

EXPERIMENTAL INVESTIGATION OF CHITOSAN FILM REINFORCED BY CHITIN FIBERS AND CHITIN WHISKERS EXTRACTED FROM SHRIMP SHELL WASTE

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Abstract

An investigation has been made to predict the effects of fore body and after body chitin and chitosan are natural polymers that have many advantages, such as biocompatibility, biodegradability, healing acceleration, non-toxicity, and anti-infection properties. However, the use of pure chitosan films in many applications is limited due to their poor tensile strength and elasticity. Nevertheless, creating biocompatible and biodegradable high-strength composites is of interest to researchers. In this study, chitosan films were reinforced by the addition of chitin fibres and chitin whiskers with 25 wt.% and 35 wt.% concentration. Chitosan films were prepared by solution casting. The physical, mechanical, chemical, and thermal properties of the synthesized composite films were investigated. Experimental results showed that 25 wt.% chitin whiskers enhanced the yield strength, fracture strength, and Young's modulus of the composites relative to pure chitosan. The thermal degradation temperature of chitosan was improved by adding chitin fibres and chitin whiskers to chitosan composites, which indicated that the composite thin films could be used in thermal applications. Thus, chitin fibre and chitin whisker chitosan composites are promising materials to replace synthetic plastic polymers.

Keywords: Biomaterial, Biopolymer, Composites, Chitin fibres, Chitosan.

1. Introduction

Natural polymers are advantageous because they are biodegradable and biocompatible. Natural polymer materials are carbohydrates and are composed of repeating monosaccharide units; thus, they naturally degrade and release safe, non-toxic chemicals with positive effects on the environment. Natural polymers are cheap and widely available. In the last years, widespread efforts have been made to improve the stability of bio-composite materials for various manufacturing applications. Polysaccharides are widely found in the environment and fulfil various important functions in living organisms and plants [1].

Natural fibres have attracted interest for their potential to replace synthetic fibres in reinforced plastics to improve sustainability at low cost. Natural fibres benefits include renewable sources, low cost of production, high specific strength and stiffness and eco-friendly properties [2]. In this study, chitin fibres and chitin whiskers were used as reinforcement materials to enhance the properties of chitosan films. Chitin is the second most abundant natural polymer after cellulose. The main sources of chitin are crustacean shells, such as the shells of shrimps, crabs and insects, some mushroom envelopes, green algae cell walls and yeasts [3]. The most important derivative of chitin is chitosan, which is obtained by partial deacetylation of chitin under alkaline conditions to form repeating β (1,4)-2-amino-2-deoxy-D-glucose and β -(1,4)-2-acetamido-2-deoxy-D-glucose units, as shown in Fig. 1. The difference between chitin and chitosan is the acetyl content of the polymer. Chitin and chitosan have been widely studied for various uses in pharmaceutical, agriculture, water treatment and food packaging applications due to their biodegradability and biocompatibility and antimicrobial and antibacterial properties.

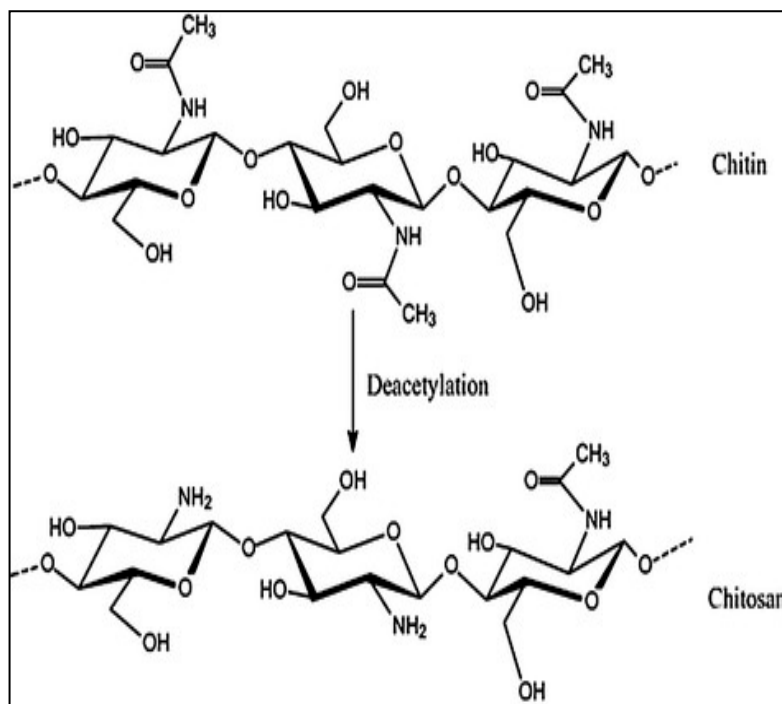


Fig. 1. Chemical structure of chitin and chitosan.

