



Pineapple Leaves Fiber (PALF) Crosslinked with Chitosan for Antimicrobial Properties

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The goal of this study is to treat pineapple leaves fiber (PALF) with chitosan for antimicrobial properties. The treated PALF with chitosan was then crosslinked with crosslinking agent; butanetetracarboxylic acid (BTCA) to preserve chitosan in the PALF structure. The effectiveness of chitosan and crosslinking agent towards PALF was determined using untreated PALF, PALF treated chitosan, PALF treated chitosan (wash), PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (wash). After the treatment with chitosan and BTCA, the treated PALF undergone the washing process to determine the effectiveness of BTCA to avoid the removal of chitosan from PALF during washing. The presence of chitosan in the PALF was confirmed using Fourier Transform Infra-red (FTIR) analysis. The treated PALF was then characterised in terms of its antimicrobial activity and mechanical properties. Antimicrobial activity test confirmed the presence of chitosan successfully retarded the growth of microbe. Tensile test analysis revealed the untreated PALF has the highest tensile strength compared to all treated PALF. In contrast, young modulus of the treated PALF shows increment relative to the untreated PALF. This study is particularly important for the further application of PALF or other natural fibers especially in medical and textile industry.

1. Introduction

Agriculture is among the important sectors that contributed to Malaysia's economic growth. It is reported that more than five million hectares of land in Malaysia is utilised with crops such as palm oil, paddy fields, rubber and others. Out of that 5 million ha, more than 11,000 ha are planted with pineapples (Rosma et al., 2005). In Malaysia, the cultivation of pineapple is mainly focusing on food production and usually commercialised as canned fruits (Upadhyay, 2004). The harvesting of pineapples fruit resulted in massive waste produced, especially the pineapples leaves. Like any other agriculture waste, the residues are usually being burnt, which cause environmental problems.

Recently, natural fibers are getting significant interest due to their unique properties and ability to function like synthetic fiber. Examples of natural fibers which commonly being used are banana leaves (Albinante et al., 2014), jute, hemp, kenaf, cotton and flax. Pineapple leaf fiber (PALF) is a multicellular lignocellulose which consists of component such as cellulose (75 %), hemicellulose (16 %) and lignin (9 %) (Nadirul et al., 2013). The presence of these actively biological compounds somehow resulted in short life span due to fast degradation (Windler et al., 2013). Therefore, to increase the life span of this natural fiber, treatment with antimicrobial agent is essential. To date, various substances have been reported to possess antimicrobial property, such as triclosan (Vasil et al., 2013), phenols, metal and salts, organometallics, quaternary ammonium compound, chitosan and many more (Joshi et al., 2008). In recent years, chitosan has attracted much attention as an antimicrobial agent due to its effectiveness, cheap and abundance (Naknean et al., 2015). Chitosan is obtained by alkaline deacetylation of the chitin which derived from the exoskeletons of crustaceans and arthropods and has been useful in many areas of application such as wastewater treatment

(Razmi et al., 2017), food and textile industries (Abramiuc et al., 2013), drug industries and recently being used as a hydrating agent in cosmetics (Lee et al., 2013). In the textile industries, issue with the chitosan removal upon washing the fabric have been highlighted and reported. Thus, the use of crosslinker to prevent the removal of chitosan can be an option to solve the problem. El-bendary and Hudson (2005) reported the treatment of cotton fabrics with BTCA in the presence of chitosan increase the antimicrobial activity of the fabrics more significant compared to other crosslinking agent.

Currently the synthetic organic compounds dominate the antimicrobials market on a weight basis, thus the discovery of the natural antimicrobials for textile and other applications is in dire need (Windler et al., 2013). In this research, the effectiveness of incorporating antimicrobial agent (chitosan) to pineapple leaf fiber (PALF) was determined using *E. coli* solution. Subsequently the ability of crosslinking agent, BTCA in preserving the antimicrobial properties after washing treatment was also investigated. Both treated samples with and without crosslinker undergone washing process to determine the efficiency of BTCA. Fourier Transform Infra-red (FTIR) analysis was used to determine the presence of chitosan and BTCA on PALF structure. Subsequently all samples were characterised in terms of mechanical and antimicrobial properties. This study is particularly important to enhance the life span of natural fiber which usually susceptible to degradation due to the presence of active compound in the structure.

