

Cross-linked Chitosan/Corn Cob Biocomposite Films with Salicylaldehyde on Tensile, Thermal, and Biodegradable Properties: A Comparative Study

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ABSTRACT: The focus of this research paper was to study the properties of uncrosslinked and cross-linked chitosan (CS)/corn cob (CC) biocomposite films with salicylaldehyde (SAL). The uncrosslinked and cross-linked CS/CC biocomposite films with SAL were prepared through solvent casting method. The SAL is used as cross-linking agent in biocomposite films. The effect of CC content and SAL on the tensile, thermal, biodegradation properties, and morphological study of CS/CC biocomposite films are investigated. The tensile strength and elongation at break of CS/CC cob biocomposite films decreases, but modulus of elasticity increases with increasing CC content. However, the addition of SAL showed 59.47% and 25.18% of improvement in average tensile strength and modulus of elasticity of cross-linked CS/CC biocomposite films, respectively, as compared to uncrosslinked biocomposite films. The cross-linked biocomposite films exhibited higher thermal stability than uncrosslinked biocomposite films. Moreover, the cross-linked biocomposite films with SAL cross-linking agent showed lower weight loss in enzyme and soil biodegradation as compared to uncrosslinked biocomposite films. As a comparison, the cross-linked biocomposite films with SAL showed the highest properties compared to others cross-linking agent such as glutaraldehyde and epichlorohydrin as reported in our previous study. © 2016 Wiley Periodicals, Inc. *Adv Polym Technol* 2016, 0, 21784; View this article online at wileyonlinelibrary.com. DOI 10.1002/adv.21784

KEY WORDS: Biocomposite films, Biodegradable, Chitosan, Corn cob, Cross-linking

Introduction

Recently, most researchers are more interested in the utilization of renewable resources in their research. These renewable resources including low value plants, energy crops, and product from food crops, sawmills, palm oil production, marine waste, and food waste as a new materials to produce biocomposites.¹ Natural filler known as renewable raw materials and their availability is unlimited. These natural fillers promote several advantages, including low density, renewability,

biodegradability, recyclability, and cost effectiveness. Corn (*Zea mays*) is Poaceae (grass family) which is one of the top three cereal crops grown in the world.² In year 2013, annual world corn production is about 964 million tons, which generated about 204 million tons of corn residues.³ Corn cob (CC) is one of the corn residues that is abundant around the world. Corn cob is the center core of a corn, which is a part of the corn kernel grow.

Chitosan (CS) is a partially deacetylated polymer of *N*-acetyl glucosamine through alkaline treatment from chitin. Chitosan is a weakly basic polymer as the primary amine

group of D-glucosamine residues that has a pK_a value in the range 6.2–7. Moreover, CS is an unbranched cationic biopolymer, which has three reactive functional groups that allow further chemical modification such as amino groups at C-2 position and both primary and secondary hydroxyl groups at C-6 and C-3 position.⁴ Besides, CS has a higher potential in various applications compared to chitin because CS contain greater amount of free NH_2 groups.⁵ CS insoluble in water, but soluble in aqueous organic acids such as formic, acetic, citric, and lactic acid to produce viscous solution.⁶ The films made from CS are non-toxic, biocompatible, antimicrobial, and have a good mechanical properties.^{7–10} Because of these excellent properties, CS has become a potential source for packaging film. Nevertheless, the tensile, thermal, and biodegradability of CS biocomposite films are dependent strongly upon dispersion filler in matrix and the filler-matrix interaction.¹¹

Cross-linking is one form of chemical modification used to improve the properties of biocomposite films by enhancing the filler-matrix interaction and filler dispersion.^{8,11} Cross-links are involve the covalent bonds linking one polymer chain to another by a cross-linking agent.¹¹ Salicylaldehyde (SAL) is an organic compound with the formula of $C_7H_6O_2$. The reaction between amino groups of CS and aromatic aldehyde groups of SAL tend to form Schiff base.^{12,13} These Schiff base compounds containing an imine groups ($-RC=N$) by the condensation reaction between primary amine from CS and an active aldehyde from SAL.^{6,13,14} Furthermore, this biopolymer Schiff base is stable in air and insoluble in common organic solvent.^{6,12}

The most of composite materials in market are made from no degradable polymeric matrices and natural fillers or fibers.¹⁵ However, these non-renewable and petroleum resources polymeric materials do not degrade easily in the environment, causing many environment pollutions such as CO_2 emission and green house.¹⁶ Therefore, the biodegradable polymer is one of solution for waste-disposal problems.¹⁷ In general, the biodegradation method of polymers can be carried out in two different ways such as enzymatic hydrolysis and soil degradation. The enzymatic hydrolysis of biopolymers is a heterogeneous process. The heterogeneous process is effected by the interaction between enzymes and polymeric chains.¹⁸ Some researchers were using the enzymatic hydrolysis method to degrade the CS films.^{19,20} In soil degradation method, the soil microbes can be initiated the depolymerization of biopolymers such as polysaccharide, cellulose, and hemicellulose. These soil microbes secrete different kind of enzymes into the soil water and then begin breakdown the polymers. There are several studies on soil degradation of CS films.^{21,22}

In this study, the CS was used as matrix, while CC was used as filler to produce CS/CC biocomposite films. The objective of this study was to determine the influence of the CC content and cross-linking agent on the properties of CS/CC biocomposite films. Besides that, the properties comparison between cross-linked CS/CC biocomposite films with SAL and cross-linked CS/CC biocomposite films with glutaraldehyde (GLA) and epichlorohydrin (EP) (as reported in previous study) were investigated.

Experimental

MATERIALS

Chitosan with degree of deacetylation (DD) of 90% was supplied by Hunza Nutraceutical Sdn. Bhd., Malaysia. The molecular weight of CS used was 200 kD. Corn cob was obtained from Kodiang Plantation, Kedah, Malaysia. The CC was process into powder form with average particle size of 38 μm was measured by Malvern particle size analyzer. Acetic acid and SAL were provided by BASF, the Chemical Company, Germany, and Aldrich Chemistry, respectively.

UNCROSSLINKED AND CROSS-LINKED CS/CC BIOCOMPOSITE FILMS PREPARATION

The biocomposite films were prepared via solvent casting method. Chitosan powder was dissolved in 1 v/v% of acetic acid and stirred for 30 min using mechanical stirrer. Then, the CC powder was added into CS solution and stirred for 15 min. However, for cross-linked CS/CC biocomposite films with SAL, the SAL was dissolved in methanol (2:1, v/v), then added to the CS solution. The mixture was stirred for 1 hour. During stirring, a deep yellow gel was appeared. This indicated that the formation of Schiff base in the CS matrix. Similar observation was reported by Santos et al.⁶ and Senna et al.²³ The formulations of uncrosslinked and cross-linked CS/CC biocomposite films with SAL is listed in Table I.

ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED

Fourier Transform Infrared (FTIR) spectroscopy (Perkin-Elmer, Model L1280044, Waltham, Massachusetts, USA) was used to investigate the different functional groups between uncrosslinked and cross-linked CS/CC biocomposite films. The spectra of specimens were recorded with 16 scans in the wavenumber (cm^{-1}) range 4000–600 cm^{-1} . A resolution of 4 cm^{-1} was recorded for each specimens.

TABLE I Formulations of Uncrosslinked and Cross-linked CS/CC Biocomposite Films with SAL

Biocomposite Film	Materials		
	Chitosan (CS), wt%	Corn Cob (CC), wt%	Salicylaldehyde (SAL), %
Uncrosslinked CS/CC biocomposite films	100 (Neat CS film)	0	
	90	10	
	80	20	–
	70	30	
	60	40	
Cross-linked CS/CC biocomposite films	90	10	
	80	20	
	70	30	1
	60	40	

TENSILE PROPERTIES

The tensile properties (tensile strength, elongation at break, and modulus of elasticity) of biocomposite films with dimension of 100 × 15 mm. The average of 10 biocomposite films recorded, were conducted using an Instron Universal Testing Machine, Model 5569, according to ASTM D 882. The testing was performed at room temperature and a cross-head speed of 15 mm/min was used.

In addition, Nicolais–Narkis equation was related to tensile strength of composites that can be identified whether any interaction occurred between CS matrix and CC filler. Nicolais–Narkis equation assumes that the filler are distributed uniformly in continuous phase of matrix and there is existed poor interaction between matrix and filler. Thus, the filler-matrix interaction can be determined by this equation. This method was suggested by.^{24,25} The Nicolais–Narkis equation was stated as:

$$\sigma_c = \sigma_o(1 - 1.21\emptyset^{2/3}) \quad (1)$$

where σ_c = yield tensile strength of CS/CC biocomposite film; σ_o = yield tensile strength of neat CS film; \emptyset = volume fraction of CC.

Furthermore, the theoretical effect of filler volume on the relative elongation at yield of biocomposite films was calculated according Nielsen equation as shown in Eq. (2). The elongation of biocomposite films depend on the interfacial interaction between the components of the composite.²⁶ According to Nielsen theory, in the case of perfect adhesion, under the assumption that the polymer breaks at the same elongation in the filled system as in the neat polymer. However, in the case of poor adhesion, the elongation is expected to decrease more gradually than in the case of perfect adhesion.²⁷ The Nielsen equation can be described as:

$$\varepsilon_c = \varepsilon_o(1 - \emptyset^{1/3}) \quad (2)$$

where ε_c = elongation at yield of CS/CC biocomposite film; ε_o = elongation at yield of neat CS film

THERMOGRAVIMETRIC ANALYSIS

The thermal analysis of both uncrosslinked and cross-linked CS/CC biocomposite films were measured using thermogravimetric analysis (TGA) Pyris Diamond (Perkin-Elmer). The testing was performed under a nitrogen atmosphere with a flow rate of 50 mL/min. The specimens were scanned from 30 to 600°C at a heating rate of 10°C/min.

ENZYMATIC BIODEGRADATION

The enzymatic degradation test of CS/CC biocomposite films were carried out using α -amylases as enzyme. The enzyme buffer solution was prepared by adding 4.8 mL of 0.2 M acetic acid to 45.2 mL of 0.2 M sodium acetate solution and 10 mg of α -amylases was added to produce a 50 mL mixture solution. Then, the specimens were soaked into buffer solution. The specimens were taken out every 2 days, then

rinsed with distilled water to remove excess of α -amylases on the surface of specimens. Next, the specimens were dried in an oven at 50°C for 24 h. Similar method was reported by Husseinsyah et al.²⁸ The weight loss of enzymatic degradation was estimated using Eq. (3).

$$W_{ED} = \left[\frac{w_i - w}{w_i} \times 100\% \right] \quad (3)$$

where W_{ED} = percentage of weight loss on enzymatic degradation; w_i = initial weight of a specimen; w = weight of a specimen after enzymatic degradation.

SOIL BIODEGRADATION

The soil degradation test was conducted according to ASTM D 5988. The specimens with size of 20 × 30 mm were dried in an oven at 50°C for 24 h and weighted before buried in soil. The test was performed at temperature of 25 ± 3°C and humidity of 40–45% to ensure aerobic conditions of degradation. The specimens were taken out from soil at different times and washed thoroughly with distilled water to remove the remaining soil before conditioning in an oven at 50°C for 24 h. The weight loss of soil degradation was calculated using Eq. (4).

$$W_{SD} = \left[\frac{w_j - w_o}{w_j} \times 100\% \right] \quad (4)$$

where W_{SD} = percentage of weight loss on soil degradation; w_j = initial weight of a specimen; w_o = weight of a specimen after soil degradation.

GEL FRACTION

The specimens were solvent extracted with 1% of acetic acid for 24 h. Then, the extracted specimens were dried in an oven at 50°C for 24 h Ramaprasad et al.²⁹ The percentage of gel fraction according to Eq. (5).

$$\text{Gel Fraction (\%)} = \frac{W_d}{W_{BS}} \times 100\% \quad (5)$$

where W_d = weight of dry gel; W_{BS} = weight of specimens before swelling.

MORPHOLOGICAL STUDY

The fracture surface morphologies of specimens were analyzed using a scanning electron microscope (SEM) (Japan), Model JEOL JSM-6460 LA, at an accelerating voltage of 5 kV. All specimen films were coated with a thin layer of palladium for conductive purpose before testing.

STATISTICAL ANALYSIS

Statistical analysis was performed with Microsoft Excel 2013 statically analysis. The single factor for analysis of variance (ANOVA) was used to determine the significance in the difference between means as well as to detect significant