Chitosan is a by-product extracted from chitin using different biological and chemical methods. The chemical method using alkali treatment involves three main steps. The first step is deproteinization (DP), which removes proteins and other undesired materials such as pigments from shrimp shells. This step is followed by demineralization (DM), which uses an acidic solution to remove all minerals. The final step is deacetylation (DD), which removes acetyl groups from the polymeric chain. The quality of chitosan produced depends on the extraction conditions, such as the alkali concentration, acid reaction time, and type of shrimp species [4]. Chitosan can be easily transformed into different forms, such as gels, membranes, beads, nanoparticles, micro particles, and scaffolds. Chitosan has been studied as an excellent biodegradable film in edible coating and food packaging applications. However, the poor mechanical properties of chitosan prevent its use in some applications. Therefore, it is important to study different techniques for improving the mechanical and physicochemical properties of chitosan. Researchers have found that the addition of reinforcement fillers or crosslinking agents may improve these properties [5].

Chitosan composites have been tried in wastewater medications for adsorption of colours and substantial metals. To frame composites with chitosan, various substances have been utilized, for example, montmorillonite, polyurethane, actuated earth, bentonite, zeolites, oil palm debris, calcium alginate, polyvinyl liquor, cellulose, magnetite, sand, cotton filaments, perlite and artistic alumina [6]. Additionally, chitosan shows antimicrobial action against a wide scope of microscopic organisms. This antimicrobial property and film-framing limit together being transparent has made chitosan a good candidate for food packaging [7]. Furthermore, Chitosan and chitosan nanoparticles are used as transporter materials in drug delivery, vaccine transfer, antibacterial mediator, and wound healing applications [8].

2. Methodology

2.1. Materials

Raw shrimp shell waste was purchased from a local fish market in Egypt. All shrimp shell waste included different species of shrimp. All the reagents used were of a high analytical grade and were purchased from Sigma-Aldrich Chemical Company, i.e., hydrochloric acid (HCl, 35-38%), sodium hydroxide pellets (NaOH, pure), and acetic acid (CH₃COOH, 99%). The combinations employed in this research are presented in Table 1.

Table 1. Combinations of chitosan composites with chitin and chitin whisker extracted from shrimp shell waste.

	Matrix	Reinforcement	Reinforcement content %	Code
1	Chitosan (CS)	Chitin	25	CS-25CH
2	Chitosan (CS)	Chitin	35	CS-35CH
3	Chitosan (CS)	Chitin whisker	25	CS-25WH
4	Chitosan (CS)	Chitin whisker	35	CS-35WH

2.2. Methods

2.2.1. Extraction of chitin powder

The shrimp shells were washed well and left to dry at room temperature Fig. 2(a). Then, the shells were subjected to deproteinization (DP) and demineralization (DM). In the deproteinization step, the shells were heated at 110 °C in a solution of 1 N of

NaOH, the solution was exchanged until the brownish colour disappeared, as shown in Fig. 2(b). In the demineralization step, chitin was produced by adding 1 N of HCl for 15 min. to the mixture, till bubbles of carbon dioxide were released. This step was repeated until no bubbles were observed, as shown in Fig. 2(c). The produced chitin was washed with distilled water (DI) until the pH was neutral and then left to dry at room temperature Fig. 2(d). [9].

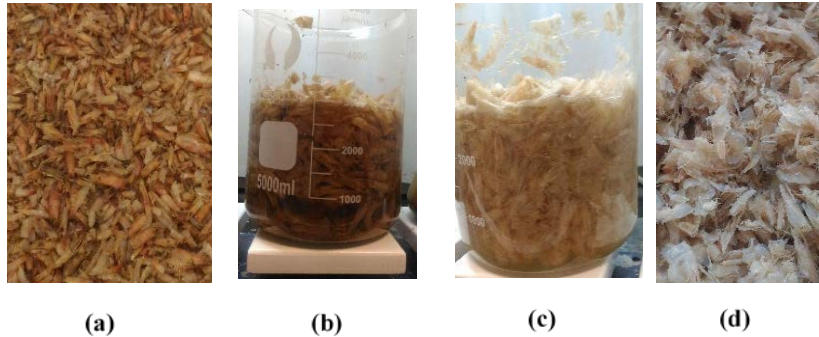


Fig. 2. Steps of chitin extraction
a) Shrimp shell waste, b) DP step, c) DM step, and d) Dried chitin.

2.2.2. Extraction of chitin whiskers (WH)

3 mole HCl was added to 1 g of produced chitin, the mixture was heated and stirred at 90 °C for 3 h. The hydrolytic process was repeated three times. For the third cycle, filtration was very difficult due to the very small size of the whiskers; centrifugation was used to wash the produced chitin whiskers to obtain a neutral pH, and the whiskers were left to dry at room temperature, as shown in Fig. 3 [10].



Fig. 3. Extraction of chitin whiskers.

2.2.3. Extraction of chitosan (CS)

Chitosan was produced by deacetylating (DD) of chitin. 50% NaOH was added to the extracted chitin and heated at 110 °C for 10 h. as illustrated in Fig. 4(a). Chitosan was synthesized at 81% degree of deacetylation and left to dry in room temperature as shown in Fig. 4(b) [9, 11].