2. Experimental

2.1 Materials

Pineapple leaves fiber, chitosan (low molecular weight, 50,000 M/V, Aldrich), hydrochloric acid (HCl; 37 %, Aldrich), butanetetracarboxylic acid (BTCA – 99 %, Aldrich), and *Escherichia Coli* (*E. Coli*) broth were used. Distilled water was used throughout the experiment.

2.2 Preparation of PALF treated chitosan

2 g of chitosan was added into 100 g of distilled water under stirring condition. While stirring, the pH of the solution was adjusted to pH 6 by titrating 0.5 M of HCl solution. The solution was left for 4 h under stirring condition at room temperature. After that, 1.7 g of pineapple leaves fiber was immersed in the solution for 30 min followed by drying process in the oven at 80 °C for 1 h 20 min. Subsequently the treated PALF was left in the oven for 24 h at 40 °C to ensure complete drying. At this stage, the prepared fiber is known as PALF treated chitosan.

Half of the PALF treated chitosan undergone washing process with distilled water followed by the drying process at 80 °C to evaluate the durability of the treatment. At this stage, the fiber is known as PALF treated chitosan (W).

2.3 Preparation of PALF treated chitosan/ butanetetracarboxylic acid

2 g of chitosan was added into 100 g of distilled water under stirring condition. While stirring, the pH of the solution was adjusted to pH 6 by titrating 0.5 M of HCl solution. The solution was left at room temperature for 4 h under stirring condition. After that 7.5 g of BTCA was added slowly into the solution and stir for 2 h. Subsequently, 1.7 g of pineapple leaves fiber was immersed in the solution for 30 min followed by drying at 80 °C for 5 min and later was cured under at 160 °C for 15 min. At this stage, the prepared fiber is known as PALF treated chitosan/BTCA.

Half of the PALF treated chitosan/BTCA undergone washing process with distilled water followed by drying process at 80 °C to evaluate the durability of the treatment. At this stage, the fiber is known as PALF treated chitosan/BTCA (W). Table 1 shows all the samples prepared in this study.

Table 1: List of samples prepared in this study

No	Samples	Criteria
A	PALF	No modification
B	PALF treated chitosan	Contained chitosan
C	PALF treated chitosan (W)	No chitosan
D	PALF treated chitosan/BTCA	Contained chitosan and BTCA
E	PALF treated chitosan/BTCA (W)	Contained chitosan and BTCA

2.4 Testing and characterisation

2.4.1 Fourier Transform Infra-Red (FTIR)

The samples were characterised using FTIR to confirm the presence of chitosan and carboxylic acid group in the fiber. For this purpose, FTIR Shimadzu Irtace-100 was used to analyse the characteristics of the samples. FTIR spectra were recorded in the transmittance mode after 40 scans.

2.4.2 Tensile strength

The tensile property was measured using Emic Universal Tensile Testing Machine (LRX 2.5KN LLYOD Tensile Tester), at a 5 mm min^{-1} of crosshead speed and in accordance to ASTM D3822 standards. In this study, three samples were taken from PALF, PALF treated chitosan, PALF treated chitosan (W), PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (W). Average values from these three samples were taken as the resultant value.

2.4.3 Antimicrobial activity test

The antimicrobial activity performance of PALF, PALF treated chitosan, PALF treated chitosan (W), PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (W) were examined based on the growth of microbes on the surface of all samples. In this method, samples with 5 cm length were placed in the inoculated bottles containing microbes and incubated at the appropriate temperature for 24 h. The samples were mixed with 0.5 mL of pure microbe solution. The appearance of the microbe growth on the samples were measured from time to time.

