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Processing and analysis of chitosan nanocomposites reinforced with chitin whiskers and tannic acid as a crosslinker

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ABSTRACT

Chitosan film reinforced with nano-sized chitin whiskers and crosslinked using tannic acid was synthesized by the casting–vaporation method. The mechanical and physicochemical properties of several film samples (consisting of different ratio of chitin and tannic acid) were compared with neat chitosan. Tensile tests show that the addition of chitin improves the nanocomposite films mechanical properties up to 137% compared to neat chitosan, but this is slightly degraded when tannic acid is introduced. However, tannic acid and chitin whisker content greatly reduced moisture content by 294% and water solubility by 13%. Transmission electron microscopy (TEM) and Fourier-transform-infrared spectroscopy (FTIR) were used to investigate the morphology and molecular interaction of film. X-ray diffraction results indicated that the samples with chitin whiskers had a more rigid structure. The addition of tannic acid changed the structure into an anhydrous crystalline conformation when compared to neat chitosan film.

1. Introduction

The production of synthetic polymers based on petroleum has dominated global commercial industries for decades, however their non-biodegradability has caused environmental concerns. The need for biocompatible material has inspired the development of "green" synthetic polymers like polylactic acid (PLA) or polyhydroxybutyrate (PHB), but they are expensive and still have problems with ecological compatibility (Šimkovic, 2013). Bionanocomposite materials are replacing petroleum based materials for many packaging and structural applications. Polysaccharide composites are abundant, biodegradable, and non-toxic. Cellulose and chitosan are types of polysaccharides used for the structural material in food packaging and other bio-based applications. Although they are biodegradable, their mechanical and physiochemical properties are inferior to petroleum based polymers (Tuhin et al., 2012). The structures inherent in many biological organisms consist of a biocomposite matrix, reinforced with fibrous biopolymers (Neville, 1993). This has inspired much research focused on modifying biopolymer matrix materials with nano-sized fillers (1–1000 nm) as a reinforcement.

Chitosan is a biodegradable and biocompatible natural polysaccharide. Its excellent film forming capability gives it great potential for use in many applications. The inspiration for the research in this paper is its potential application as a wing membrane in a biomimetic micro air vehicle (BMAV). This is a type of miniaturized aircraft that generates lift by mimicking the flapping wing motion of flying organisms (e.g. insects or small birds). It is envisioned that future BMAV will be mass produced and used in one-way (disposable) missions. This requires that the wings be composed of biodegradable and biocompatible materials so not to pose risk to the biosphere. However, the poor mechanical properties of neat chitosan make it unsuitable for this and other applications (e.g. packaging). Therefore it is important to study methods of improving its mechanical and physicochemical properties. Past research has indicated that the addition of reinforcement fillers or crosslinking agents may improve these properties (Liu, Su, Lee, & Lai, 2005). The addition of non-biodegradable fillers, such as carbon nanotubes, parallel-aligned graphene oxide and unzipped multiwalled carbon nanotube oxides into a chitosan membrane, will yield a higher mechanical strength that is unique to the filler material selected (Fan et al., 2012; Pan, Wu, Bao, & Li, 2011). But since these fillers are not biodegradable, its applications are limited.

Polysaccharides, such as cellulose and chitin, are one of the most abundant biomaterials on earth with added advantage of low cost, environmental friendly, renewable and biodegradable properties (Muzzarelli, 2012). Chitin is usually found in stiff extracellular coatings typified by the arthropod exoskeleton and developed as

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robust structures for insect exoskeletons and wings (Muzzarelli, 2011a). Research has been done examining the many potential beneficial biomedical applications of chitin (Muzzarelli, 2011b). The use of chitin is also of interest in BMAV materials research and development. The polymers form a highly-structured crystalline network, consisting of three types of polymorphs: α, β, and γ. The α-polymorph is the most abundant and it is also more stable than the other two types (Mathew, Laborie, & Oksman, 2009). Despite its wide availability, the utilization of chitin has been restricted by its intractability and insolubility. Chitin whiskers have been successfully prepared from crab shells (Marchessault, Morehead, & Walter, 1959). Nano-sized whiskers exhibit a high crystallinity and rigidity that can elevate a composite film's mechanical property if it is used as a filler material. Both chitin and chitosan are composed of glucosamine and N-acetylated glucosamine (2-acetyamino-2-deoxy-D-glucopyranose) units linked by (1–4) glycosidic bonds (Möller, Grelier, Pardon, & Coma, 2004).

A polymer matrix can also be modified by introducing a crosslinking agent (Chambi & Grosso, 2006; Sung, Huang, Huang, & Tsai, 1999). This chemical process alters the stability and the mechanical properties of the polymer. Two different reactions are associated with crosslinking: Schiff base or Michael-type adducts (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Glutaraldehyde is a highly effective crosslinking agent for biopolymers, yielding superior mechanical properties and water resistivity than most other agents (Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001). However, its cytotoxicity hinders its biocompatibility. Several chemicals have been studied as alternative crosslinking agents. These include ferulic, tannic acid, genipin and citric (Cao, Fu, & He, 2007; Muzzarelli, 2009; Reddy & Yang, 2010). Tannic acid is extracted from plants and microorganisms. It is fully biodegradable and less expensive to produce, compared to the other chemical derivatives. It is a high molecular weight, polyphenolic compound containing a central carbohydrate core that is esterified by phenol acids (Božič, Gorgieva, & Kokol, 2012). Tannic acid has a high antioxidant capacity and can interact with other biological macromolecules (Shutava, Prouty, Kommireddy, & Lvov, 2005).