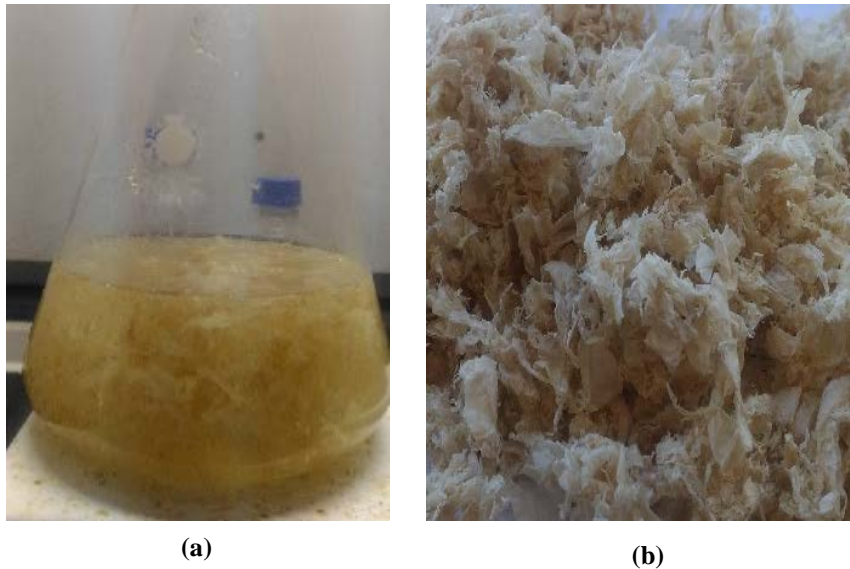


Fig. 4. Steps of chitosan extraction. (a) DD step. (b) Dried chitosan.

2.2.4. Preparation of composites by solution casting

Preparation of CS films with chitin fibres (CH) and WH by casting. A solution of CS was prepared by dissolving 2 g of chitosan powder in 1% acetic acid with heating at 100 °C and magnetic stirring at 300 rpm. Once the CS was dissolved, 25 wt. % and 35 wt. % of both CH and WH were added to the CS solution and left for 1 h. Then, each solution was poured into a flat flexible plastic container and left to dry at room temperature.

3. Characterization Techniques

3.1. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectral analysis was performed to detect and confirm the functional groups of extracted chitins. Additionally, this technique was used to detect changes in the functional groups of the composites due to the presence of CH and WH in the CS matrices. Attenuated total reflectance (ATR) mid-infrared spectra were acquired using a Tensor 27 (Bruker Optics) spectrometer equipped with a DTGS detector and a heated single reflectance diamond ATR accessory with a sealed sapphire anvil. Spectra were obtained with 128 scans at a 4 cm⁻¹ resolution for samples at room temperature. The spectra were vector normalized over the entire wavelength range of 400-4000 cm⁻¹.

3.2. X-ray diffraction

X-ray powder diffraction (XRD) is an analytical technique primarily used for phase identification of a crystalline material. The samples were scanned on a Siemens D500 instrument, which uses Cu-k-alpha X-rays at 40 kV and 25 mA. Specimens were 15×15 mm².

3.3. Mechanical strength

Mechanical tests were carried out using an Instron table top machine with a load cell and a 50 kN maximum load following the European Standards for determination of tensile properties; the method was fully described in our previous work [12]. In summary, specimens of 50×50 mm² were cut immediately prior to testing using laser equipment and mounted on a paper frame. After applying a pre-load (with stress not exceeding the Young's modulus/2000), the specimens were loaded in displacement control mode (5 mm/min) until fracture. Stress vs. strain plots were recorded during the test through the extension recorded from the load cell crosshead measurements with a data acquisition frequency of 10 Hz.

3.4. Cryogenic scanning electron microscopy (C-SEM)

Cryogenic scanning electron microscopy (SEM) was used to study the morphological properties of the films. The SEM images revealed the homogeneity of the composites, the presence of voids, and the dispersion level of the particles within the continuous matrix. Small pieces of the thin films were glued into a holder using Tissue-Tek O.C.T.TM compound and cooled to solid nitrogen temperature under vacuum. Under vacuum, they were fractured by a blade from the side, brought up to -90 °C, platinum coated and then imaged.

3.5. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis (TGA) was used to assess the thermal stability of chitosan and the chitosan composite films using a Mettler Toledo TGA/DSC 3+ star system model. In the tests, the pan was inserted at 25 °C and held at that temperature for 5 min. before ramping up from 25 °C to 450 °C at 10 °C/min [12].

3.6. In Soil degradation test

Soil degradation test is a standard method for degradation because it is similar to actual waste disposal conditions. The degradation test was performed on chitosan and the chitosan composites to study the effect of fillers on the degradation rate of pure chitosan. Biodegradability of the samples was studied by weight loss over time. Small randomly dug holes were augured in the soil. The recorded temperature was 20 °C. The initial weight of each sample was measured before separately burying the samples in soil at a depth of 10 cm. The soil was kept moisturized by injecting water to maintain the microorganisms active. The biodegradation of the specimen was monitored by measuring the weight of each sample every five days. The samples were dried in a vacuum oven at 50±1 °C for 24 h before measuring the weight, and then the weight loss was determined [13].

3.7. Solubility tests

For the solubility tests, each film was dried in an oven at 70 °C for 24 h to determine the initial dry mass (*M1*); then, the films were placed in 30 ml of distilled water. The beakers were covered with plastic wrap and stored at 25 °C for 24 h. Each film was again weighed to find (*M2*). Lastly, each film was dried at 70 °C in an oven to determine the final dry mass (*M3*). Three measurements were taken for each sample for statistical confidence in the collected data. The film solubility and swelling degree were calculated by using the following Eq. (1):

$$\text{Solubility (\%)} = ((M1 - M3)/M1) * 100 \quad (1)$$

All films were neutralized before they were buried in soil. The neutralization was performed by adding 1% NaOH solution to each film for 30 min., washing the film with deionized water, and finally drying the film before use [14].

