

## Research Article

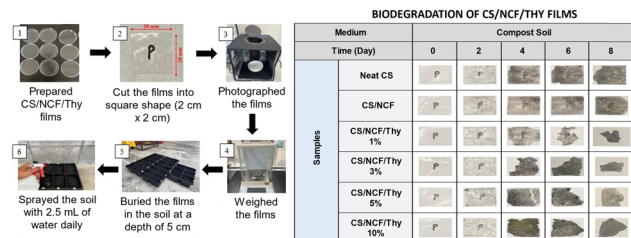
Norhazirah Nordin, Muhamad Farhan Fisal, Siti Hajar Othman\*,  
Intan Syafinaz Mohamed Amin Tawakkal, and Roseliza Kadir Basha

# Biodegradability of corn starch films containing nanocellulose fiber and thymol

<https://doi.org/10.1515/ntrev-2024-0110>

received April 14, 2024; accepted September 26, 2024

**Abstract:** The biodegradability of corn starch (CS), CS/nanocellulose fiber (NCF), and CS/NCF/thymol (Thy) films produced *via* a solvent casting method was investigated by soil burial test (SBT) in two types of soils (compost and planting). The films were evaluated in regard to weight loss, visual appearance, morphological properties, and structural properties. Based on SBT, CS/NCF films exhibited the least weight loss and changes in visual appearance, morphology, and structural properties. The inclusion of Thy into CS/NCF films increased substantially the degree of degradation of films in both soils. The microscopy images of films after SBT show that all of the films had voids, holes, and rough surfaces especially films containing Thy, indicating that the films degraded. Fourier-transform infrared spectroscopy results revealed shifting of peaks and changes in peak intensity of some functional groups of films, confirming the degradation phenomenon. Compost soil has more organic material, active microbes, nutrients, and humidity than planting soil, thus resulting in films with greater weight loss and changes in visual appearance, morphology, and



## Graphical abstract

structural properties. It can be concluded that the films have a high potential for applications and can help to lower the amount of usage of non-degradable materials.

**Keywords:** starch, nanocellulose fiber, thymol, degradation, soil burial

## 1 Introduction

Plastics are used extensively in the manufacturing of food packaging materials due to their versatility for various applications. However, conventional plastics are mostly made from non-degradable materials, thus causing disposal problems and threatening the environment. Synthetic-based plastic materials such as polypropylene, polystyrene, polyamide, and poly(vinyl chloride) are extremely difficult to degrade by microbes in the environment. According to a study by Meijer *et al.* [1], around 0.8–2.7 million metric tons of plastic are released each year from 1,000 rivers around the world. Hence, increasing concerns about environmental sustainability, depletion of petroleum reserves, and desire for green products and bio-economy have resulted in noteworthy advancement of bio-based plastics.

Plastic made from biopolymer is more environmentally friendly compared to conventional plastic since it can be manufactured using natural resources rather than synthetic petroleum resources. Vegetable oil, starch, cellulose, and lignin, as well as animal-derived polymers like protein and lipids, are the most frequently used natural biopolymers in the development of environmentally

\* **Corresponding author: Siti Hajar Othman**, Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia; Nanomaterials Processing and Technology Laboratory, Institute of Nanoscience and Nanotechnology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, e-mail: s.hajar@upm.edu.my, tel: +603-9769-6350, fax: +603-9769-4440

**Norhazirah Nordin:** Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, e-mail: gs48541@student.upm.edu.my

**Muhamad Farhan Fisal:** Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, e-mail: muhdfarhan12921@gmail.com

**Intan Syafinaz Mohamed Amin Tawakkal:** Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, e-mail: intanamin@upm.edu.my

**Roseliza Kadir Basha:** Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia, e-mail: roseliza@upm.edu.my

friendly plastics [2]. Furthermore, when compared to synthetic plastics which have tremendous drawbacks, specifically in terms of degradation, they exhibit extremely favorable degradation characteristics, including the ability to degrade faster and emit less hazardous carbon dioxide, making them more environmentally friendly. In addition, bioplastic can decompose up to 100% without producing any harmful residue with the final product being carbon dioxide and water [3].

In this work, corn starch (CS) was chosen as the base material for the production of bioplastic due to its ability to form free-standing films with acceptable performance and good processing capabilities [4]. However, films made from starch have serious limitations due to their inherent hydrophilicity which affects their utilization for most common uses. To address this issue, reinforcing starch-based films with natural fiber is a viable strategy to increase the mechanical characteristics of these films while retaining their biodegradability. The incorporation of nanocellulose fiber (NCF) into starch-based films, as well as integrating plasticizing agents such as glycerol and essential oil into the starch biopolymer matrix forming bionanocomposite films can overcome the limitation. Thymol (Thy), commonly known as *Thymus vulgaris*, is an essential oil compound extracted from thyme that can function as a plasticizer by changing the hydrophilicity of film matrix and acts as an effective antimicrobial agent with the potential to reduce the rate of microbial growth and food deterioration [5]. There are two works done by Othman *et al.* [5,6] that have investigated the influences of NCF and various amounts of Thy addition on the physical, thermal, mechanical, and water vapor barrier characteristics of CS films. They found that the films exhibited improved properties. Othman *et al.* [6] have also investigated the antibacterial properties of the films and found improvement in the inhibition of films against *Escherichia coli* with the increase in the concentration of Thy. The bacterial count of *E. coli* on fresh meat samples that were in direct contact with CS/NCF/Thy films reduced significantly demonstrating the potential of the films for active food packaging applications.

Previous research on the biodegradation of natural biopolymer films has been conducted using soil as a degradation medium because dumping on the soil is one of the common methods of handling municipal waste. For example, Othman *et al.* [7] revealed that the inclusion of chitosan nanoparticles into tapioca starch films improved the resistance of films to degradation in both compost and planting soils and that the degradation decreased with the rise in amounts of nanoparticles in films. Ong *et al.* [8] reported that the incorporation of NCF in *Citrus aurantifolia* crosslinked tapioca starch films reduced the biodegradation rate of films in compost soil. Thy, on the other hand, was found to increase the degradation rate of poly (lactic) acid-based films in solid synthetic wet waste by facilitating hydrolysis processes through the film matrix [9].

To the best of knowledge, no research has yet been carried out to determine the biodegradation properties of CS/NCF films added with several amounts of Thy at 1, 3, 5, and 10 wt% in compost soil and planting soil. The different amounts of Thy in films and the different types of soils will influence the biodegradation properties of CS/NCF films. Hence, this study aims to explore the effects of Thy amounts on the biodegradability of CS/NCF films, as Othman *et al.* [5,6] have proved that various Thy concentrations also affected the mechanical, thermal, and barrier attributes of CS/NCF films. Two different types of soils particularly compost and planting soils were chosen as soil burial test (SBT) media to examine the effects of various SBT surrounding conditions on the biodegradation properties of films because the soils contain different relative humidity (RH), amount of microbe, and total organic matter. The biodegradation properties of the films were explored in regard to weight reduction, appearance, morphological characteristics, and structural characteristics.

## 2 Materials and methods

### 2.1 Materials

Food-grade CS consisting of 33% amylose and 67% amylopectin, Thy in crystal form, ethanol, glycerol, and magnesium nitrate ( $Mg(NO_3)_2$ ) were bought from R&M Marketing, Semenyih, Malaysia. NCF in suspension form with a concentration of 2% w/v, length of ten-hundreds  $\mu m$ , diameter less than 80 nm, pH of 7, and crystallinity of 40% was obtained from the Institute of Tropical Forestry and Forest Product, Universiti Putra Malaysia, Serdang, Malaysia. A surfactant, Tween 20 (poly-sorbate 20) was bought from Sigma-Aldrich, USA. Planting soil (Brand Ninso Organic Soil) was purchased from a local nursery in Serdang, Malaysia. Compost soil (Brand GE Compost 3333) was bought from Sin Seng Huat Seeds Sdn. Bhd., Serdang, Malaysia. The compositions of the two types of soils used in this work based on the specifications from manufacturers were similar as reported by Othman *et al.* [7].