Despite its high potential, little has been published on the use of tannic acid as a crosslinking agent for chitosan nanocomposites. Therefore, the objective of this research is to synthesize a chitosan based biopolymer and study the effects of tannic acid and chitin whiskers on its mechanical and physicochemical properties.

2. Materials and methods

2.1. Materials

High molecular weight chitosan powder (with minimum deacetylation degree of 75%) and chitin flakes (from crab shell) were purchased form Sigma–Aldrich (Malaysia). Glacial acetic acid grade AR and tannic acid were purchased from Friendemann Schmidt. Hydrochloric acid (HCI 36–38 wt%) and other agents were provided by Fisher Scientific (and used as received).

2.2. Preparation of chitin whiskers

Chitin whiskers were prepared by the hydrolysis method to remove the amorphous region of the chitin flakes, using a modification of the method reported in Gopalan Nair and Dufresne (2003a). The preparation begins by dispersing 5 g of chitin flakes into 150 ml of a 3 N HCI solution (weight ratio of 1:30) in a flat bottom flask, fitted with a magnetic stirrer under reflux for 3 h at 90 °C. After acid hydrolysis, the suspension was centrifuged at 10,000 rpm for 10 min. This process was repeated three times followed by removal of the HCI solution from the container. The samples were diluted with distilled water for each run. Next, the chitin whiskers slurry was collected and transferred into a dialysis bag. The slurry was dialyzed in running water for 2 h and then soaked in distilled water for 24 h. Once neutralized, the pH of the chitin whiskers suspension was corrected to 2.5 by adding diluted HCI. The dispersion was completed by 10 min of ultrasonic treatment for every 30 cm^3 aliquot. Finally, the chitin whiskers suspension was refrigerated in an air tight container at 6 °C with a final concentration of 2.5 wt% of chitin whiskers in the suspension.

Table 1
Nomenclature of the sets of composite films studied.

Sample set	Sample code	Chitosan (%)	Chitin (%)	Tannic acid (mg TA/chitosan)
I	Control	100		
II	A	100		20
	B	100		40
III	A	90	10	
	B	80	20	
	C	70	30	
IV	A	90	10	20
	B	80	20	20
	C	70	30	20
	D	90	10	40
	E	80	20	40
	F	70	30	40

2.3. Preparation of chitin whiskers/chitosan composite film

The chitosan solution was prepared by adding 2 wt% of chitosan powder into a 2% (v/v) acetic acid solution under magnetic stirring for 3 h at 35 °C. Then, the chitin whiskers were slowly added into the chitosan solution for 1 h, until a mixed suspension was obtained. The desired mass ratio of chitin whiskers to chitosan was controlled at 0:100, 10:90, 20:80, and 30:70. The mixed suspension was cast onto a plastic Petri dish. The casted suspensions were left overnight in a dry cabinet to remove bubbles and later evaporated in a drying oven for 48 h to obtain dry composite films. Theoretical dry weight of each sample was 0.4607 g.

2.4. Preparation of crosslinked chitin whiskers/chitosan film

Chemical crosslinking was achieved by incorporating different amounts of tannic acid (20 and 40 mg of tannic acid per 1 g of chitosan into the suspensions under vigorous magnetic stirring for another 1 h at 35 °C until it was homogenized. The mixed suspensions were cast with the same procedure described in Section 2.3. Table 1 shows twelve different types of composite films (Set I: neat chitosan film, serving as the control sample; Set II: crosslinked chitosan film; Set III: chitosan film embedded with chitin whiskers; Set IV: crosslinked chitosan film embedded with chitin whiskers). All samples have an average thickness of 0.1 mm.

2.5. Microstructural studies by transmission electron microscope (TEM)

The size and morphology of chitin whiskers were examined using a Zeis EFTEM Libra 120 transmission electron microscope (TEM). The chitin whiskers were dispersed in distilled water after ultrasonic treatment and dropped on a carbon coated copper grid, as reported in Ma, Qin, Li, Zhao, and He (2014). The copper grid was stored in dry cabinet for 48 h before viewing.

2.6. Fourier-transform-infrared spectroscopy (FTIR)

The Fourier-transform-infrared (FTIR) spectra of the films were recorded on a Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer. A total of 32 scans were performed at 4 cm^{-1} resolution. Measurements were recorded between 4000 cm^{-1} and 400 cm^{-1}.

2.7. X-ray diffraction (XRD)

Composite films were analyzed by an X-ray diffractometer (Siemens D5000) equipped with Cu Kα radiation source (k = 1.540600 Å) operating at 40 kV and 40 mA (at room temperature). The relative intensity was recorded in the scattering range of (2θ) 5–80° with a step size of 0.1°.

2.8. Mechanical properties

Tensile properties were measured at room temperature using a Shimadzu AGS-X series with 100 N load cell and a crosshead speed of 1 mm/min. Composite films were cut in a rectangular shape (80 mm by 8.5 mm). Tensile strength, Young's modulus and elongation at the break were calculated according to the ASTM D882-02 method. At least five samples were tested for each film.

