of the films was carried out to determine the molecular interaction between CNP and starch matrix. The FTIR spectra for CNP were taken in the liquid state, and potassium bromide (KBr) pellets were used with a 50:1 weight ratio of KBr to CNP. FTIR spectra for neat starch and starch/CNP films were taken in the solid state. All the spectra were recorded in the range of 4,000–400 cm⁻¹.

2.5 Morphological properties

A transmission electron microscope (TEM; Hitachi HT7700, Japan) was utilized to determine the morphology of the films and the dispersion of CNP within the starch films. The starch films that were incorporated with 15 and 30% w/w of CNP were chosen for this analysis to compare the morphology of the films and dispersion behavior of CNP in the starch films, with a low concentration of CNP, and with a high concentration of CNP. A cryo-ultramicrotome (RMC PowerTome PC, USA) was utilized to cut and attain a thin cross-section of the films. The thin films were then mounted on the 300-mesh copper and examined under the TEM (×7k and ×50k magnifications).

2.6 Mechanical properties

TS, EAB, and YM were determined by a tensile test performed using a texture analyzer (TA.XT2 Stable Micro Systems, UK) following the ASTM D882 [18]. Film strips (100 mm × 15 mm) were cut from the sample film and located between the grips. Test speed and initial grip separation were set to 0.5 mm/s and 60 mm, respectively.
2.7 Thermal properties

Thermogravimetric analysis (TGA) was carried out under a nitrogen atmosphere using a thermogravimetric analyzer (Mettler Toledo, Switzerland). The neat starch, 15% w/w starch/CNP, and 30% w/w starch/CNP films were subjected to the TGA analysis to compare the thermal stability of the films without CNP, with a low concentration of CNP, and with a high concentration of CNP. Approximately 20 mg sample film was subjected to the TGA analysis in the temperature range of 25–600°C, with an increased rate of 10°C/min. TGA data were analyzed using STARe Thermal Analysis Software Version 12.10.

2.8 Optical properties

Color parameters ($L^*$, $a^*$, $b^*$) and light transmittance of the films were measured using a color spectrophotometer (Hunterlab, Ultrascan Pro, USA) with D65 illuminant and 10° observer angle. The color difference ($\Delta E$) was calculated according to equation (1):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2},$$

where $\Delta L^*$ is the differential values of $L^*$ of neat starch and starch/CNP films, $\Delta a^*$ is the differential values of $a^*$ of neat starch and starch/CNP films, and $\Delta b^*$ is the differential values of $b^*$ of neat starch and starch/CNP films.

The percentage of light transmittance of the films was recorded at the wavelength range from 200 to 800 nm. Then, the opacity values of films were calculated using equation (2) [19]:

$$\text{Opacity} = (T_{600}/100) \times L,$$

where $T_{600}$ is the value of transmittance at 600 nm and $L$ is the film thickness (mm).

2.9 Barrier properties

Barrier properties of the films were investigated in terms of water vapor permeability (WVP) and oxygen permeability (OP). Determination of WVP was carried out using a modified dry cup method according to ASTM E96-95 [20]. The films were cut into circular shapes using the cutter (diameter: 7 cm) and placed on the cup containing 10 g of anhydrous calcium chloride (RH = 0%). The mixture of paraffin wax and bee wax (8:2) was heated using the magnetic stirring hotplate until the wax melted. The mixture of wax was used as a sealant around the circular cup. To maintain the surrounding humidity of the cup, each cup was stored in a desiccator containing saturated MgNO$_3$ to provide a constant RH of 51% at 30°C. A digital temperature humidity meter (Proskit NT-312) was used to monitor the relative humidity and temperature. Changes in the weight of the cup were recorded every 24 h for 10 days and plotted to obtain a weight loss versus time graph.

The water vapor transmission rate (WVTR) was calculated as equation (3):

$$\text{WVTR} = \frac{(W/t)}{A},$$

where $W/t$ is the slope of weight changes versus the time graph (g/h) and $A$ is the transmission area of the film (28 cm$^2$).

The water vapor permeability (WVP) was calculated as equation (4):

$$\text{WVP} = \frac{(WVTR \times L)}{(P_1 - P_2)},$$

where $L$ is the average of film thickness (m), $P_1$ is the water vapor partial pressure in a desiccator at RH = 51%, $21.64 \times 10^5$ Pa, and $P_2$ is the water vapor partial pressure in the cup at RH = 0%.

Oxygen permeability was measured using an oxygen permeation system equipped with a calorimetric sensor (MOCON, USA), following ASTM F1927-07 [21]. The films (diameter: 50 cm$^2$) were preconditioned at 30°C and 51% RH for 3 days.

The preconditioned film was sealed tightly between two chambers of the oxygen permeation system. The oxygen in the chamber was flushed out using nitrogen gas until the percentage of oxygen in the chamber dropped to 0%. Then, oxygen gas (12.5 kPa) was allowed to enter the chamber and transmitted through the film. The oxygen permeability was recorded every 5 s until the line of oxygen permeate versus time curve became a plateau. Then, the average values of oxygen permeability at plateau were calculated.

