

Article

Biodegradability of Starch Nanocomposite Films Containing Different Concentrations of Chitosan Nanoparticles in Compost and Planting Soils

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Abstract: Starch-based nanocomposite films containing chitosan nanoparticles (S/CNP films) are biodegradable and promising alternatives for non-biodegradable synthetic plastics. Nonetheless, limited work has been conducted to investigate the biodegradability of the films in soil. Thus, this work is aimed at investigating the biodegradation of starch-based films containing different concentrations of CNP (0, 10, 15, 20, 25% *w/w* solid starch) via a soil burial test using compost and planting soils. The biodegradability was investigated in terms of weight loss, visual appearance, morphology, and structural changes. It was found that S/CNP films biodegraded slower than neat starch films, and the degradation rate was reduced by 46 and 44% in compost and planting soils, respectively, with the increase in the concentrations of CNP from 10 to 25% *w/w* incorporated into the films. The degradation rate of films in compost soil after 8 days was found to be higher (0.0617 g/day) than in planting soil (0.0266 g/day). Visual appearance, morphology, and structural change results also supported these findings. The biodegradable S/CNP films can be used to reduce the usage of synthetic plastic.

Keywords: biodegradation; chitosan nanoparticle; film; nanocomposite; soil burial



Citation: Othman, S.H.; Ronzi, N.D.A.; Shapi'i, R.A.; Dun, M.; Ariffin, S.H.; Mohammed, M.A.P. Biodegradability of Starch Nanocomposite Films Containing Different Concentrations of Chitosan Nanoparticles in Compost and Planting Soils. *Coatings* **2023**, *13*, 777. <https://doi.org/10.3390/coatings13040777>

Academic Editor: Elena Torrieri

Received: 17 March 2023

Revised: 12 April 2023

Accepted: 15 April 2023

Published: 17 April 2023



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1. Introduction

Polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS) are the most often used food packaging materials because of their low cost, wide availability, high tensile and tear strength, superior barrier properties to oxygen and fragrance compounds, and heat sealability [1–3]. However, these synthetic plastics are non-biodegradable, which can result in environmental pollution associated with the piling of discarded plastic in landfills [4,5]. These plastics are made from petroleum and are generally stable, and will remain undegraded in the environment for a long time after being discarded.

As an alternative, biopolymer films are promising alternatives to reduce the usage of synthetic plastics due to their biodegradable properties. Among the biopolymer films, starch-based films are an excellent replacement for some synthetic plastics. Starch can be used to produce food packaging films that can extend the shelf life of food due to its good transparency, adequate strength, and low moisture absorption [2]. Starch is naturally available from wheat, corn, yam, potatoes, and tapioca [6]. Among the many starch sources, tapioca starch is promising to create starch-based films, since it is widely available in tropical climate countries such as Thailand, Indonesia, and Malaysia [7]. However, starch-based films possess limited properties, mainly due to water solubility, brittleness, and poor mechanical properties [8]. These limited properties can be encountered by blending the films with either plasticizers or other mechanically robust polymers to improve the

structure and enhance the elasticity of the films. The advancement of nanotechnology also reveals that the incorporation of nanoparticles (1 to 100 nm) into the matrix of biopolymers can effectively increase the competitiveness of biopolymers against non-renewable plastics due to performance improvement [9].

Among the many nanoparticles, chitosan nanoparticles (CNP) are natural materials that exhibit high antibacterial activity against a wide spectrum of pathogens [9,10], as well as antioxidant activity [11]. CNP has been used in food packaging, food coating, textiles, and other sectors [12]. Several nanocomposite films have been created by incorporating CNP into the polymeric matrix [10]. Nanocomposites with antimicrobial and antioxidant functions can help to reduce the growth of post-processing contaminating microorganisms, lengthen the shelf life of food, and improve food safety [9,10,13]. Apart from that, the incorporation of different concentrations of CNP into starch-based films to produce S/CNP films has been proven to result in remarkably improved mechanical, thermal, and barrier properties of the films to be used for food packaging applications [10,11,14]. Nonetheless, despite knowing that S/CNP films are biodegradable, to the best of our knowledge, limited work has been conducted on investigating the biodegradability properties of the films containing different concentrations of CNP. Only Babae et al. [15] have investigated the biodegradation of S/CNP films loaded with different concentrations of CNP using white rot fungus. It is vital to ensure that the films can degrade naturally, despite the numerous advantages of CNP incorporation in starch biopolymer films.

Meanwhile, biopolymer films can also be degraded in soil by microorganisms. Soil is the most convenient degradation medium due to its high availability and low cost, and soil is primarily constituted of organic components dissolved by the aerobic decomposition of plant and animal matter [16]. Thus, soil is a promising degradation medium for S/CNP films. There are two types of biodegradation processes including aerobic and anaerobic biodegradation [17]. Aerobic biodegradation occurs with the presence of oxygen and produces carbon dioxide. When no oxygen is present, anaerobic biodegradation occurs, and methane is produced instead of carbon dioxide. The conversion of biopolymer films to by-products such as gases (carbon dioxide, methane, and nitrogen compounds), water, salts, minerals, and to the main product, particularly biomass, results in the biodegradation of the films. Microorganisms will then consume all the biomass and convert carbon to carbon dioxide, leaving no accumulation of the films in the soil [17]. Thus, biodegradation can protect soil health and prevent soil pollution.

To date, no work has been carried out to investigate the biodegradability of the S/CNP films containing different concentrations of CNP in different types of soils via the soil burial test. The concentration of CNP added to starch films is one of the important parameters to be investigated, since it affected the dispersion of CNP inside the films and the many properties of the films [10,11,14]. The biodegradability of the films will be affected by the types of soils due to the different content of microorganisms, humidity, temperature, and other variables. Thus, this study is directed to investigate the biodegradation properties of the starch films containing different concentrations of CNP (0, 10, 15, 20, and 25% *w/w*) in different types of soils (compost and planting soils) in terms of weight loss, visual appearance, morphology, and structural changes.

2. Materials and Methods

2.1. Materials

Chitosan (CH) (low molecular weight: 50 kDa, viscosity: 20–300 cP in 1% *w/v* of acetic acid, deacetylation: 75–85%) and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Tapioca starch (amylose: 19%, amylopectin: 81%) was obtained from Thye Huat Chan Sdn Bhd, Bangkok, Thailand (Brand Kapal ABC). Acetic acid, glycerol, sodium hydroxide (NaOH), and saturated magnesium nitrate were purchased from R&M Marketing, ENG, UK. Planting soil was purchased from TCT Trading Sdn Bhd, Sabah, Malaysia (Brand Ninso). Compost soil was purchased from Sin Seng Huat

Seeds Sdn Bhd, Selangor, Malaysia (Brand GE Compost 3333). The contents of both soils are tabulated in Table 1.

Table 1. Contents of compost and planting soils.

Contents (% <i>w/w</i>)	Compost Soil	Planting Soil
Humic Acid (Ha)	16.00	NA
Total Organic Matter (OM)	50.00	26.3
Total Organic Carbon (OC)	26.00	14.00
pH	8.20	6.50
Humidity	<45	<25
Material	Palm oil waste, soya bean waste, decanter cake, pome sludge, cocoa, and 20 million units of active microbes.	Coco peat, red burnt soil, sand, rice husk, humus organic compound, and topsoil.