2.9. Water uptake test

The film was cut into 15 mm × 12 mm pieces. Samples were weighed to the nearest 0.0001 g in a dry state after heating in oven at 105 °C for 1.5 h. Water uptake was measured by immersing dried film pieces in 75 ml of distilled water (under constant agitation) for 1 h at 25 °C. Then samples were recovered and dried with filter paper to remove excess surface water and weighed again. The average value of water uptake was calculated using Eq. (1):

$$\%\ \text{water uptake} = \left[\frac{W_s - W_i}{W_i}\right] \times 100 \quad (1)$$

where W_s is the weight of the swollen sample and W_i is the weight of the sample after heating it in a convection oven to make sure the film is in a completely dry state.

2.10. Solubility test

Immediately after recording the swollen weight (Section 2.9), the samples were dried in a convection oven at 105 °C for at least 1 h 30 min until it reaches a constant dry weight. The samples were weighted to the nearest 0.0001 g. This dry weight was then used in Eq. (2) to find the solubility:

$$\%\ \text{solubility} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \quad (2)$$

3. Results and discussion

3.1. Microstructural studies by transmission electron microscope (TEM)

The morphology of the chitin whiskers was studied using a transmission electron microscope (TEM). Fig. 1 shows TEM images of nano-scale chitin whiskers in dilute HCI. It was found that chitin suspension is composed of individual and aggregated nanocrystals in a lump state. The individual nanocrystals are shaped like long, slender rods with an average aspect ratio of 8.8. The TEM image in Fig. 1a shows that the chitin whiskers have a length of 200–300 nm (with the average value being ~246 nm). Fig. 1b shows that the diameters of the individual chitin whiskers range from 20 to 40 nm (with an average value being ~28 nm). These measurements accord with the dimensions of crab shell chitin whiskers

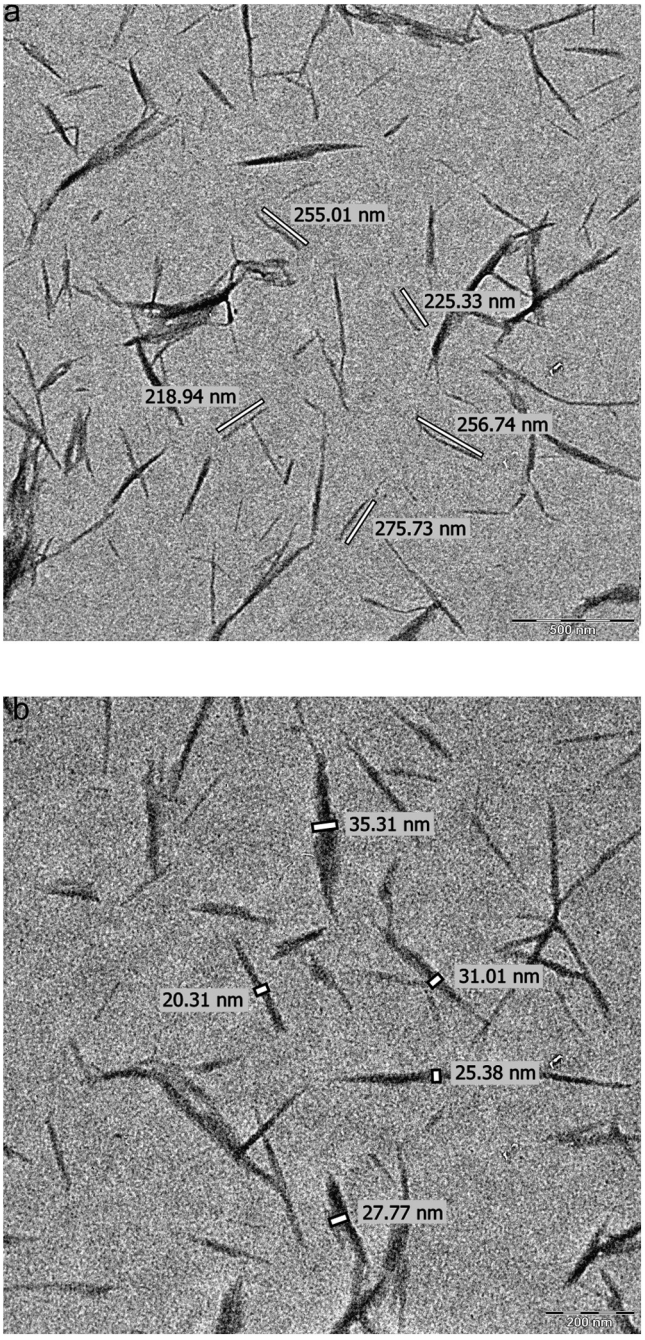

Fig. 1. TEM images of nano-scale chitin whiskers in dilute HCI, (a) magnified at 500 nm with measured length and (b) magnified at 200 nm with measured width.

reported in past studies (Zeng, He, Li, & Wang, 2011). The chitin whisker suspension exhibits colloidal behavior, due to the protonation of the amino groups $(-NH_3)^+$ which induces positive charges on the surface of the crystallites and promotes the stability of the suspension as described by Watthanaphanit, Supaphol, Tamura, Tokura, and Rujiravanit (2008).

