

Research Article

Water Sorption and Mechanical Properties of Starch/Chitosan Nanoparticle Films

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The usage of biopolymer as food packaging material has been limited due to high water sorption and poor mechanical properties of the biopolymer. Thus, this study is aimed at improving the water sorption and mechanical properties of biopolymer particularly starch films by incorporation of a natural filler particularly chitosan nanoparticle (CNP) and investigating the properties of starch/CNP films at different storage conditions (relative humidity: 23, 50, and 75%; temperature: 4, 30, and 40°C). The water sorption behavior and isotherms of the films were investigated by fitting the water sorption data to the Peleg model and Guggenheim, Anderson, and de Boer model. Both the models were well fitted to the experimental data, thus proving the reliability of water sorption behavior prediction. It was found that different storage conditions of the films significantly affected the mechanical properties of the films due to the sensitivity of the films towards moisture. Water sorption and mechanical properties of the films were best improved at relative humidity of 23% and temperature of 30°C. The water sorption and mechanical properties of the films are worth to be investigated because the properties affected the stability, shelf life, and application of the films in the food packaging field.

1. Introduction

Food packaging is important to preserve, protect, market, and distribute food. The common used materials for food packaging are metal, glass, paper, and plastic [1]. Among all these materials, plastic particularly synthetic plastic is commonly used in the food packaging industry due to its lightweight, durability, and resistance to breakage [2]. Synthetic plastic is usually derived from petroleum and natural gas which are nonrenewable resources. Synthetic plastic made from nonrenewable resources is nonbiodegradable and thus can affect the environment because they take thousands of years to decompose as they were unable to be broken down by bacteria in the environment [3]. Although synthetic plastic can be recycled, the solid waste generated from synthetic plastic has become a problem as the solid waste tends to increase the landfill space [4]. Alternatively, the extensive

usage of synthetic plastic as food packaging material can be replaced by using biodegradable packaging material from renewable resources such as biopolymer. Biopolymer is environmental friendly because they can be decomposed by the microorganism such as bacteria, enzymes, and fungi [3–5].

Most biopolymers are naturally existing polymers including polysaccharides and proteins [6]. Among them, starch, a polysaccharide, is one of the most abundant biopolymer source occurring substance which is usually found in the cereal (wheat, corn, and rice) and tuber (tapioca, potato, and sweet potato) of the plants [7]. In this study, tapioca starch was chosen to produce starch-based films due to the high availability, cheapness, biodegradability, and edibility [8, 9]. Tapioca starch can be extracted from the cassava plant tuber (*Manihot esculenta*) which is easy to be cultivated in a tropical climate country [10]. Tapioca starch contains 19% of amylose and 81% of amylopectin which are sufficient to

produce the starch-based films [11]. Starch-based films are also generally safe, odourless, and colourless, thus promising for food packaging application [8].

However, films produced from biopolymer present poor barrier and mechanical properties compared to synthetic plastic [7]. Starch-based films have some drawbacks including limited long-term stability and thus short shelf life caused by water absorption. The strong tendency of intermolecular associations between starch chains due to their hygroscopic characteristic makes starch films more brittle and highly or rapidly soluble in water thus limiting its application [8]. Water sorption of starch-based films will affect the properties of the films resulting to poor mechanical properties in terms of tensile strength, Young's modulus, and elongation at break [12]. Nonetheless, these properties can be improved by the incorporation of reinforcing a nanosized particle or nanofiller to form a bionanocomposite film [5].

To date, research in the application of nanofiller into a biopolymer film for food packaging application has received growing interest. Synthetic nanofillers such as silver and zinc oxide are being incorporated into the food packaging film because of their unique properties such as antimicrobial properties and the ability to improve mechanical, barrier, and thermal properties of the film [1, 5]. Despite the explosion of growth in this field, migration of synthetic nanofillers from the food packaging film into food might occur due to the very small dimensions of the nanofillers (1-100 nm) [1, 13]. Ingestion of synthetic nanofillers may result to long-term adverse effect to a human although there are limited scientific data available on their toxicology [14]. Thus, this research is directed towards synthesizing natural nanofiller particularly chitosan nanoparticle (CNP) and developing bionanocomposite films particularly starch/CNP films with enhanced water sorption and mechanical properties.

Chitosan has a great potential in food packaging application due to its value-added properties which are biodegradability, biocompatibility, good antimicrobial activity, and nontoxicity, and it improves chemical and physical properties of biopolymer [15, 16]. Chitosan can be used to produce films and coating to enhance the shelf life of food product [16]. Although there are numerous studies of incorporating micron-sized chitosan into starch-based films, the incorporation of nanosized chitosan or CNP into starch-based films has not been studied extensively. Sodium tripolyphosphate (TPP) can be used as a cross-linker to synthesize the CNP via the ionic gelation process [16, 17]. A negative charge of phosphate anions in TPP molecules tend to cross-link with a positive charge of amino cations in chitosan, thus forming a spherical shape of CNP. CNP provides a large contact surface area that favours strong matrix-filler interaction compared to micron-sized chitosan. Their tiny size allows them to occupy the empty spaces in the pores of the starch matrix [17]. Filled matrix pores and a large contact surface area provide the strong interaction between matrix and CNP; thus, the composite structure become compact and rigid [18]. The rigid structure may improve mechanical and water vapor barrier stability compared to the pure starch film [19, 20]. They also exhibit antimicrobial properties to the

food packaging film [16]. A safe edible food packaging film with improved properties can be developed by incorporating CNP into a starch-based film.

Since starch/CNP films are made up of natural resources, they have high sensitivity towards surrounding conditions such as temperature and relative humidity [12, 21]. Therefore, investigation on water sorption behavior of the films at different temperatures and relative humidities towards mechanical properties of the films is essential to develop food packaging films with excellent properties. To the best of our knowledge, no studies have been conducted to investigate the water sorption behavior and mechanical properties of starch and starch/CNP films at different relative humidities and temperatures. Moisture content and mechanical properties of the films play an important role in the performance of the films because moisture is a transferrable element which can pass through the film and hence change the properties of the film as well as the product being packaged [8]. Water sorption isotherms provide information on the moisture content-binding capacity of the packaging films at determined environment relative humidity and temperature and are useful for the analysis of moisture sorption and their effect on mechanical properties [7, 22].