2.2. Preparation of CNP

The preparation of CNP was done according to the work of Shapi'i et al. [18]. Firstly, chitosan solutions (5, 10, 15, 20, 25% *w/w* solid starch) were prepared by dispersing the different amounts of chitosan flakes (0.15, 0.30, 0.45, 0.60, 0.75 g, respectively) in 50 mL aqueous acetic acid solution (1% *v/v*) using a magnetic stirrer (FAVORIT HS0707V2, PLT Scientific Sdn Bhd, Selangor, Malaysia) for 30 min. It is worth noting that the percentages of chitosan were chosen based on the work of Shapi'i et al. [18], whereby the 25% *w/w* CNP in solid starch was adequate to improve the mechanical and barrier properties of the starch films. The pH of the solution was then adjusted with NaOH to the optimal pH of 4.6. At the same time, the TPP powder (0.03, 0.06, 0.09, 0.12, 0.15 g, respectively) was dissolved in 50 mL of distilled water according to the optimum ratio of CH to TPP (5:1).

CNP was produced spontaneously by adding 50 mL of TPP solution drop by drop to 50 mL of chitosan solution while stirring vigorously at room temperature (25 °C) for 30 min using a magnetic stirrer. The CNP suspension was then dispersed for 15 min using an ultrasonic probe (Q500, QSonica LLC0, CT, USA) with a sequence of 1 min sonication and 10 s of rest at a 50% amplitude. During ultrasonication, the beaker containing CNP was put in an ice bath to keep the temperature of the CNP suspension in the desired range (30–35 °C).

2.3. Preparation of S/CNP Films

The preparation of S/CNP films was done according to the work of Shapi'i et al. [18]. To obtain 3% *w/w* suspensions, 3 g tapioca starch was dispersed in 100 mL distilled water-glycerol solutions. Next, 25% *w/w* glycerol of the dry starch solid weight was added to the starch solution. The solution was then heated at 75 °C with constant stirring until gelatinized. Before mixing with the CNP suspension, the starch solution was cooled to 50 ± 2 °C.

The S/CNP film solution was prepared by mixing the 100 mL CNP suspension with 100 mL gelatinized starch solution and stirring for 30 min. The solution was then sonicated for 5 min to produce a homogenous solution. An amount of 50 mL of the solution was poured into an acrylic petri dish (diameter: 14 cm) and placed on a flat table in an air-conditioned room (20 °C) for 48 h. The petri dish containing the S/CNP film was dried to constant weight in a ventilated oven (Universal Oven U, Memmert GmbH + Co. KG, Bavaria, Germany) at 40 °C for 5 h. A neat starch (NS) film without the addition of CNP was also prepared as a control.

After drying, the film was peeled from the petri dish and conditioned in a desiccator with saturated magnesium nitrate solution (Relative humidity (RH): 51%, Temperature: 30 °C). The thickness of the films was measured using a digital micrometer (Mitutoyo, Yokohama, Japan) at five random positions around the film. The average value of the film thickness was measured (0.07 ± 0.002 mm) and used to determine the biodegradation of the film by a soil burial test (SBT). It is worth noting that the mechanical, thermal, and

barrier properties of the films have been investigated by Shapi'i et al. [18]. The antimicrobial properties of the films have also been explored by Shapi'i et al. [10].

2.4. Soil Burial Test (SBT)

The SBT was carried out based on an adaptation from Bonilla and Sobral [19] with a slight modification. First, the films were cut into a square shape (20 × 20 mm) and weighed before being buried (W_0). Approximately 0.04 to 0.06 g of the sample films were used for SBT. Next, the compost and planting soils were prepared in plastic containers, and films were planted at depths of 5 cm. The plastic containers were then placed in the laboratory, and the moisture of the soil was maintained by spraying water for about 2.5 mL daily in order to maintain the activity of the microorganisms [20]. All the films were then analyzed after 2, 4, 6, and 8 days of degradation in soils. The same procedure was repeated for the SBT in the planting soil.

2.5. Weight Loss of the Films

The NS and S/CNP films were removed from the soils after 2, 4, 6, and 8 days, and were cleaned carefully using a paintbrush to remove adherent soil, and then dried in an oven at 40 °C for 15 min. The films were then placed in a desiccator for 24 h until a constant weight was attained. The films were weighed using an analytical scale to determine the weight after degradation (W_A). The percentage of weight loss (%) and degradation rate (g/d) were then calculated using Equations (1) and (2), respectively:

$$\text{Weight loss (\%)} = (W_0 - W_A)/W_0 \times 100\% \quad (1)$$

where W_0 is the initial weight of the films (g) and W_A is the weight of the films after each testing period (g).

$$\text{Degradation rate (g/d)} = \text{Weight loss}/t \quad (2)$$

where t is the time of degradation (days).

2.6. Visual Appearance of the Films

After 2, 4, 6, and 8 days of degradation, the films were photographed from a height of 15 cm with 4× magnification using a phone camera (Model: iPhone 7 Plus) in the photo light box (Shenzhen Puluz Technology Limited, Shenzhen, China). The photos were reported in chronological sequence to observe the degradation of the NS and S/CNP films over time.

2.7. Surface Morphology of the Films

The surface morphologies of the NS and S/CNP films were analyzed using scanning electron microscopy (SEM, JSM-IT100 InTouchScope, JEOL Ltd., Tokyo, Japan). The films were observed before and after 8 days of degradation in the soils at 10.0 kV of an acceleration voltage and 250× magnification.

2.8. Structural Properties of the Films

Fourier transform infrared spectroscopy (FTIR, Nicolet 6700, Thermo Fischer Scientific, Waltham, MA, USA) was used to determine the structural changes of the NS and S/CNP films. The FTIR spectra of the films were recorded before and after 8 days of degradation in the soils in the range of 400 to 4000 cm^{-1} .

2.9. Statistical Analysis

Statistical analyses of the obtained experimental data were performed using the Minitab software (Minitab Inc, State College, PA, USA). The data were represented as a mean value ± standard deviation of a triplicate of each measurement ($n = 3$). A significant difference between means was evaluated using the analysis of variance (ANOVA). A

Tukey's test was used for comparing mean values, and the differences between means were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. S/CNP Films

The CNP produced in this study was spherical in shape, as reported by Shapi'i et al. [21]. They also reported that the average diameter sizes increased from 46.76 to 126.51 nm with the increase in the initial concentration of chitosan from 5 to 20% *w/w* solid starch, and the particle size distribution increased as well. At a lower concentration, the intermolecular interaction forces are not strong because the distances between chitosan molecules are far apart. Hence, chitosan molecules are inclined to be stable as a single molecule. A tiny size of CNP formed spontaneously when added with the TPP solution due to the cross-linkage of the anions of TPP phosphate with the cations of the single chitosan molecules. At the high initial concentration of chitosan, strong intermolecular interactions between chitosan molecules occurred because of the short distance between each molecule, leading to the chitosan molecules entangling among themselves. This consequently formed a single larger particle of CNP after ionic gelation. Apart from that, a high initial concentration of chitosan resulted in the agglomeration of CNP [21].

When CNP is added to starch films, there exists an intermolecular interaction between starch and CNP that involves the formation of hydrogen bonding between amino cation (NH_3^+) of the chitosan backbone and hydroxyl anion (OH^-) of the starch backbone [22]. The amino groups (NH_2) of chitosan are protonated to NH_3^+ in the acetic acid solution. The ordered crystalline structures of starch granules are destroyed by the gelatinization process, resulting in the formation of OH^- , which is exposed to form the hydrogen bonds with NH_3^+ .