4. Results and Discussion

4.1. FTIR of chitin powder, chitosan films with CH and WH

The FTIR spectrum of chitin in Table 1 shows a characteristic band at 3446 cm^{-1} , which is attributed to the stretching vibration of -NH and -OH groups, and a band 3102 cm^{-1} , which is assigned to the vibrational modes of NH in the amide, (intermolecular hydrogen bond C=O H-N, and the NH groups intermolecular bonded by H). The characteristic carbonyl C = O stretching of chitin at 1654 cm^{-1} is attributed to the vibrations of the amide I band. The band at 1560 cm^{-1} corresponds to the N-H deformation of amide II. The band at 1378 cm^{-1} is assigned to the CH- deformation band. The vibration band at 1074 cm^{-1} indicated C-O-C vibration inside the chitin ring [15]. These peaks are crucial to differentiate the formation between chitin and chitosan from the extracted sample as shown in Table 2. Moreover, the vibration of -CH₃ group in chitin spectrum was absent in the FTIR spectrum of chitosan after treating in concentrated NaOH thus the COCH₃ in chitin backbone was eliminated during the deacetylation process and chitosan was produced [16].

Table 2. FTIR spectral value of chitin, chitosan and chitosan composites.

Assignment	Frequency			
	Chitin	CS	CS-25CH	CS-25WH
Hydroxyl, O-H stretch	3446			
Amine, N-H stretch	3102	3263	3249	3234
Aliphatic compound, -CH ₂ stretch		2919	2916	2914
Secondary amide, C=O	1654	1643	1637	1621
Amide, N-H bend	1560	1541	1540	1533
Aliphatic compound -CH ₂ bend	1378	1403	1401	1401
C-O-C Glucosidic linkage	1074	1151	1150	1155

Figure 5 shows the spectrum of CS films and CS composite films. CS films show peaks at 3263, 2919, 1643 cm^{-1} which corresponds to the stretching vibrational of -NH stretching, C-H and C=O stretching of amide I band respectively. Absorption peaks at 1541, 1403 are associated with the presence of bending vibrations of the N-H (N-acetylated residues, amide II band), C-H bending. Peaks at 1155 and 1063 cm^{-1} are assigned to ant-symmetric stretching of (C-O-C) bridge and skeletal vibration involving (C-O-C) stretching [17]. The FTIR spectrum of Chitosan composite film with chitin (CS-25CH) show that main peaks of chitosan shifted to lower wavenumber due to the intermolecular interaction between amino group of chitosan with chitin, and shift more with chitin whisker.

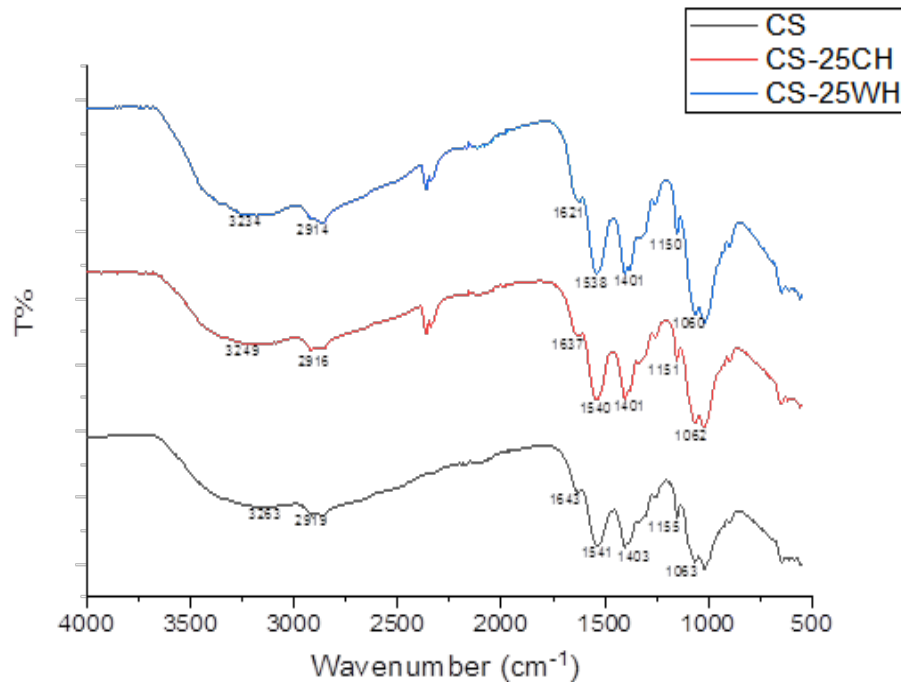


Fig. 5. FTIR of pure chitosan and chitosan composites with 25% chitin and 25% Chitin whisker.