The aims of this work have been directed towards developing bionanocomposite films particularly starch/CNP films and investigating the water sorption characteristic of the films and their relationship to mechanical properties at different temperature (4, 30, and 40°C) and relative humidity (23, 50, and 75%) conditions. This research work brings the potential to develop new food packaging materials with improved properties that meet increasing demands in society for food safety.

2. Materials and Methods

2.1. Materials. Low molecular weight chitosan powder (molecular weight: 50 kDA, degree of deacetylation: 75-85%) and sodium tripolyphosphate (TPP) have been purchased from Sigma-Aldrich, USA. Glacial acetic acid, sodium hydroxide (NaOH), and glycerol have been purchased from R&M Chemical, UK. Tapioca starch powder (Cap Kapal ABC, Malaysia) has been purchased from Thye Huat Chan Sdn Bhd. Note that all the materials were food grade materials.

2.2. Synthesis of CNP. CNP was produced using the ionic gelatin process as reported by Gokce et al. [23] with modification of the initial amount of chitosan. The initial amount of chitosan of 15% *w/w* per 100 g of starch was chosen to produce CNP due to the good mechanical, thermal, barrier, and antibacterial properties of the 15% *w/w* of the starch/CNP film [24]. An amount of 0.45 g (15% *w/w* of starch) of chitosan powder was dissolved in 50 mL aqueous solution of 1% *v/v* acetic acid with constant stirring (stirring speed: level 6) for 30 minutes at room temperature (20°C) using a stirring hot plate (Favorit HS0707V2, Malaysia). After that, the initial pH of the chitosan solution (pH 3.7) was adjusted to pH 4.6 using 10% *v/v* sodium hydroxide (NaOH).

Next, TPP solution was prepared by dissolving 0.09 g of TPP powder (ratio TPP to chitosan: 1:5) in 50 mL distilled water. The TPP solution was added dropwise to the chitosan solution under vigorous magnetic stirring (stirring speed: level 10) at room temperature (20°C) for 30 minutes using a magnetic stirrer. CNP was formed spontaneously via the ionic gelation mechanism upon the addition of TPP. Then, the CNP suspension underwent ultrasonication using an ultrasonic probe (Qsonica, USA) for 15 minutes at amplitude of 50% with pulse on at 60 seconds and pulse off at 10 seconds to break any aggregations and reduce particle size of the CNP. The beaker containing CNP suspension was placed in the ice bath during ultrasonication to ensure that the temperature of CNP suspension was in the favourable range (25-30°C). Then, CNP suspension (average size of CNP: 53.93 ± 0.07 nm [24]) was stored in the chiller (4°C \pm 2) prior to the preparation of starch/CNP films.

2.3. Preparation of Starch/CNP Films. Starch/CNP films were prepared by the solution casting method. Film-forming solution (FFS) was prepared by mixing 3 g of tapioca starch (Cap Kapal ABC, Malaysia) and 100 mL of distilled water. An amount of 0.75 g (25% *w/w* of starch) glycerol (R&M Marketing, UK) was added dropwise as the plasticizer. Then, FFS was heated with constant stirring (stirring speed: level 6) using a magnetic stirrer until gelatinized completely at 75°C. The starch solution was then cooled down until 40°C before mixing with CNP suspension.

CNP suspension was dispersed into FFS with constant stirring for 30 minutes using a stirring hot plate, then underwent ultrasonication for 5 minutes using an ultrasonic probe to produce a homogeneous solution. An amount of 50 mL mixture of starch/CNP FFS was poured into an acrylic petri dish (diameter: 14cm) and dried in air-conditioned room at temperature of 20°C for 48 hours on a flat table to ensure that the film thickness was uniform. Then, the petri dish containing the mixture of starch/CNP FFS was continued to be dried at temperature of 40°C in a ventilated oven to constant weight for about 3 hours. The starch film without addition of CNP was also prepared as the control film. Composition of starch films and starch/CNP films was summarized in Table 1.

After drying, the film was peeled off from the petri dish and conditioned in a desiccator containing saturated magnesium nitrate (R&M Marketing) solution (relative humidity (RH): 51%, temperature: 30°C). Film thickness was measured using a micrometre (Mitutoyo Digimatic Model MDC-1" SX, Japan). Average thickness of the films was found to be around 0.06 mm.

2.4. Morphology of Starch/CNP Films. Transmission electron microscopy (TEM) (Model 300 kV HF-3300 TEM/STEM, Hitachi, Japan) was utilized to observe the morphology of starch/CNP films. The films were cut using cryoultramicrotome (RMC PowerTome PC, USA) to obtain thin cross-section of the film. Then, the thin films were mounted on the 300-mesh copper and viewed using TEM.

TABLE 1: Composition of the films.

Composition	Starch films	Starch/CNP films
Starch	3 g	3 g
Glycerol	0.75 g	0.75 g
CNP	0	0.45 g (15% <i>w/w</i> of starch)

2.5. Moisture Curve and Rates. The films were cut into dimension of 30 mm \times 30 mm, and the initial weight of the films (W_i) was measured. Then, the films were predried in a desiccator containing dried silica gel (0% RH) for 3 days at 30°C, and the final weight of the predried films (W_d) was measured. The initial moisture content was calculated using

$$M_i = \frac{W_i - W_d}{W_d}, \quad (1)$$

where M_i is the initial moisture content (% dry basis), W_i is the initial weight of the film (g), and W_d is the weight of the film after the predrying process (g).

The predried films were placed in separate desiccators in triplicate samples containing different saturated salt solutions. The saturated salt solutions included potassium acetate (23%), magnesium nitrate (50%), and sodium chloride (75%) providing different relative humidity conditions in the desiccators. The water sorption properties were investigated at three different temperatures which were 4, 30, and 40°C whereby the desiccators containing films were conditioned separately in the chiller (4°C) and incubator (30°C and 40°C). The digital temperature humidity meter with a probe (Pro'sKit, USA; standard deviation of RH: \pm 6%) was placed in each desiccator to monitor the temperature and RH.

The weight gained of each film was determined for every 2 hours on the first day. Then, the weight of each film was determined every 24 hours until two consecutive weightings were almost equal or constant. The equilibrium moisture content of the film that correspond to the different temperatures and relative humidities was calculated using

$$M_e = \frac{W_e}{W_i} (M_i + 1) - 1, \quad (2)$$

where M_e is equilibrium moisture content of the film (% dry basis), W_i is the initial weight of the film (g), and W_e is the equilibrium weight of the film (g).