Shapi'i et al. [18] revealed that the addition of a lower concentration of CNP resulted in CNP that was well-dispersed in the starch films. However, a higher concentration of CNP resulted in the poor dispersion and agglomeration of CNP in the starch films. Their morphological studies also revealed that there are nanovoids between the CNP in well-dispersed films, as observed using a transmission electron microscope. In terms of structure, it was found that CNP films exhibited uniform and smooth structures despite the different concentrations of CNP added [18]. In fact, the good interaction between starch and CNP resulted in the homogenous structure of the S/CNP films compared to NS films. The large size of the granule structure in starch film disintegrated to the smaller size when augmented with chitosan [23]. On the other hand, it can be speculated that the structure of the starch matrix evolved during the storage of the films before the SBT due to the biodegradability properties of starch films. Thus, this work fixed the storage time of the films to not more than a week in order to ensure consistent biodegradability results.

3.2. Weight Loss of the Films

Figure 1 shows the weight loss of NS and S/CNP films after each soil burial interval (2, 4, 6, and 8 days) in two soil types: (a) compost soil, and (b) planting soil. It can be observed that all of the films experienced weight loss during the SBT period. This was due to the hydrophilic nature of the starch, which tended to absorb water [24], and the water was used by microbes in soils to thrive and release hydrolytic enzymes to break down biopolymers [20]. It can also be seen that the weight losses of S/CNP films were generally lower than NS films in the compost and planting soils due to the existence of CNP.

The weight losses of the films also decreased with the increase in CNP concentrations. These findings can be attributed to the improvement in the crystallinity and tensile strength, and the reduction in the water vapor permeability (WVP) of the films with the addition of CNP [18], which impeded the degradation of the films. Although chitosan is hydrophilic in nature, the presence of amine groups on chitosan molecules formed strong hydrogen bonds with the hydroxyl groups in starch, thus reducing the hydrophilicity of the S/CNP films. This consequently reduced the WVP of the films, whereby the tiny size of CNP reinforced

the matrix empty spaces between the starch interchain and formed a good intermolecular interaction between CNP and starch, thus decreasing the empty spaces. The structure of S/CNP films became more compact than that of NS films, which resulted in a difficult tortuous pathway for moisture to permeate, thus delaying the moisture permeation across the films and decreasing the WVP [18]. Meanwhile, the weight losses of the films were also found to be higher in compost soil compared to the planting soil due to more nutrients, humidity, active microorganisms, and organic matter contained in the compost soil (Table 1) that favored biodegradation.

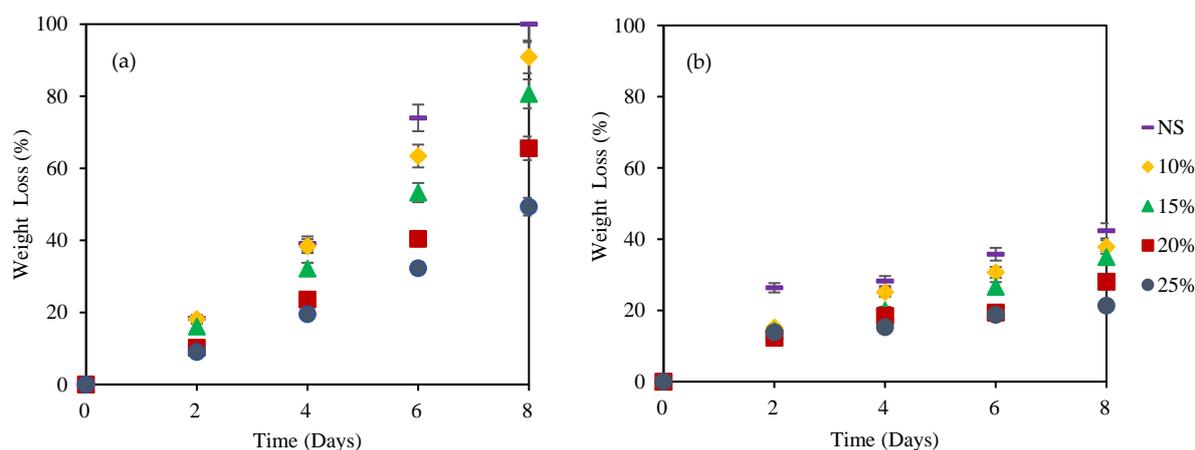


Figure 1. The weight loss of films after SBT for 8 days in (a) compost soil and (b) planting soil.

For the purpose of clarity, the percentage weight loss and degradation rate of the NS and S/CNP films after SBT for 8 days in compost soil and planting soil obtained from Figure 1 are tabulated in Table 2. After 8 days of SBT in compost and planting soils, the degradation rates of all S/CNP films were lower than NS films. These findings were consistent with the work by Babae et al. [15], where they found that the incorporation of CNP into plasticized starch decreased the weight loss of films. Hence, the degradation rate of the films also decreased. The addition of CNPs into starch films provided a superior resistance in response to degradation compared with NS films. The dense percolating network and crystallinity property of CNP entrapped inside the starch matrix resisted water from the soil from diffusing into the starch matrix [15]. This inhibited not only the susceptibility of water towards the matrix, but also the microorganism's growth, and restricted the damaging enzymatic performances to promote degradation [25,26]. Shapi'i et al. [18] also found that the WVP of the films decreased with the addition of CNP, which contributed to the decrease in weight loss and degradation rate found in this study.

Table 2. Percentage of weight loss and degradation rate of the S/CNP films after SBT for 8 days.

Samples	(a) Compost Soil		(b) Planting Soil	
	Weight Loss (%)	Degradation Rate (g/Day)	Weight Loss (%)	Degradation Rate (g/Day)
NS	100.0 ± 0.6 ^a	0.1250 ± 0.0008 ^a	42.4 ± 0.8 ^a	0.0529 ± 0.0010 ^a
S/CNP (10% w/w)	90.9 ± 0.1 ^b	0.1136 ± 0.0001 ^b	37.8 ± 0.2 ^b	0.0473 ± 0.0003 ^b
S/CNP (15% w/w)	80.7 ± 1.0 ^c	0.1008 ± 0.0013 ^c	35.0 ± 0.1 ^c	0.0437 ± 0.0001 ^c
S/CNP (20% w/w)	65.5 ± 1.5 ^d	0.0819 ± 0.0019 ^d	28.0 ± 3.4 ^d	0.0350 ± 0.0043 ^d
S/CNP (25% w/w)	49.3 ± 0.3 ^e	0.0617 ± 0.0004 ^e	21.3 ± 0.2 ^e	0.0266 ± 0.0003 ^e

Values in the same column showing the different superscript letters are significantly different ($p < 0.05$).

Table 2 also demonstrates a correlation between the concentrations of CNP added and the weight loss and degradation rate of the films after 8 days of SBT in compost and planting soils. There was a trend of decreasing weight loss and degradation rate with the increase in the concentrations of CNP added into the films in both types of soils. The S/CNP 10% w/w films experienced the highest weight loss in compost and planting soils, while S/CNP 25% w/w films experienced the least weight loss. These results were attributed

to the formation of denser and less permeable structures when the concentration of CNP was increased [12–14]. The denser CNP assembly and the crystallinity property of CNP inside the starch matrix reduced the permeability of water through the starch films. This reduced the water absorption from the soil into films, making the films more resistant to degradation. Indeed, Shapi'i et al. [18] also discovered that the WVP of the films decreased with the increase in the concentrations of CNP added to the starch films. When less water was able to permeate through the films, this resulted in a reduction in weight loss and degradation rate, as discussed earlier.