### 2.2 Preparation of films

#### 2.2.1 CS/NCF bionanocomposite films

CS/NCF films were fabricated *via* a solvent-casting method. An amount of 80 mL of distilled water containing glycerol (25 wt% of starch) was used to dissolve 4 g of CS. Using a hot

plate magnetic stirrer (Favorit, Indonesia), the solution was heated for 30 min till the mixture was gelatinized at 90°C. At the same time, a fixed amount of NCF (1.5 wt% of starch) was dispersed in 20 mL of distilled water and then incorporated into the film-forming solution. The film mixture underwent stirring for another 5 min. The film mixture was subjected to ultrasonication using an ultrasonic probe (Qsonica, 20 kHz, 500 W) for 5 min of 50% amplitude before casting into the Petri dish with a diameter of 140 mm. The neat CS films were also fabricated using the same procedures except that without the addition of NCF.

### 2.2.2 CS/NCF/Thy bionanocomposite films

The NCF/Thy emulsion was obtained by mixing different weight percentages of Thy (0, 1, 3, 5, and 10 wt% of starch) with 20 mL of distilled water that contained 1.5 wt% of NCF, at 50°C. Next, a predetermined quantity of Tween 20 (20 wt% of Thy) was introduced into the mixture to produce an emulsion, and the emulsion was kept stirring for 15 min utilizing the hot plate magnetic stirrer. This emulsion was administered to the starch solution that was gelatinized and cooled to 40°C in a dropwise manner using a Pasteur pipette. After that, the solution was dispensed into Petri dishes to produce films.

### 2.2.3 Casting of films

The films were dried at room temperature of 21°C for 48 h. After that, the films were dried for 15 min at 45°C using a ventilated oven (Mettler universal oven UN110, Germany). Finally, the films were peeled and kept in a desiccator at a temperature of 30°C with saturated ( $\text{Mg}(\text{NO}_3)_2$ ) solution to provide RH of 51% for 48 h before undergoing characterization.

## 2.3 SBT

The prepared films were first cut into square shape (2 cm × 2 cm) and then weighed and photographed. After that, the films were buried in compost soil and planting soil. The soils were placed in a container (30 cm × 40 cm × 15 cm) with 6 compartments and the films were buried 5 cm below the soil. Then, the container was placed outside the laboratory where it was subjected to open conditions, including temperature, sunlight, wind, and humidity. The soil was kept moist by providing around 2.5 mL of water every day *via* spraying. The films were examined after designated periods of 2, 4, 6, and 8 days of burial in soils.

## 2.4 Biodegradation analysis

### 2.4.1 Weight loss

After 2, 4, 6, and 8 days, the films were removed from soils. A total period of 8 days for SBT was chosen because there were difficulties in evaluating some of the films after 8 days of degradation since they broke into small pieces. Films were first cleaned gently by means of a paintbrush in order to get rid of adherent soil. The films were then kept at 40°C in a ventilated oven for 15 min to dry. Then, the dried films were placed in a desiccator that contained saturated  $\text{Mg}(\text{NO}_3)_2$  solution at RH of 51% and temperature of 30°C for 24 h till the weight became constant. The weight loss (%) of films was calculated using equation (1).

$$\text{Weight loss (\%)} = (M_0 - M_1)/M_0 \times 100\%, \quad (1)$$

where  $M_0$  is the weight of films before burial and  $M_1$  is the weight of residual films after 2, 4, 6, and 8 days of burial. The degradation rate ( $g/d$ ) of films was calculated based on the previous study by Adelaja and Daramola [8] as shown in equation (2).

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

where  $W_L$  is the weight loss of films after 8 days of burial and  $t$  is the period of burial (days).

### 2.4.2 Visual appearance

Following 2, 4, 6, and 8 days of burial, photographs of the films were taken from 15 cm height *via* a phone camera (Model: iPhone 11) with 3.5× magnification inside Puluz lightbox (Shenzen Puluz Technology Limited, China). The photos were chronologically arranged to visually evaluate the degradation of films over time.

### 2.4.3 Structural properties

The Fourier transform infrared spectroscopy (FTIR) spectra of films were acquired by means of FTIR spectroscopy (Thermo Fischer Scientific, model Nicolet 6700, USA) before and after 4 days of SBT. The analysis was conducted after 4 days of SBT because the films were still in shape and not broken into small fragments allowing for more precise FTIR results. The spectra were taken from 400 to 4,000  $\text{cm}^{-1}$  wavelength, to determine changes in the structure of films.

### 2.4.4 Morphological properties

The surface morphologies of films were investigated *via* scanning electron microscopy (SEM, JSM-IT100 InTouch

Scope, Japan). The films were examined at 500 $\times$  magnification and 10.0 kV voltage prior to and following 4 days of burial. A double-sided adhesive tape was used to mount films on aluminum stubs.

## 2.5 Statistical analysis

Minitab 19 (Minitab Inc., USA) software was used to perform the analysis of variance (ANOVA) of the experimental data. Tukey's test was used to compare the means at the 0.05 level of significance.

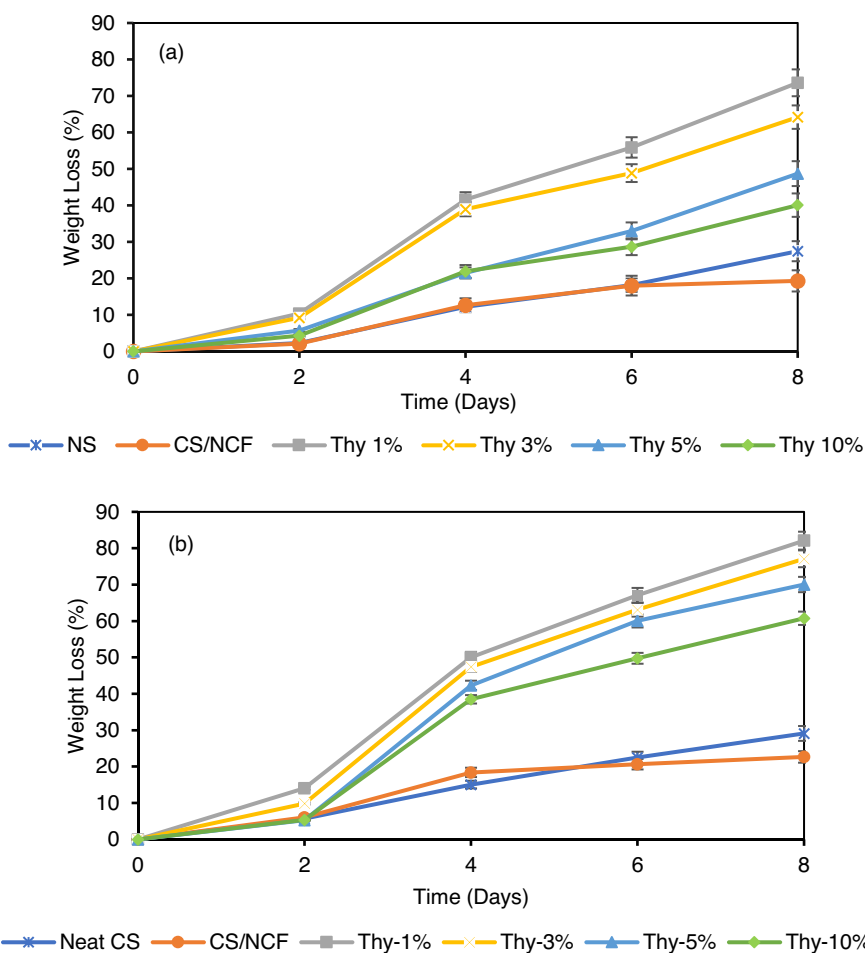
## 3 Results and discussion

### 3.1 Weight loss

The SBT was carried out by burying films in soils for 8 days and leaving the soils in an open environment. Figure 1

presents the percentage weight loss of CS-based films with the incorporation of NCF and Thy upon SBT in compost soil and plan. The percentage of weight loss and rate of degradation of films after being buried for 8 days in both soil mediums are tabulated in Table 1. Based on Figure 1, the degradation of all films is approximately linearly correlated with time. The weight losses of neat CS films after being buried for 8 days in compost soil and planting soil were 29.12 and 27.47%, respectively (Table 1). The weight losses were slightly reduced with the addition of NCF into the films but the degradation rates did not significantly differ. CS/NCF films exhibited the lowest weight losses among other films in compost soil and planting soil which were 22.67 and 19.34 wt%, respectively.