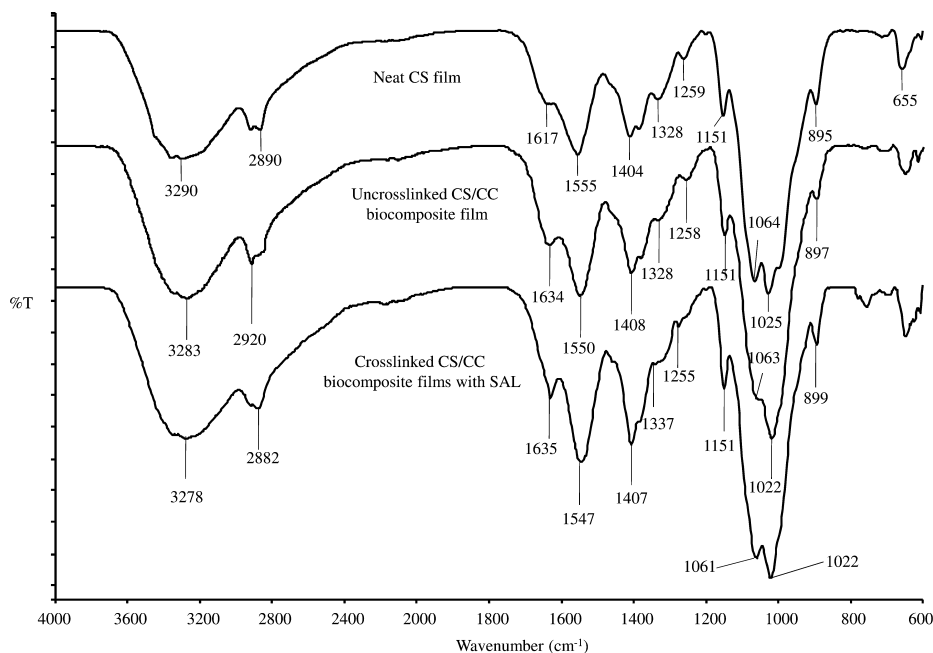


FIGURE 1. FTIR spectra of neat chitosan (CS) film, uncrosslinked and cross-linked CS/corn cob (CC) biocomposite films.

differences in film properties. The significance level used was $P \leq 0.05$.

Results and Discussion

FTIR ANALYSIS

Figure 1 shows the FTIR transmission spectra of uncrosslinked and cross-linked CS/CC biocomposite films with SAL. The major functional groups and their specific wavenumbers of uncrosslinked and cross-linked CS/CC biocomposite film with SAL is listed in Table II. The peaks at 3283 and 2920 cm^{-1} are related to stretching OH and C-H groups, respectively. The peaks at 1634 and 1550 cm^{-1} are assigned to amide I and amide II, respectively. Moreover, the peak at 1408 cm^{-1} is correlated with $-\text{CH}_2$ deformation from cellulose or C-H deformation from lignin. The peak at 1328 cm^{-1} is attributed to C-N amino group. The C-H group deformation is attributed by the peak at 1258 cm^{-1} . The peaks in range of 1000–1150 cm^{-1} are corresponded to C-O-C and C-O groups. The CS showed a saccharide structure because the presence of peaks at 1151, 1063, 1022, and 897 cm^{-1} . Again, there is no change in main backbone of saccharide structure of neat CS in both uncrosslinked and cross-linked CS/CC biocomposite films.

From Figure 1, the peak intensity at 1634 cm^{-1} increased about 1.3% from 1634 to 1635 cm^{-1} , which was due to the presence of C=N vibration characteristic of imines. The presence of C=C stretching in the aromatic ring of the aldehyde cause the peak intensity at 1547 cm^{-1} increased. Furthermore, the peak intensity at 1151 cm^{-1} increased around 1.6% due to overlapping of C-O-C groups from CS with C-N groups from CS-SAL interaction. The intensity band at 899 cm^{-1} was also increased because the presence of C-H stretching in the

TABLE II
The Major Spectra of Uncrosslinked and Cross-linked Chitosan (CS)/Corn Cob (CC) Biocomposite Films with Salicylaldehyde (SAL)

Material	Wavenumber (cm^{-1})	Functional Group	Intensity (%)
Uncrosslinked CS/CC biocomposite film	3283	Hydroxyl (-OH), amine (N-H)	
	2920	CH group	
	1634	C=O stretching	
	1550	N-H bending	
	1408	C=C stretching	
	1328	C-N amino group	
	1258	CH group	
	1151	C-O-C bridge	
	1063	C-O stretching	
	1022	C-O stretching	
Cross-linked CS/CC biocomposite film with SAL	897	CH bending	
	1635	C=N (imine linkages)	Increased (1.3)
	1547	C=C (Ethylenic bonds)	Increased (2.7)
	1151	C-O-C stretching	Increased (1.6)
	899	C-H stretching (Aromatic ring of SAL)	Increased (0.3)

aromatic ring of aldehyde from SAL. Similar observation was found by Guinesi and Cavalheiro,³⁰ who studied the preparation of Schiff base from CS and SAL.

TENSILE PROPERTIES

Figure 2(a) displays the tensile strength of uncrosslinked and cross-linked CS/CC biocomposite films with SAL.

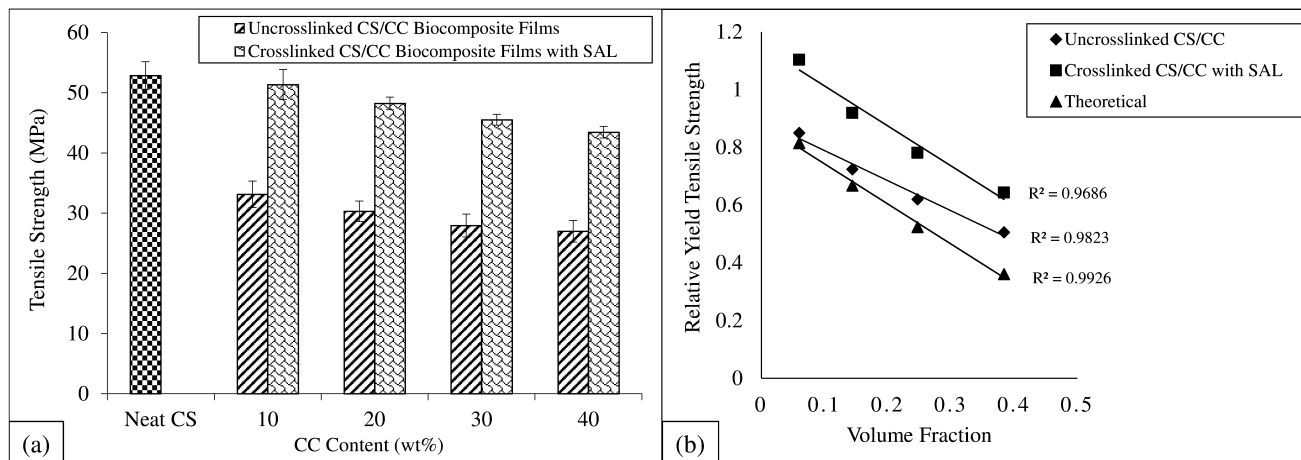


FIGURE 2. (a) Tensile strength of uncrosslinked and cross-linked chitosan (CS)/corn cob (CC) biocomposite films with salicylaldehyde (SAL); and (b) relative tensile strength of theoretical and experimental results on uncrosslinked and cross-linked CS/CC biocomposite films with SAL.