2.10 Statistical analysis

A Minitab (Minitab Inc., USA) was utilized to conduct the statistical analysis of the collected experimental results. A triplicate of each measurement ($n = 3$) was used to calculate the mean value and standard deviation. The analysis of variance (ANOVA) was used to evaluate the significant difference between means. Mean values were compared using the Tukey test whereby the mean values were considered significant when $p < 0.05$. 
3 Results and discussion

3.1 Molecular interaction of CNP and starch

FTIR analysis was conducted on the CNP, neat starch films, and starch/CNP films whereby the FTIR spectra are shown in Figure 1. The FTIR spectrum of CNP (Figure 1(a)) shows a broad peak and strong absorption band at 3256.5 cm\(^{-1}\), which was attributed to the hydroxyl group (O–H stretching) due to the presence of water. This was because the FTIR spectrum of CNP was taken in the liquid form. The strong absorption band at 1635.54 cm\(^{-1}\) indicates the presence of amino groups (N–H) in CNP. The small peaks at 2361.31 and 2335.47 cm\(^{-1}\) correspond to the alkane groups (C–H deformation and C–H stretching). The assignments of various bands and peaks for CNP in this study were consistent with those reported in the literature for similar functional groups [12,22].

FTIR spectrum of the neat starch films (Figure 1(b)) exhibit two peaks at 3259.27 and 2917.39 cm\(^{-1}\), which indicate the presence of hydroxyl groups (O–H stretching) and alkane groups (C–H stretching), respectively. FTIR spectrum for starch/CNP films (Figure 1(c)) also exhibit two peaks at 3264.82 and 2926.29 cm\(^{-1}\), which indicated the hydroxyl groups (O–H stretching) and alkane groups (C–H stretching), respectively, consistent with Figure 1(b). There were hydroxyl groups (O–H stretching) in both films due to the high number of hydrogen bonds in the films.

The main chain elements of the neat starch films (Figure 1(b)) and starch/CNP films (Figure 1(c)) were similar except for the existence of amino group at 1560.17 cm\(^{-1}\) for starch/CNP films, which proved the existence of CNP in the films. When two or more polymers were mixed, the chemical interactions can be determined by the changes of spectra peaks [19]. The band at 1646.20 cm\(^{-1}\) in the spectrum of neat starch films (Figure 1(b)) was assigned to the hydroxyl group (O–H bending). The O–H bending in the starch/CNP films shifted from 1646.20 to 1645.69 cm\(^{-1}\) due to the hydrogen bonds that occurred between starch and CNP at the hydroxyl group. The spectrum of starch/CNP film also shows that the amino peak of CNP shifted from 1635.54 to 1560.17 cm\(^{-1}\), thus confirming the formation of the hydrogen bond between hydroxyl groups of starch and the amino group of CNP [23].

3.2 Morphological properties

TEM analysis was carried out to determine the morphological properties of the films and the dispersion of CNP in the starch matrix. Figure 2(a) and (b) show the TEM micrographs of 15 and 30 % w/w starch/CNP films at ×7k magnification, respectively, while Figure 2(c) and (d) show 15 and 30% w/w starch/CNP films at ×50k magnification, respectively. As shown in Figure 2(a) and (b), both films had a uniform and smooth structure with an
undulating appearance due to the shrinkage of the films. Antoniou et al. [12] also found the undulate appearance of tara gum/CNP films, which was a wavelike form surface. They suggested that the undulate appearance of tara gum films occurred due to the stress exerted on the films during freezing in liquid nitrogen when conducting ultramicrotome preparation, which corroborated with this work.

Moreover, the TEM images of starch/CNP films show the solid black and round shape, which were CNP particles incorporated in the films. This indicated that 5 min of sonication process using the ultrasonic probe did not promote any rupture of the nanoparticles. It can be observed from Figure 2 that CNP in 15% w/w starch/CNP films (Figure 2(a)) was dispersed well in the starch matrix compared to CNP in 30% w/w starch/CNP films (Figure 2(b)). The agglomeration of CNP at a high concentration (30% w/w) resulted in the poor distribution of CNP (Figure 2(b)). The dispersion and agglomeration of CNP in the starch films could be observed clearly from the TEM images of starch/CNP films at high magnification (×50k) (Figure 2(c) and (d)).

Figure 2(c) shows that CNP in 15% w/w starch/CNP films were in the size range between 20 and 50 nm. The CNP was dispersed well in the starch films without the formation of agglomeration. This was due to the steric stabilization of the CNP formed by the starch matrix and glycerol. Note that 25% w/w of glycerol was blended with the starch/CNP films prepared in this work, which might be sufficient to maintain the steric stabilization of CNP at a low concentration. Lee et al. [24] also found that the presence of glycerol could lead to steric stabilization among nanoparticles. A protective layer was formed around the CNP due to the combination of starch matrix and glycerol, causing the steric repulsive forces and resulting in steric stabilization. Meanwhile, Figure 2(d) shows the agglomeration of CNP in 30% w/w starch/CNP films with a single-particle size range of 50–100 nm and bulk particle size around 400 nm. Bridging forces created by starch matrix and glycerol might be not sufficient to maintain the steric stabilization of CNP at high concentration, thus allowing the agglomeration of CNP in the starch films to occur.