This finding was in line with the finding of Ong *et al.* [9] who found that incorporating NCF into *Citrus aurantiifolia* crosslinked tapioca starch films led to greater resistance to degradation of films, hence lower weight loss. This was due to the fact that incorporating NCF into the starch biopolymer matrix increased the crystallinity of the



**Figure 1:** The weight loss of CS, CS/NCF, and CS/NCF/Thy films during 8 days of degradation (a) in compost and (b) in planting soils.

biopolymer matrix [5], which created a dense structure that restricted the enzymatic hydrolysis and destructive enzyme activities [9]. Othman *et al.* [5] also revealed an improvement in the tensile strength (TS) of CS/NCF films compared to neat CS films due to good intermolecular forces between CS and NCF, leading to higher rigidity of films. This improvement might contribute to higher resistance to film degradation. Apart from that, the integration of NCF into the starch biopolymer matrix caused an increase in the hydrophobicity of the film matrix which affected biodegradation, hence the weight loss. NCF increased the hydrophobicity of starch biopolymer matrix due to the numerous hydroxyl groups available on its surface that resulted in hydrogen bonding among –OH groups of hydrophilic polymer matrices and non-polar matrices [10]. Indeed, Othman *et al.* [5] found a decrement in water vapor permeability of CS/NCF films, supporting the increment in hydrophobicity reasoning.

Generally, the addition of Thy into bionanocomposite films resulted in a drastic increase in weight losses of the films, and the degradation rates in both compost and planting soils. Based on Figure 1(a), CS/NCF/Thy-1% film demonstrated the highest weight loss in compost soil following 8 days of SBT (82.09%), followed by CS/NCF/Thy-3% (77.10%), CS/NCF/Thy-5% (70.03%), and CS/NCF/Thy-10% (60.76%). A similar trend was also obtained for the weight loss of films containing Thy in planting soil as shown in Figure 1(b). These occurrences can be described by the influence of Thy on water molecule diffusion in the biopolymer matrix, leading to a notable increase in weight loss. The enhancement of the water diffusion process by Thy was a result of the increased chain mobility caused by the presence of additives [11]. The enhancement of the water diffusion process favored the microbial attack on the film matrix [12,13], leading to the increment in the destruction of film structure, hence higher weight loss.

It is important to note that as the amount of Thy added to the films increased, the weight loss and rate of degradation of

the films decreased. Among the Thy-incorporated films, CS/NCF/Thy 1% films experienced the greatest weight loss in both degradation media, whereas CS/NCF/Thy 10% films had the smallest weight loss. These were ascribed to the hydrophobic nature of Thy as an essential oil compound. Othman *et al.* [6] demonstrated that the hydrophobicity of films rose with the increment in the amount of Thy added to CS/NCF films, depicted by the reduction in water vapor permeability. At a high concentration of Thy and adequate dispersion, Thy promoted an effective cross-linking of the components of films, particularly, CS and NCF. The hydrophobic group of Thy led to a rise in the hydrophobic properties of films. Besides, Othman *et al.* [6] also found that the incorporation of a low amount of Thy did not have any substantial effect on the hydrophobicity of films. In fact, this promoted the hydrolysis process at a maximum rate, which directly increased the degradation rate of films. It is important to note that Othman *et al.* [5,6] also revealed a decrement in the TS of CS/NCF/Thy films with the rise in the amount of Thy included to the films. Logically, this would contribute to higher degradation of films at higher Thy concentrations. Nonetheless, it can be deduced that hydrophobicity has a greater impact on film degradation than the strength of films.

Figure 1 and Table 1 demonstrate that the weight losses of films differed significantly between the two media, with compost soil exhibiting greater weight losses than planting soil. The results proved that the degradation process was affected by the varieties of soil utilized. Compost soil resulted in higher film degradation because of the large quantity of nutrients and microbes composition compared to planting soil [14]. Furthermore, humidity and the total organic matter in soil also influence the degradation of films. In the previous work of Ahimbisibwe *et al.* [15], they revealed that when the films were buried in soil with a high RH, the films absorbed water molecules in the soil quicker than that buried in the soil at a low RH; hence enhanced the microbial growth on the surface of

**Table 1:** Percentage weight loss and degradation rate of CS, CS/NCF, and CS/NCF/Thy films after being buried for 8 days in compost soil and planting soil

Samples	Compost soil		Planting soil	
	Weight loss (%)	Degradation rate (g/day)	Weight loss (%)	Degradation rate (g/day)
Neat CS	29.12 ± 0.17 <sup>dA</sup>	0.036 ± 0.021 <sup>aC</sup>	27.47 ± 0.07 <sup>aB</sup>	0.034 ± 0.008 <sup>aC</sup>
CS/NCF	22.67 ± 0.03 <sup>bA</sup>	0.028 ± 0.003 <sup>aC</sup>	19.34 ± 0.02 <sup>bB</sup>	0.024 ± 0.003 <sup>aC</sup>
CS/NCF/Thy-1%	82.09 ± 0.11 <sup>cA</sup>	0.103 ± 0.014 <sup>bC</sup>	73.61 ± 0.08 <sup>cB</sup>	0.092 ± 0.010 <sup>bC</sup>
CS/NCF/Thy-3%	77.10 ± 0.03 <sup>dA</sup>	0.096 ± 0.004 <sup>bC</sup>	64.20 ± 0.13 <sup>dB</sup>	0.080 ± 0.016 <sup>bC</sup>
CS/NCF/Thy-5%	70.03 ± 0.09 <sup>eA</sup>	0.088 ± 0.015 <sup>bC</sup>	48.72 ± 0.17 <sup>eB</sup>	0.061 ± 0.021 <sup>bCC</sup>
CS/NCF/Thy-10%	60.76 ± 0.20 <sup>fA</sup>	0.076 ± 0.025 <sup>bC</sup>	40.09 ± 0.06 <sup>fB</sup>	0.050 ± 0.007 <sup>cD</sup>

Values in a similar column with different lowercase superscripts are significantly different ( $p < 0.05$ ). Values in a similar row with different uppercase superscripts are significantly different ( $p < 0.05$ ).



films which led to the increase in degradation extent or weight loss of films. The observation aligns with the findings of this study, whereby the weight loss was greater in compost soil that contained a larger quantity of nutrients, moisture, active microbial, and total organic matter than in planting soil. Table 1 also demonstrates that the degradation rates did not significantly differ between the types of soil, suggesting that in this work, the contents of nutrients, humidity, microbes, and organic matter in the soil had a substantial influence on the degradation amount of films but not the degradation rates.