Table III lists the comparison of tensile properties between uncrosslinked and cross-linked CS/CC biocomposite films with different cross-linkers. As CC content increased from 10 to 40 wt%, the tensile strength of uncrosslinked CS/CC biocomposite films decreased. The decrement trend of tensile strength is due to poor interfacial interaction between CS and CC. The poor interactions between CC filler and CS matrix generated stress concentration points and agglomeration, which led to low tensile strength and elongation at break. The tensile strength of cross-linked CS/CC biocomposite films with SAL is significantly higher ($P \leq 0.05$) compared to uncrosslinked CS/CC biocomposite films. The cross-linked CS/CC biocomposite films showed 59.47% of improvement in average tensile strength from 10 to 40 wt% of CC content as compared to uncrosslinked biocomposite films. This finding is the evidence of the formation of imine linkages between amino groups of CS and aromatic aldehyde groups of SAL through Schiff base reaction. These covalent bonds resisted the chain plasticity of CS matrix. The formation of imine bonds in biocomposite films improved the interfacial interaction between CC filler and CS matrix due to the cross-linked biocomposite film shrinks more than uncrosslinked biocomposite film. Thus, there is a compressive forces as polymer matrix clamps down around the CC filler particles, providing a good contact between filler and matrix and thus significant adhesion. Hence, the addition of SAL as a crosslinking agent in biocomposite films was significantly improved the tensile strength.

In addition, the tensile strength of cross-linked CS/CC biocomposite films with SAL at 20 and 40 wt% of CC content showed 12.86% and 9.22%, respectively, higher in comparison with tensile strength results that obtained from cross-linked CS/CC with GLA,¹¹ as reported in previous study. However, cross-linked CS/CC biocomposite films with SAL exhibited 2.69% and 15.44% higher than cross-linked CS/CC biocomposite films with EP at 20 and 40 wt% of CC content, respectively¹⁰ (as reported in previous study). This result implies that the cross-linked CS/CC with SAL showed more significant effect as compared to cross-linked CS/CC with GLA.

TABLE III
Comparison of Tensile Properties Between Neat Chitosan (CS), Uncrosslinked and Cross-linked CS/Corn Cob (CC) Biocomposite Films at 20 and 40 wt% of CC Content with Different Cross-linking Agent

Biocomposite Films	Tensile Strength (MPa)	Modulus of Elasticity (MPa)	Elongation at Break (%)
Neat CS	52.8 ± 2.3	2269 ± 186	13.8 ± 0.4
Uncrosslinked CS/CC (80:20)	33.1 ± 1.7	2571 ± 121	8.7 ± 0.7
Uncrosslinked CS/CC (60:40)	27.0 ± 1.8	2754 ± 142	5.2 ± 0.2
Cross-linked CS/CC (80:20) with SAL	48.2 ± 2.5	3177 ± 55	5.2 ± 0.7
Cross-linked CS/CC (60:40) with SAL	43.4 ± 1.0	3256 ± 175	3.6 ± 0.4
Cross-linked CS/CC (80:20) with GLA ^a	42.0 ± 1.6	3178 ± 174	7.7 ± 0.2
Cross-linked CS/CC (60:40) with GLA ^a	39.4 ± 1.3	3288 ± 200	3.2 ± 0.2
Cross-linked CS/CC (80:20) with EP ^b	46.9 ± 0.2	2703 ± 177	8.2 ± 0.4
Cross-linked CS/CC (60:40) with EP ^b	36.7 ± 1.0	3006 ± 203	3.1 ± 0.3

The numerical value in the parenthesis is standard deviation.

^aResults were obtained from Ref. [11].

^bResults were obtained from Ref. [10].

The Nicolais–Narkis equation (Eq. 1) is one of the method to measure the filler-matrix interaction of composites.^{24,31} The Nicolais–Narkis equation plots of predicted and experimental relative tensile strength results of uncrosslinked and cross-linked CS/CC biocomposite films with SAL is displayed in Fig. 2(b). Obviously, the relative tensile strength of CS/CC biocomposite films decreased linearly as the CC content increased. From Fig. 2(b), it can be observed that the Nicolais–Narkis slope of uncrosslinked CS/CC greater as compared to the slope of predicted. The Nicolais–Narkis plots suggest there was some degree of adhesion, if not good adhesion between matrix and filler.³² Furthermore, the cross-linked CS/CC

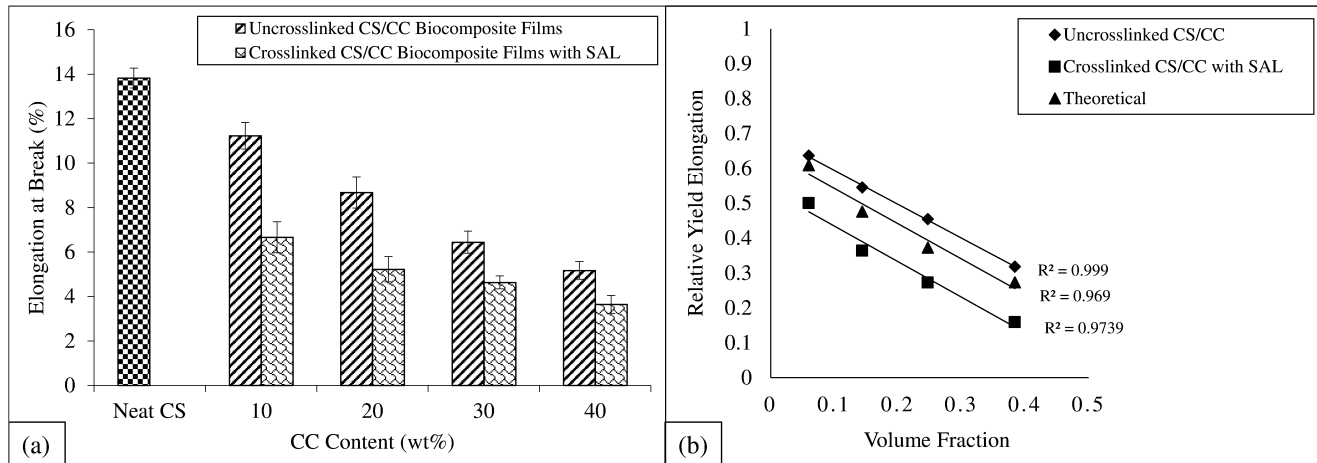


FIGURE 3. (a) Elongation at break of uncrosslinked and cross-linked chitosan (CS)/corn cob (CC) biocomposite films with salicylaldehyde (SAL); and (b) relative elongation of theoretical and experimental results on uncrosslinked and cross-linked CS/CC biocomposite films with SAL.

biocomposite films with SAL appeared more adhere between CS and CC, as evidenced by a greater slope than the slope of uncrosslinked biocomposite films and theoretical. In other words, the SAL cross-linking agent improved the adhesion between CS and CC, and this is consistent with the tensile strength results of cross-linked CS/CC biocomposite films with SAL as illustrated in Fig. 2(a).