3.2. Fourier-transform-infrared spectroscopy (FTIR)

A Fourier-transform-infrared (FTIR) spectroscopy identifies unknown materials and the chemical components in a mixture by emitting infrared radiation through a sample. The resulting spectrum creates a unique representation of the molecular absorption and transmission. This was used to characterize the

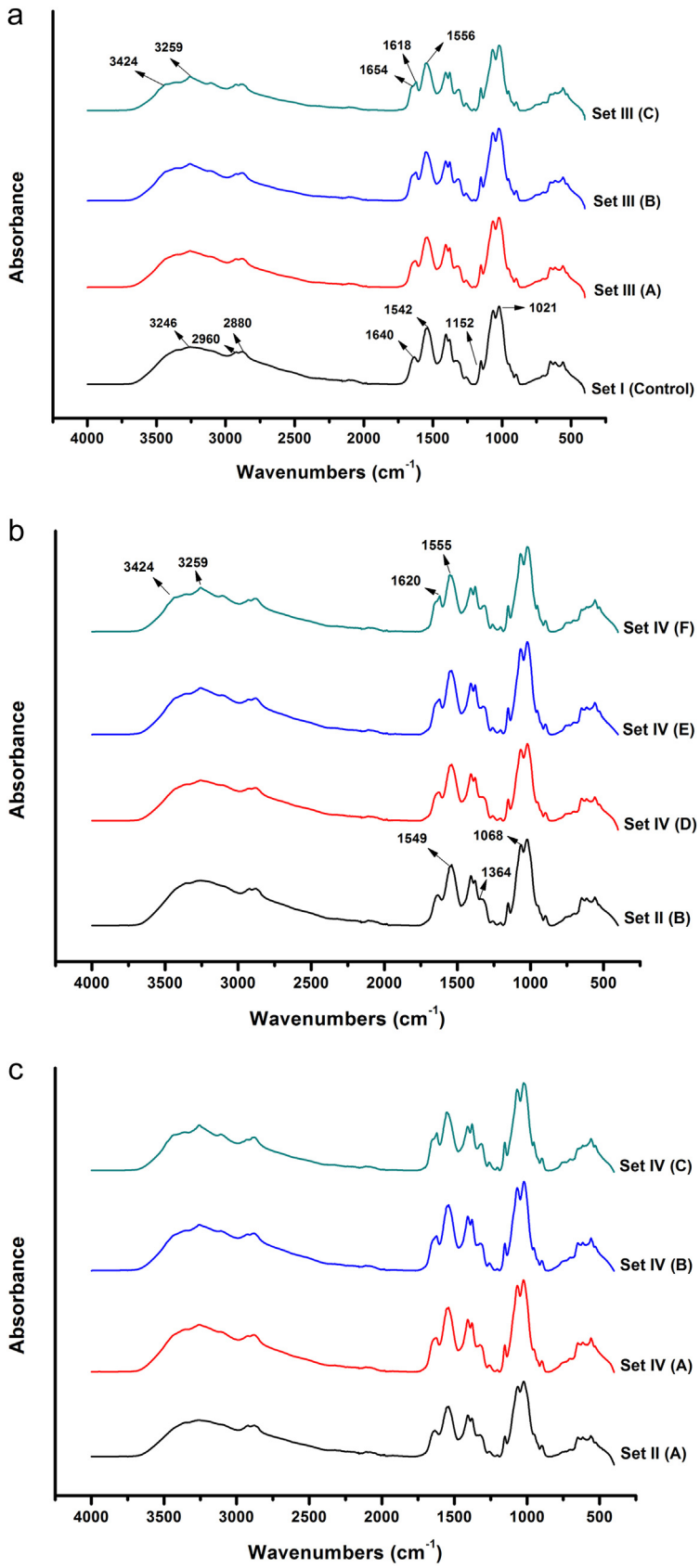

Fig. 2. FTIR spectra of all sample sets, (a) chitosan film without crosslinker, (b) 40 mg tannic acid crosslinked chitosan film, and (c) 20 mg tannic acid crosslinked chitosan film.

microstructure of the composite film. Fig. 2 shows FTIR spectra of all sample sets. The neat chitosan film Set I (Fig. 2a) exhibited a broad band across 3000–3600 cm^{-1} with a maximum absorption 3246 cm^{-1}. These results attributed from the O—H and N—H stretching vibrations of the functional group engaged in hydrogen bonding between the chitosan molecules. Bands at 2880 cm^{-1} and 2960 cm^{-1} are the characteristic of methyl (CH_3) anti-symmetric and symmetric stretching vibrations, in accordance with the past findings of Pradal, Kithva, Martin, Trau, and Grondahl (2011). This film also exhibits distinctive absorption bands at 1640 cm^{-1} (C=O stretching in amide group, amide I band) and 1542 cm^{-1} (NH bending vibration in amide group). An absorption band at 1152 cm^{-1} is the characteristic of anti-symmetric stretching of the C—O—C bridge and 1024 cm^{-1} is the vibration involving C—O group stretching of the chitosan film as reported in Pawlak and Mucha (2003).

Film Set III (C) exhibited split peaks at 1654 cm^{-1} and 1618 cm^{-1} (signature of α chitin) attributed to the stretching of C=O stretching amide I O=C—$NHCH_3$. Traces of chitin validated by the N—H bond of N-acetyl group (amide II) were observed at 1556 cm^{-1} (Saravanan, Hemalatha, & Sudha, 2011). The peaks at 3424 cm^{-1} and 3259 cm^{-1} show a shift to a higher wave number when compared to the neat chitosan (Set I) film spectra. Past reporting relates these peaks with O—H and N—H stretching vibrations (Ifuku, Shervani, & Saimoto, 2013, chap. 3). These observations indicate that there is good miscibility between the chitin whiskers and the chitosan matrix.