Furthermore, the weight loss and degradation rate of all films in compost soil were higher than in planting soil (Table 2). NS films were completely degraded (100 wt% loss) after 8 days of SBT in compost soil, but NS films exhibited 42.4 wt% loss after 8 days of SBT in planting soil. NS, S/CNP 10% *w/w*, S/CNP 15% *w/w*, and S/CNP 20% *w/w* films had more than 50 wt% loss after 8 days of SBT in compost soil. However, the weight losses of all samples in planting soil did not exceed 50 wt%. These results proved that the weight loss and degradation rate were influenced by the types of soils used.

Compost soil accelerated the degradation of the films because it contains a high amount of active microbes, high humidity, and a high total of organic matter compared to the planting soil [27,28]. The high amount of active microbes and total organic matter favors the degradation of the films. When the films are being kept under high relative humidity conditions, water molecules from the environment could be absorbed into the films faster than the films under low relative humidity conditions due to the saturated water molecules at high relative humidity [14]. This increases the rate of absorption of water molecules in the surrounding area into the films, resulting in a faster degradation through microorganism growth [29].

3.3. Visual Appearance of the Films

The visual appearance changes of NS and S/CNP films after SBT for 8 days in compost and planting soils are shown in Figures 2 and 3. The films' deterioration in size, appearance, and color change patterns demonstrate biological action and can be the earliest indicator of any surface degradation [24,30]. After 8 days of SBT, all of the films lost their integrated appearance, with NS film degrading faster than the others. The degradation of films in soil was regulated by the hydrolysis reaction, where microorganisms digested and degraded components such as starch in the presence of water. The water from the soil diffused into the films, causing the films to become soft and swell [24]. Hence, after 8 days of SBT, the starch-based films became hard, brittle, and easily broken, with an eventual decrease in size.

After 8 days of SBT, the NS film in compost soil was fully biodegraded with minor traces (Figure 2a). However, the NS film in planting soil was not fully biodegraded but exhibited cracking in the middle of the film, a noticeable crumbling effect, and color changes to a darker tone compared to previous days of SBT (Figure 3a). These were due to the contents of compost soil that favored biodegradation compared to planting soil, as discussed in Section 3.2. The darkening of the film was mainly due to starch consumption by microorganisms [20].

From Figures 2 and 3, there seemed to be non-noticeable changes in the opacity of the films incorporated with different concentrations of CNP before SBT. The film opacity indicated the transparency of the film, whereas when the film's opacity increased, the film's transparency decreased. According to Shapi'i et al. [18], adding CNP into starch films increased the film's opacity due to the undissolved CNP in the starch matrix. However, the film opacity between different concentrations of CNP was only slightly affected and non-noticeable when observed visually because of the decent interaction between the starch and the CNP.

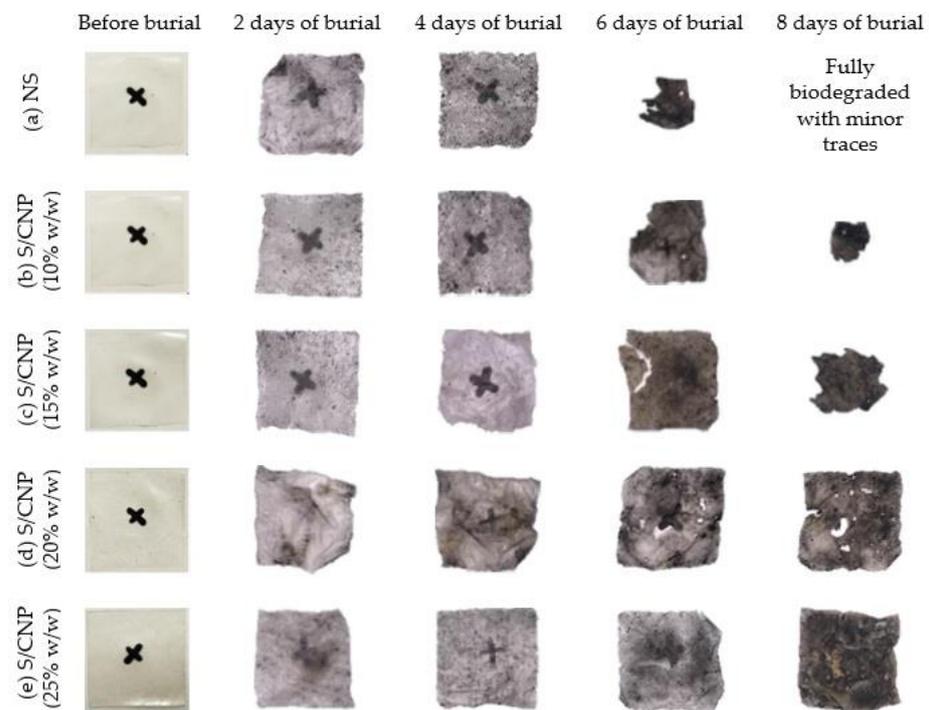


Figure 2. The visual appearance of (a) NS, (b) S/CNP (10% *w/w*), (c) S/CNP (15% *w/w*), (d) S/CNP (20% *w/w*), and (e) S/CNP (25% *w/w*) films subjected to SBT for 2, 4, 6, and 8 days in compost soil.

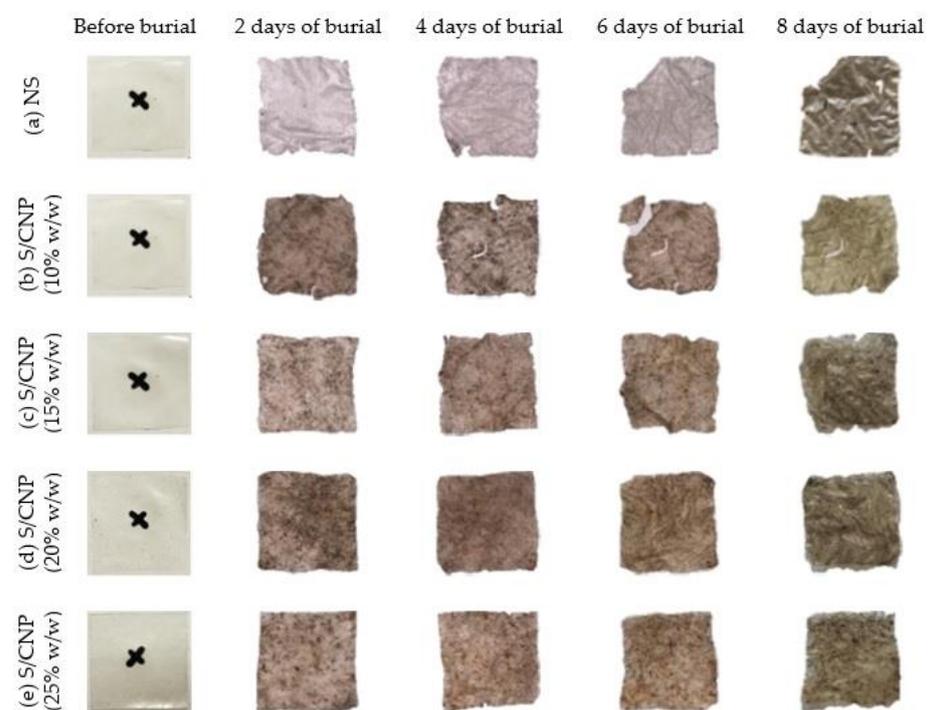


Figure 3. The visual appearance of (a) NS, (b) S/CNP (10% *w/w*), (c) S/CNP (15% *w/w*), (d) S/CNP (20% *w/w*), and (e) S/CNP (25% *w/w*) films subjected to SBT for 2, 4, 6, and 8 days in planting soil.