Figure 3(a) presents the elongation at break of uncrosslinked and cross-linked CS/CC biocomposite films with SAL with different amount of CC filler. The elongation at break of both uncrosslinked and cross-linked CS/CC biocomposite films significantly decreases ($P \leq 0.05$) as increasing of CC content. As the CC content increased, the chain flexibility of biocomposite film was highly restricted by CC filler, and the elongation at break is drastically decreased. Besides, the effective cross-sectional area of the CS continuous phase was reduced, resulting in decreased elongation at break. As expected, the CC filler in CS matrix acted as stress concentrators, which inducing crack and results in low elongation at break. However, the elongation at break of cross-linked CS/CC biocomposite films with SAL is decreased as compared to uncrosslinked biocomposite films. The average elongation at break of cross-linked CS/CC with SAL reduced around 48.96% compared uncrosslinked CS/CC biocomposite films. In this case, the lower elongation at break of cross-linked CS/CC biocomposite films due to the presence of imine bonds between amino groups of CS and aromatic groups of SAL hindered the chain plasticity of CS, which increase the adhesion and rigidity of CS/CC biocomposite films. Moreover, based on Table III, as can be seen that the elongation at break of cross-linked CS/CC biocomposite films with SAL is lower about 32.47% and 57.69% as compared to cross-linked CS/CC biocomposite films with GLA¹¹ and cross-linked CS/CC biocomposite films with EP¹⁰ at 20 wt% of CC content, respectively. The SAL cross-linking agent increased the tensile strength of biocomposite films with enhancement in filler-matrix adhesion as discuss earlier, which consequently reduced the elongation at break of biocomposite films.

Figure 3(b) depicts the Nielsen plots of theoretical, uncrosslinked and cross-linked CS/CC biocomposite films with SAL. The Nielsen equation (Eq. 2) is related to the elongation at break or yield of composites, which also can be determine the filler-matrix interaction in composites.^{24,26} The slope of uncrosslinked CS/CC biocomposite films greater than the slope of theoretical due to there are some degree of adhesion between CS and CC in uncrosslinked CS/CC biocomposite films, as mentioned earlier. Apparently, the slope of cross-linked CS/CC biocomposite films with SAL is the lowest compared to the slope of theoretical and uncrosslinked biocomposite films. This finding was due to the formation of imine linkages in cross-linked CS/CC biocomposite films, which improved the interaction between CS matrix and CC filler. These results were consistent with elongation at break result as shown in Fig. 3(a).

The modulus of elasticity of uncrosslinked and cross-linked CS/CC biocomposite films with SAL is described in Fig. 4. It was found that the addition of CC has increased the modulus of elasticity, resulting increased the stiffness of biocomposite films. The modulus values of both uncrosslinked and cross-linked biocomposite films change significantly ($P \leq 0.05$) with CC content increases. Aside from that, the CC filler with higher stiffness than that CS matrix can increase the modulus of elasticity of CS/CC biocomposite films, as the modulus of elasticity was a measure of the materials stiffness. Thus, the inclusion of rigid CC filler into CS matrix was reduced the chain mobility of CS matrix, resulting in increases stiffness and reduced ductility of CS/CC biocompsite films. Furthermore, the modulus of elasticity of cross-linked CS/CC biocomposites films higher than uncrosslinked biocomposite films. The cross-linked CS/CC with SAL exhibited higher enhancement on modulus of elasticity with around 25.18% higher than uncrosslinked biocomposite films. This is due to the fact of the formation of imine linkages through Schiff base reaction and consequently increases the stiffness of biocomposite films. However, as comparison the modulus of elasticity of cross-linked CS/CC biocomposite films with SAL exhibited about 0.97% lower

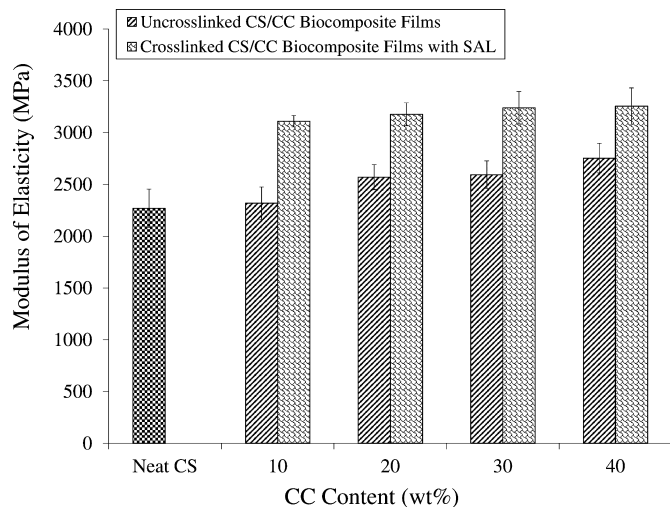


FIGURE 4. Modulus of elasticity of uncrosslinked and cross-linked chitosan (CS)/corn cob (CC) biocomposite films with salicylaldehyde (SAL).

than cross-linked CS/CC biocomposite films with GLA¹¹ but 5.38% higher than cross-linked CS/CC biocomposite films with EP¹⁰ at 20 wt% of CC content, as listed in Table III. The increases in modulus of elasticity on biocomposite films were most likely caused by the presence of cross-linking agent³³ and the higher stiffness of natural filler.³⁴ Figure 5 illustrates the proposed cross-linking reaction of CS and different cross-linking agent (SAL, GLA, and EP), respectively. The reaction between CS and SAL formed imine linkages. The cross-linking reaction between CS and GLA were also formed imine bonds. However, the reaction between CS and EP formed amide linkages.

THERMOGRAVIMETRY ANALYSIS OF BIOCOMPOSITE FILMS

Figures 6 (a) and 6(b) illustrate the thermogravimetry analysis (TGA) and derivative thermogravimetry analysis (DTG) curves of uncrosslinked and cross-linked CS/CC biocomposite films with SAL, respectively. Table IV presents the

comparison of TGA and DTG data between neat CS, uncross-linked and cross-linked CS/CC biocomposite films with different cross-linking agents. It can be seen that the neat CS film showed two weight loss stages: (i) first stage starts at 50°C with a weight loss of around 16%, which related to the evaporation of absorbed moisture; and (ii) second weight loss of 33% from 200 to 340°C, which correlated with the decomposition of CS, vaporization, and elimination of volatile products. However, the TGA and DTG curve of uncrosslinked and cross-linked CS/CC biocomposite films with SAL show three degradation stages: (i) first stage degradation occurred below 100°C with weight loss due to the loss of absorbed water; (ii) second stage degradation of modified CC/CC biocomposite films with weight loss at temperature of about 300°C related to the thermal decomposition of cellulose, hemicellulose, and lignin in CC; and (iii) decomposition product around 400°C and above was characterized as carbonaceous residue. Moreover, from DTG curve, the temperature in range 310–350°C showed a shoulder peak for each biocomposite films due to the decomposition of hemicellulose.