The moisture sorption data of the films was then fitted to the Peleg model to determine the sorption process in the starch films [26]. The following is the equation (equation (3)) by Peleg [25]:

$$M_t = M_i + \frac{t}{k_1 + k_2 t}, \quad (3)$$

where M_t is the moisture at time t (%), M_i is the initial moisture content (%), k_1 is the Peleg rate constant, k_2 is the Peleg

capacity constant, and t is time. Rearranging equation (3), it becomes

$$\frac{t}{M_t - M_i} = k_1 + k_2 t. \quad (4)$$

From the Peleg model, a curve plot of $t/(M_t - M_i)$ versus t was constructed, and a straight line was obtained from the plot where k_1 is the intercept value and k_2 is the gradient of the graph. The parameter of k_1 is related to the water sorption rate and k_2 is related to the water adsorption capacity.

2.6. Water Sorption Isotherm. The GAB model was used to determine the water sorption isotherm of the films at different storage conditions. This model was selected because it has been broadly used to determine the sorption behavior of food products [12]. The model is as

$$\frac{M_e}{W_m} = \frac{Cka_w}{(1 - ka_w)(1 - ka_w + Ca_w)}, \quad (5)$$

where M_e is equilibrium moisture content of the sample in dry basis (g), W_m is the GAB monolayer moisture content, C is the Guggenheim constant, a_w is the water activity (%), and k is the factor correcting the properties of multilayer molecules corresponding to bulk liquid.

The parameters (k , W_m , and C) in GAB models were obtained from its second order polynomial form:

$$\frac{a_w}{M_e} = \alpha a_w^2 + \beta a_w + \gamma, \quad (6)$$

where

$$\alpha = \frac{k}{W_m(1 - 1/C)}, \quad (7)$$

$$\beta = \frac{1}{W_m(1 - 2/C)}, \quad (8)$$

$$\gamma = \frac{1}{W_m k C}. \quad (9)$$

This model was solved using the linear regression analysis technique to determine α , β , and γ , and afterward, the parameter values of W_m , C , and k were determined from the substitution calculation technique using equations (7)–(9).

The accuracy of the GAB model was determined by calculating the percentage of root mean square error (RMSE) using

$$\text{RSME} = \sqrt{\frac{\sum((M_{\text{exp}} - M_{\text{cal}})/M_{\text{exp}})^2}{N}} \times 100, \quad (10)$$

where M_{exp} is moisture content of the film determined from the experimental data, M_{cal} is equilibrium moisture

content predicted using the GAB model, and N is the number of the sample film. RMSE value of 10% and less indicate an excellent fit using the model [27].

2.7. Mechanical Analysis. Mechanical properties of the films were determined using a texture analyzer (TA.XTPlus, UK). The films were cut into rectangular shape (100 mm × 15 mm) using a specimen cutter following the ASTM D882. Before proceeding with the mechanical analysis, the sample films were predried in a desiccator containing silica gel for 3 days at 30°C to remove the moisture content. After 3 days, the films were placed in a separate desiccator containing different salt solutions at different relative humidities (23, 50, and 75%) for 7 days at 4, 30, and 40°C whereby the desiccators containing samples were placed separately in conditioning equipment that include a chiller set at 4°C and incubator set at 30°C and 40°C, respectively. A digital temperature humidity meter was placed in each desiccator to monitor the temperature and RH. The mechanical test was run with the clamp distance of 60 mm and the crosshead speed at 0.85 mm/sec, respectively [24]. The parameters such as tensile strength, elongation at break, and Young's modulus were determined.

3. Results and Discussion

3.1. Morphology of Starch/CNP Films. Figure 1 shows the TEM micrograph of 15% w/w starch/CNP film at ×20k magnification. Black particles in Figure 1 represent the CNP whereby the diameter size range was found to be between 20 and 100 nm. Most of the CNP was dispersed well in the starch film due to the steric stabilization created by the starch matrix and glycerol to the CNP [28]. Combination of the starch matrix and glycerol forms a protective layer around the CNP thus inducing the steric repulsive forces and leading to the steric stabilization. Well dispersion of CNP in the films resulted to the improvement of water sorption and mechanical properties of the starch/CNP films compared to starch films. This was due to the good intermolecular interaction between starch and CNP thus improving the structure and properties of the films.

3.2. Moisture Curve. Figures 2(a)–2(c) and 2(d)–2(f) show the moisture content of starch films and starch/CNP films, respectively, at different temperatures (4, 30, and 40°C) and relative humidities (23, 50, and 75%). It can be seen from Figure 2 that the moisture content of the films increased rapidly during the first 2 to 8 hours due to the high partial pressure of saturated vapor at the initial stage [29] which indicated the early stage of water sorption activity of the film. At some stage, there seems to be a “shoot” in moisture content probably due to the very high difference in pressure between the film and the water surroundings at the initial stage of water sorption activity. Then, the rate of water sorption declined with time as the moisture content inside the film was increased [30]. In general, moisture content of the films then reached a plateau indicating that moisture content of the films became equilibrium with the relative humidity [12].

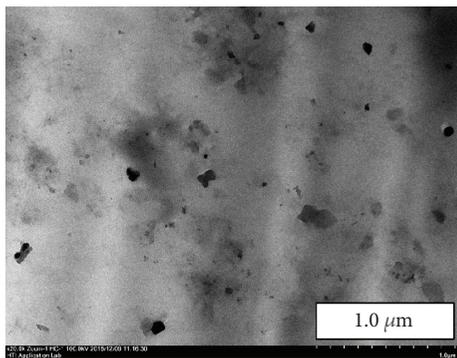


FIGURE 1: TEM image of starch/CNP films (magnification at $\times 20k$).

Generally, in Figure 2, moisture content of the films was seen to be influenced by relative humidity. Films stored at high relative humidity condition contained higher amount of moisture or water compared to films stored at lower humidity condition. For example, from Figure 2(a), it can be seen that at temperature of 4°C , the amount of moisture content of starch films at 75% RH was much higher than at 23% RH. Equilibrium moisture content of starch films at 75% RH was around 15% while that of 23% RH was around 3.5%. At high relative humidity condition, water molecules were adsorbed into the films faster than the films stored at low relative humidity condition. This was due to the saturated water molecules at high relative humidity condition, thus increasing the rate of adsorption of water molecules in the surrounding into the films [31].