After the SBT, from visual observation there was no significant difference in the color of NS and S/CNP films in compost soil (Figure 2) in the designated period. However, in planting soil (Figure 3), the color change of NS films was lighter than S/CNP films. This might be due to the NS films that did not have crosslinking interaction, which could trap the moisture absorption from the planting soil in the starch matrix and affect the appearance of

the NS films. The obvious increase in opacity with the increase in the SBT period can be seen in all the films. According to Shahrin et al. [24] and Tai et al. [31], the opaqueness of the films when conducting soil burial occurred due to the receiving of moisture from the surrounding soil and microbial activity during soil burial. Moreover, there were obvious color changes from light grey to dark grey for the films buried in compost soil. Meanwhile, the color of the films in the planting soil changed from slightly yellow to dark brown. The contents of compost soil, which consists of the decanter cake, pome sludge, and cocoa, make the soil's color greyish black, while the planting soil contains red burnt soil and coco peat; hence the color of the soil is dark brown. This affected the color changes of the films after SBT in compost soil and planting soil.

The NS film in compost soil showed apparent degradation and was fully biodegraded after 8 days (Figure 2a), while other S/CNP films degraded into small sized films (Figure 2b–e). This proved that S/CNP films degraded slower than NS films because the incorporation of CNP made the films more resistant to degradation. In compost soil, the S/CNP 10% *w/w* film started to degrade after 2 days because the film's edges slightly diminished, and the film became smaller with the increase in SBT period time (Figure 2b). S/CNP 15% *w/w* film was degraded after 4 days due to the apparent changes at the edge of the film where it had diminished (Figure 2c). The film exhibited cracking after 6 days and became smaller after 8 days in compost soil. As for S/CNP 20% *w/w* in compost soil, the film exhibited some small cracking after 6 and 8 days, but the size of the film after 8 days remained the same (Figure 2d). Meanwhile, the S/CNP 25% *w/w* films showed no sign of cracking but exhibited a slight trace of crumbling after 8 days of SBT in compost soil (Figure 2e).

In planting soil, the S/CNP 10% *w/w* film crumbled after 2 days and exhibited cracking in the middle of the film and at the edge of the film from 4 days onwards (Figure 3b). S/CNP 15% *w/w* in planting soil crumbled after 4 days (Figure 3c), but S/CNP 20% *w/w* started to crumble after 6 days (Figure 3d). The sizes of the films were reduced during SBT, whereby the edges of the films slightly diminished. S/CNP 25% *w/w* showed no sign of crumbling or cracking, but the size slightly reduced over time in planting soil (Figure 3e).

These findings proved that the films degraded slower with the increase in the concentration of CNP, due to the dense structure of the CNP, which lowered moisture uptake and decreased microbial activity to promote degradation. Based on the appearance variations in Figs. 2 and 3, the order of degradation rate was determined as follows: NS > S/CNP 10% *w/w* > S/CNP 15% *w/w* > S/CNP 20% *w/w* > S/CNP 25% *w/w*, which corroborates the previous discussion on weight loss and degradation rate (Section 3.2), whereby the increase in the concentrations of CNP resulted in a lower weight loss and degradation rate of films.

Comparing the visual appearances of NS and S/CNP films in compost soil and planting soil, rapid degradation of the sample films in compost soil (Figure 2) was observed after 6 days compared to degradation in planting soil (Figure 3). Higher weight loss and a more significant appearance change indicated a higher degradation rate. Compost soil has higher humidity, which could increase the water absorption rate from the soil into the films. As a result, the absorbed water facilitated the microbial digestion of film, causing a fracture or changes in physical properties such as color and size [31].

3.4. Surface Morphology of the Films

The morphological changes of NS, S/CNP 15% *w/w*, and S/CNP 25% *w/w* films before and after 8 days of SBT in compost and planting soils were observed by SEM, and the micrographs are presented in Figure 4. The surface morphology of NS film before SBT, as in Figure 4a, was rough, and exhibited a disordered microstructure, withered with large granules of starch. A disordered microstructure was due to a lack of regularity or organization in the arrangement of the large starch granules in the films. In contrast, S/CNP 15% *w/w* and S/CNP 25% *w/w* films in Figure 4d,g, respectively, exhibited smoother surfaces and more homogenous microstructure films compared to the NS film due to the

good interaction between starch and CNP, and that CNP can disintegrate the large starch granules to smaller sizes, as discussed in Section 3.2.