### 3.2 Visual appearance

The visual appearance of all samples in different soils was investigated by capturing images of films at the designated period and the results were tabulated in Tables 2 and 3. Before the SBT, neat CS films were clear and transparent but the transparency and color of films changed proportionally with degradation time. In compost soil, all colors of films changed from transparent to dark grey due to the greyish-black color of the compost soil which contains decanter cake, pome sludge, and cocoa [7]. Whereas in planting soil, the colors of all films transitioned from

transparent to light brown due to the planting soil being dark brown in color because it comprises coco peat and red burnt soil. Furthermore, in compost soil, the transition of films from clear and transparent to more opaque was glaringly obvious, denoting that films degraded to a greater extent compared to planting soil. The degradation and color change trends indicated biological action on the films [16]. There were cracks and a light trace of crumbling of neat CS films observed following 8 days of burial in compost soil and planting soil.

Based on Tables 2 and 3, the inclusion of NCF and Thy in CS films did not affect the transparency of films before SBT. However, according to Othman *et al.* [5,6], a slight decrement in the transparency of films was observed with the incorporation of NCF and Thy. Apart from that, they also found an increase in the total color difference of films due to the incorporation of NCF/Thy as an oil-in-water emulsion into the film matrix. Perhaps in this work, there was a slight decrement in the transparency of films when added with NCF and Thy, but the changes were non-noticeable when observed visually. Meanwhile, CS/NCF films exhibited less cracking and crumbling compared to neat CS films after 8 days of SBT. This finding indicated that CS/NCF films were less degraded than neat CS films because of the crystallinity effect of NCF when incorporated into the starch biopolymer matrix [8] and the enhancement in TS of films [5].

**Table 2:** The visual appearance of CS, CS/NCF, and CS/NCF/Thy films after being buried for 8 days in compost soil

Medium		Compost soil				
Time (days)		0	2	4	6	8
Samples	Neat CS					
	CS/NCF					
	CS/NCF/Thy 1%					
	CS/NCF/Thy 3%					
	CS/NCF/Thy 5%					
	CS/NCF/Thy 10%					

Following SBT in compost soil, the inclusion of Thy in CS/NCF films has led to changes in transparency and color whereby the color turned from transparent to dark grey, indicating that the films were degraded more compared to other neat CS and CS/NCF films. Transparency changes of films throughout SBT indicated that films underwent surface modifications. These surface modifications were due to the initiation of biopolymer hydrolytic decomposition processes, which were correlated with moisture absorption of the biopolymer [11]. The increase in opacity was caused by several simultaneous events, such as the development of crystallinity in the polymer matrix or the formation of low molar-mass breakdown by-products during hydrolysis [17]. The films containing Thy started to crumble and crack after being buried for 2 and 4 days in compost soil, respectively.

Besides, the degradation of films containing Thy in planting soil resulted in a slight color change from transparent to light brown. The cracking and crumbling of CS/NCF/Thy films started after 2 days of being buried in planting soil. The cracking and crumbling process started early in the planting soil in comparison to compost soil because of the coarse texture of planting soil that could not retain the water content in the soil [18]; hence it made the films dry and brittle. Apart from that, film degradation was lesser in planting soil compared to compost soil as can

be observed from the transparency, color, and shape of films after 8 days of SBT. From the results obtained, it can be observed that films containing lower concentrations of Thy degraded to a greater extent compared to those containing high concentrations of Thy because Thy hindered the interactions between biopolymer chains and decreased cross-linking, thus the soil moisture could easily penetrate biopolymer network, weakening biopolymer chain, and hydrolyzed by soil microorganisms [19].

### 3.3 Morphological properties

Figure 2 depicts the SEM micrographs of neat CS, CS/NCF, and CS/CNF/Thy 3% before and after 4 days of burial in different types of soils. Neat CS film (Figure 2(a)) exhibited a rough surface due to the disorder of starch microstructure and was covered with big starch granules. Restu *et al.* [20] found that the SEM image of the neat starch film exhibited a rough structure and large granule formation. After 4 days of soil burial in both soils, the surface of neat CS films exhibited a worm-like structure indicating that the films started to fracture, signifying the beginning of the degradation process (Figure 2(b) and (c)). However, the surface of neat CS film was relatively smooth after

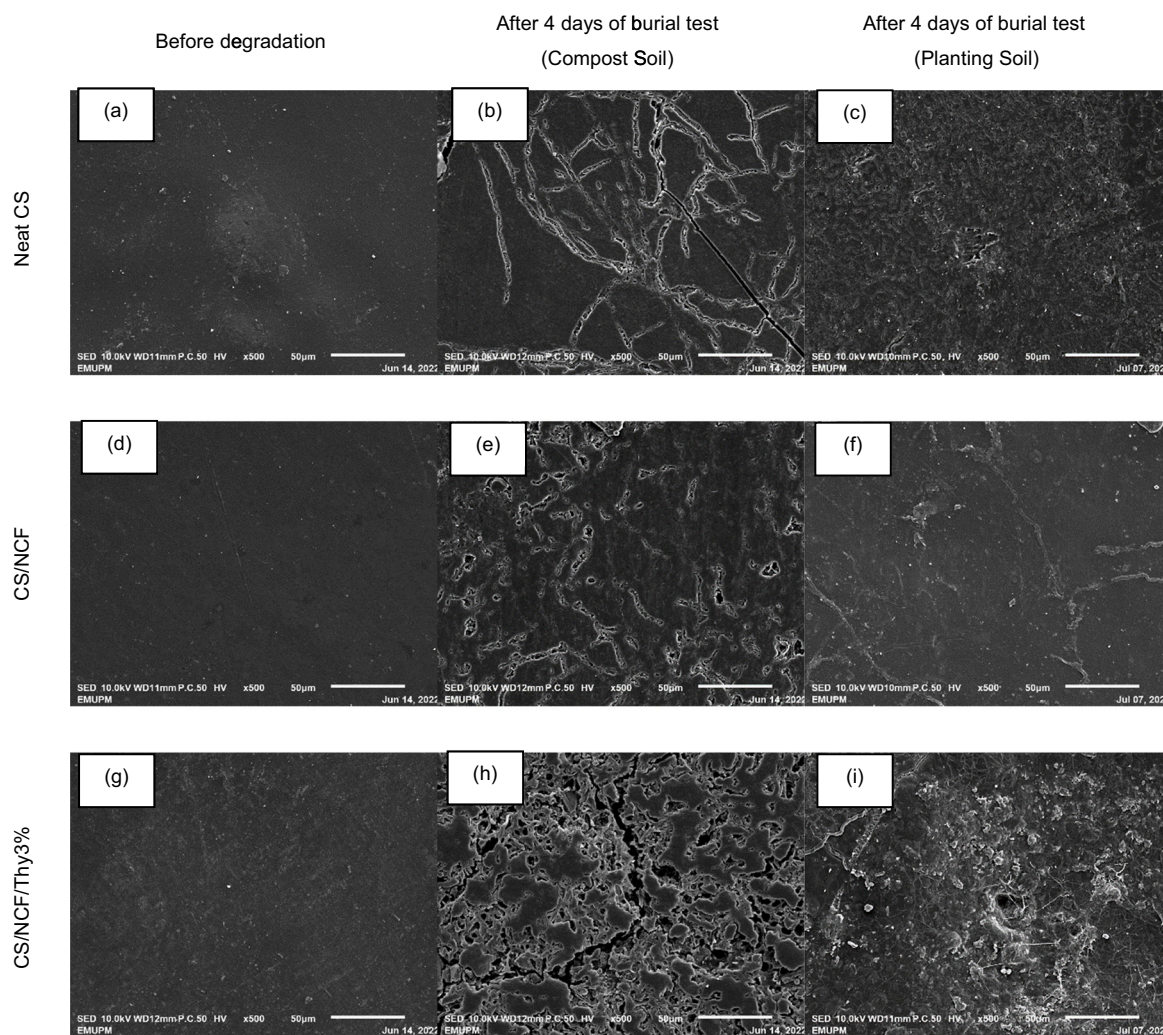
**Table 3:** The visual appearance of CS, CS/NCF, and CS/NCF/Thy films after being buried for 8 days in planting soil

Medium		Planting soil				
Time (days)		0	2	4	6	8
Samples	Neat CS					
	CS/NCF					
	CS/NCF/Thy 1%					
	CS/NCF/Thy 3%					
	CS/NCF/Thy 5%					
	CS/NCF/Thy 10%					

degradation in planting soil compared to compost soil due to the different composition and structure of soils, resulting in the lesser degradation extent of neat CS film in planting soil.