Spectra of film Set II (B) (Fig. 2b) showed a band peak located at 1549 cm^{-1}, which is due to the symmetrical deformation of NH_3^+ resulted from ionization of the primary amine group in the presence of carboxylic group. This is caused by dissociative interactions between chitosan and tannic acid under acidic conditions. A weak band at 1364 cm^{-1} replaces the band at 1320 cm^{-1} from neat chitosan (Set I) spectra. The 1068 cm^{-1} band (attributed to the C—O—C bonding vibration) and the 1338 cm^{-1} band both disappear, when compared to the neat chitosan (Set I) film spectra. These changes indicate interactions between the chitosan amino group and the carboxyl group located in tannic acid, as reported by Aelenei, Popa, Novac, Lisa, and Balaita (2009). Film Set IV (F) (Fig. 2b) has significant peaks at both 3259 cm^{-1} and 3424 cm^{-1}, resulting from chitin whiskers in the crosslinked film (this did not appear in Sets I and II). The band shift from 1549 cm^{-1} to 1555 cm^{-1} was caused by the addition of chitin whiskers in the crosslinked film.

The FTIR results show distinct changes in the chemical interaction between Set I, Set II (B), Set III (C) and Set IV (F). Samples with lower concentrations of chitin whiskers and tannic acid show similar changes (Fig. 2c) without any significance.

3.3. X-ray diffraction (XRD)

An X-ray diffraction study was conducted to investigate the microstructure and crystalline structure. Fig. 3 shows X-ray diffractograms of five samples, one from each of the four sets and a sample of pure chitin whiskers. The four samples shown were selected because each had the highest concentration of additives within their particular set, these include: Set I (control), Set II (B) 100% chitosan with 40 mg of tannic acid, Set III (C) 30% of chitin whiskers, Set IV (F) 30% of chitin whiskers with 40 mg of tannic acid and pure chitin whiskers. Neat chitosan exhibited 2 major peaks located at $2\theta = 22.6^\circ$ and 11.4° and two other minor peaks located at 18.1° and 9.3°. These two major peaks are associated with the hydrated "tendons" conformation of chitosan as reported by Ogawa, Yui, and Okuyama, 2004). According to Rivero, García, and Pinotti (2010), addition of tannic acid (Set II (B)) raises the peak in the region at $2\theta = 15^\circ$ that exhibits the characteristic of anhydrous ("annealed") crystalline conformation. In present work, all films in Sets II and IV exhibited similar behavior at $2\theta = 15^\circ$.

Sets III and IV both have a significant rise in the peak than Set I due to the introduction of chitin whiskers. Set III (C), Set IV (F) and chitin whiskers exhibit two major peaks located at $2\theta = 19.3^\circ$ and 9.3° and minor peaks at 23.5° and 21.1°. According to past research (Kadokawa, Takegawa, Mine, & Prasad, 2011) this is a typical diffraction pattern observed with chitin powder. It indicates that the crystallinity of α-chitin powder is the same as the embedded chitin whiskers. Chitin whisker possess highest peak intensity which is far higher than Set I. This indicates that chitin whiskers have high apparent degree of crystallinity. Addition of chitin whiskers showed strong scattering peak of chitin whiskers being more significant with increasing whisker content. However, with the addition of whisker content, the relative intensity and width of scattering peaks belonging to Set I did not change. This indicates that the addition of chitin whiskers does not affect the apparent degree of crystallinity of the chitosan matrix. Similar trend reported by Sriupayo, Supaphol, Blackwell, and Rujiravanit (2005) where the increasing α-chitin whisker does not change the intensity and the width of the scattering peaks of the chitosan matrix and indicated that the presence of α-chitin whisker did not affect the crystallinity of the matrix.

3.4. Mechanical properties

Fig. 4 shows the mechanical properties of all sample sets. The control film (Set I) exhibits a low tensile strength of 22.02 MPa, with a maximum elongation of break of 50.3% (Fig. 4a and b). The addition of chitin whiskers sharply increases the tensile strength and significantly reduces the elongation at break. Set III (C) had the highest tensile strength of 52.23 MPa, which is slightly greater than 49.90 MPa measured for Set III (B) and has an elongation at break of 21.32%. By analyzing the nanocomposite films, increasing the whiskers content makes the tensile strength increase exponentially until it reaches a threshold. There is a possibility that it will reach a maximum value and gradually decrease with further addition of chitin whiskers as reported by Sriupayo et al. (2005).

Crosslinking the chitosan film also increases the tensile strength. Set II (A) recorded a tensile strength of 39.75 MPa with an elongation at break of 26.78%. However, the further addition of tannic acid, as done in Set II (B), yielded an insignificant increase in tensile strength (41.88 MPa). A similar behavior was observed in past research involving gelatin film crosslinked by tannic acid (Peña, de la Caba, Eceiza, Ruseckaite, & Mondragon, 2010). Addition of tannic acid appears to have no effects on the elongation of the Set IV nanocomposite films.

The addition of the chitin whiskers (Set III) clearly increases the Young's modulus (stiffness) of the chitosan matrix (Fig. 4c). The Set III films have much greater stiffness than Set I. In fact Set III (B and C) are both more than twice as stiff as Set I. However, the addition of tannic acid on the nanocomposite increases the stiffness of the chitosan matrix in Set II, but decreases the tensile strength and Young's modulus of Set IV films especially for the Set III (B) and Set IV (B and E).