It is worth discussing that the single-particle size range of the CNP at low concentration (15% w/w) was smaller than that at high concentration (30% w/w) owing to the weak intermolecular interaction forces during the CNP formation because the chitosan molecules were
separated by a large distance (less amount of chitosan used: 0.45 g/50 mL distilled water). Therefore, chitosan molecules are usually stable as single-unit molecules. The anions of TPP phosphate from the TPP solution added into the solution were then cross-linked with the cations of single chitosan molecules and formed small sizes of CNP instantly. The intermolecular interaction between chitosan molecules became stronger as the concentration of chitosan increased (0.90 g/50 mL distilled water) due to the short distance between each molecule. Subsequently, the chitosan molecules entangled and formed a single larger particle during the ionic gelation process [10,25].

3.3 Mechanical properties

Figure 3 shows the effects of CNP concentrations on the mechanical properties of the films. As shown in Figure 3(a), the addition of CNP into neat starch films increased the TS, thus improving the strength of the starch films. CNP tends to improve the intermolecular interaction in the starch matrix, bringing adjacent chains of starch closer and reducing the free volume, thereby raising the TS of the nanocomposite films. Improvement in TS of starch/CNP films with the addition of CNP was attributed to the interactions between the starch chain and CNP as proven from the FTIR spectra (Figure 1). When CNP was added to the gelatinized starch solution, the amino group and hydroxyl group in CNP tend to form hydrogen bonds with the hydroxyl group in the starch chain. The hydrogen bonds produced strong intermolecular bonding, thus increasing the tensile strength of starch/CNP films [23].

There is a trend of increasing TS with the increase in the CNP concentration added to the films. As the CNP concentrations in the starch films were increased from 0 to 35% w/w, the TS values increased significantly (7.96-fold increment, \( p < 0.05 \)) from 1.12 to 10.03 MPa. The trend of this finding was consistent with the work reported by Moura et al. [26] whereby they studied the effect of CNP addition on the mechanical properties of carboxymethylcellulose (CMC) films. They found that the TS value of CMC films increased from 5 to 32 MPa with the increase in the concentration of CNP from 0 to 40% w/w.

It is important to note that although the TEM analysis of starch/CNP films incorporated with 30% w/w CNP shows agglomeration of CNP (Figure 2(b) and (d)), the agglomeration did not hinder the role of CNP as the reinforcing agent whereby there was still improvement in the TS value of the films. This result was in line with the work of Hosseini et al. [13] who found that the agglomeration of CNP in the fish gelatin films did not hinder the role of CNP as the reinforcing agent, thus improving TS. The increase in CNP concentration led to the higher molecular

![Figure 3: Effects of CNP concentrations on mechanical properties of starch films: (a) tensile strength, (b) elongation at break, and (c) Young’s modulus.](image-url)
interaction among the CNP, which produced a more dense and compact CNP with an overall positive surface [27]. The high density of positive charge around the agglomerated CNP exposed to the starch chain increased the number of hydrogen bonds and improved the TS of the films. High molecular interaction between agglomerated CNP and starch chain resulted in more complex hydrogen bonding between starch and CNP compared to single CNP. Note that the TS values of the films incorporated with 30 and 35% w/w CNP (9.33 and 10.32 MPa, respectively) were comparable with the TS of common food packaging material particularly low-density polyethylene (LDPE; range: 8.3–31.4 MPa) [28].

The incorporation of CNP in the starch films also affected the EAB values of the films as shown in Figure 3(b). When the concentration of CNP was increased from 0 to 15% w/w, the EAB values of the films increased (0.35-fold increment, p < 0.05) from 67.00 to 90.77%. It is worth noting that the EAB values of these films were comparable with the EAB of commercial LDPE film (68.70%) [29]. At 15% w/w concentration of CNP, most of the empty spaces within the starch matrix were filled up with CNP. CNP and starch formed strong hydrogen bonding, which increased both TS and EAB. Moura et al. [26] discovered that the incorporation of 40% w/w CNP increased the EAB of neat CMC films from 6.0 to 8.1% (0.35-fold increment). They reported that the improvement of films flexibility was attributed to the particle size of CNP at 40% w/w, which was around 110 nm (measured by differential light scattering (DLS) method), thus comparable with the particle size of 15% w/w CNP produced in the present work, which was 106.34 nm (measured by DLS) as reported in our previous study [10].

Owing to the hydrophilic nature of CNP, the size of CNP measured using TEM as reported in the current study was smaller than the size measured using DLS. The CNP absorbed water and swell; therefore, the size in the hydrated state as measured by DLS (106.34 nm) was greater than the actual size measured via TEM (size range: 20–50 nm) without water molecules.

Meanwhile, the incorporation of 20–35% w/w CNP to the starch films decreased the EAB, which was consistent with the trend of EAB of fish gelatin films incorporated with chitosan as reported by Gomez-Estaca et al. [30]. They explained that this phenomenon was attributed to the high intermolecular forces between chitosan and biopolymer. The increase in intermolecular interaction of starch and CNP led to the strong attractive forces between both matrix and filler, thus bringing the adjacent spaces in the starch films closer. This phenomenon led to a decrease in the free volume of starch matrix and produced high-density films, which were difficult to elongate.