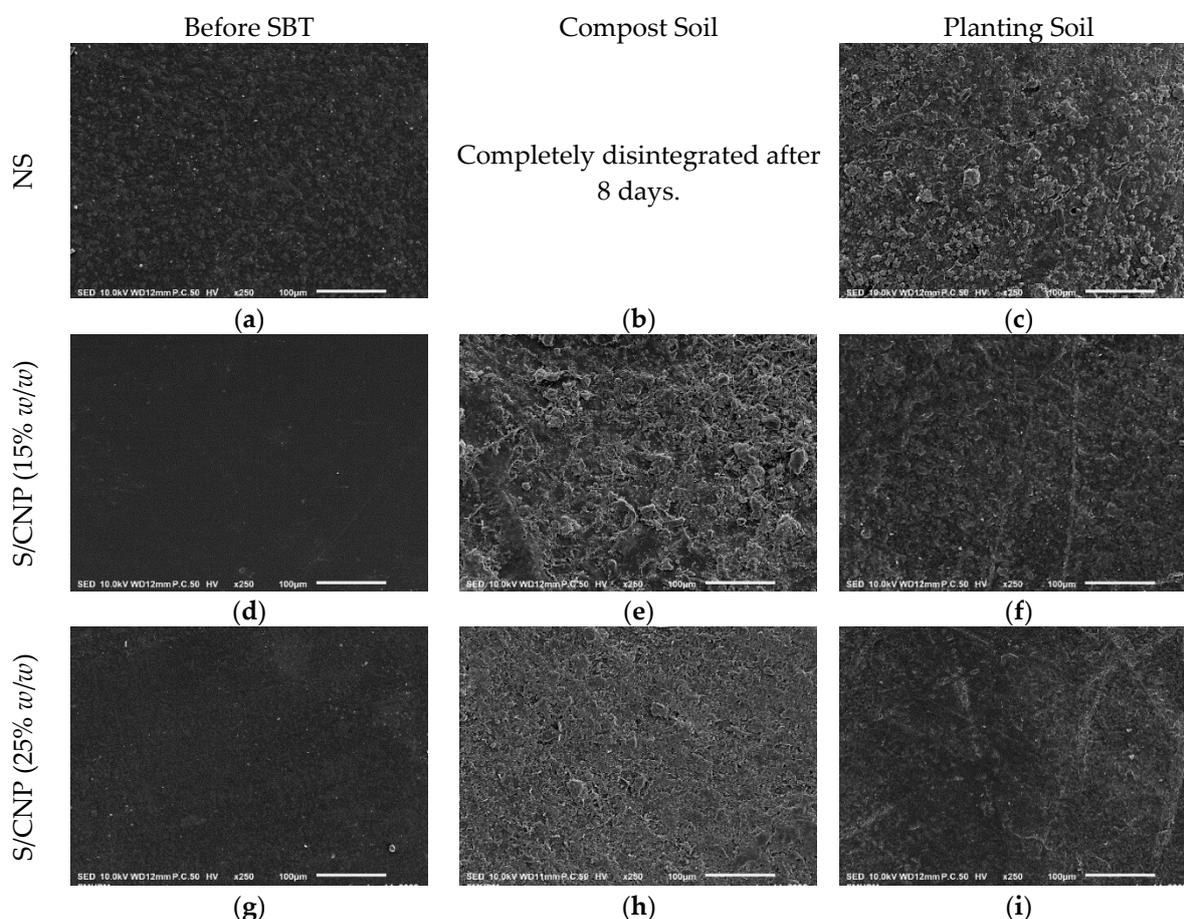


Figure 4. SEM micrographs of NS, S/CNP (15% *w/w*), and S/CNP (25% *w/w*) films (a,d,g) before and after the SBT for 8 days in (b,e,h) compost soil and (c,f,i) planting soil.

This finding is in agreement with the finding of Shapi'i and Othman [32], whereby the SEM micrograph of the neat starch film shows a very rough surface with granules of starch, while starch films containing chitosan had a smoother and more homogenous structure. Singh and Kamlesh [23] also reported that when the concentrations of chitosan increased, the large size of the pebbles structure in the starch film fragmented to a smaller size, hence the smoother and more homogeneous structure. Similarly, Restu et al. [11] also revealed that the SEM micrograph of the neat starch film has a large and rough granule structure. They also reported that when the starch matrix was incorporated with chitosan, the films exhibited a smoother surface than neat starch film due to the good intermolecular bonding between the chitosan and starch molecules.

After being buried in soil for 8 days, the formation of cracks and an increase in surface roughness were observed on all the films' surfaces. There was no SEM image for NS films buried in compost soil after 8 days because the film was completely degraded after 8 days. The degradation of NS films in planting soil (Figure 4c) was much faster compared to S/CNP films, as indicated by higher surface irregularities, more visible hollows and cracks, and rougher surfaces compared to SEM images of S/CNP films. The presence of many hydrophilic groups on the neat starch molecular structure increased the absorption of water molecules [18], thus NS film could easily absorb moisture from the soil, resulting in swelling phenomena which led to a crack on the NS film surface and faster degradation.

When comparing NS films (Figure 4c) with the films containing CNP after SBT, the surface morphology of the S/CNP films in Figure 4f,i show a less eroded and random

degradation demonstrated by less large size cavities and irregularities on the surface of the films. Furthermore, the S/CNP 25% *w/w* films (Figure 4h,i) exhibited less surface erosion, fractures, and hollows than S/CNP 15% *w/w* films (Figure 4e,f), indicating slower degradation than S/CNP 15% *w/w*. Surface erosion occurred in the course of biodegradation because of the contact and absorption of water. Many tiny voids were observed on the film surface, implying that microorganisms degraded the surface of the films in the soil surrounding [33]. These microorganisms produced enzymes catalyzing hydrolysis for degradation. The order of degradation rate determined from the SEM images was consistent with that determined by the visual appearance in Figures 2 and 3. According to Othman et al. [14], adding CNP to the starch matrix increased the hydrophobicity of films. Hence, when the concentration of CNP incorporated into films increased, water penetration into the films reduced, which slowed down the degradation process, and consequently the films had a lower percentage of weight loss.

The films underwent swelling and shrinkage when buried in the soil, which caused a rougher surface and cracking of the surface of the films depending on the soil humidity [34–36]. The SEM results are consistent with the visual appearance evaluation and weight loss measurement, where the films in compost soil degraded faster than the films buried in planting soil. After 8 days of SBT, all films in compost soil had much rougher surfaces, large voids, and more surface degradation and deterioration compared to all films in planting soil. This is because compost soil has high soil humidity and contains the richest microorganisms and organic matter, therefore providing optimal conditions for degradation [37].

3.5. Structural Properties of the Films

The structural properties of NS, S/CNP 15% *w/w*, and S/CNP 25% *w/w* films before and after degradation were determined using FTIR. Figures 5–7 depicted the FTIR spectra of the films in the range of 500 to 4000 cm^{-1} for all three samples before and after 8 days of SBT in compost and planting soils. The FTIR spectrum of NS film before the SBT (Figure 5a) shows a broad absorption area between 3000 to 3600 cm^{-1} , indicating the stretching vibration of the hydroxyl group (O-H) of starch, glycerol, and water [38]. The absorption peak at 2930 cm^{-1} corresponded to the alkane group's (C-H) stretching vibration because of the establishment of intramolecular and intermolecular bonds between the starch and glycerol hydroxyl groups [24]. At the wavelength of 1644 cm^{-1} , there was a strong absorption peak which correlates to a carbonyl group (C=O) stretching vibration. The strong absorption peak at 1026 cm^{-1} corresponds to the stretching vibration of carboxylic groups (C–O).

After the SBT in planting soil, the peak of the O-H groups of NS film (Figure 5c) from 2900 to 3700 cm^{-1} was found to be broader compared to NS film before SBT (Figure 5a). This indicates the existence of a higher amount of OH groups that could easily vibrate, which is possibly produced by the hydrolysis of starch [38]. The absorption peak intensity of the C-H group of NS film after SBT in planting soil (Figure 5c) reduced, indicating the fracture of the starch chain in the soil [39]. The intensity of the absorption peak of the C=O group of NS film also decreased, and the C-O stretching vibration absorption band of NS film shifted from 1026 to 1016 cm^{-1} after SBT in planting soil (Figure 5c). The changes in the intensity at absorption peaks and the shifting wavelength of O-H, C-H, C=O, and C-O groups indicated that the degradation process had indeed occurred. These results were consistent with the finding by Zain et al. [40], where there were changes in functional groups, such as the emergence of new peaks and changes in the absorption peaks of the thermoplastic starch polymer matrix after 30 days of degradation, which indicated that the films were degraded. In this study, there were only small changes in the absorption peaks of the NS film that was undergoing degradation in the planting soil, demonstrating that NS films degraded slowly in the planting soil as compared to the NS film before SBT.