The results show that the tensile properties and Young's modulus of Sets II, III, and IV are superior to those of neat chitosan film (Set I), due to the reinforcement effect of the crosslinker (Sets II and IV) and chitin whisker fillers (Sets III and IV). Chitin whiskers are rod-like short fibers that have a high aspect ratio and possess a high degree of crystallinity, as can be seen from XRD pattern. This acts as contribution factor that increases the mechanical properties. According to past research (Ma et al., 2014) hydrogen bonding causes the composite film to exhibit higher modulus and strength. However, Fig. 4a shows that the difference in tensile strength between Set III (A and B) is higher when compared to the difference between Set III (B and C). This is due to the aggregation of

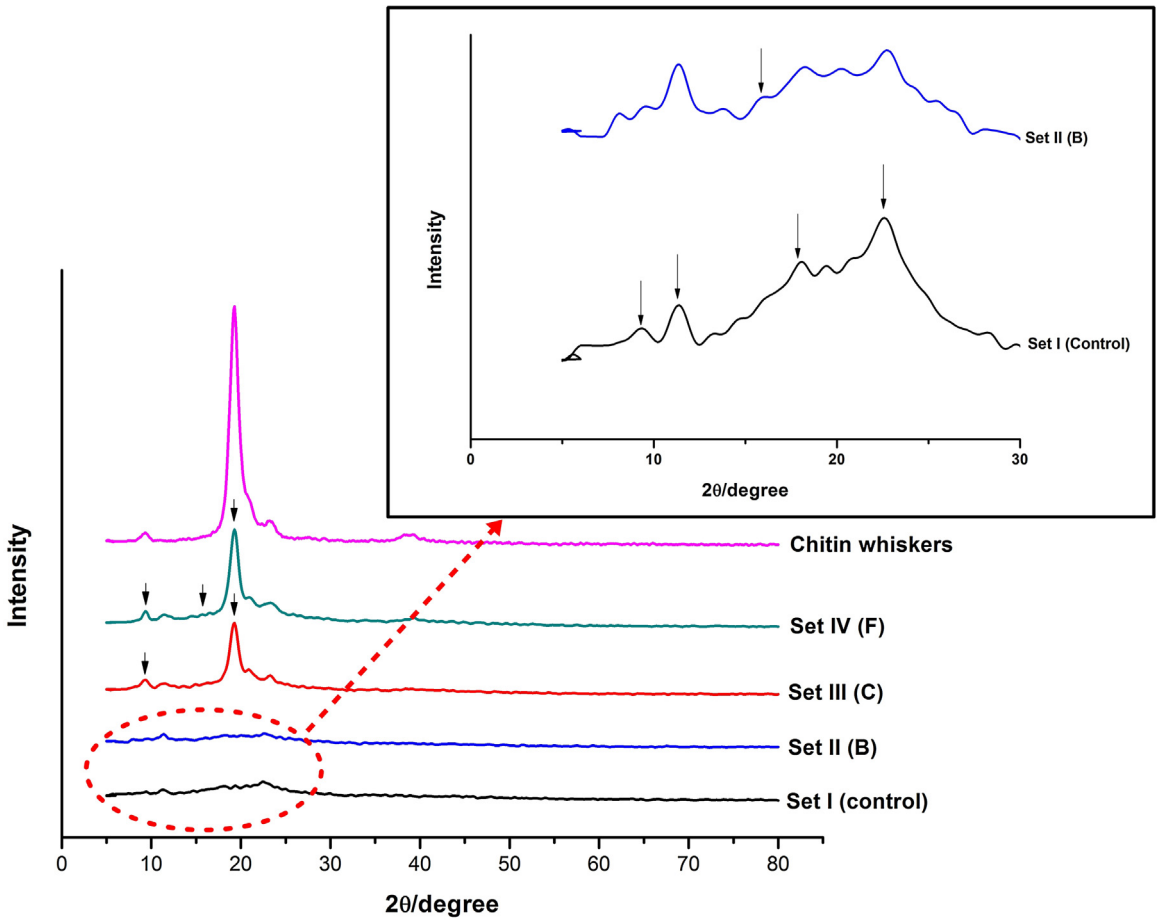

Fig. 3. Comparison of X-ray diffractograms of samples that had the highest concentration of additives in each set and chitin whiskers.

chitin whiskers. The composite film tends to be more rigid, due to higher crystalline structure introduced by chitin whisker, and as a result, the percentage of elongation at break decreases. A similar behavior was reported by Lu, Weng, and Zhang (2004) where the percentage of elongation at break decreases with increasing chitin whisker content for soy protein isolate films. The increase in tensile strength and Young's modulus by crosslinking is probably due to the formation of a more stable network between chitosan and tannic acid. Fig. 4a shows that a further increase of tannic acid results in an insignificant increase in tensile strength for Set II films. This suggests that a slight phase separation occurs, which will reduce the tensile strength as further increments of tannic acid is added. A similar trend was reported by Peña et al. (2010), where a slight phase separation reduced the tensile strength as more tannic acid was added into the gelatin film. The transparency of the film also deteriorated with a higher loading of tannic acid, which agrees well that a slight phase separation took place. The transparency of our film also reduced with the higher loading of tannic acid in Set II (B) and Set IV (D, E and F). Introduction of tannic acid to the Set IV films resulted in a marked decrease in tensile strength and Young's modulus. This is likely due to crosslinking effect of the tannic acid that jeopardizes the formation of the chitin network in the chitosan matrix. Similar results obtained from past research (Gopalan Nair & Dufresne, 2003b) found that strength of the chitin network in the crosslinked natural rubber matrix is lower than that in unvulcanized rubber. They concluded that the chemical crosslinking of the matrix most probably interferes with the formation of the chitin network, thereby a lower tensile modulus in vulcanized samples compared to unvulcanized samples.

