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Optimization Study of Decolorization Process of Oyster Mushroom's Chitin Thin Film

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Abstract

This study focuses on decolorizing the thin film using sodium hypochlorite (NaClO) to produce highly transparent chitin nanocomposite from chitin nanofiber. Preparation of the thin film from chitin extract of oyster mushroom (*Pleurotus ostreatus*) demonstrated in this study. Optimization of decolorization of chitin thin film in sodium hypochlorite (NaCIO) was done by one-factor-at-time (OFAT) and statistical approach using Response Surface Methodology (RSM) in Design expert Software. This is to determine the optimal process conditions of decolorization based on three parameters which are concentration of NaCIO, duration and temperature of the process. The optimization was conducted on the basis of response mechanical properties (tensile strength) of chitin thin film. The OFAT method indicated that the optimum condition for decolorization of thin film was at concentration of 6%, duration of 4 hours and temperature of 30ºC with the tensile stress value of 52.57 MPa.

Keywords: chitin nanofiber, pleurotus ostreatus,decolorization, optimization, Response Surface Methodology, tensile stresss

1. INTRODUCTION

Chitin is a tough, semi-transparent substance which acts as the building material that gives strength to the exoskeletons of crustaceans, insects and the cell walls of fungi. It can be found abundantly in nature apart from cellulose. Due to their huge sources in nature, research field on chitin based materials has been widened and expanded. Chitin nanofiber has properties of biodegradable, biocompatible, relatively inert and high mechanical properties [1]. Due to its nano- sized structure, higher surface area, outstanding mechanical properties such as high Young's modulus and fracture strength, the chitin nanofiber have strong potential in developing the nanofiber and chitin technology [2]. Chitin is a linear polymer consisting of *β*-(1-4) *N*-acetyl-D-glucosamine structure.Chitin nanofiber network in the cell walls of mushrooms is embedded in the matrices mainly of β-1, 3 glucans. Chitin can be disintegrated into nanofibers by several mechanical treatments such as grinder [1], high speed blender [4] and high pressure homogenizer [5].The width of the nanofibers in the mushroom are ranging from 20-28 nm depends on its type [2]. Since this biopolymer can be found abundantly in nature and has biocompatible properties, it contributes in major application of biotechnology including biomedicine, pharmaceutical and agro-biosciences as well as food industry.

2. MATERIALS AND METHODS

A. Materials

Oyster mushrooms (*Pleurotus ostreatus*) were used as the raw material in this study. The oyster mushrooms were purchased at NSK Selayang in Selangor .The collection of the mushroom was made on 4th February 2018.

B. Chitin extraction from Pleurotus ostreatus

The oyster mushrooms were blended using high speed blender (Vita Mixer Innofood, 19000 rpm, power consumption 1500W) for 5 minutes after rinsed with distilled water. Chitin extraction process began with the hot water extraction. The blended mushroom slurry was heated up to 85°C for 1 hour during hot water extraction. Water-soluble compounds were removed in this process. Deproteinization step was carried out for protein removal by adding 1 M NaOH to the mushroom slurry and been heated up to 65°C for 3 hours. The suspension then was filtered and neutralized using distilled water until pH becomes neutral. The stock chitin extract placed in a container and stored in 4 °C.

C. Preparation of Chitin Thin Film

Preparation of chitin thin film started with filtration of chitin solution for 1.5 hours using filter paper (Sartorius 1288) by setting up vacuum pump (GAST, DOA-P604-BN). After filtration, the chitin wet cake was placed in between cotton cloth to reduce moisture content. Two metal plates are to be placed on the top and the bottom of cotton cloth to make sure the chitin

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cake does not move. The chitin wet cake then left completely dried under the 5 kg weight at 120ºC for 3 hours in the oven. After the drying process, 80 gsm of chitin thin film with the diameter of 110 mm and 0.76g are prepared.

D. Decolorization of chitin thin film

The chitin thin films with 60 mm length and 10 mm width were bleached in NaClO solution under three different process parameters which are concentration, duration and temperature of the process. In first OFAT, the concentration is varied 4%, 6%. 8%, 10% but fixed the duration at 4 hours and temperature of 28ºC. For the second OFAT, the duration is varied for 2 h, 4 h, 6 h and 8 h but fixed concentration at 6% and temperature 28 ºC. In third OFAT, the temperature is varied at 25ºC, 30ºC, 35ºC, 40ºC but the concentration and the duration is fixed at 6% and 4 hours respectively.

E. Tensile test of chitin thin film

The specimens were cut into rectangular pieces with the dimension of 60 mm long and 10 mm wide. Both ends of each specimen were tabbed with mounting board (1 mm thick) using epoxy adhesive glue (Araldite® Standard) to avoid sample from damage at grip point and slippage during the test. The samples were kept inside the silica chamber to ensure the constant humidity at room temperature (28% RH, 28ºC). Universal tester (SHIMADZU, AGS-X, 5 kN load cell) with sample grip pressure of 4 bar was used for the tensile test according to ASTM D882-12. The specimen gage length and test speed is set to 30 mm and 1 mm/min, respectively. Five replicates of each thin film were tested for each run.