From Table IV, it can be found that the T_{dmax} of uncross-linked and cross-linked CS/CC biocomposite films decreases with amount of CC increases. The T_{dmax} shift to lower temperature as increasing CC content, indicated that the incorporation of CC reduced the thermal stability of CS/CC biocomposite films. However, the presence of SAL cross-linking agent enhanced the thermal stability of CS/CC biocomposite films, as evidenced by the T_{dmax} shift to higher temperature compared to uncrosslinked CS/CC biocomposite films. It was found that the neat CS film was more stable with higher T_{dmax} than cross-linked CS/CC biocomposite films with SAL due to the presence of free amino groups in the cross-linked biocomposite films. Since, the free amino groups of CS were provided a stabilizing effect on biocomposite film.¹² Besides that, the char residue at temperature of 200, 500, and 600°C of uncrosslinked and cross-linked CS/CC biocomposite films with SAL decreased with increasing CC content. However, the cross-linked biocomposite films with SAL showed higher char residue at temperature of 200, 500, and 600°C compared to uncrosslinked biocomposite films at 20 wt % of CC content, as exhibited in Table IV. These results reveal the imine bonds from SAL-CS promoted char formation in

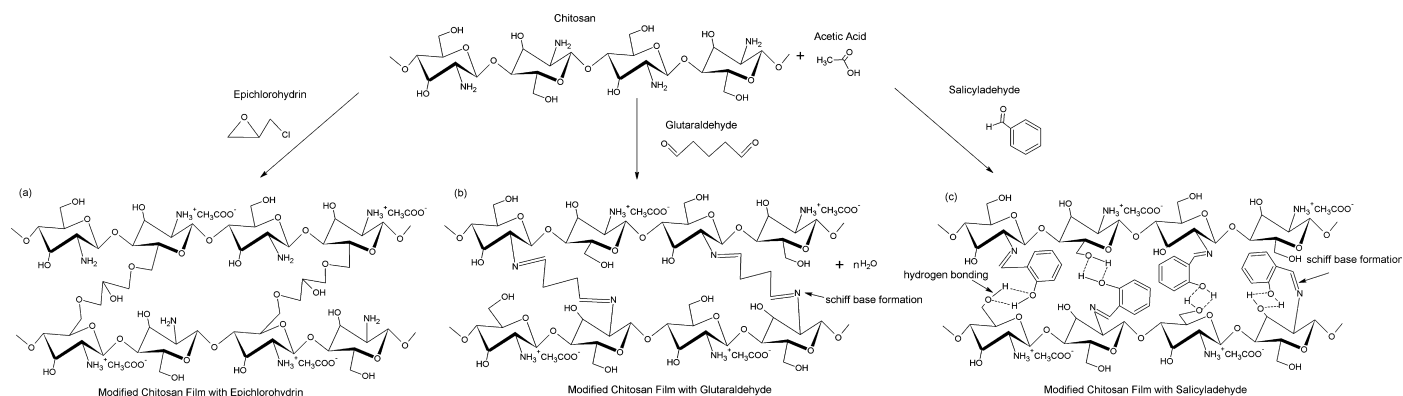


FIGURE 5. Proposed schematic cross-link reaction between (a) chitosan (CS) and epichlorohydrin (EP); (b) CS and glutaraldehyde (GLA); (c) CS and salicylaldehyde (SAL).

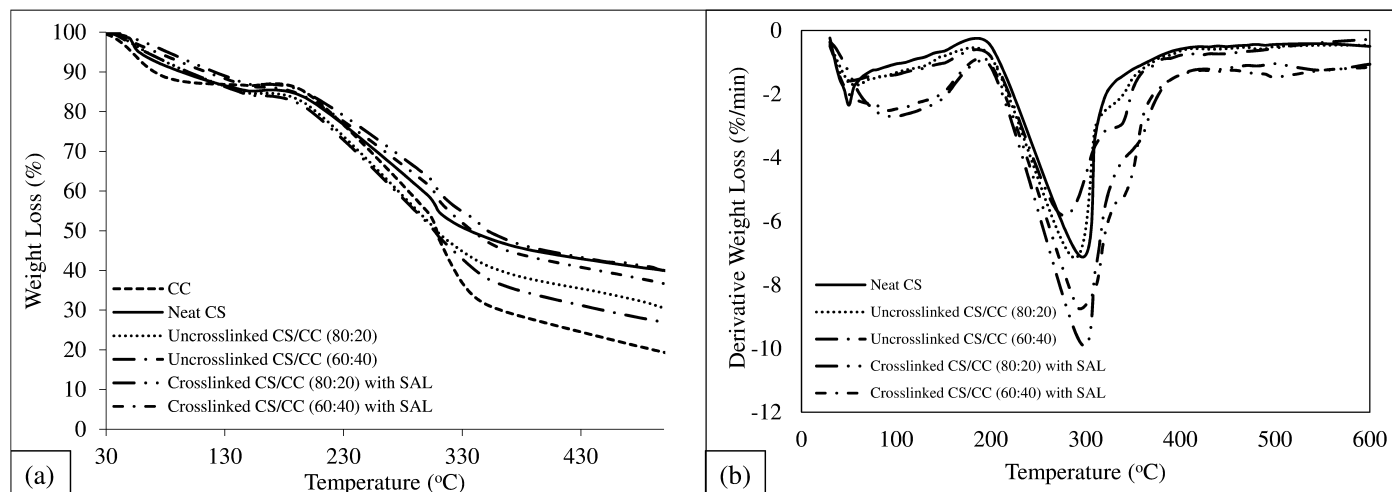


FIGURE 6. Thermal decomposition properties of uncrosslinked and cross-linked chitosan (CS)/corn cob (CC) biocomposite films with salicylaldehyde (SAL) (a) thermogravimetric analysis (TGA); and (b) derivative thermogravimetry analysis (DTG).

pyrolysis of biocomposite films. The char layer act as protective layer and reduce the diffusion of gaseous that producing during thermal decomposition to polymer. Thus, the presence of imine linkages in biocomposite films delayed the degradation temperature and improved the thermal stability of biocomposite films. As a comparison, the cross-linked CS/CC biocomposite films with SAL showed more char residue layer compared to cross-linked CS/CC with GLA.¹¹ However, cross-linked CS/CC biocomposite films with EP¹⁰ showed similar char residue at 600°C with cross-linked CS/CC biocomposite films with SAL, as presented in Table IV. Hence, the modified CS/CC biocomposite films with SAL showed better thermal stability than cross-linked CS/CC biocomposite films with GLA but similar thermal stability with cross-linked CS/CC biocomposite films with EP.