3. Result and Discussion

3.1 Modification of PALF using Chitosan and butanetetracarboxylic acid

Reaction of PALF with chitosan was successfully conducted under condition explained in experimental part. At pH 6, chitosan is fully dissolved in water to enable the reaction between chitosan and PALF. The charges present in chitosan chains are generated by protonation of amino groups when in acid medium. Fourier transform infra-red (FTIR) analysis was used to determine the presence of chitosan on the PALF structure. Figure 1 shows the FTIR spectrum of untreated PALF. The broad peak at $3,200$ to $3,600 \text{ cm}^{-1}$ correspond to the stretching vibration of OH in the PALF structure. The peak at $1,700 \text{ cm}^{-1}$ attributed to C-O stretching bond of carbonyl groups in hemicellulose. The peaks centred at around $1,254$ to $1,436 \text{ cm}^{-1}$ indicated the existence of lignin and hemicellulose structure. Strong peak observed at $1,060 \text{ cm}^{-1}$ is assigned to the binding of C-O-H and C-O-C bindings in chitosan. The assignment of peaks observed are consistent with the FTIR analysis reported by Lopattanon et al. (2006).

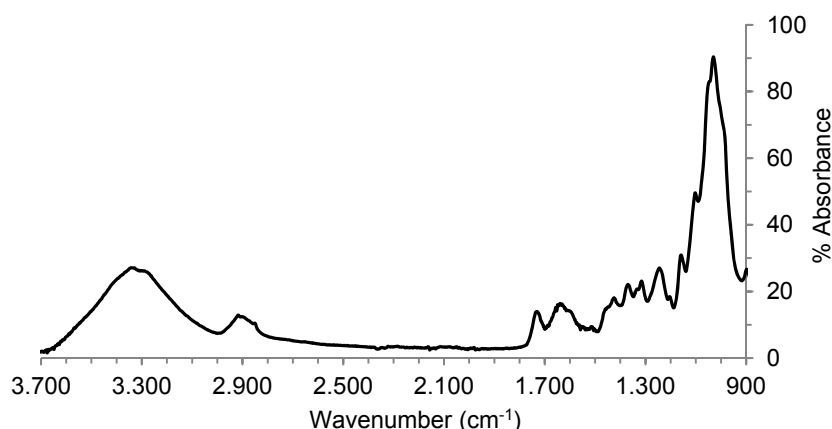


Figure 1: FTIR spectrum of untreated PALF

The treated PALF samples on the other hand (Figure 2) shows spectra with slight peak differences in comparison to the untreated PALF spectrum. The peak for chitosan is around $3,200 - 3,600 \text{ cm}^{-1}$ which overlapped with the peak of OH stretching bond from PALF. The differences in peak intensity suggested the presence of other compound; in this study it is referred to the presence of chitosan. The addition of BTCA in

sample D and E have decreased the OH peak due to the esterification reaction between the carboxylic acid group from BTCA and OH group from both PALF and chitosan to form ester (Sennur and Ebru, 2015). The increase of carbonyl peak (ester) at $1,700\text{ cm}^{-1}$ confirms the occurrence of esterification reaction between BTCA and PALF/chitosan. Strong peak $1,060\text{ cm}^{-1}$ which assigned to the binding of C-O-H and C-O-C bindings in chitosan also decreased accordingly.