Meanwhile, the incorporation of NCF into the starch biopolymer matrix decreased the surface roughness of films before burial (Figure 2(d)), showing the compatibility of NCF between CS molecules. The similarities in the chemical structure of CS and NCF, as well as the nano-sized effect of the NCF, were the main reasons for the compatibility of the two components [5]. After 4 days of burial, the surface of CS/NCF films shows voids and worm-like structures (Figure 2(e) and (f)), indicating the start of degradation. The visible voids, worm-like structures, and surface roughness of CS/NCF films were less than neat CS films and CS/NCF/Thy 3% films due to the high crystallinity and TS of CS/NCF films that caused greater resistance to the degradation compared to other films [6,8].

Apart from that, adding Thy to the films altered the surface characteristics from smooth to rough (Figure 2(g)). This finding is similar to the previous work by Davoodi *et al.* [21], where in the absence of Thy, the potato starch film had a compact and heterogeneous structure, but with the presence of Thy, the film had a coarser and more heterogeneous structure with fissures dispersed throughout the matrix. The rough surface of CS/NCF/Thy-3% films was caused by conformational rearrangements, as discussed by Othman *et al.* [5], where they discovered that the inclusion of Thy in films attributed to readjustment of the order of molecular in the starch matrix that significantly lowered hydroxyl group interactions with starch molecules. Acosta *et al.* [22] also discovered that the introduction of essential oil into cassava starch-gelatin films increased surface irregularities of the films because of the limited miscibility of lipid components in the starch biopolymer matrix. After 4 days of SBT, the surface



**Figure 2:** The SEM micrographs of neat CS ((a) to (c)), CS/NCF ((d) to (f)), and CS/NCF/Thy-3% ((g) to (i)) before and after 4 days of burial in compost soil and planting soil.



roughness increased significantly due to the formation of voids and holes (Figure 2(h) and (i)). CS/CNF/Thy-3wt% films had the highest surface irregularities and formation of voids and visible hollows compared to CS and CS/NCF films. These findings were consistent with the results found from the degradation study *via* weight loss and visual appearance changes of CS/NCF/Thy films. The voids and holes are signs of the degradation process. According to Ramos *et al.* [11], the formation of surface holes in all samples indicated the start of the hydrolytic degradation process, and the inclusion of Thy contributed to more degraded materials subjected to composting conditions.

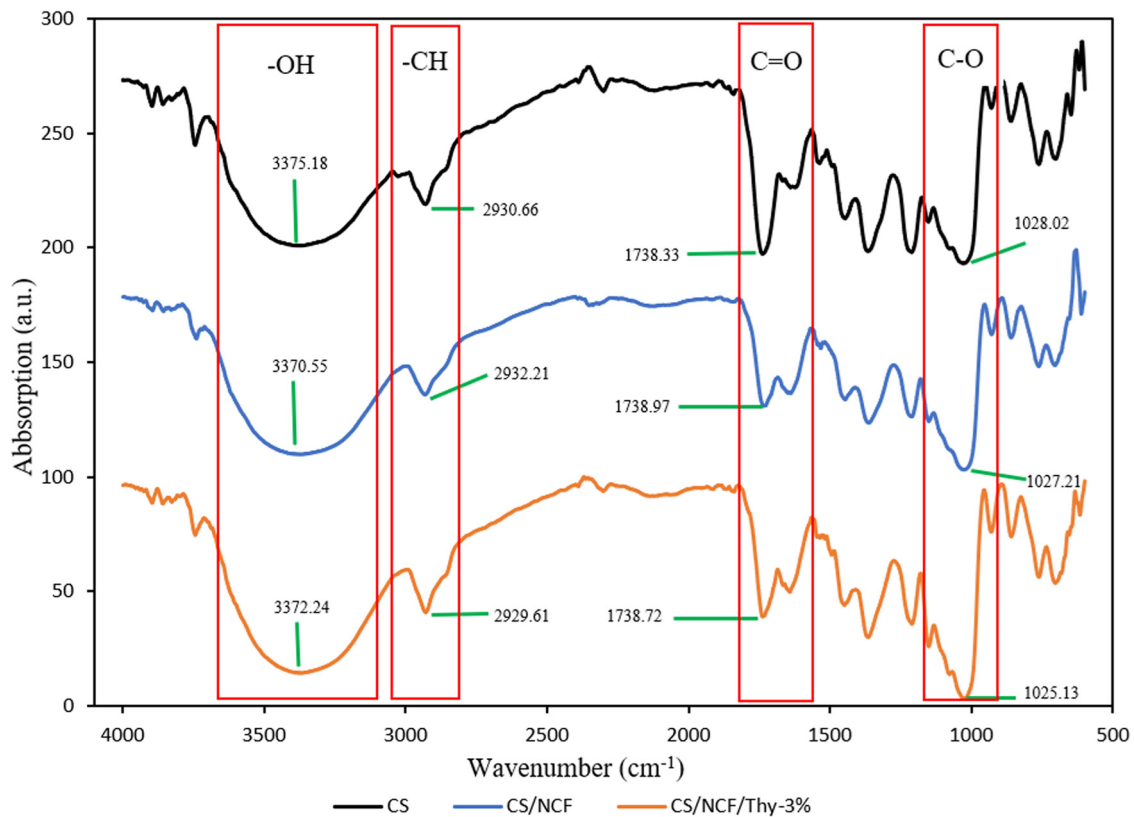
Based on SEM micrographs of sample films, all films underwent the degradation process, but there was a difference in the extent of degradation of the biopolymer matrix. As discussed previously, different types of soils contain different types and amounts of microbes and nutrients, which have significant roles in affecting the degree of degradation of films. The results revealed that films buried in compost soils exhibited surfaces that were rougher with large void formation and high surface deterioration in contrast to films buried in planting soil, indicating greater degradation. This was due to the fact that compost soil usually contains high humidity, the most vibrant microorganisms,

and hence a high load of organic matter and perfect conditions for deterioration [23].