GEL FRACTION

The presence of cross-linkages in CS/CC biocomposite films can be estimated from gel fraction determination.³⁵ Table V represents the percent gel fraction of uncrosslinked and cross-linked CS/CC biocomposite films with different cross-linkers. The gel fraction of uncrosslinked and cross-linked CS/CC biocomposite films increased as increasing CC content. In contrast, the cross-linked CS/CC biocomposite films presented higher gel fraction than uncrosslinked CS/CC biocomposite films. The cross-linked CS/CC biocomposite films with SAL exhibited the highest gel fraction compared to cross-linked biocomposite films with GLA and EP, respectively. Meanwhile, the SAL cross-linker showed higher cross-linking efficiency than GLA and EP. The result was expected because the SAL is well-known reactive cross-linking agent to form cross-linkages. These cross-linkages could be also attributed to reduce the chain mobility due to formation of three-dimensional network structure. The formation of these three-dimensional network structure was restricted the chain mobility because of the bulky structure of SAL, that became sterically hindered.

TABLE IV
Thermogravimetric analysis (TGA) and Derivative Thermogravimetry Analysis (DTG) Data of Neat Chitosan (CS), Uncrosslinked and Cross-linked CS/Corn Cob (CC) Biocomposite Films with Different Cross-linking Agent

Biocomposite Films	T_{dmax} (°C)	Char Residue (%)		
		$T_{200^{\circ}C}$	$T_{500^{\circ}C}$	$T_{600^{\circ}C}$
Neat CS	293	83	39	36
Uncrosslinked CS/CC (80:20)	285	81	30	29
Uncrosslinked CS/CC (60:40)	272	80	26	23
Cross-linked CS/CC (80:20) with SAL	292	85	40	38
Cross-linked CS/CC (60:40) with SAL	290	83	36	35
Cross-linked CS/CC (80:20) with GLA ^a	287	84	38	35
Cross-linked CS/CC (60:40) with GLA ^a	274	81	36	31
Cross-linked CS/CC (80:20) with EP ^b	290	86	40	38
Cross-linked CS/CC (60:40) with EP ^b	283	84	38	35

^aResults were obtained from Ref. [11].

^bResults were obtained from Ref. [10].

ENZYMATIC BIODEGRADATION

Table VI represents the weight loss of uncrosslinked and cross-linked CS/CC biocomposite films with different cross-linking agents on enzymatic biodegradation. The biodegradability of each biocomposite films increases with time incubated in α -amylases solution. Besides, the biodegradation of uncrosslinked and cross-linked CS/CC biocomposite films increased as increasing of CC content. Hence, the CC natural filler was enhanced the biodegradation of CS/CC

TABLE V
Gel Fraction of Uncrosslinked and Cross-linked Chitosan (CS)/Corn Cob (CC) Biocomposite Films with Different Cross-linking Agent

Biocomposite Films	Gel Fraction (%)
Neat CS	44.39
Uncrosslinked CS/CC (80:20)	50.34
Uncrosslinked CS/CC (60:40)	57.51
Cross-linked CS/CC (80:20) with SAL	79.30
Cross-linked CS/CC (60:40) with SAL	83.21
Cross-linked CS/CC (80:20) with GLA	72.36
Cross-linked CS/CC (60:40) with GLA	77.31
Cross-linked CS/CC (80:20) with EP	76.00
Cross-linked CS/CC (60:40) with EP	79.12

biocomposite films. However, the weight loss of neat CS lower than uncrosslinked CS/CC biocomposite films due to the neat CS with high degree of deacetylation (DD) (90%) was resistance degradation process.^{11,36} Based on Table VI, it can be seen that the biodegradation of uncrosslinked CS/CC biocomposite films was greater than those of cross-linked CS/CC biocomposite films with SAL. The uncrosslinked CS/CC biocomposite films show 44.17% and 31.14% higher weight loss of degradation than cross-linked biocomposite films with SAL at 20 and 40 wt% of CC content, respectively. This indicated that the cross-linked CS/CC biocomposite films with SAL formed the imine cross-linking structure have a greater stereo-hindrance for penetration of α -amylases. The structure of stereo-hindrance prevent the amylases from binding the N-acetylglucosamine residues on CS. Similar finding was reported by Mi et al.³⁷ They studied the enzymatic degradability of aglycone geniposidic acid (aGSA) and glutaraldehyde cross-linked CS film, respectively. They noted that the bulky cross-linking structure of aGSA cross-linked CS film restricted the penetration of enzyme in film than glutaraldehyde cross-linked CS film, resulting aGSA cross-linked CS film showed lower enzymatic degradability than glutaraldehyde cross-linked CS film. Besides that, it can be seen that from Table VI, the cross-linked CS/CC biocomposite films with SAL showed more resistance to hydrolyze in buffer solution as compared to others two cross-linking agents.

SOIL BIODEGRADATION

The data on percent weight loss of uncrosslinked and cross-linked CS/CC biocomposite films with different cross-linking agents, as a function of biodegradation time in soil are shown in Table VII. Within 14 weeks, the weight loss of biodegradation in soil of uncrosslinked and cross-linked CS/CC biocomposite films increased with CC increases. As discuss earlier in enzymatic degradation, the natural filler was help in biodegradation of biocomposite films. Nevertheless, the cross-linked CS/CC biocomposite films with SAL had a slower degradation rate than uncrosslinked biocomposite films. The uncrosslinked CS/CC biocomposite films exhibit 29.9% and 27.13% higher weight loss as compared to cross-linked biocomposite films at 20 and 40 wt% of CC content, respectively. However, weight loss of neat CS on biodegradation in soil is lower than uncrosslinked CS/CC biocomposite

TABLE VI
Weight Loss of Uncrosslinked and Cross-linked Chitosan (CS)/Corn Cob (CC) Biocomposite Films with Different Cross-linking Agent After 14 Days on Enzymatic Biodegradation

Biocomposite Films	Weight Loss of CS/CC Biocomposite Films After 14 Days (%) on Enzymatic Biodegradation
Neat CS	12.32 \pm 0.47
Uncrosslinked CS/CC (80:20)	14.76 \pm 0.51
Uncrosslinked CS/CC (60:40)	16.83 \pm 0.39
Cross-linked CS/CC (80:20) with SAL	8.24 \pm 0.62
Cross-linked CS/CC (60:40) with SAL	10.50 \pm 0.35
Cross-linked CS/CC (80:20) with GLA	11.12 \pm 0.91
Cross-linked CS/CC (60:40) with GLA	13.32 \pm 0.44
Cross-linked CS/CC (80:20) with EP ^a	9.83 \pm 0.23
Cross-linked CS/CC (60:40) with EP ^a	11.72 \pm 0.43

^aResults were obtained from Ref. [10].