Figure 3(c) presents the effects of CNP addition on the YM of the starch films. The addition of 0–15% w/w CNP to the films slightly reduced the YM from 5.96 to 5.70 MPa. This was due to the increase in flexibility of the films as proven from the EAB result (Figure 3(b)), thus decreasing the stiffness of the films. This was due to the existence of strong intermolecular interaction of starch and CNP that facilitated the movement of the starch chain against stress. Although a few studies have reported the effects of CNP on the TS and EAB of biopolymer films, however, there is still a lack of study done on YM. Note that it is essential to measure the YM of the films as YM determines the stiffness and flexibility properties of the films, which are important for food packaging applications.

Meanwhile, the addition of 20–35% w/w CNP increased the YM of neat starch films from 7.46 to 39.87 MPa. This indicates that the addition of 20–30% w/w CNP led to an increase in the stiffness behavior of starch films because the starch and CNP exhibited strong intermolecular interaction. Incorporation of 20–35% w/w CNP to the starch films resulted in a higher number of hydrogen bonding sites exposed to the starch chain compared to the addition of 5–15% w/w CNP. An increase in the concentration of CNP led to strong intermolecular interactions with the starch chain, thus increasing the density of the starch matrix [31]. The produced starch/CNP films became stronger and more compact, which increases the stiffness of the films.

3.4 Thermal properties

TGA was performed to investigate the thermal stability and degradation temperature of the films. The thermogravimetric curves of the neat starch, 15% w/w starch/CNP, and 30% w/w starch/CNP films are shown in Figure 4.

Figure 4: Thermogravimetric curves of (a) neat starch, (b) 15% w/w starch/CNP, and (c) 30% w/w starch/CNP films.
TGA records the weight loss of all the samples over a range of temperatures from 25 to 600°C.

Figure 4 shows the two stages of weight loss of both neat starch and starch/CNP films. The first weight loss occurred around 50–120°C due to the loss of moisture in the films, which was evaporated at the boiling point of water. The weight loss for the second stage occurred in the range of 288–316°C, which indicated the degradation of starch due to the depolymerization of film compositions mainly starch, CNP, and glycerol. Liu et al. [32] also discovered that the main degradation temperature of neat corn starch and corn starch films with the addition of starch nanoparticles was in the range of 250–350°C, which is in line with the results in this study. The details of TGA parameters such as onset temperature ($T_{\text{onset}}$), the temperature at maximum degradation ($T_{\text{max}}$), and weight of remaining ash (%) for the second stage of thermal decomposition are tabulated in Table 2.

From Table 2, the $T_{\text{onset}}$ of starch/CNP films decreased from 164.22 to 154.79°C with the addition of 15% w/w CNP into the starch matrix. This indicated that the addition of CNP resulted in a lower requirement of energy (low $T_{\text{onset}}$) for the films to start to degrade. This was due to the high polar group of TPP in the cross-linked backbones of chitosan, which provoked the separation of CNP and starch films [33], thus requiring less energy to degrade. As the concentration of CNP in the starch matrix was further increased up to 30% w/w, the $T_{\text{onset}}$ decreased slightly from 154.79 to 153.97°C. Note that chitosan is a natural biopolymer that is unstable at an extreme temperature and therefore can lose its structural role as a reinforcing agent of starch films [34], hence lower $T_{\text{onset}}$.

Meanwhile, $T_{\text{max}}$ indicates the temperature at which the maximum degradation of the films occurred. When the films reached maximum degradation, the films are considered to be completely degraded and remain as residues [35]. Table 2 shows that the $T_{\text{max}}$ of the films decreased from 316.22 to 289.11°C when 15% w/w CNP was added into the starch matrix. When the concentration of CNP was increased up to 30% w/w in starch films, the $T_{\text{max}}$ was slightly decreased to 288.29°C. This result indicated that the addition of CNP in starch films reduced the thermal stability of the films because of the interruption of CNP between the starch matrix.

Table 2 shows that the ash residual of 15% w/w starch/CNP films (23.48%) was greater than neat starch films (7.91%) speculated to be due to the crystallinity of the CNP, which enhanced the packing structure of the films. Further increase in the CNP concentration of up to 30% w/w increased the ash residual of the films to 24.56% owing to the increase in the packing structure of the films. This result was consistent with the study by Liu et al. [32]. They found that the addition of starch nanoparticles into corn starch films increased the ash residual from 10 to 23%. They suggested that strong crystal structures of the starch nanoparticles were more difficult to decompose compared with the corn starch matrix, thus increasing the percentage of ash residual. CNP exhibited higher crystallinity and compact structure, and thus, it is more difficult to decompose compared with starch, which consisted of a higher content of the amorphous region. The incorporation of CNP into the starch matrix also hindered the diffusion of volatile materials that were trapped within the starch matrix [36], thus high ash residual value.