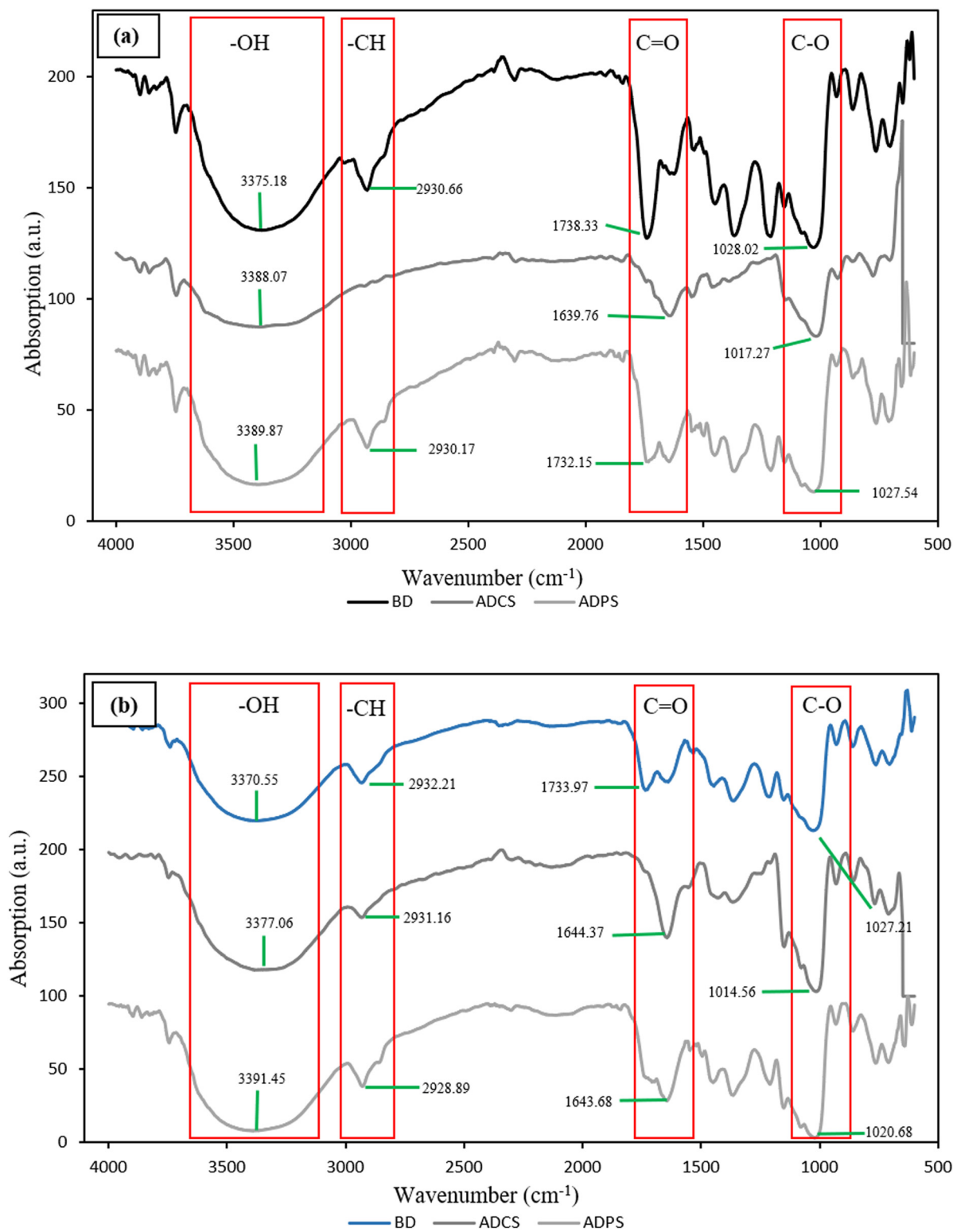
### 3.4 Structural properties

Structural properties of neat CS, CS/NCF, and CS/NCF/Thy films before SBT were determined using FTIR as shown in Figure 3. Several identical peaks were identified for all films before SBT. There were strong and broad absorption peaks at around  $3,370\text{ cm}^{-1}$ , corresponding to the stretching vibration of  $-\text{OH}$  groups. Medium absorption peaks can be seen at around  $2,930\text{ cm}^{-1}$  representing the stretching vibration of the  $\text{C}-\text{H}$  groups. Furthermore, there were strong absorption peaks at about  $1,738\text{ cm}^{-1}$  revealing stretching vibration of  $\text{C}=\text{O}$ . The peaks at around  $1,025\text{--}1,028\text{ cm}^{-1}$  demonstrated the stretching vibration of  $\text{C}-\text{O}$ . All the peaks found in this work were in line with the studies by Othman *et al.* [5] and Khairuddin *et al.* [24].

The inclusion of NCF in the starch biopolymer matrix did not alter any structural properties particularly functional groups of CS films. FTIR spectrum before the SBT of CS/NCF films shows that films have almost similar chemical



**Figure 3:** FTIR spectra of neat CS, CS/NCF, and CS/NCF/Thy-3% films before SBT.



**Figure 4:** FTIR spectra of (a) neat CS film, (b) CS/NCF, and (c) CS/NCF/Thy 3% before degradation (BD), after 4 days of burial in compost soil (ADCS), and after 4 days of burial in planting soil (ADPS).

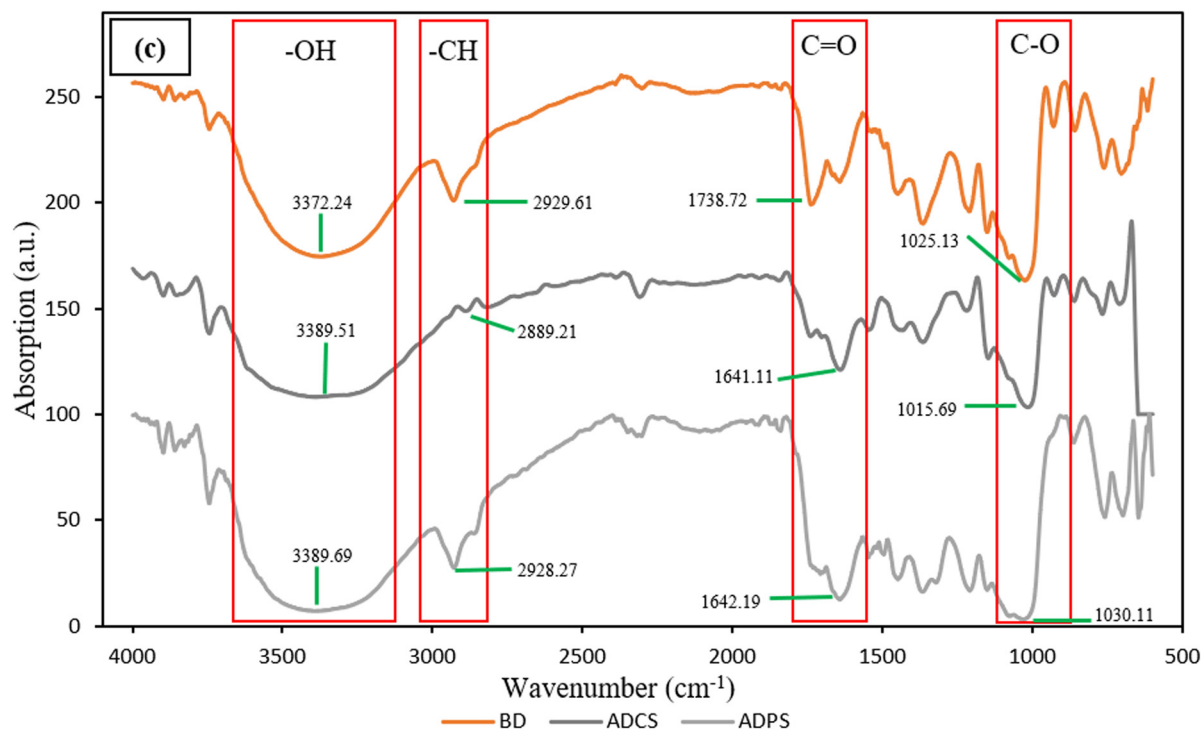


Figure 4: (Continued)

groups to the neat CS films, proving that NCF and CS were compatible with each other. This finding was consistent with the investigation of Othman *et al.* [5], whereby they discovered the presence of intermolecular and/or intramolecular interactions between CS and NCF by hydrogen bonds due to the potential compatibility between the two components. In addition, Vigneshwaran *et al.* [25] also revealed that the FTIR spectrum of potato starch and potato starch/NCF films have many characteristics that overlap, indicating that starch and NCF have remarkably similar chemical properties.