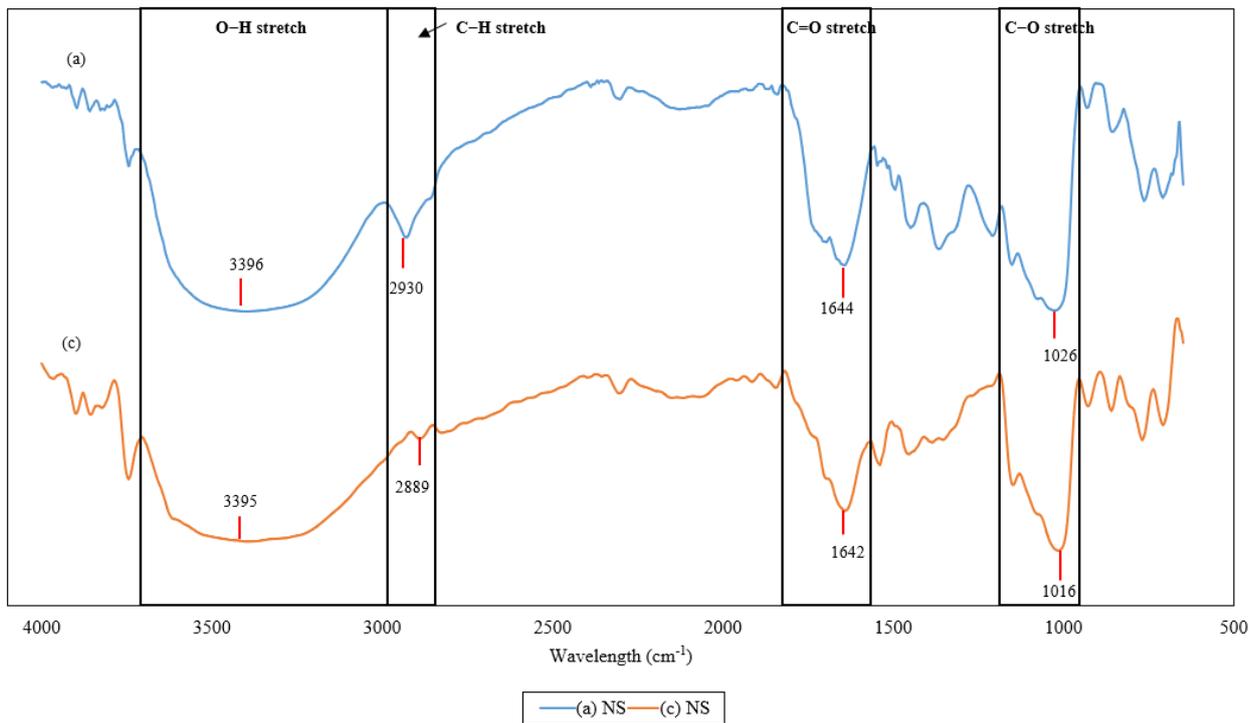


Figure 5. The FTIR spectra of NS films (a) before SBT and after SBT for 8 days in (c) planting soil. Note that no FTIR spectrum of NS film after SBT for 8 days in compost soil is shown because the film was fully biodegraded, with minor traces remaining.

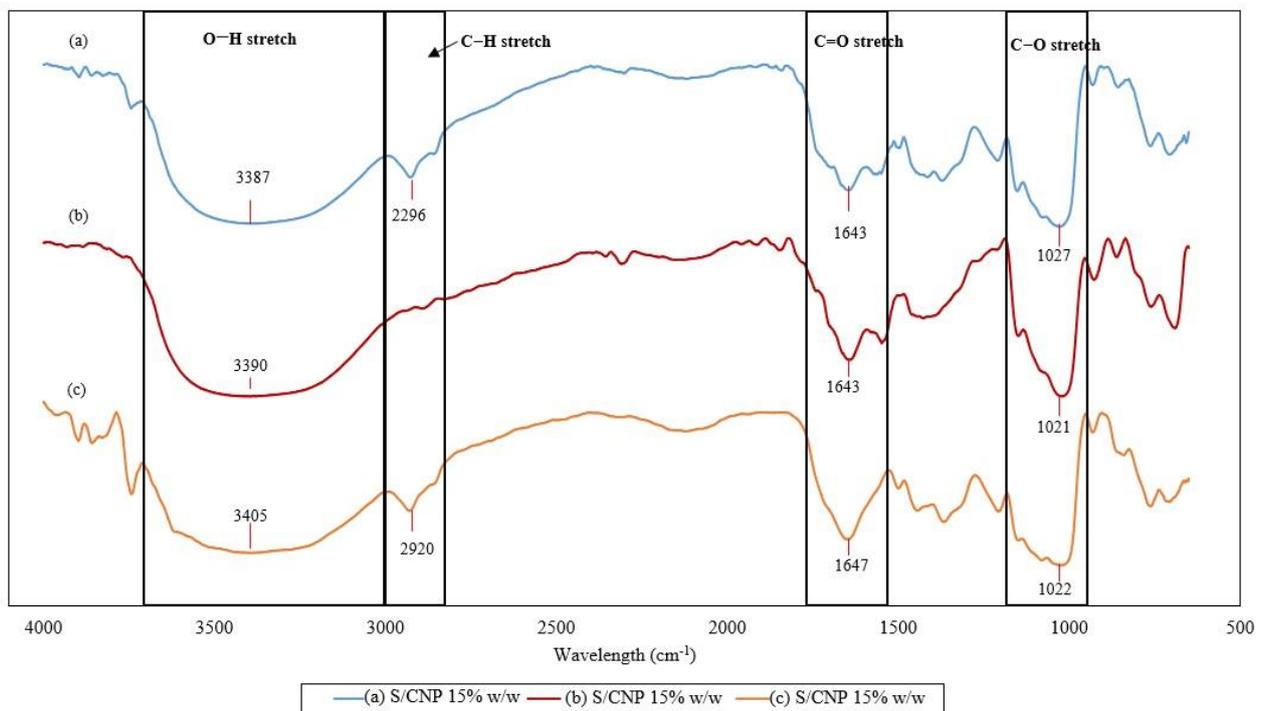


Figure 6. The FTIR spectra of S/CNP 15% w/w films (a) before SBT and after SBT for 8 days in (b) compost soil and (c) planting soil.

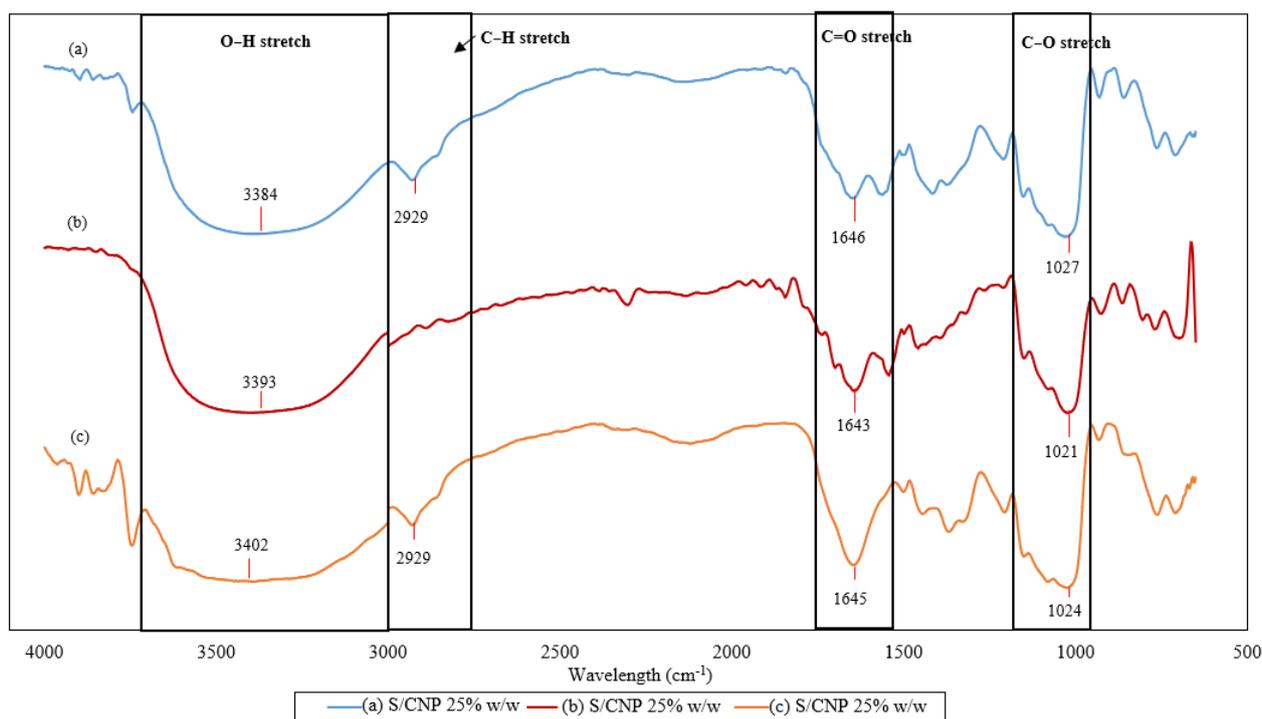


Figure 7. The FTIR spectra of S/CNP 25% *w/w* films (a) before and after SBT for 8 days in (b) compost soil, and (c) planting soil.