Antoniou et al. [12] have investigated the effect of CNP on the thermal properties of tara gum films via thermogravimetric analysis. They found that an increment in the concentration of CNP from 5 to 15% reduced the weight loss of tara gum film, thus proving the improvement in the thermal stability of the films. The current study also demonstrated a reduction in weight loss or an increase in ash residual. Nonetheless, the thermal stability of the starch/CNP films was considered slightly lower than neat starch films due to the slight decrease of $T_{\text{onset}}$ and $T_{\text{max}}$ values. It is worth discussing that starch/CNP films start to degrade at a high temperature ($T_{\text{onset}}$), which was above 150°C, demonstrating that the films are suitable to be used below 150°C during the application.

3.5 Optical properties

The optical properties of food packaging material could influence the food product appearance through the package as well as determine the consumer acceptance toward food products [37]. Optical properties parameters that include opacity and $\Delta E$ of starch/CNP films at different concentrations (0–35% w/w) were determined and tabulated in Table 3.

Based on Table 3, it can be seen that the addition of CNP into neat starch film increased the opacity of the
film, which indicated a decrease in the transparency of the film. It was found that the different concentrations of CNP did not affect much the trend of film opacity, whereby only small changes in the film’s opacity between each concentration of CNP could be observed. The opacity of neat starch film increased from 8.07 to 14.67 with the addition of 15% w/w CNP. This was due to the mechanism of CNP as filler in the starch matrix, which was not dissolved in the matrix, thus resulting in a decrease in the transparency of the films [13]. Apart from that, CNP in the starch matrix tends to block the light that can pass through the starch/CNP film and resulted in a decrease in film transparency. A study by Hosseini et al. [37] also found that the addition of 8% w/w of CNP powder into fish gelatin film resulted in an increase in the film opacity from 0.97 to 3.65, which also indicated a decrease in film transparency, thus supporting current findings. Although the addition of CNP into starch films reduced the transparency of the films, CNP provides other advantages whereby CNP exhibits excellent barrier property against UV light. This property could prevent lipid oxidation in the food composition, thus able to extend the shelf life of food products [13].

Meanwhile, the addition of CNP into starch films only slightly affected the ΔE value, whereby the values ranged from 1.20 to 1.57, which indicated a very small difference of color between starch/CNP films and neat starch films. Only small changes of ΔE were observed with varying concentrations of CNP. The difference in ΔE value of neat starch films and starch/CNP films was attributed to the addition of CNP as filler into the starch matrix, which was yellowish in nature compared to the white color of starch.

The transparency of neat starch film, 15% w/w starch/CNP film, and 30% w/w starch/CNP film were compared as demonstrated in Figure 5. It can be seen that all the films were transparent as the alphabets were seen through the films, thus confirming the low values of film opacity as discussed earlier. It is worth noting that good interaction between starch and CNP resulted in the homogenous structure of the films, thus maintaining the transparency of starch/CNP films [38].

### 3.6 Barrier properties

Figure 6 shows the effects of CNP addition (0–35% w/w based on the solid starch) on WVP and OP of starch/CNP films. From Figure 2(a), the highest WVP was exhibited by neat starch films (1.1 × 10^{-11} g/Pa.h.m) due to the hydrophilic property of starch and glycerol. The addition of glycerol into starch film increased the interchain spacing of the starch molecules [12], thus creating empty spaces between interchain spacing. Owing to the hydrophilic nature property of starch, starch molecules tend to bind with water vapors, thus allowing the moisture to absorb and permeate through the empty spaces in the starch matrix. It can be seen that the addition of CNP into starch films decreased the WVP due to the compact

| Concentration of CNP (%) w/w | Opacity (T\text{500}) | Total color difference (ΔE) |
|-----------------------------|----------------------|----------------------------|
| 0                           | 8.07 ± 0.00a         | 0a                         |
| 5                           | 13.68 ± 0.02b        | 1.20 ± 0.03b               |
| 10                          | 12.71± 0.01c         | 1.41 ± 0.16c               |
| 15                          | 14.67± 0.01d         | 1.57 ± 0.06d               |
| 20                          | 12.48 ± 0.03e        | 1.64 ± 0.09d               |
| 25                          | 13.53 ± 0.01f        | 1.22 ± 0.03b               |
| 30                          | 13.11± 0.00g         | 1.39 ± 0.01d               |
| 35                          | 13.46 ± 0.01h        | 1.50 ± 0.05e               |

The data are reported a mean ± SD and n = 3. Different letters indicate a significant difference at p < 0.05.

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**Figure 5:** Physical appearance of the films: (a) neat starch film, (b) 15% w/w starch/CNP film, and (c) 30% w/w starch/CNP film.
structure of starch/CNP films [12]. The decrease in WVP of starch/CNP films can be explained by the decrease of empty spaces between starch interchain due to the tiny size of CNP particles that were reinforced in matrix empty spaces. CNP tends to fill in the empty spaces between the starch matrix due to the tiny size of CNP and form good intermolecular interaction between CNP and starch. The structure of starch/CNP films became more compact compared to neat starch film. As a result, a compact structure of starch/CNP films created a difficult tortuous pathway for moisture to diffuse and permeate through the starch/CNP films. The formation of a tortuous pathway in the starch matrix created by CNP was able to delay the moisture to permeate through the film and hence reduced the WVP of the starch film [26].