Furthermore, more time was needed for the moisture content of the films to reach equilibrium at high relative humidity than low relative humidity [22]. For example, it can be clearly seen from Figure 2(d) that at temperature of 4°C , more time was needed for moisture content of the starch/CNP films to reach equilibrium at 75% RH than at 23% RH whereby at 75% RH, around 96 hours was needed for the moisture content of the films to reach equilibrium while that of 23% RH was around 8 hours. Note that saturated water molecules in the surrounding also lead to the longer time for the water sorption activity to reach equilibrium. Hence, the time required for the moisture content to reach equilibrium increases with increasing relative humidity [31].

Besides, the effect of temperature on water sorption activity of the films can also be observed from the value of equilibrium moisture content of starch and starch/CNP films at different temperatures. According to Lei et al. [30], temperature influences the binding characteristic of water molecules in the film matrix. It can be observed from Figures 2(a)–2(f) that the amount of equilibrium moisture content for both starch and starch/CNP films at 75% RH and temperature of 4°C was the highest followed by at 75% RH and temperatures of 30°C and 40°C . This indicated that if water activity is maintained constant, an increase in temperature leads to a decrease in the amount of water absorbed. This was due to the fact that at high temperature, some water molecules are activated to energy levels that allow them to break away from their sorption sites in the film. This resulted to a decrease of equilibrium moisture content [32].

Furthermore, comparing between Figures 2(a)–2(c) and 2(d)–2(f), it can be seen that starch/CNP films contained higher moisture content than starch films. For example, at 4°C and 75% RH (Figure 2(d)), starch/CNP films contained around 28% moisture compared to that of 15% (Figure 2(a)) in starch films. This was due to the strong hygroscopic nature of chitosan [33] which tend to absorb moisture from the surrounding air. The hygroscopic characteristic of food packaging films may be an advantage to wrap fresh food products such as fruits and vegetables but may be disadvantage for dry food products such as bread and cereal.

3.3. Water Sorption Analysis Using the Peleg Model. Water sorption data of starch and starch/CNP films obtained at different temperatures and relative humidities was then fitted using rearranged Peleg's model (equation (4)). The plot of $t/(M_t - M_i)$ versus time t allowed determination of the Peleg constants. The constant k_1 (water sorption rate) and constant k_2 (water adsorption capacity) as well as the R^2 (regression square) values were determined from the intercept, gradient, and R^2 of the plot, respectively. The values obtained were tabulated in Table 2. It can be observed from Table 2 that the R^2 values were varied from 0.96 to 0.99 which confirmed the adequacy of the Peleg model in describing the water sorption behavior of starch and starch/CNP films within the range of temperature and relative humidity.

In general, k_1 and k_2 decreased with the increase in relative humidity. Low value of k_1 indicates that the films exhibit a high initial water sorption rate [25] while low value of k_2 indicates that the films exhibit high water adsorption capacity [12]. Note that saturated water molecules in the surrounding also result in high kinetic energy, thus increasing the sorption of the water into the films [31]. This resulted to an increase in the rate of sorption (low k_1) and water adsorption capacity (low k_2) with an increase in relative humidity whereby the films absorbed faster and stored more water when kept at high relative humidity surroundings [12]. This finding seems to be consistent with the previous discussion whereby the increase in relative humidity resulted to an increase in equilibrium moisture content. Meanwhile, based on the results obtained in Table 2, it was found that starch/CNP films exhibited lower k_1 and k_2 values at any temperature and relative humidity compared to starch films. This trend was due to the hygroscopic nature of chitosan [33] that resulted to a high water sorption rate and high water adsorption capacity compared to starch films without addition of CNP.

In terms of temperature, no specific trend of k_1 values with the change in temperature was observed for various levels of relative humidity. According to Othman et al. [31], at any specific relative humidity, temperature increment may result to a reduction in water sorption. This was due to the high kinetic energy caused the water molecules to become unstable and escape from the binding sites in the films, thus reducing the water sorption rate. On the other hand, increment in temperature at any specific relative humidity may lead to an increment in the water sorption rate, owing to additional new binding sites opening for water to be