With the addition of CNP to the starch films, the peaks observed from NS film before SBT had displacements where the peaks at 1026 of the C-O group, 1644 of the C=O group, 2930 of the C-H group, and 3396 cm^{-1} of the O-H group (Figure 5a) shifted slightly to other wavenumbers (Figures 6a and 7a). These changes confirmed the possible interaction between CNP and the film components. This result was in accordance with the work of Hosseini et al. [41], where the incorporation of magnesium oxide nanoparticles to the pure modified starch films caused the peaks of the films to be shifted or removed to other wavenumbers. Furthermore, the absorption band of the O-H group of NS film in Figure 5a moved to a lower wavenumber from 3396 cm^{-1} to 3387 cm^{-1} for S/CNP 15% *w/w* (Figure 6a) and 3384 cm^{-1} for S/CNP 25% *w/w* (Figure 7a). These shifts in wavelengths confirmed the bond formation between the O-H group of starch and CNP.

After 8 days of SBT in planting soil, there were only slight changes in the intensity peak of C-H, C=O, and C-O groups of S/CNP films (Figures 6c and 7c) compared to NS film (Figure 5c), demonstrating that the S/CNP films underwent a slower degradation process. The high crystallinity of the CNP inhibited the microbial attack of the S/CNP film matrices compared to the NS film [15]. This finding was in line with the weight loss, degradation rate, visual appearance, and surface morphology results of the starch films incorporated with CNP in planting soil.

The FTIR spectrum of films containing different concentrations of CNP before SBT, S/CNP 15% *w/w* (Figure 6a), and S/CNP 25% *w/w* (Figure 7a), show that both films have similar functional groups, but with a slight shifting of the absorption peaks. The intensity peak of the O-H group of S/CNP films before SBT was lower than that of the NS films due to the fact that the addition of CNP reduced the water vapor permeability of the films [18], hence the lower amount of O-H groups. Moreover, after SBT, the intensity peak of the O-H group of S/CNP 25% *w/w* films (Figure 7b,c) was lower than S/CNP 15% *w/w* (Figure 6b,c) indicating less degradation of S/CNP 25% *w/w* films compared to S/CNP 15% *w/w*. The O-H groups have a tendency to bind with water molecules and increased the hydrophilicity of films. Thus, the decrement in the intensity of the O-H group with the increase in the concentration of CNP indicated the lower degradation rate of S/CNP 25% *w/w* films due

to the lower propensity of CNP to bind with water molecules to result in the degradation of the films.

There were several changes in the peaks for the films buried in both types of soils, such as changes in the intensity of certain absorption peaks and the shifting of the peaks. After 8 days of SBT, the absorption peaks of O-H groups for S/CNP 15% *w/w* and S/CNP 25% *w/w* in compost soil (Figures 6b and 7b) were higher in intensity and wider when compared to the S/CNP 15% *w/w* and S/CNP 25% *w/w* in planting soil (Figures 6c and 7c) due to the hydrolysis of starch, which produced more O-H groups [38]. Furthermore, the absorption peak of C=O groups for both S/CNP 15% *w/w* and S/CNP 25% *w/w* films in compost soil was slightly lower in intensity as compared to that in planting soil due to the higher changes in the crosslinking chain between starch and CNP. Moreover, it was found that the peak of the C-H stretching position of the S/CNP 15% *w/w* and S/CNP 25% *w/w* films in compost soil (Figures 6b and 7b) diminished, but there were no obvious changes in the peak of the C-H stretching position of the S/CNP 15% *w/w* and S/CNP 25% *w/w* in planting soil (Figures 6c and 7c). These findings confirmed that the S/CNP films degraded faster under compost soil than in planting soil, thus supporting the previous findings.

4. Conclusions

In this work, the effects of CNP concentrations in starch films and types of soils on the biodegradability of starch films were investigated in terms of weight loss, visual appearance, morphological properties, and structural properties. Compared to NS films, the addition of CNP to starch films resulted in films with higher resistance to degradation, whereby NS films exhibited a higher percentage of weight loss and a higher degradation rate after 8 days of SBT in compost and planting soils compared to S/CNP films. The degradation of the films reduced with the increase in the concentrations of CNP incorporated into the films. The S/CNP 25% *w/w* films had lower weight losses and degradation rates in both types of soils compared to S/CNP 10% *w/w* films. These findings were supported by changes in the morphological properties observed by SEM and changes in the structural properties observed by FTIR. The morphological studies proved that NS films have the most visible hollows and cracks, and an increase in surface roughness, which indicated that the films underwent a rapid degradation compared to those containing CNP. When the concentration of CNP decreased, the formation of fractures and hollows of the films became highly visible. From FTIR, all films exhibited changes in the intensity and the shifting of the absorption peaks of the O-H, C=O, C-O, and C-H groups due to the fracture of the films' chain in the soil during SBT for 8 days. All of the films were found to degrade more rapidly in compost soil compared to planting soil due to the high content of active microbes, soil humidity, and total organic matter of the soil.

Although the weight loss and degradation rates of S/CNP films were lower than NS films, by observing the linear trend of degradation, it can be postulated that the S/CNP films, even at the highest concentration of CNP (25% *w/w*), can be fully degraded within 16 and 32 days in compost and planting soils, respectively, which is good compared to synthetic plastic films such as those made from petroleum. If the weight loss and degradation rate were too high, there would be no room for the application of the films. The addition of 25% *w/w* CNP in starch films has been proven to improve mechanical properties and food safety, as well as application efficacy. The development of S/CNP films in this study is promising for applications designed to reduce the usage of non-degradable materials, hence addressing some of the environmental problems.

Author Contributions: Conceptualization, S.H.O.; methodology, N.D.A.R.; software, N.D.A.R.; validation, S.H.O., N.D.A.R., R.A.S., M.D., S.H.A. and M.A.P.M.; formal analysis, N.D.A.R.; investigation, N.D.A.R.; resources, S.H.O.; data curation, S.H.O.; writing—original draft preparation, N.D.A.R.; writing—review and editing, S.H.O.; visualization, S.H.O.; supervision, S.H.O., N.D.A.R., R.A.S., M.D., S.H.A. and M.A.P.M.; project administration, S.H.O.; funding acquisition, S.H.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education Malaysia [Project number FRGS/1/2021/TK0/UPM/02/5, vote number 5540542].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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