4.2. X-Ray diffraction

Figure 6 shows the XRD patterns of the chitosan powder and chitosan composites. Chitosan is a semi-crystalline polymer with peaks at 9° , 11° and 19° . However, the peak observed for chitosan at $2\theta = 9^\circ$ and the very broad peak at $2\theta = 19^\circ$ changed upon the addition of fillers. These results suggest that chitosan has decent compatibility and led to the formation of a porous network [18]. In the CS-CH composite, the characteristic peaks of chitin and chitosan shifted, and the peak at 11° had a reduction in intensity. Additionally, the intensities of the CS-CH peaks decreased as the content increased from 25% to 35%. The incorporation of chitin caused a decrease in the crystallinity degree of the samples and this indicates the complexation between the filler and chitosan [19]. The numbers of Bragg reflections with 2θ values of 19° , 20° corresponding to (1, 0, 2), (0, 2, 2) respectively indicated that the chitosan was crystalline in nature (JCPDS card no: 39-1894). The numbers of Bragg reflections with 2θ values of 20° corresponding to (1, 2, 0) and (1, 0, 1) indicated the presence of chitin in the composite (JCPDS card no: 35-1974).

For chitin whiskers, they were obtained by acid hydrolysis of chitin, and this process results in cleavage of amorphous content and the extraction of highly crystalline content, which was confirmed by the increasing intensity of the XRD spectrum. The sharp peaks at $2\theta = 20^\circ$ confirmed the crystalline structure of the chitin whiskers. This led to an enhancement of the crystallinity of the CS-WH composites compared to chitin filler [20] In the CS-WH composites, there was an overlap between

the peaks of CS and WH, and the main chitin peak at 26° was not present in the spectra of the mixtures, which suggested the formation of new phases. Moreover, no noticeable changes occurred upon increasing the WH content from 25% to 35%.

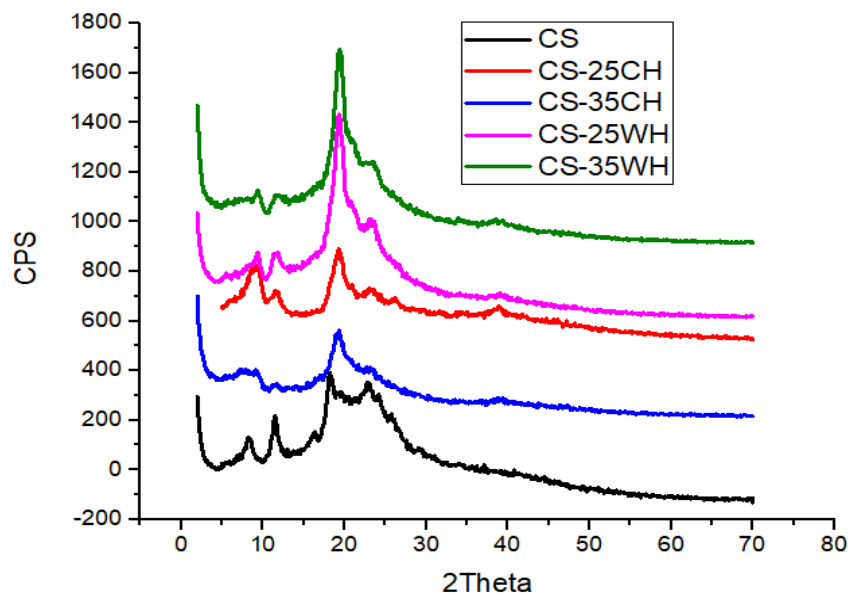


Fig. 6. XRD of chitosan and chitosan composites with chitin and chitin whisker.

4.3. Mechanical tests

Figure 7 shows the effect of CH and WH on the mechanical properties of the prepared composites compared to those of pure chitosan. CS-25WH showed the highest yield strength (30.3 MPa), Young's modulus (1383.5 MPa), fracture strength (32 MPa) and ductility (16.7%). The mechanical properties decreased with increasing WH content, from 25 to 35%, in the composite. However, CS-25CH showed the lowest yield strength (8.4 MPa), fracture strength (9.5 MPa), Young's modulus (522 MPa) and ductility (4.1%).

Pure CS showed a yield strength of 17.5 MPa, fracture strength of 20.2 MPa, Young's modulus of 1064 MPa, and ductility of 15.9%. The improvement in the mechanical properties of the films obtained by adding WH is may be due to the fibre network that formed in the CS-WH filler, which was confirmed by SEM images, as well as the strong bonds that formed between CS and WH.

The factors that controlled the mechanical properties of the composites were the type of the filler in composites, and wt. % of the filler. At high reinforcement content, a failure in the mobility of the polymer chains took place making it harder to adjust to deforming forces. The filler suffered debonding, leading to development of holes, which eventually decreased the mechanical properties of the composite due to pulling out of fillers. The slight addition of the WH filler that did not inhibit deforming forces and was well hydrogen bonded with the chitosan and at the same time did not cause debonding from the chitosan matrix [21].