Furthermore, when the concentration of CNP in the film was increased up to 35% w/w, WVP value was further decreased to $6.30 \times 10^{-5}$ g/Pa h m, which was fourfold lower than neat starch films. There was a trend of a decrease in WVP of the films with the increase in CNP concentration due to more CNP that was available, thus creating a more tortuous pathway compared to film added with a low concentration of CNP. The trend in Figure 6(a) was consistent with the study by Chang et al. [11] who found that the increase in CNP powder concentration from 0 to 6% w/w into potato starch film led to a decrease in WVP from $2.09 \times 10^{-6}$ to $1.13 \times 10^{-6}$ g/Pa h m (0.51-fold decrement). The latest study by Hosseini et al. [13] also found that the increase in the concentration of CNP powder from 0 to 6% w/w decreased the WVP of fish gelatin film from $1.42 \times 10^{-6}$ to $0.88 \times 10^{-6}$ g/Pa h m due to the good intermolecular interaction between matrix and filler, thus supporting the present findings.

The formation of the intermolecular hydrogen bond between starch and CNP reduced the number of free hydroxyl groups that were able to interact with water molecules [39], thus resulting in low WVP. As proved by the FTIR analysis (Figure 1), starch and CNP formed good molecular interactions, thus resulting in good affinity and compact structure of starch/CNP films. The strong affinity between starch and CNP provoked the difficulty of moisture to absorb and permeate through the films. It is worth noting that although CNP is categorized as a hydrophilic polymer [40], CNP is still able to improve the WVP of the films. Note that chitosan molecules have a lot of free amino and hydroxyl groups that tend to bind with water molecules, thus indicating high hydrophilicity. Meanwhile, the synthesis process of CNP allows the amino and hydroxyl group of chitosan to bind with the phosphate group of TPP. Thus, CNP has less free hydroxyl site compared to chitosan, which reduces the tendency of CNP to bind with water molecules [12].

The difference in the chemical structure between starch and CNP also hinders moisture absorption and permeation through the starch/CNP films. The presence of acetyl groups at the second carbon of the chitosan pyranose ring contributed to a less hydrophilic property of chitosan compared with the hydroxyl groups of starch [38]. Furthermore, improvement in water barrier properties of starch/CNP films was also attributed to the formation of hydrogen bonds at $\beta$-D-(1-4) glucopyranosyl linkages between each chitosan monomer, thus forming the packing structure and producing higher crystallinity of starch/CNP films compared to $\alpha$-D-(1-4) glucopyranosyl linkages of starch [39].

Figure 6(b) shows the effects of CNP addition (0 to 35% w/w based on the solid starch) on the OP of starch/CNP films. The highest OP was exhibited by neat starch films ($6.924 \times 10^{-3}$ cm$^3$/Pa day m) due to the semi-crystalline structure of starch, which consisted of high amorphous

![Figure 6: Effect of CNP concentration on the barrier properties of starch films: (a) water vapor permeability (WVP) and (b) oxygen permeability (OP).](image-url)
content that was highly permeable [41]. Furthermore, poor mechanical properties of the neat starch film might cause the film to tear when oxygen was flushed through the film, thus allowing a high amount of oxygen to diffuse through the film. Tee et al. [42] reported that the oxygen permeability of flaxseed mucilage film incorporated with glycerol could not be determined due to its viscosity and tore when flushed with oxygen.

From Figure 2(b), it can be seen that the addition of CNP into the starch films decreased the OP due to the ability of CNP to occupy the empty spaces in the starch matrix, thus creating a difficult tortuous pathway for the oxygen to diffuse and permeate. Good intermolecular bonding between starch and CNP contributed to the good compatibility between starch and CNP, thus facilitating the CNP to occupy empty spaces between the starch chain [41]. The tortuous pathway created by the incorporation of CNP in the starch matrix forced the oxygen to wiggle around CNP, and thus, oxygen took a longer path to permeate through the films, thus reducing the film permeability. Figure 2(b) also shows a decreasing trend of OP with an increase in the concentration of CNP. As the concentration of CNP was increased up to 35% w/w, OP of starch/CNP films decreased significantly to $3.59 \times 10^{-3}$ cm$^3$/m day Pa whereby 0.51-fold decrement of OP compared to 5% w/w CNP. The decrement of OP value demonstrated improvement in barrier properties of starch/CNP films toward oxygen.

Shen et al. [41] also reported a similar trend of results whereby the incorporation of 15% (w/w) chitosan into sweet potato starch films led to a decrease of OP from $3.520 \times 1,940$ cm$^2$/m day Pa due to the increase in the crystallinity of the films. The trend of this work was also consistent with the work reported by Dang and Yoksan [39]. It was found that the OP of the thermoplastic starch films incorporated with chitosan (TPS/chitosan film) decreased from $6.1 \times 10^{-10}$ to $3.9 \times 10^{-14}$ mol/m s Pa with the increase in the concentration of chitosan from 0.37 to 1.45%. They explained that a reduction in OP of TPS/chitosan films was due to the surface coverage of the film with a relatively higher crystallinity of the chitosan-rich layer. It can be speculated that bulk chitosan has a different mechanism in improving the barrier properties of the starch film compared to CNP whereby bulk chitosan formed intermolecular bonding with starch at the surface while CNP formed inter-molecular bonding within the starch matrix. High crystallinity of chitosan and CNP in the starch matrix led to the reduction of film permeability, thus improving the oxygen barrier properties of the starch film.