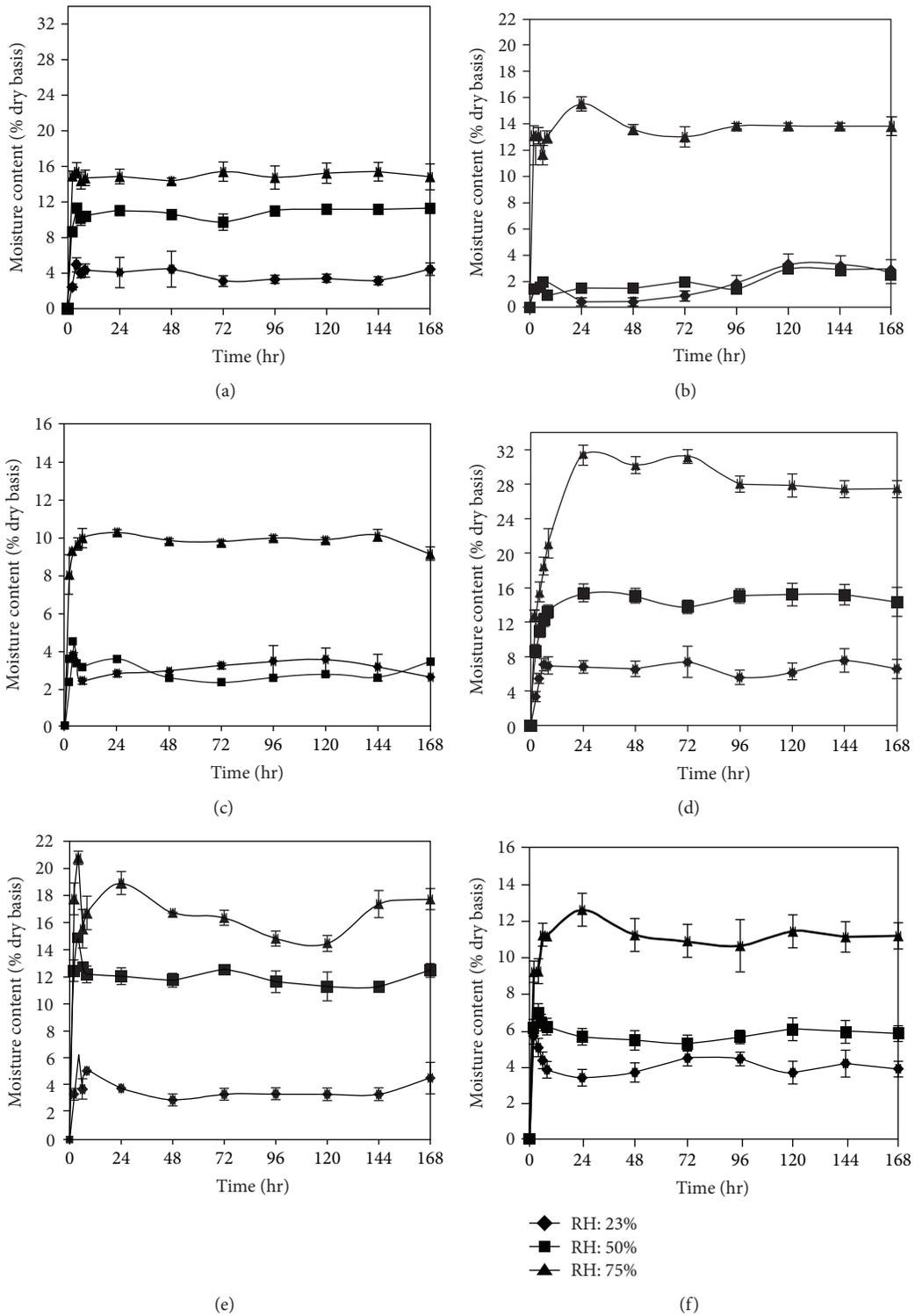


FIGURE 2: Moisture content of starch films at different relative humidities (23%, 50%, and 75%) at temperatures of (a) 4°C, (b) 30°C, and (c) 40°C as well as moisture content of starch/CNP films at different relative humidities (23%, 50%, and 75%) at temperatures of (d) 4°C, (e) 30°C, and (f) 40°C.

adsorbed. However, for various temperatures, a linear trend of k_1 and k_2 can be observed from Table 2 whereby k_2 decreased with decrement in k_1 and k_2 increased with increment in k_1 . A high water sorption rate (low k_1) offers a higher chance for the films to hold more water, hence the high water

sorption capacity (low k_2). Meanwhile, the low water sorption rate (high k_1) offers a lower chance for the films to hold water, hence low water sorption capacity (high k_2).

It is recommended from Table 2 that in order to reduce the water sorption rate and water adsorption capacity hence

TABLE 2: Water sorption rate (k_1), water sorption capacity (k_2), and regression square (R^2) obtained from water sorption data fitted to the Peleg model at different temperatures for (a) starch films and (b) starch/CNP films.

Relative humidity	Temperature 4°C			Temperature 30°C			Temperature 40°C		
	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Starch films									
23%	0.0651	0.0832	0.9966	0.2193	0.0897	0.9954	1.0547	0.1966	0.9607
50%	0.0366	0.0507	0.9992	0.0867	0.0740	0.9999	0.1229	0.1471	0.9927
75%	0.0336	0.0423	0.9993	0.0092	0.0422	0.9997	0.1070	0.0700	0.9965
Starch/CNP films									
23%	0.0072	0.0541	0.9984	0.1114	0.0856	0.9949	0.0271	0.0785	0.9995
50%	0.0062	0.0377	0.9993	0.0206	0.0466	0.9984	0.0200	0.0623	0.9997
75%	0.0016	0.0252	0.9983	0.0111	0.0390	0.9938	0.0168	0.0582	0.9992

increase stability and shelf life of both the films, the films need to be kept at both low relative humidity and high temperature.

3.4. Water Sorption Isotherm Using the GAB Model. Water sorption data of starch and starch/CNP films at different temperatures and relative humidities was then fitted using the GAB model (equation (5)). The plot of a_w/M_e versus a_w (equation (6)) resulted in a second order polynomial equation, and the constant values determined from the equation were used to calculate the GAB constant parameters (k , W_m , and C) using equations (7)–(9). The GAB constant parameters as well as RMSE values were tabulated in Table 3.

Among all the GAB constant parameters, the monolayer moisture content (W_m) is the most important parameter to be studied [34]. W_m indicates quantity of water that is strongly adsorbed to the specific sorption site of the film [35]. It also indicates the number of sorption sites. From the tabulated data, it was found that the W_m values of starch and starch/CNP films decreased with the increase in temperature from 4°C to 40°C. According to Akoy et al. [34], the decrease in the monolayer moisture content of the films at higher temperatures is expected since the total number of active sorption sites for water binding decreases owing to physical or chemical changes in the film that are caused by the temperature rise.

Monolayer moisture content also corresponds to the minimum moisture to avoid autooxidation and to improve the stability of the films. However, at the level of the monolayer moisture content, there is still the possibility for some trace elements to be exposed, which resulted in a browning effect [36]. Thus, the higher the temperature, the lower the amount of moisture required to cause autooxidation as well as to impair the stability of the film. It can be deduced that at high temperature and high relative humidity condition, the lower are the chances to prevent autooxidation and the higher the chances of the film to become unstable. Consequently, for high temperature condition, it is recommended for the films to be kept at as low relative humidity as possible to avoid autooxidation.

Meanwhile, W_m of starch/CNP films was found to be lower than starch films except at a temperature of 40°C. This was due to the tortuous water pathway created by the CNP in the films which lower the water permeability of the films, and

TABLE 3: GAB constant parameters for (a) starch films and (b) starch/CNP films at different temperatures.

Constant	Temperature 4°C	Temperature 30°C	Temperature 40°C
Starch films			
k	-0.0154	-0.4644	-0.0588
W_m	5.5179	2.3575	0.0036
C	-1.9994	-1.6135	-0.5169
RSME (%)	0.8523	1.0122	0.9999
Starch/CNP films			
k	-0.3684	-0.5593	-0.275
W_m	0.0416	0.0325	0.0302
C	-1.5775	-1.5826	-1.0107
RSME (%)	0.8523	0.9924	1.000

thus, less water was able to adsorb to the sorption site of the films. However, at 40°C, W_m of starch films was higher than starch/CNP films. This finding was most probably due to the fact that chitosan was not stable at high temperature and hence may lost its function as a water barrier. According to Viljoen et al. [37], exposure of chitosan at elevated temperature (40°C) resulted to a decrease in hardness and mechanical strength of chitosan.