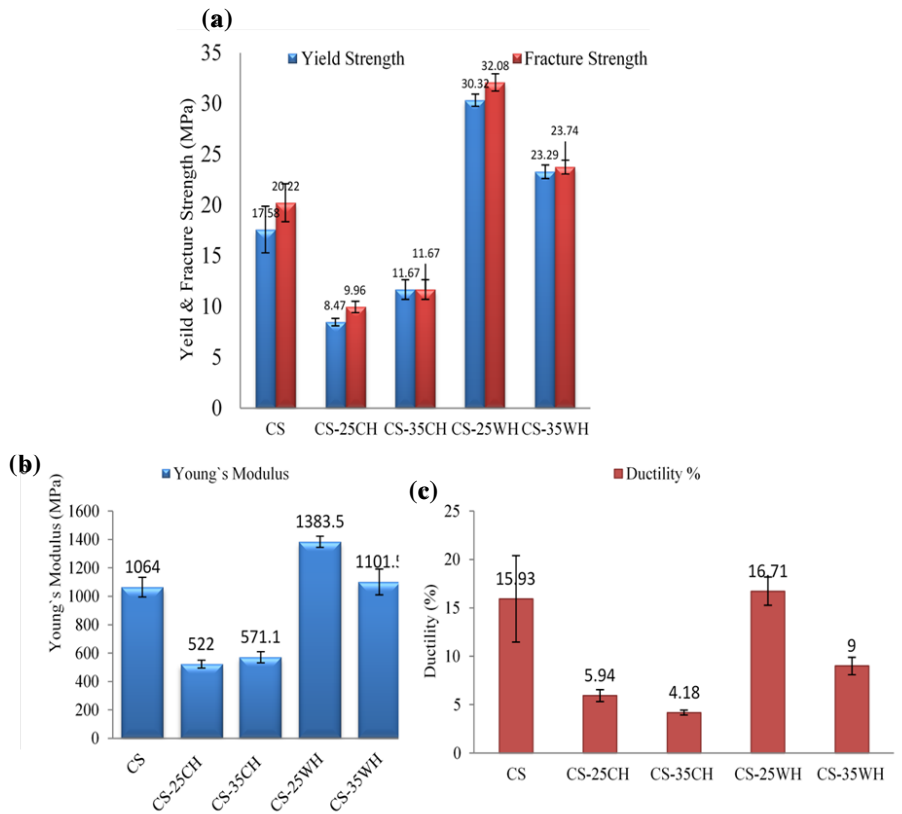


Fig. 7. Mechanical test results of chitosan and chitosan composites: (a) yield strength (MPa), (b) Young's modulus (MPa), and (c) ductility (%).

4.4. SEM of chitosan composite films reinforced with CH and WH

Figure 8 shows the SEM images of the chitosan and chitosan composites. The pure CS film showed homogeneity in the form of smooth surfaces without cracks or pores, indicating good miscibility in the synthesized films Fig. 8(a). CS-25CH has a rough surface with swirls, indicating inhomogeneity, and the chitin particles did not mix well with chitosan, which led to a weak, cracked film that was easily broken, as demonstrated by the mechanical results for CS-25CH in Fig. 8(b).

In contrast, in the CS-25WH composite, the chitin whiskers were uniformly dispersed within the chitosan, forming a fibrous network structure with a random orientation, and the tight network is shown in Fig. 8(c). Whisker fibres are produced during the chemical treatment of chitin to obtain chitin whiskers, and the fibres increase the intermolecular bond interactions between chitosan and the chitin whiskers. This fibrous network structure endows the composites with more strength, which significantly enhances the mechanical properties of the composites.

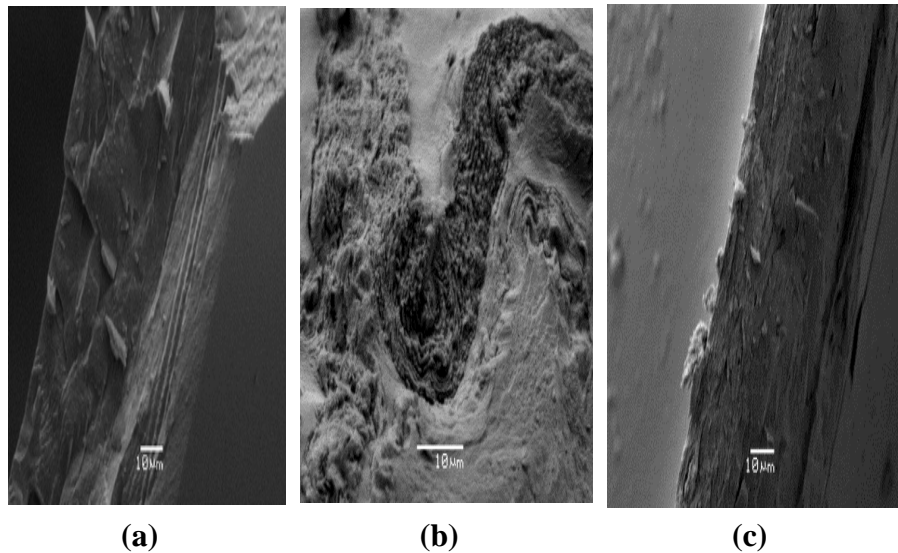


Fig. 8. SEM images of (a) CS, (b) CS-25CH, and (c) CS-25WH..

4.5. Thermal gravimetric analysis

The TGA results for the pure chitosan film show that chitosan decomposes in two stages. The first stage was in the range of 45 °C to 105 °C, and the observed weight loss was due to the loss of moisture, indicating the hygroscopic nature of chitosan linked with the evaporation of acetic acid and water that is physically absorbed and strongly hydrogen bonded to chitosan.