It can be concluded that the addition of CNP into starch films contributed to the improvement of barrier properties of starch/CNP films toward moisture and oxygen. Improvement in the barrier properties of starch/CNP films could lead to the wide application of the films as food packaging materials. The transfer of oxygen and moisture through a food packaging material packaged will be very much affected the quality and shelf life of food packaged in the materials.

4 Conclusion

The incorporation of CNP as the reinforcing agent into the starch films appears to be a promising route for the preparation of bionanocomposite films that are environmental friendly and sustainable for potential food packaging applications. The incorporation of the suitable amount of CNP into the starch films (15% w/w of CNP) resulted in improved mechanical properties and barrier properties with satisfactory opacity, color, and thermal properties. It is recommended to evaluate the biodegradability, antioxidant, and antimicrobial properties as well as real application of the starch/CNP films in future work, for efficient application of the films as food packaging materials.

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References

[1] Cottaz A, Bouarab L, De Clercq J, Oulahal N, Degraeve P, Joly C. Potential of incorporation of antimicrobial plant phenolics into polyolefin-based food contact materials to produce active packaging by melt-blending: proof of concept with isobutyl-4-hydroxybenzoate. Front Chem. 2019;7:1–13.

[2] Mendes JF, Paschoalin RT, Carmona VB, Sena AR, Marques ACP, Marconcini JM, et al. Biodegradable polymer blends based on corn starch and thermoplastic chitosan processed by extrusion. Carbohydr Polym. 2016;137:452–8.
[3] Imre B, Pukánszky B. Compatibilization in bio-based and biodegradable polymer blends. Eur Polym J. 2015;49:1215–33.

[4] Othman SH, Majid NA, Tawakkal ISMA, Basha RK, Nordin N, Shapi’i RA. Tapioca starch films reinforced with microcrystalline cellulose for potential food packaging application. Food Sci Technol. 2019;2061:1–8.

[5] Caetano KDS, Lopes NA, Costa TMH, Brandelli A, Rodrigues E, Filãres SH, et al. Characterization of active biodegradable films based on cassava starch and natural compounds. Food Packag Shelf Life. 2018;16:138–47.

[6] Nazri MSM, Tawakkal ISMA, Khairuddin N, Talib RA, Othman SH. Characterization of jackfruit straw-based films: effect of starch and plasticizer contents. Pertanika J Sci Technol. 2019;27:1–14.

[7] Shapi’i RA, Othman SH, Naim MN, Basha RK. Mechanical properties of tapioca starch-based film incorporated with bulk chitosan and chitosan nanoparticle: a comparative study. Pertanika J Sci Technol. 2019;29:95–107.

[8] Othman SH, Othman NFL, Shapi’i RA, Ariffin SH, Yunos KFM. Corn starch/chitosan/nanoparticles/thymol bio-nanocomposite films for potential food packaging applications. Polymers. 2021;3:390.

[9] Singh T, Shukla S, Kumar P, Wahlia V, Bajpai VK. Application of nanotechnology in food science: perception and overview. Front Microbiol. 2017;8:1–7.

[10] Shapi’i RA, Othman SH, Naim MN, Basha RK. Effect of initial concentration of chitosan on the particle size of chitosan nanoparticle. Int J Nanotechnol. 2016;16:680–91.

[11] Chang PR, Jian R, Yu J, Ma X. Fabrication and characterization of chitosan nanoparticles/plasticised starch composites. Food Chem. 2010;120:736–40.

[12] Antoniou J, Liu F, Majeed H, Zhong F. Characterization of tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: a comparative study. Food Hydrocoll. 2015;44:309–19.

[13] Hosseini SF, Rezaei M, Zandi M, Farahmandghavi F. Fabrication of bio-nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles. Food Hydrocoll. 2015;44:172–82.

[14] Lorevice MV, Otoni CG, de Moura MR, Mattoso LHC. Chitosan nanoparticles on the improvement of thermal, barrier, and mechanical properties of high- and low-methyl pectin films. Food Hydrocoll. 2016;52:732–40.

[15] Shojaei M, Eshaghi M, Nateghi L. Characterization of hydroxypropyl methyl cellulose–whey protein concentrate biocomposite films reinforced by chitosan nanoparticles. J Food Process Preserv. 2019;43:1–12.

[16] Jamnathy N, Shojaee-Alibadi S, Moslehi shad M, Moradi E. Comparing mechanical, barrier and antimicrobial properties of nanocellulose/CMC and nanochitosan/CMC composite films. Int J Biol Macromol. 2020;164:2323–8.

[17] Gökçe Y, Cengiz B, Yıldız N, Calmil A, Aktas Z. Ultrasonication of chitosan nanoparticle suspension: influence on particle size. Colloids Surf A Physicochem Eng Asp. 2014;462:75–81.