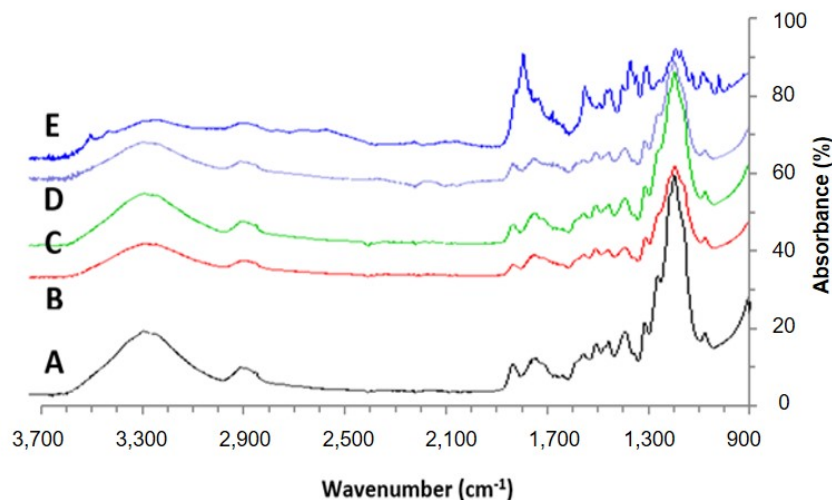


Figure 2: FTIR spectra of PALF (Sample A), PALF treated chitosan (Sample B), PALF treated chitosan (W) (Sample C), PALF treated chitosan/BTCA (Sample D) and PALF treated chitosan/BTCA (W) (Sample E)

3.2 Tensile analysis

The effect of chitosan and BTCA addition on the strength property of PALF was investigated. Figure 3 shows the tensile strength and Young Modulus for the prepared five samples. From Figure 3a, tensile strength of PALF decreased with the addition of chitosan and BTCA on the fiber. Tensile strength is the stress needed to break a sample which is related to the material's flexibility. PALF is known to have higher tensile strength compared to rigid chitosan. The addition of chitosan is expected to lower the tensile strength of the material. The Young Modulus of PALF treated chitosan as shown in Figure 3b, increased in comparison to the untreated PALF.

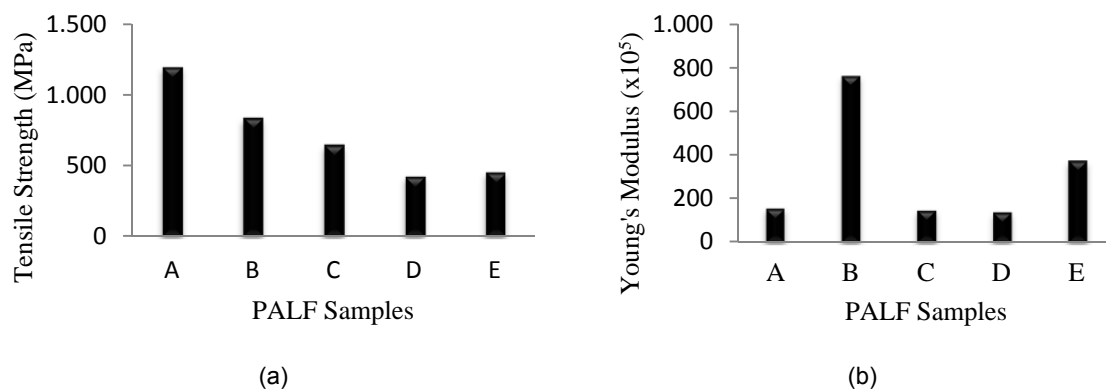


Figure 3: (a) Tensile strength and (b) Young Modulus of A) PALF, B) PALF treated chitosan, C) PALF treated chitosan (W), D) PALF treated chitosan/BTCA and E) PALF treated chitosan/BTCA (W)

Young Modulus is the parameter that characterises the rigidity of a material. The higher the Young Modulus, the higher the resistance to deformation and rigidity which resulted in lower flexibility. The addition of rigid chitosan to PALF improved the Young Modulus of the material (Jeong et al., 2002). Consistent result was reported by Tanjung et al. (2014) whereby they investigated the mechanical properties of chitosan filled polypropylene composites. However, the PALF treated chitosan which undergone washing shown decrement

in both tensile strength and Young Modulus. This observation might due to the possibility of PALF degradation upon washing with water.

3.3 Antimicrobial activity of PALF modified Chitosan/BTCA

The antimicrobial activity of PALF, PALF treated chitosan, PALF treated chitosan (W), PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (W) were conducted in a pure *E. coli* solution for 20 d. After 20 d, samples with no chitosan (PALF and PALF treated chitosan (W)) were found to comprise of microbe while sample with chitosan (PALF treated chitosan, PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (W)) show no traces of microbe as shown in Figure 4.