TABLE VII
Weight Loss of Uncrosslinked and Cross-linked Chitosan (CS)/Corn Cob (CC) Biocomposite Films with Different Cross-linking Agent After 14 Weeks on Soil Biodegradation

Biocomposite Films	Percentage Weight Loss of CS/CC Biocomposite Films After 14 Weeks on Soil Biodegradation (%)
Neat CS	71.68 \pm 5.62
Uncrosslinked CS/CC (80:20)	81.40 \pm 3.83
Uncrosslinked CS/CC (60:40)	87.34 \pm 4.35
Cross-linked CS/CC (80:20) with SAL	57.05 \pm 3.21
Cross-linked CS/CC (60:40) with SAL	63.64 \pm 4.10
Cross-linked CS/CC (80:20) with GLA	67.51 \pm 2.91
Cross-linked CS/CC (60:40) with GLA	70.50 \pm 5.21
Cross-linked CS/CC (80:20) with EP ^a	61.71 \pm 4.60
Cross-linked CS/CC (60:40) with EP ^a	64.81 \pm 3.73

^aResults were obtained from Ref. [10].

films but higher than cross-linked CS/CC biocomposite films with SAL. Consequently, the imine cross-linking reduced the degradation of CS/CC biocomposite films. The presence of Schiff base cross-linkages in CS/CC biocomposite were restricted the cellulases and α -amylases from microorganisms to attack CS/CC biocomposite films, resulting in SAL cross-linking agent had reduced the biodegradation of CS/CC

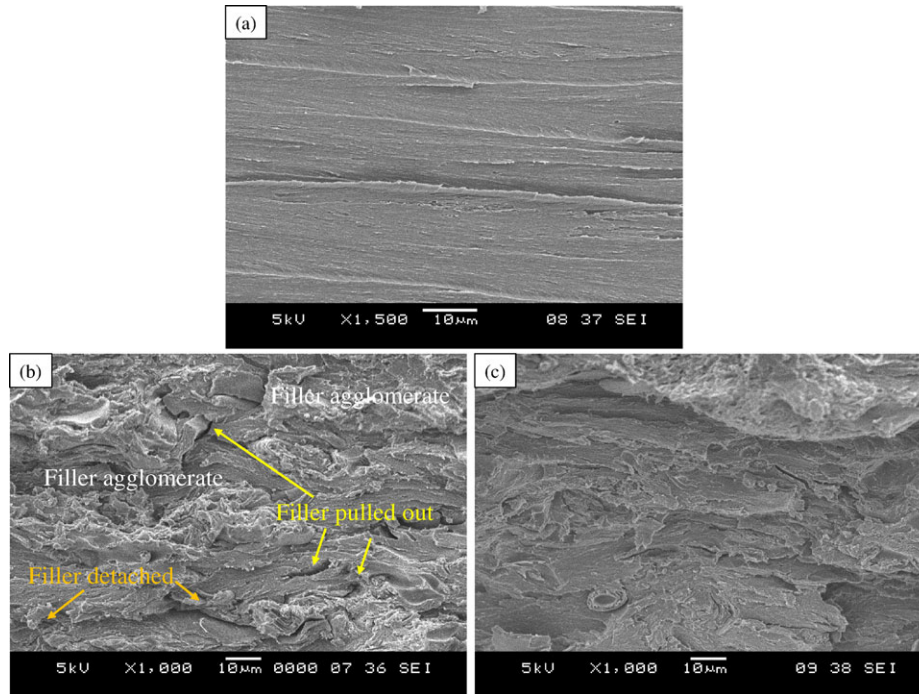


FIGURE 7. SEM micrograph of (a) neat chitosan (CS) film; (b) uncrosslinked CS/corn cob (CC) biocomposite films; and (c) cross-linked CS/CC biocomposite films with salicylaldehyde (SAL) at 40 wt% of CC content.

biocomposite film in soil. As a comparison (Table VII), the cross-linked CS/CC biocomposite films with SAL also exhibited the lowest weight loss on soil biodegradation compared to cross-linked CS/CC with GLA and cross-linked CS/CC biocomposite films with EP.

MORPHOLOGICAL STUDY

The SEM micrograph of neat CS film is illustrated in Fig. 7(a). The tensile fracture surface of neat CS film exhibited smooth and homogeneous tearing surface. Besides, the SEM micrograph of uncrosslinked CS/CC biocomposite film at 40 wt% of CC content is demonstrated in Fig. 7(b). From Fig. 7(b), it can be seen that there are some filler pulled out and detached from CS matrix. Moreover, the formation of agglomeration at higher CC content, as presented in Fig. 7(b). On the other hand, the cross-linked CS/CC biocomposite films with SAL at 40 wt% of CC content is shown in Fig. 7(c). After chemical modification with SAL cross-linking agent, the fracture surface showed less CC pulled out and detached from CS matrix. From morphological study, it could be confirmed that the chemical modification of biocomposite film with the presence of imine bonds had improved the filler-matrix interfacial interaction of CS/CC biocomposite films. Moreover, the improvement of interfacial adhesion on modified CS/CC film with cross-linking agent was also proven by Nicolais–Narkis and Nielsen equations. Moreover, the SEM micrograph of cross-linked CS/CC biocomposite film with SAL (Fig. 7c) showed the brittle fracture surface due to the formation cross-linkages in biocomposite films.

Conclusion

As summary, the utilization of SAL as cross-linker in CS/CC biocomposite films, exhibited higher tensile and thermal properties compared to biocomposite films without cross-linker due to the formation of imine linkages. The improvement of filler-matrix interfacial interaction is proven by morphological study. Moreover, the gel fraction of CS/CC biocomposite films increases as CC content increases. However, after chemical modification with SAL cross-linking agent, showed the highest gel fraction in comparison with uncrosslinked biocomposite films. The linkages in biocomposite films were resisted the penetration of enzymes from buffer solution and soil in biocomposite films. Thus, the cross-linked CS/CC biocomposite films were resistance to hydrolyze in α -amylases buffer solution and biodegrade in soil condition. Among three cross-linking agent such as glutaraldehyde (GLA), epichlorohydrin (EP) and SAL, the cross-linked CS/CC biocomposite films with SAL showed the better tensile and thermal properties compared to others cross-linking agent. However, cross-linked CS/CC biocomposite films with SAL is the most resistance to hydrolyze in enzyme and soil degradation as compared to GLA and EP.

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