From Table 3, the RMSE values for all temperatures were found to be less than 10% which indicated excellent fitting of the data using the GAB model [27]. This demonstrates that the GAB model is reliable and good at predicting the moisture sorption behavior of the films.

3.5. Mechanical Analysis

3.5.1. Tensile Strength (TS). According to Javadian et al. [38], TS plays an important role in determining the mechanical properties of the films developed for usage in many food applications because tensile strength is an indication of film strength. Film strength is important for food packaging application because it determines the protection of the food from external forces. Figure 3 shows the tensile strength for starch and starch/CNP films at various relative humidities and temperatures.

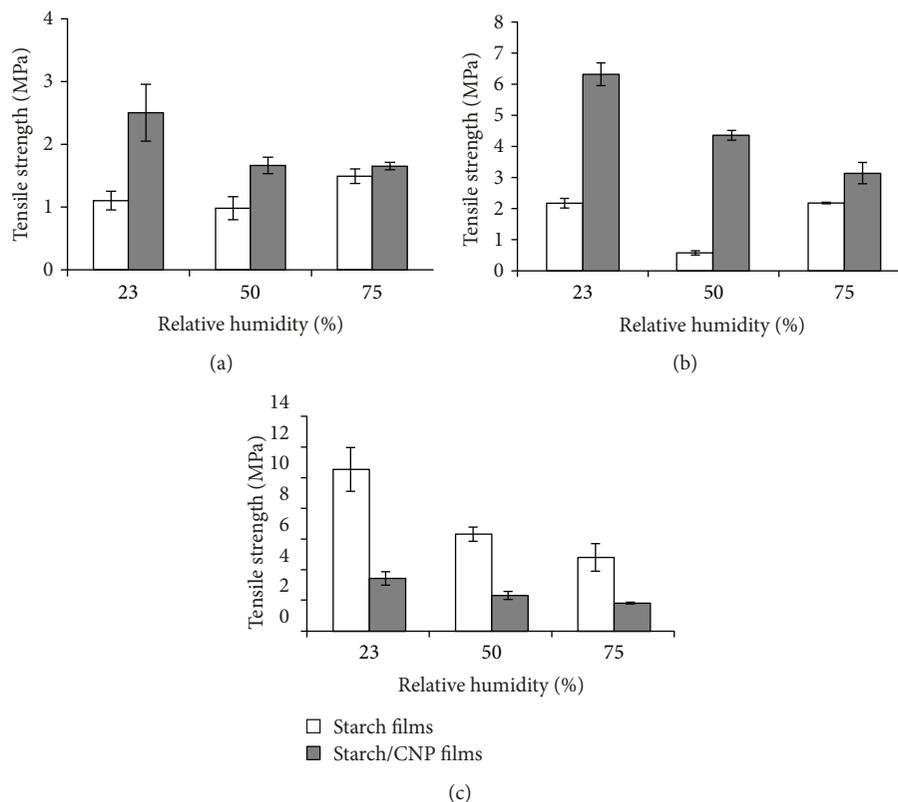


FIGURE 3: Tensile strength for starch films and starch/CNP films at different relative humidities (23%, 50%, and 75%) and temperatures (4°C, 30°C, and 40°C). The error bars represent the standard deviation of measurements for 3 sample films in three separate sample runs.

TS for both films was found to decrease with the increase in RH except for starch films at temperatures of 4°C and 30°C, where TS decreased with the increase in RH from 23% to 50% and then increased with the increase in RH from 50% to 75%. Decrement in TS with the increase in RH was observed because as the moisture equilibrium increased at high RH, the concentration of water absorbed by the films increased thus resulting to plasticizing effect in the film matrix. Mali et al. [12] also found that decrease in tensile strength with the increase in RH was due to the films stored at high RH conditions that presented high equilibrium moisture content, which exerted a plasticizing effect. Water exerted a plasticizing effect and acted as a mobilizer enhancer. However, sometimes, if the concentration of water is too high (high RH condition), the water cannot exert a plasticizing effect on the film; thus, it will increase the TS [22].

Meanwhile, Figure 3 also shows that TS of starch films increased with the increase in temperature at constant relative humidity. For example, at 23% RH, TS of starch films increased from 1.10 MPa to 2.17 MPa as temperature was increased from 4 to 30°C, and TS was further increased to 10.58 MPa as temperature was further increased to 40°C. The amount of water absorbed by the films at constant relative humidity decreased with the increase in temperature as supported by previous findings. Thus, less water was bound at higher temperatures resulting to a decrease in plasticizing effect of the films and causing less weakening of film structure thereby improving TS of the films [29].

However, at 50% RH, there was a decrease in TS of starch films as the temperature was increased from 4 to 30°C (TS decreased from 0.98 to 0.58 MPa) and an increase in TS as the temperature was increased from 30 to 40°C (TS increased from 0.58 to 6.32 MPa). On the other hand, TS of starch/CNP films at all constant RH increased when temperature was increased from 4 to 30°C and then decreased from 30 to 40°C. Bertuzzi et al. [39] reported that water can play a dual role as a plasticizer and antiplasticizer depending on the property measured. As discussed before, at any specific relative humidity, temperature increment may result to increment in the water sorption rate, owing to additional new binding sites opening for water to be adsorbed. This leads to water exerting plasticizing effect on the films thus reducing TS. On the other hand, increment in temperature at any specific relative humidity may result to reduction in water sorption because the high energy level caused the water molecules to become unstable and escape from the binding sites, thus reducing the water sorption rate. The lower water sorption rate may increase the TS due to lower water content, and thus, water exerts antiplasticizing effect on the films.