The second stage was at 175 °C due to the scission (C-O-C) of the ether linkage in the chitosan backbone as highlighted in Fig. 9(a). This poor thermal stability of chitosan is overcome by addition of fillers and Nano fillers [22]. The CS-25CH and CS-25WH composite films showed the highest degradation onset temperature of 244 °C to decomposition of the acetylated units of chitin and the degradation of polysaccharide structures.

By increasing the amount of filler from 25 wt. % to 35 wt. %, the onset temperature slightly decreased, and the onset temperatures for CS-35CH and CS-35WH were 230 °C and 233 °C, respectively in Fig. 9(b). Thus, the reinforcement content affects the thermal stability. Even if the filler had more free-end chains at 35 wt. %, there was poor dispersion of filler in matrix with the lack of the hydrolysis being performed [22].

The weight loss for CH and WH was approximately 30%, while for the pure chitosan films, the weight loss was over 60%. For the chitosan composites, the addition of WH resulted in less weight loss (29.7%) and more residues (43.5%), while the addition of CH resulted in weight loss (approximately 30.7%) and a higher residue weight (approximately 37.5%).

This enhancement may be because chitin fibres and chitin whiskers result in many chains at the surface due to the small particle size and the high specific surface area restricts the mobility chain of the chitosan matrix. Overall, these results indicate a positive enhancement in the thermal degradation rate of all chitosan composites compared to pure chitosan due to the higher chitin content [23].

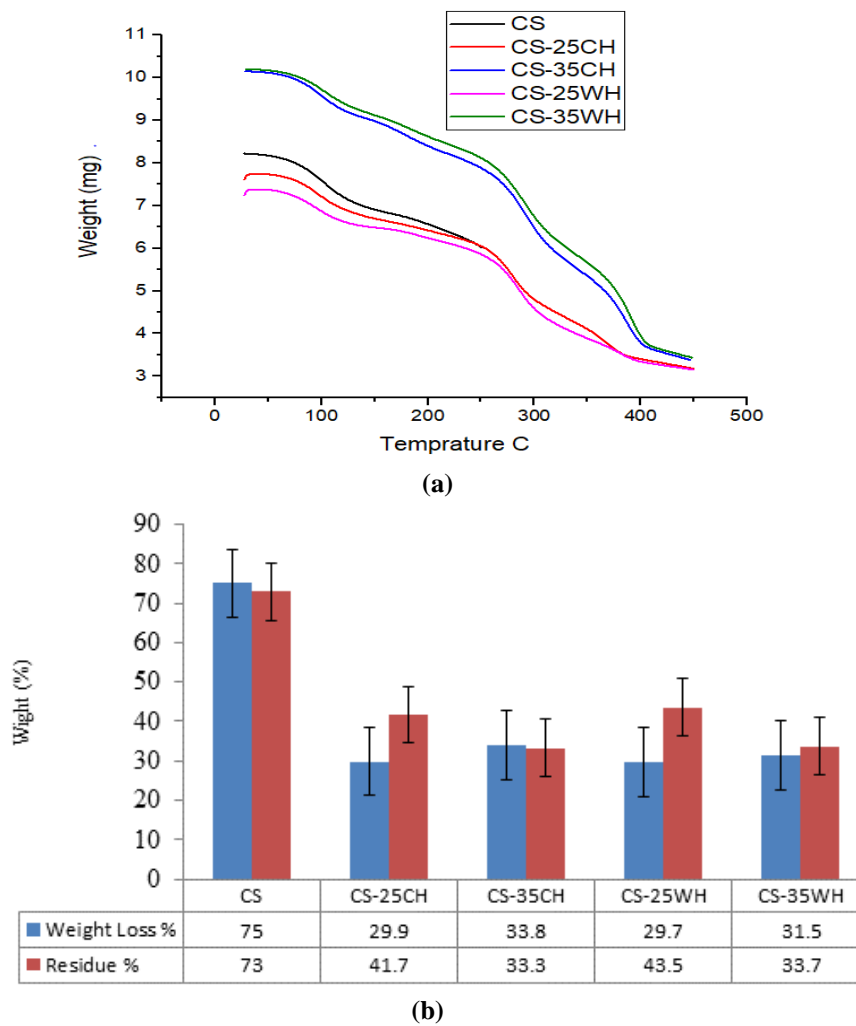


Fig. 9. Thermal properties of CS composites: (a) Degradation onset temperature, (b) Weight loss and residue weight percentages.

4.6. In soil degradation test

Figure 10 illustrates the in-soil degradation for CS, CS-25CH, and CS-25WH films. The CS and CS-25WH films were completely degraded after 75 and 85 days with weight losses of approximately 59%, 60%, respectively, while the CS-25CH films were degraded after 100 days with an average weight loss of 40%, which is lower than the degradation rate of pure chitosan. Composites reinforced with fillers such as chitin showed higher degradation rate due to its crystalline structure. The addition of fillers gives microorganisms larger surfaces to consume and results in higher degradation rate [24]. Figure 11 showed the shape of films during the test, after 40 days the samples were brittle and bended. The tests revealed a noticeable improvement in the biodegradability of the CS composites after adding 25 wt. % of CH.