3.5. Water uptake and solubility studies

Water solubility and moisture content are an important factor in determining the application of bio-based films. Fig. 5 shows the physicochemical properties of all the sample sets. Set I has a water uptake equal to 555% of its original dry mass (Fig. 5a). This is unacceptably high for any application that requires water resistivity. (Applying this material as the wing membrane in a BMAV is one such example.) The addition of chitin whiskers drastically reduces the swelling behavior and solubility of neat chitosan film (Fig. 5a and b). This is due to the behavior of chitin whiskers that increases the concentration of nanocrystals, in the chitosan matrix which have lower affinity and reactivity to water. This also reduces the number of amino groups that facilitate the interaction with water molecules. The addition of tannic acid on neat chitosan and film and nanocomposite film (Sets II and IV) also decreased the water uptake and solubility. According to Kubota, Kikuchi, Mizuhara, Ishihara, and Takita (1993) N-acetylation with some crosslinking agents can be formed on chitosan molecules with the presence of water, where the reduction of hydrophilic groups would decrease the solubility and dissolution of the crosslinked film. One report observed that (at high temperatures) chitosan film crosslinked with tannic acid has reduced moisture uptake (Rivero, García, & Pinotti, 2011). They postulated that this is due to the carboxylic acids reacting with the amine group to form an amide, eliminating water in the reaction. Since this test was conducted after exposing the samples to a temperature of 105 °C for 1.5 h, the effectiveness of the crosslink increases the hydrophobic and/or hydrogen interaction between chitosan and tannic acid. Therefore this experiment shows that the

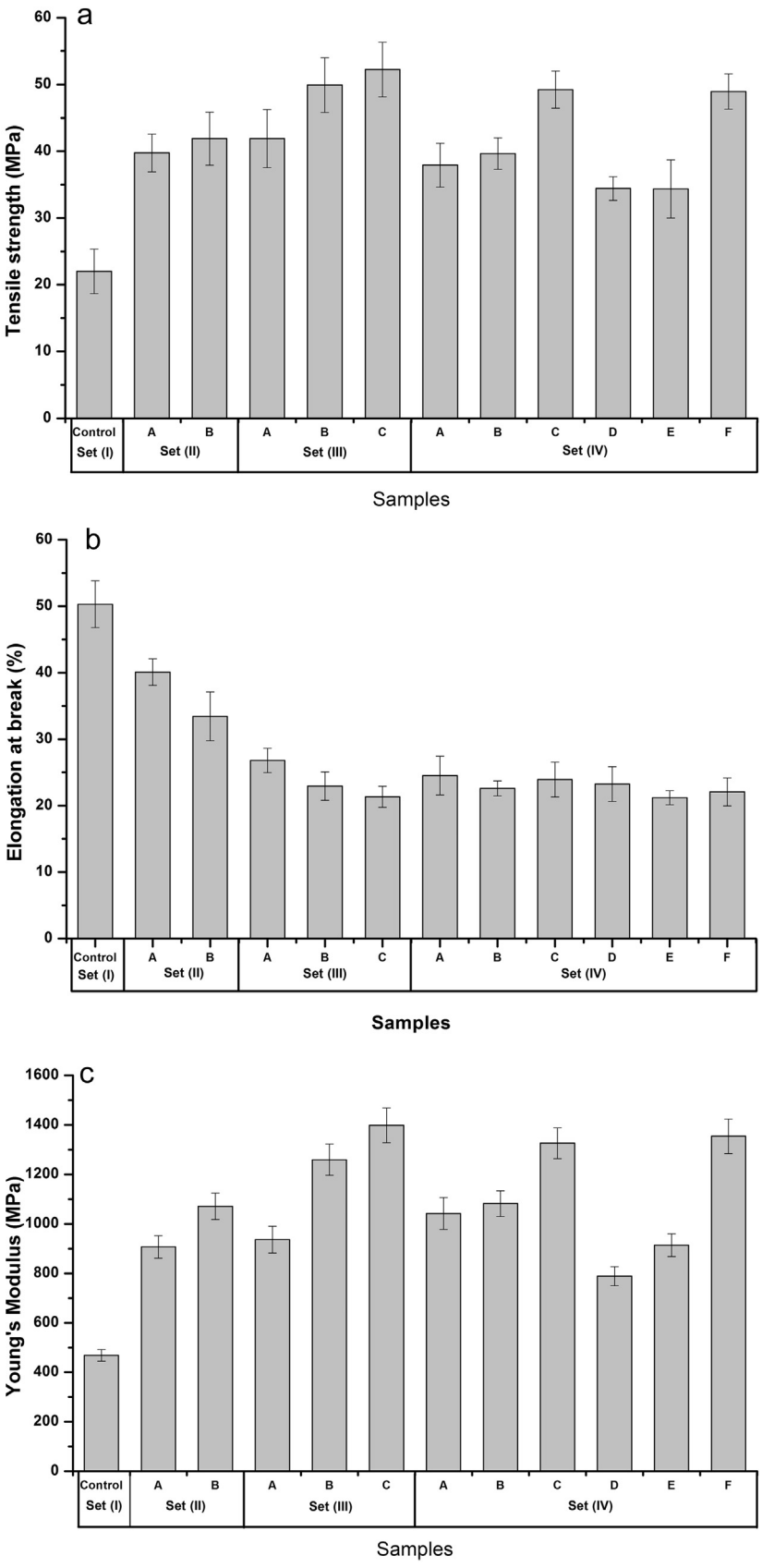

Fig. 4. Mechanical properties of all sample sets, (a) tensile strength, (b) elongation at break, and (c) Young's modulus.