Upon the addition of Thy, Figure 3 clearly shows an increase in the intensity of the hydroxyl group at a peak of around  $3,372\text{ cm}^{-1}$ . This signified an increment in the quantity of hydroxyl group associated with molecular bonds with the introduction of Thy in CS/CNF films. Although Thy is hydrophobic in nature, at a lower concentration of Thy ( $<7\text{ wt}\%$ ), Thy could not surpass the dominance of hydrophilic molecules in the CS/NCF film matrix, leading to the water diffusion process enhancement [6]. The intensity of peak at about  $1,738\text{ cm}^{-1}$  corresponding to the stretching vibration of  $\text{C}=\text{O}$  decreased slightly compared to neat CS film in line with the finding by Souza *et al.* [26] whereby interactions among hydroxyl groups in starch and ether bond ( $\text{C}=\text{O}$ ) in carvacrol essential oil caused the change in peak intensity at around  $1,000\text{ cm}^{-1}$ . Research by Othman *et al.* [5] and Nordin *et al.* [27] found the

decrement in intensity of the  $\text{C}=\text{O}$  absorption peak indicated the successful incorporation of Thy into the starch biopolymer matrix and that intermolecular interactions among the film constituents occurred. The influence of Thy was noticeable at a peak around  $1,025\text{ cm}^{-1}$  attributed to the  $\text{C}-\text{O}$  stretching where the intensity of this peak increased significantly because of the existence of an aromatic ring in Thy [5].

Meanwhile, Figure 4 depicts FTIR spectra for the three films in the range of  $500\text{--}4,000\text{ cm}^{-1}$  prior to and following 4 days of burial in compost soil and planting soil. Generally, after 4 days of burial in different soils, there was a huge reduction in the intensity of several absorption peaks as well as some shifting of peaks for all films due to the breaking of the starch chain under the degradable environment. The changes in intensity and shifting of peaks of  $-\text{OH}$ ,  $\text{C}=\text{O}$ ,  $\text{C}-\text{O}$ , and  $\text{C}-\text{H}$  suggested the occurrence of the degradation process. These results corroborated with the findings of Zain *et al.* [28], who discovered changes in peaks of several functional groups, including the appearance of distinct peaks and variation in absorption peaks in thermoplastic starch biopolymer matrix after being buried for 30 days in compost soil, indicating that films had degraded.

From Figure 4(a), it can be obviously seen that there was a significant intensity reduction and slight shifting of  $-\text{OH}$  peaks in the range of  $3,200\text{--}3,500\text{ cm}^{-1}$  after 4 days of

burial of neat CS film in both soil mediums. Changes in the –OH peaks of the FTIR spectrum of neat CS films following SBT were more pronounced in compost soil than in planting soil, indicating that neat CS films degraded to a greater extent in compost than in planting soil. According to Veliz *et al.* [29], the intensity changes of peak that correspond to the hydroxyl group could be referred to as the interaction between the degradation medium and hydrophilic components of films that enhanced the hydrolysis and degradation of starch. Figure 4(a) also depicts that absorption peaks for –CH groups almost completely disappeared after 4 days of burial in compost soil, while in planting soil, there was a slight decrement in the absorption peak intensity of the C–H group at around  $2,930\text{ cm}^{-1}$ , further supporting the previous finding.

After 4 days of burial in compost soil, there was a shift in absorption peaks of C=O from  $1,738$  to  $1,639\text{ cm}^{-1}$ , showing that the molecular structure of films was rearranged possibly due to hydrolytic degradation and/or microbial attack. Nandiyanto *et al.* [2] discovered that after 2 weeks of immersing pure CS films in ultrapure water, there were several shifts in absorption peaks, indicating that fungi on the surface of films were changing the chemical structure of bioplastics. The minor variation in absorption peak of C=O was seen after 4 days of burial in planting soil compared to compost soil, showing that films underwent a lesser extent of degradation process in planting soil. Additionally, there were minor shifts in the C–O group absorption peak intensity after 4 days of burial in both soil mediums, but a significant decrease in the absorption peak intensity was observed after 4 days of decomposition in compost soil.

From Figure 4(b), after 4 days of being buried in both planting and compost soils, the absorption peaks of CS/NCF films only changed slightly in contrast to neat CS films (Figure 4(a)) and CS/NCF/Thy films (Figure 4(c)) indicating that the films experienced minor degradation due to crystallinity of NCF. This result was consistent with the degradation study *via* weight reduction, appearance, and morphological properties in both soil mediums. Only absorption peaks of C=O and C–O had significant shifts where in compost soil the C=O and C–O peaks shifted from  $1,733$  to  $1,644\text{ cm}^{-1}$  and from  $1,027$  to  $1,014\text{ cm}^{-1}$ , respectively, while in planting soil, the peaks shifted from  $1,733$  to  $1,643\text{ cm}^{-1}$  and from  $1,027$  to  $1,020\text{ cm}^{-1}$ , respectively. The other functional groups did not change much after burial in terms of peak shifting and intensity reduction, suggesting that the degradation of films was minor for CS/NCF films.

Furthermore, after 4 days of burial, the FTIR spectrum (Figure 4(c)) of CS/NCF/Thy 3% shows more variations in absorption peaks of films in compost soil in comparison to planting soil. Even though the appearance of some new

peaks between  $1,500$  and  $1,000\text{ cm}^{-1}$  of films in planting soil could be evidenced, there are substantial changes in intensity and shifting of peaks could be evidenced in compost soil than in planting soil. This finding shows that films were degraded further when buried in compost soil than when buried in planting soil. Films in both degradation mediums experienced some variations in peaks, including changes in intensity and shifting of certain absorption peaks. A huge intensity reduction and shifting of peaks related to carbonyl group C=O from  $1,738$  to  $1,641\text{ cm}^{-1}$  and from  $1,738$  to  $1,642\text{ cm}^{-1}$  were revealed in both compost and planting soils, respectively. These findings were in accordance with that of Ramos *et al.* [11] who discovered that degradation of poly (lactic acid)/silver nanoparticles films containing Thy was accompanied by a significant decrease in the intensity of the carbonyl group at a peak around  $1,750\text{ cm}^{-1}$  due to the formation of carbonyl groups of carboxylic acids by hydrolytic scission of ester groups. The FTIR spectrum of films containing Thy illustrates more changes than neat CS and CS/NCF films implying that the Thy addition enhanced the degradation process, mainly because of the ability of Thy to increase the diffusion process, resulting in higher hydrolysis and release of small molecules of starch such as glucose and fructose that could be attacked by microorganisms.

## 4 Conclusions

In this study, the influences of NCF and Thy on the degradation of starch biopolymer films were discussed based on weight loss, visual appearance, morphological properties, and structural properties prior to and following burial in compost and planting soils. Based on the analysis, it can be concluded that the incorporation of NCF and Thy into the starch biopolymer matrix significantly affected the biodegradation properties of films. The inclusion of NCF into the starch biopolymer matrix reduced the weight loss of films attributed to the increase in hydrophobicity and TS as well as the crystallinity effect of NCF. The Thy inclusion into the films increased weight loss and degradation in comparison to neat CS and CS/NCF films because Thy promoted the hydrolysis process. Nevertheless, increasing the amount of Thy decreased the weight loss because Thy is hydrophobic. The morphological studies have proven that films containing Thy have the most visible voids and holes, indicating that the films underwent hydrolytic degradation. In addition, results on weight loss, appearance, and morphology were supported by the structural property findings from FTIR as observed from the most significant



variations in the intensity and shifting of absorption peaks of certain functional groups. Based on the results obtained from all analyses, the degradation of films buried in compost soil was higher in contrast to planting soil due to the compost soil being composed of high nutrients, humidity, quantity of active microbial, and a load of organic material. The development of CS/NCF/Thy films in this study opens up the prospect for the application of films to lower the utilization of non-degradable materials, thus solving various environmental problems.