[18] ASTM D882. Standard test method tensile properties of thin plastic sheeting. https://www.astm.org/Standards/D882/, 2018 (accessed 1 January 2021).

[19] Oleyai SA, Almasi H, Ghanbarzadeh B, Moayed AA. Synergistic reinforcing effect of TiO2 and montmorillonite on potato starch nanocomposite films: thermal, mechanical and barrier properties. Carbohydrate Polym. 2016;152:253–62.

[20] ASTM E96–95. Standard test methods for water vapor transmission of materials. https://www.astm.org/e0096–95.html, 2017 (accessed 1 January 2021).

[21] ASTM F1927–07. Standard test method for determination of oxygen gas transmission rate, permeability, and permeance at controlled relative humidity through barrier materials using a coulometric detector. https://www.astm.org/f1927–07.html, 2014 (accessed 1 January 2021).

[22] Ashori A, Bahrami R. Modification of physico-mechanical properties of chitosan-tapioca starch blend films using nano-graphene. Polym-Plast Technol Eng. 2014;53:312–8.

[23] Xu YK, Kim KM, Hanna MA, Nag D. Chitosan-starch composite film: preparation and characterization. Ind Crops Prod. 2005;21:185–92.

[24] Lee JE, Chung K, Jang YH, Jang YJ, Ko chu veedu SL, Li D, et al. Bimetallic multifunctional core@shell plasmonic nanoparticles for localized surface plasmon resonance-based sensing and electrocatalysis. Anal Chem. 2012;84:6494–500.

[25] Hussain Z, Sahudin S. Preparation, characterisation and colloidal stability of chitosan-tripolyphosphate nanoparticles: optimisation of formulation and process parameters. Int J Pharm Pharm Sci. 2016;8:297–308.

[26] Mora MR, Lorevice MV, Mattoso LHC, Zucolotto V. Highly stable, edible cellulose films incorporating chitosan nanoparticles. J Food Sci. 2011;76:25–9.

[27] Gan Q, Wang T, Cochrane C, McCarron P. Modulation of surface charge, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery. Colloids Surf B Biointerfaces. 2005;44:65–73.

[28] Chin AW. Polymers for innovative food packaging; 2010. https://web.wpi.edu/Pubs/E Project/Available/E project–050610–124822/unrestricted/Polymers_for_Innovative_Food_Packaging.pdf.

[29] Cirillo G, Spizzirri UG, lemma F. Functional polymers in food science. Massachusetts: Scrivener Publishing; 2015.

[30] Gomez–Estaca J, Gomez–Guillem MC, Fernandez–Martin F, Montero P. Effects of gelatin origin, bovine hide and tunaskin, on the properties of compound gelatin-chitosan films. Food Hydrocoll. 2011;25:1461–9.

[31] Othman SH, Hassan N, Talib RA, Basha RK, Risyon NP. Mechanical and thermal properties of PLA/halloysite biocomposite films: effect of halloysite nanoclay concentration and addition of glycerol. J Polym Eng. 2017;37:381–9.

[32] Liu C, Jiang S, Zhang S, Xi T, Sun Q, Xiong L. Characterization of edible corn starch nanocomposite films: he effect of self-assembled starch nanoparticles. Starch. 2015;68:239–48.

[33] Ali SW, Rajendran S, Joshi M. Synthesis and characterization of chitosan and silver loaded chitosan nanoparticles for bioactive polyester. Carbohydr Polym. 2011;83:438–46.

[34] Othman SH, Khichik N, Shapi’i RA, Talib RA, Tawakkal ISMA. Water sorption and mechanical properties of starch/chitosan nanoparticle films. J Nanomater. 2019;2019:3843949. doi: 10.1155/2019/3843949.

[35] Arun S, Kumar KAA, Sreekala MS. Fully biodegradable potato starch composites: effect of macro and nano fiber reinforcement on mechanical, thermal and water-sorption characteristics. Int J Plast Technol. 2012;16:50–66.

[36] Iman M, Manhar AK, Maji TK. Preparation and characterization of zinc oxide and nanoclay reinforced crosslinked starch/jute green nanocomposites. RSC Advances. 2014;4:33826–39.
Hosseini SF, Rezaei M, Ghavi FF. Preparation and functional properties of fish gelatin-chitosan blend edible films. Food Chem. 2013;3–4:1490–5.

Bangyekan C, Aht-Ong D, Srikulkit K. Preparation and properties evaluation of chitosan-coated cassava starch films. Carbohydr Polym. 2006;63(1):61–71.

Dang KM, Yoksan R. Morphological characteristics and barrier properties of thermoplastic starch/chitosan blown film. Carbohydr Polym. 2016;150:40–7.

Pereda M, Amica G, Marcovich NE. Development and characterization of edible chitosan/olive oil emulsion films. Carbohydr Polym. 2012;87(2):1318–25.

Shen XL, Wu JM, Chen Y, Zhao G. Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. Food Hydrocoll. 2010;24(4):285–90.

Tee YB, Tee LT, Daengprok W, Talib RA. Chemical, physical, and barrier properties of edible film from flaxseed mucilage. Bioresources. 2017;12(13):6656–64.