3. RESULTS AND DISCUSSION

A. Chitin Extraction from Oyster Mushroom

The dry weight of the mushroom was determined to be 7% and this value was used throughout this project since the mushroom are originated from the same species and purchased at the same place. After blending, light brown mushroom slurry was obtained and then was transferred into beaker for hot water extraction. The mushroom slurry containing fiber became more viscous as a result of water removal. The extract slurry changed from pale brown to dark brown after addition of NaOH during the deproteinization process. As the protein was removed in this process, the slurry became less viscous. During neutralization process the dark brown colour started to become light brown colour again as the pH becomes neutral (6-7) after the addition of distilled water. Chitin content from 1 kg of waste oyster mushroom was found to be in the range of 3-5% (w/w).

B. Preparation of Chitin Thin Film

The process started by filtering 0.7% chitin solution for 1.5 hours. Fabrication of thin film completed by drying in the oven at 120°C for 3 hours under 5 kg weight. The thin film left dried to remove the water completely. The weight press is important to ensure the final chitin thin film produce no wrinkles. 1 kg of mushroom produced about 20 pieces of 80 g/m^2 thin film approximately with a mass of 0.76 g and diameter of 110 mm.

C. Decolorization of chitin thin film

Chitin thin film shows translucent properties upon decolorize in sodium hypochlorite solution (refer Fig.1). The transparency of this natural polymer also does not lose since fibers which have a diameter less than one-tenth of the visible light are not affected by light scattering [3].

Figure 1: Translucent chitin thin film: treated with 6% NaClO for 4 h at 28°C

D. Mechanical properties of thin film

Table 1-3 shows the response of tensile properties for the three OFAT. The first OFAT was to determine the influence of different NaClO concentration at room temperature (28 °C) for 4 hours. From Table 2, decolorization of thin film in 6% NaClO gave the highest tensile strength (51.33 MPa). As the NaClO concentration increased, the strength of chitin decreased. This is possibly due to chitin chain degradation. However, no significant difference for tensile modulus among the concentrations was observed except only for 10% concentration which had very low tensile modulus. The second OFAT show the influence of process duration at fixed NaClO concentration (6%) and temperature (28 °C). From Table 3**,** it was observed that 4 hours of duration yielded highest tensile stress (51.71 MPa). Longer duration of decolorization caused the chitin thin films lose its mechanical strength. The significant difference was in tensile modulus comparison. The influence of temperature during decolorization using 6% NaClO for 4 hours for the final OFAT was summarized in Table 4**.** Increase of temperature during decolorization resulting in an increase of both stress and strain until maximum at 30 °C reached (54.66 MPa and 8.32%). The mechanical strength started to decline at temperature of 35 °C. At this temperature, the thin film as in a fragile condition and easily teared up. Based on the result of these three OFAT, the optimum condition for decolorization process in sodium hypochlorite solution

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Table 1: Mechanical properties of chitin thin film in different concentration of NaCIO for 4 h at 28° C

Conc. (%)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m³)
4	27.09± 0.36	1.38±0.02	21.20±2.28	16.20±0.53
6	51.33± 2.51	6.4 ±0.19	22.50±3.42	66.99±2.42
8	41.57±2.46	4.55±0.004	15.34±3.66	42.21±1.66
10	21.24±0.88	6.81 ±0.45	5.70±0.31	52.09±1.07

Table 2: Mechanical properties of chitin thin film at different duration in 6% concentration of NaCIO at 28°

Duration (h)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m³)
2	30.96± 4.10	4.19±0.79	16.58±1.61	61.48±1.44
4	51.71± 2.22	2.83±0.46	29.84±2.89	80.63±3.65
6	23.17±0.48	4.91±1.79	7.89±2.30	51.90±0.45
8	34.61±11.60	4.58 ±0.38	15.22±5.78	122.07±1.43

Table 3: Mechanical properties of chitin thin film at different temperature of 6% NaClO concentration at 4 hour

Temp. (°C)	Tensile Stress (MPa)	Tensile strain[%]	Tensile Modulus[MPa]	Tensile Toughness (MJ/m³)
25	45.61± 6.63	3.88±0.13	23.07±2.62	117.09±6.93
30	54.66± 0.72	4.73 ±0.89	25.81±2.05	125.15±3.44
35	24.57±0.70	3.64±1.02	11.14±3.42	64.61±1.76
40	20.81±10.44	1.69±0.42	14.21±5.63	18.94±1

(NaCIO) was at 6% concentration, 4 hours duration of process and 30 °C temperature of process. These results become the basis for the optimization process later on.

4. CONCLUSION

Chitin nanofibers were extracted from the oyster mushroom, *Pleurotus ostreatus* and thin films were prepared from the extracted chitin. This study aimed to determine the effect of decolorization of the chitin thin film in sodium hypochlorite (NaClO) towards its mechanical properties. The chosen parameters during decolorization were the concentration of NaClO, duration and temperature of the process with the response of mechanical properties which is tensile strength. The optimum decolorization condition by OFAT method was found to be 6% NaClO concentration, 4 hours at 30 °C with the value of tensile stress 52.57 MPa. The range of chitin content in 1 kg of mushroom was 3.0-5.0 % (w/w). Fabricated chitin thin film possesses translucent properties upon been decolorized due to nano effect of fiber. Chitin that has been undergoing decolorization process has high mechanical properties and it can be applied in making strong and transparent nanocomposite.

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