Besides, Figure 3 also demonstrates that the incorporation of CNP into the films improved the TS of the films stored at 4°C and 30°C but decreased that stored at 40°C. Addition of CNP increased the contact surface area within the biopolymer matrix which enhanced the affinity between the biopolymer matrix and nanosized filler thus improving the TS of the films [40]. At high temperature (40°C), TS of

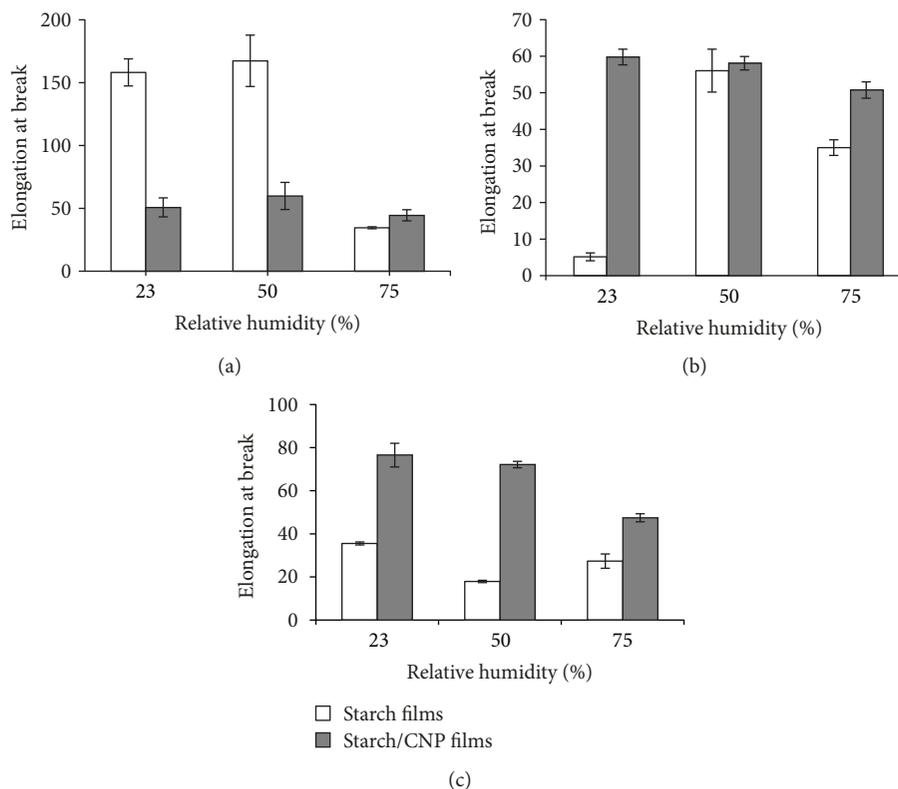


FIGURE 4: Elongation at break for starch films and starch/CNP films at different relative humidities (23%, 50%, and 75%) and temperatures (4°C, 30°C, and 40°C). The error bars represent the standard deviation of measurements for 3 sample films in three separate sample runs.

the starch films was found to be higher than that of starch/CNP films which probably related to the water sorption of the film. At high temperature, water sorption decreased because some water molecules are activated to energy levels that allow them to break away from their sorption sites in the films [32]. The high TS values were observed in starch films attributed to the numerous hydrogen bonding between the starch chains [39] attributed to less water bound with polymer thus causing less weakening of the film structure thereby improving TS of starch films [29]. Apart from that, as discussed previously, chitosan was not stable at high temperature and hence may lost its structural role to act as reinforcement to improve the mechanical properties thus low TS of starch/CNP films.

3.5.2. Elongation at Break (EAB). Elongation at break helps to determine the flexibility and stretch ability of films whereby desired flexibility of packaging films is dependent on their intended applications and subsequent transportation, handling, and storage of packaged foods. Figure 4 shows the EAB of films at different temperatures and relative humidities.

For starch films, at 4°C and 30°C, EAB seems to increase with the increase in RH from 23% to 50% and decrease with the increase in RH from 50% to 75%. This trend seems to be reciprocal to the trend of TS as can be observed from Figures 3(a) and 3(b), as expected. For example, the EAB for starch films at 30°C increased from 5.17 to 56.05% with the increase in RH from 23 to 50% and decreased from

56.05 to 35.02% with the increase in RH from 50 to 75%. Meanwhile, TS for starch films at 30°C decreased from 2.17 to 0.58 MPa with the increase in RH from 23 to 50% and increased from 0.58 to 2.18 MPa with the increase in RH from 50 to 75%. EAB decreased with the increase in TS due to the numerous hydrogen bonding between the starch chains that led to high TS thus reducing the molecular mobility of the starch films and vice versa.

Besides that, these findings can be explained by the same reasoning of plasticizing and antiplasticizing effects as discussed earlier. Increment in EAB with the increase in RH was observed because as the moisture equilibrium increased at high RH, the concentration of water absorbed by the films increased thus resulting to plasticizing effect in the film matrix. However, if the concentration of water in surroundings is too high, the water cannot exert a plasticizing effect on the film; thus, it will increase the TS [22] and decrease the EAB.

However, at 40°C, the EAB for starch films shows an opposite trend to the EAB trend of starch films at 4 and 30°C whereby EAB decreased from 35.52% to 17.90% when RH was increased from 23 and 50% and EAB increased from 17.90% to 27.34% when RH was increased from 50 to 75%. The reduction in EAB from RH 23 to 50% can be explained by the water sorption behavior of the water whereby at high temperature, there was a decrement in water sorption of water as can be seen from Table 2 thus reducing the plasticizing effect of water and decreasing the EAB. However, at high