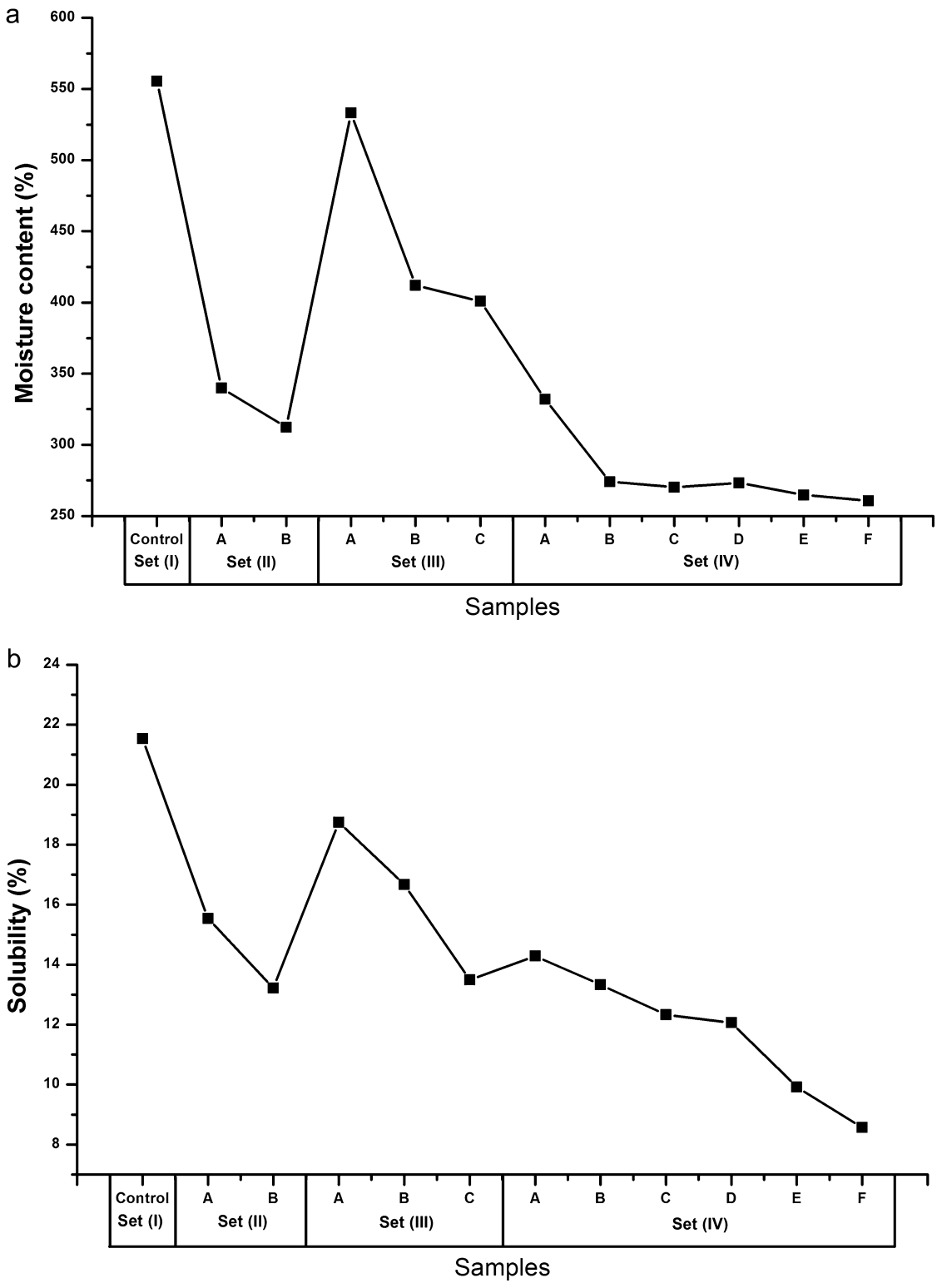

Fig. 5. Physicochemical characterization of all sample sets, (a) moisture content of samples under equilibrium condition at 25 °C and 65% relative humidity and (b) solubility of samples.

water solubility and uptake on chitosan film can be controlled by addition of tannic acid and chitin whiskers.

4. Conclusion

The use of chitin whiskers as an additive, filler material in a chitosan film matrix improves its mechanical properties. Observation of the XRD shows the presence of chitin whiskers did not appreciably affect the apparent degree of crystallinity of the chitosan matrix. Chitin whiskers increase the rigidity and stiffness of the chitosan film. The addition of chitin whiskers also reduced the water uptake and solubility of the film. Crosslinking the chitosan film with tannic acid increases the rigidity of the film structure and increases the mechanical properties of chitosan film. However, tannic acid

reduces these properties in the nanocomposite film. The XRD pattern shows that the introduction of tannic acid changes the film into an anhydrous crystalline conformation. Tannic acid also reduces the swelling behavior and solubility. In summary, the mechanical properties and water resistivity of chitosan film can be controlled by the addition of chitin whiskers and tannic acid as a crosslinking agent. It is hoped that the results of this study will encourage an increased application of chitosan film in commercial industries that produce or consume bio-based polymers.

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