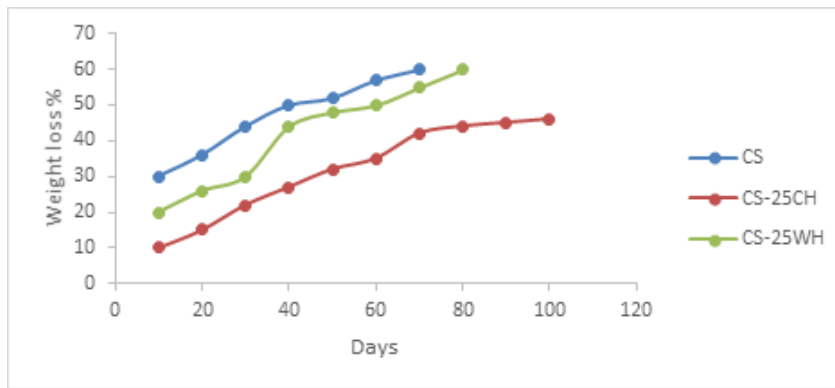


Fig. 10. In soil degradation test of chitosan and chitosan composites.

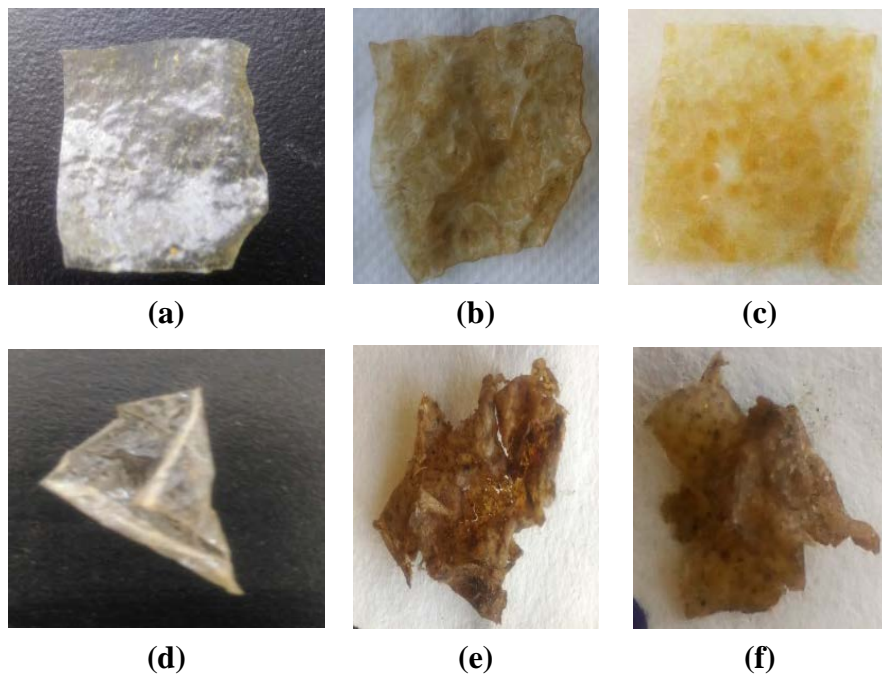


Fig. 11. Degradation test samples before and after burial for 40 days in soil (a), (d) CS, (b), (e) CS-25CH, and (c), (f) CS-25WH.

4.7. Solubility tests

The presence of CH and WH in the matrix evidently changed the solubility of the films. The pure CS films showed 3.8% solubility. Reinforcement of CS films with CH and WH resulted in a significant reduction in the solubility values. The CS- 25CH film showed 0.3% solubility, while the CS-35 CH films exhibited 0.7% solubility. For the CS-25WH films was 0.2% for solubility. This enhancement can be attributed to the presence of acetic acid that promoted the formation of strong interactions between polysaccharide chains, making the films resistant to water.

5. Conclusions

The present study developed a good approach for improving the physiochemical properties of chitosan films created using chitin fibres and chitin whiskers extracted from shrimp shell waste. Furthermore, the mechanical, thermal and biological properties of chitosan films were improved by the addition of 25 wt.% and 35 wt.% of fibres to chitosan films. Chitin whiskers resulted in the highest mechanical strength by preventing the loss of strength by forming bonds with chitosan and crosslinking chains. The films possessed high strength and high Young's modulus, while chitin fibres enhanced the biological properties of the chitosan films. In general, chitin and chitin whiskers provide mechanical properties, thermal stability and biodegradability suitable for biodegradable eco-friendly polymer films, which may be good candidates for thermal applications.

Abbreviations

CH	Chitin
CH ₃ CO	Acetic acid
CPS	Counts per second
CS	Chitosan
DI	Distilled water
DM	Demineralization
DP	Deproteinization
FTIR	Fourier-transform infrared spectroscopy
HCL	Hydrochloric Acid
JESTEC	Journal of Engineering Science and Technology
<i>M</i>	Mass
<i>N</i>	Mole
NAOH	Sodium hydroxide pellets
OH	Hydroxide
<i>T</i>	Transmittance
WH	Whisker
<i>Wt.</i>	Weight
XRD	X-Ray Diffraction

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