**Funding information:** This research was supported by the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education Malaysia (Vot no.: 5540711, Project no. FRGS/1/2024/TK05/UPM/02/9).

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Conflict of interest:** The authors state no conflict of interest.

## References

- [1] Meijer L, Van Emmerik T, Van Der Ent R, Schmidt C, Lebreton L. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Sci Adv.* 2021;7(18):1–13.
- [2] Nandiyanto ABD, Fiandini M, Ragadhita R, Sukmafutri A, Salam HA, Triawan F. Mechanical and biodegradation properties of corn starch-based bioplastic material. *Mater Phys Mech.* 2020;77(3):380–91.
- [3] Syuhada M, Sofa SA, Sedyadi E. The effect of cassava peel starch addition to bioplastic biodegradation based on chitosan on soil and river water media. *Biol Med Nat Prod Chem.* 2020;9(1):7–13.
- [4] Nordin N, Romzi NAS, Manaf YNA, Tawakkal ISMA, Ariffin SH, Othman SH, et al. Characterization of active sweet potato-based films containing thymol at different varieties: VitAto and Anggun. *Food Packag Shelf Life.* 2021;30:100750.
- [5] Othman SH, Nordin N, Azman NAA, Tawakkal ISMA, Basha RK. Effects of nanocellulose fiber and thymol on mechanical, thermal, and barrier properties of corn starch films. *Int J Biol Macromol.* 2021;183:1352–61.
- [6] Othman SH, Wane BM, Nordin N, Hasnan NZN, Talib RA, Karyadi JNW. Physical, mechanical, and water vapor barrier properties of starch/cellulose nanofiber/thymol bionanocomposite films. *Polymers.* 2021;13(23):4060.
- [7] Othman SH, Ronzi NDA, Shapi'i RA, Dun M, Ariffin SH, Mohammed MAP. Biodegradability of starch nanocomposite films containing different concentrations of chitosan nanoparticles in compost and planting soils. *Coatings.* 2023;13(4):777.
- [8] Adelaja OA, Daramola OE. Preparation and characterization of low density polyethylene-chitosan nanoparticles biocomposite as a source of biodegradable plastics. *Eur J Adv Chem Res.* 2022;3(1):25–38.
- [9] Ong HL, Villagrancia AR, Owi WT, Sam ST, Akil HM. Revealing the water resistance, thermal and biodegradation properties of citrus aurantifolia crosslinked tapioca starch/nanocellulose bionanocomposites. *J Polym Environ.* 2020;28(12):3256–69.
- [10] Nazrin A, Sapuan SM, Zuhri MYM, Syafiq RMO, Sherwani SFK. Nanocellulose reinforced thermoplastic starch (TPS), polylactic acid (PLA), and polybutylene succinate (PBS) for food packaging applications. *Front Chem.* 2020;8:1–12.
- [11] Ramos M, Fortunati E, Peltzer MA, Dominici F, Jiménez A, Garrigós MC, et al. Influence of thymol and silver nanoparticles on the degradation of poly(lactic acid) based nanocomposites: Thermal and morphological properties. *Polym Degrad Stab.* 2014;108:158–65.
- [12] Ilyas RA, Sapuan SM, Ishak, Zainudin ES. Development and characterization of sugar palm nanocrystalline cellulose reinforced sugar palm starch bionanocomposites. *Carbohydr Polym.* 2018;202:186–202.
- [13] Ilyas RA, Sapuan SM, Ibrahim R, Abrial H, Ishak MR, Zainudin ES, et al. Thermal, biodegradability and water barrier properties of bionanocomposites based on plasticised sugar palm starch and nanofibrillated celluloses from sugar palm fibres. *J Biobased Mater Bio.* 2020;14(2):234–48.
- [14] Anunciado MB, Hayes DG, Astner AF, Wadsworth LC, Cowan-Banker CD, Gonzalez JELY, et al. Effect of environmental weathering on biodegradation of biodegradable plastic mulch films under ambient soil and composting conditions. *J Polym Environ.* 2021;29(9):2916–31.
- [15] Ahimbisibwe M, Banadda N, Seay J, Nabuuma B, Atwijukire E, Wembabazi E, et al. Influence of weather and purity of plasticizer on degradation of cassava starch bioplastics in natural environmental conditions. *J Agric Chem Environ.* 2019;08(04):237–50.
- [16] Hasan M, Gopakumar DA, Olaiya NG, Fitri Z, Alfian A, Aprinasari C, et al. Evaluation of the thermomechanical properties and biodegradation of brown rice starch-based chitosan biodegradable composite films. *Int J Biol Macromol.* 2020;156:896–905.
- [17] Fukushima K, Tabuani D, Arena M, Gennari M, Camino G. Effect of clay type and loading on thermal, mechanical properties and biodegradation of poly(lactic acid) nanocomposites. *React Funct Polym.* 2013;73(3):540–9.
- [18] Brady NC, Weil RR. The nature and properties of soils. 14th edn. New Jersey: Prentice Hall; 2008.
- [19] He X, Li M, Gong X, Niu B, Li W. Biodegradable and antimicrobial CSC films containing cinnamon essential oil for preservation applications. *Food Packag Shelf Life.* 2021;29:100697.
- [20] Restu WK, Sondari D, Setiasih, Ghazali M, TriwulandariEvi, Sampora Y, et al. Preparation and characterization of edible films from starch nanoparticles and chitosan. *Bioinspired Biomimetic Nanobiomater.* 2021;10(1):1–7.
- [21] Davoodi M, Kavosi G, Shakeri R. Preparation and characterization of potato starch-thymol dispersion and film as potential antioxidant and antibacterial materials. *Int J Biol Macromol.* 2017;104:173–9.
- [22] Acosta S, Chiralt A, Santamarina P, Roselló J, Chiralt A, Cháfer M. Antifungal films based on starch-gelatin blend, containing essential oils. *Food Hydrocoll.* 2016;61:233–40.
- [23] Oberlintner A, Bajić M, Kalčíková G, Likožar B, Novak U. Biodegradability study of active chitosan biopolymer films enriched

- with Quercus polyphenol extract in different soil types. *Environ Technol Innov.* 2021;21:101318.
- [24] Khairuddin N, Muhamad II, Rahman WAWA, Siddique BM. Physicochemical and thermal characterization of hydroxyethyl cellulose - wheat starch based films incorporated thymol intended for active packaging. *Sains Malays.* 2020;49(2):323–33.
- [25] Vigneshwaran N, Ammayappan L, Huang Q. Effect of gum arabic on distribution behavior of nanocellulose fillers in starch film. *Appl Nanosci.* 2011;1(3):137–42.
- [26] Souza AG, Santos NMAD, Da Silva Torin RF, Rosa DDS. Synergic antimicrobial properties of carvacrol essential oil and montmorillonite in biodegradable starch films. *Int J Biol Macromol.* 2020;164:1737–47.
- [27] Nordin N, Othman SH, Rashid SA, Basha RK. Effects of glycerol and thymol on physical, mechanical, and thermal properties of corn starch films. *Food Hydrocoll.* 2020;106:105884.
- [28] Zain AHM, Wahab MKA, Ismail H. Biodegradation behaviour of thermoplastic starch: The roles of carboxylic acids on Cassava starch. *J Polym Environ.* 2017;26(2):691–700.
- [29] Veliz ABL, Jiménez JC, López PP, De Gáscue BR. Biodegradability study by FTIR and DSC of polymers films based on polypropylene and cassava starch. *Orbital.* 2019;11(2):71–82.