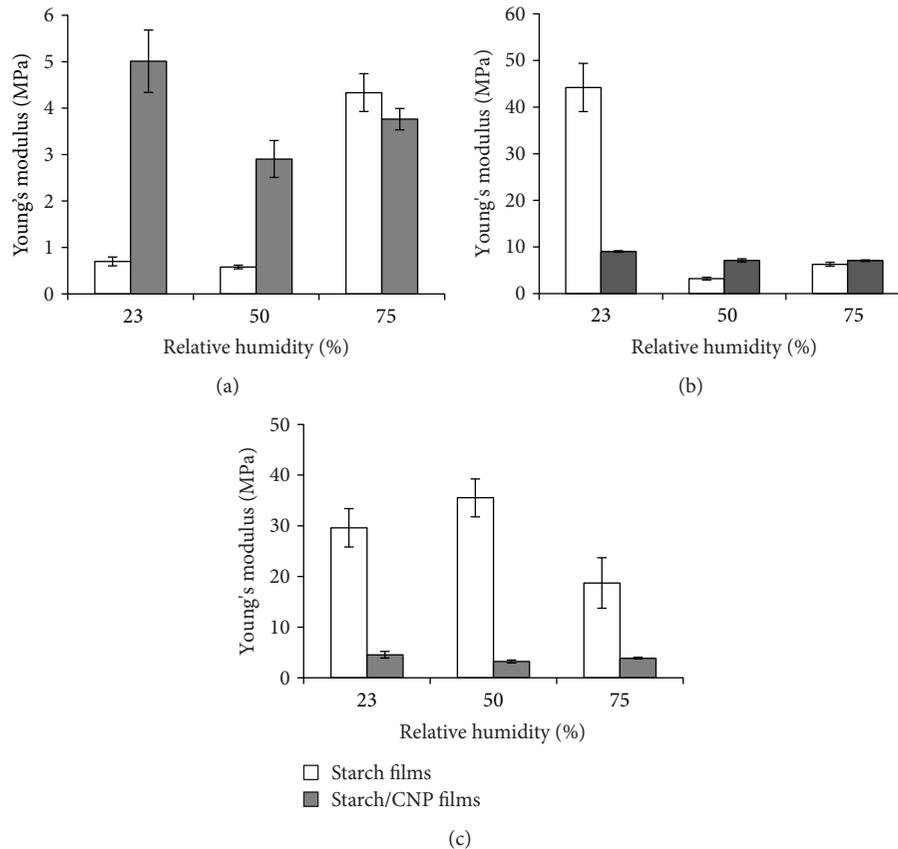


FIGURE 5: Young's modulus for starch films and starch/CNP films at different relative humidities (23%, 50%, and 75%) and temperatures (4°C, 30°C, and 40°C). The error bars represent the standard deviation of measurements for 3 sample films in three separate sample runs.

relative humidity (RH 75%), an increase in equilibrium moisture content resulted to the increment in water content thus increasing the plasticizing effect of water and increasing EAB.

In general, elongation at break of starch/CNP films decreased slightly with the increase in RH. According to Chinma et al. [29], decrease in elongation at break may be due to the increase in starch crystallinity induced by the high relative humidity in starch films. Suppakul et al. [22] stated that decrease in EAB was due to the antiplasticizing effect of the high level of water plasticizer indicating stronger interactions between the water and amylose and amylopectin in biopolymer that induced a loss of macromolecular mobility. The level of plasticizer decreased with the increase of relative humidity due to the increment in water absorption of the film.

Comparing between EAB of starch films and starch/CNP films, it was found that EAB values of starch films were higher than starch/CNP films at 4°C. Addition of CNP reduced the water permeability rate of the film due to the tortuous pathway created by CNP inside the film matrix. Thus, at low temperature, less water was absorbed by the starch/CNP films than starch films which led to less plasticizing effect of water, and this lowered the EAB of starch/CNP films. On the other hand, EAB of starch/CNP films was higher than starch films at higher temperature presumably due to chitosan that was not stable at high temperature and hence could not create a tortuous pathway in the starch

matrix. As a result, water permeability increased and water content increased and acted as plasticizer in the starch/CNP film matrix; thus, starch/CNP films exhibited high EAB than starch films.

3.5.3. Young's Modulus (YM). Young's modulus or elastic modulus is the fundamental measure of the film stiffness or rigidity of the material [38]. High YM indicates high stiffness of material. Figure 5 shows the YM for starch films and starch/CNP films at different relative humidities and temperatures. Overall, YM of both films decreased with the increase in RH from 23 to 50% and then increased with the increase in RH from 50 to 75% except for starch films at 40°C. The decrease in YM when RH was increased from 23 to 50% was due to the increase of water absorbed by the films and thus lowering the hydrogen bonding between the film molecules which made the films to become less stiff. Meanwhile, as previously discussed, at higher concentration of water surroundings (RH 75%), the water cannot exert a plasticizing effect on the film thus decreasing the EAB and increasing YM since the films became more stiff.

However, at temperature of 40°C, YM of starch films increased from 29.61 MPa to 35.56 MPa with the increase in RH from 23% to 50% and then decreased from 35.56 MPa to 18.70 MPa with the increase in RH from 50% to 75%. This can be explained by EAB findings since YM has a reciprocal relation with elongation at break [40]. It can be seen from

Figure 4(c) that EAB decreased from 35.52% to 17.90% with the increase in RH from 23% to 50% and increased from 17.90% to 27.34% with the increase in RH from 50% to 75%. Thus, it is expected for YM to increase with the decrease in EAB and to decrease with the increase in EAB due to enhancement in stiffness and decrement in stiffness, respectively.

In general, at constant relative humidity, YM of starch films increased with the increase in temperature from 4 to 40°C due to less water absorption at high temperature and hence antiplasticizing effect of water. YM of starch/CNP films also increased with the increase in temperature from 4 to 30°C due to the same reason but decreased with the increase in temperature from 30 to 40°C due to the instability of chitosan at high temperature which hindered the CNP to act as a reinforcing compound and thus lowering TS and stiffness.

The addition of CNP into starch films improved the YM at temperature of 4°C for RH of 23% and 50% and at temperature of 30°C for RH of 50 and 75% because the CNP provided a large contact surface area which favours strong interaction between the biopolymer matrix and CNP thus enhancing the YM of the films. YM has linear and reverse relations with TS and EAB, respectively [38]. At 4°C and RH of 75% as well as 30°C and RH of 25%, the percentage difference in TS for starch/CNP films was not significant than starch films, but the percentage difference in EAB for starch/CNP films was more significant than starch films, hence lowering YM than starch films.

On the other hand, the value of YM of starch films was higher than starch/CNP films especially at temperature of 40°C because chitosan fails to create a tortuous pathway at high temperature due to the instability, hence high water permeability and water sorption which lead to less stiffness of starch/CNP films than starch films.

4. Conclusions

In this work, it was found that relative humidity and temperature play an important role in determining the water sorption and mechanical properties of starch and starch/CNP films. Thus, for food packaging application, it is vital to control both factors to ensure the efficiency of the films as an alternative food packaging material as both factors will determine the stability and shelf life of the films. This study proves that at certain relative humidity and temperature, addition of CNP into starch films was able to improve the water sorption and mechanical properties of the starch films. To reduce the water sorption and enhance mechanical properties hence increase stability and shelf life of the starch/CNP films, the films need to be kept at a low relative humidity and high temperature, but the temperature must not be extremely high (40°C) because addition of CNP was able to create a tortuous pathway within the film matrix and act as reinforcement at lower temperature of 4 and 30°C but not at 40°C due to the instability of the chitosan at extreme temperature. With the growing awareness on the uses of moisture-sensitive biobased films as food packaging, the study on the effects of relative humidity and temperature towards water sorption

and mechanical properties provides prediction of the durability of starch and starch/CNP films.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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