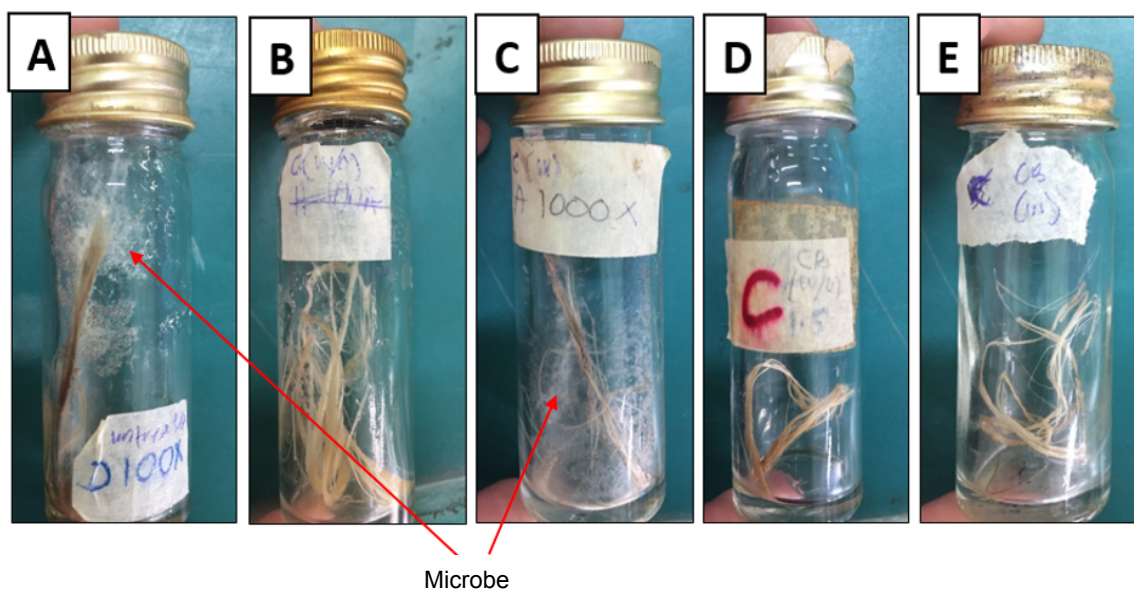


Figure 4: Photos of antimicrobial activity of (a) PALF, (b) PALF treated chitosan, (c) PALF treated chitosan (W), (d) PALF treated chitosan/BTCA and (e) PALF treated chitosan/BTCA (W)

This observation is consistent with the role of chitosan as natural antimicrobial agent which reported previously. Furthermore, the presence of BTCA also help to preserve chitosan on PALF even after washing process as evidenced by PALF treated chitosan/BTCA (W) sample.

4. Conclusions

PALF has been successfully treated with chitosan for the incorporation of antimicrobial property. The addition of BTCA as crosslinker also proven to inhibit the removal of chitosan in the PALF during washing process. The presence of chitosan and BTCA on the PALF structure was confirmed using FTIR analysis. Tensile test was conducted to determine the effect of chitosan on the strength of the treated PALF. From this study it was observed that the addition of chitosan decreases the tensile strength and increases the Young Modulus of PALF due to the nature of chitosan itself. The effectiveness of chitosan as an antimicrobial agent was demonstrated via antimicrobial activity in *E. coli* solution for 20 d. Samples containing chitosan (PALF treated chitosan, PALF treated chitosan/BTCA and PALF treated chitosan/BTCA (W)) show no traces of microbe in comparison to samples without chitosan. As for PALF treated chitosan/BTCA (W), the presence of no microbe in the sample has shown the effectiveness of the crosslinker to retain chitosan in the PALF structure during washing process. This study is particularly important for the further application of PALF or other natural fibers especially in medical and textile industry.

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