BIODEGRADABLE MUSHROOM-BASED TRANSPARENT PAPER

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ABSTRACT: There is growing interest towards biopolymer-based nanofibres due to its superior mechanical properties and environmentally friendly functions. Chitin nanofibres may be of interest as a component for nanocomposites. Decolourisation effectively enhance the desirability of chitin nanofibres as it can be used without changing the natural colour of base materials.Decolourised chitin nanofibres from waste Pleurotus ostreatus (oyster mushroom) is biocompatible, biodegradable and sustainable. It combat issues existing with the current commercial source of chitin, crustacean shells, which are more difficult to culture, limited to seasonal and geographical aspects and have inconsistent quality due to water pollution. In the present study, chitin nanofibre was extracted using hot water extraction and through a deproteinisation process using NaOH. Chitin thin film was prepared using a filtration method and decolourised using H2O2 at different concentrations. The mechanical properties of decolourised films were tested using a tensile machine. The results showed that the most optimum condition for chitin thin film was 60% H2O2 for 18 h at 40 •C with a tensile strength, modulus and toughness of 26 MPa, 10 GPa and 108 MJm-3 respectively. It was found that fungal-based chitin have a higher toughness than commercial crustacean-based chitin. Chitin nanofibres derived from mushrooms have a high potential in developing nanomaterials and biocomposites.

KEY WORDS: Decolourisation, chitin nanofibre, chitin thin film, tensile machine

I. INTRODUCTION

Henri Broconnot, a French chemist and pharmacist was the first to discover chitin while doing research on mushrooms in 1811. He concluded that the nitrogen rich polysaccharide is very different to other substances found in plants as it did not dissolve in sulfuric acid. He called it fungine. The word "chitin" is retrieved from the Greek etymology meaning "a coat of mail" and the name was coined in 1823 by Odier when he found the same chitin structure in insects. In 1843, Payen fuelled the discussion on differences between chitin and cellulose, a subject which continues to be discussed until today. By 1878, Ledderhose identified that chitin is composed of acetic acid and glucosamine [1]. Chitin (β-1,4-N-acetyl-D-glucosamine) is an important natural polysaccharide known to be the most abundant renewable, non-toxic biopolymer after cellulose. Chitin exists as ordered crystalline microfibrils or nanofibres, is insoluble in acidic aqueous media and can be formed into beads, membranes and hydrogels [2]. Nanofibres are fibres which have the diameter range between 1-100 nm and are used to strengthen materials [3]. Research on nanofibre is getting more attention due to its much larger surface to volume ratio and superior mechanical properties resulting from extensive hydrogen bonds. There has been growing interest to produce nanofibres from biopolymers such as chitin nanofibres because of its biodegradability, renewability and sustainability [3]. Chitin is found in a wide variety of species as an important structural polysaccharide. It is the major structural component in the exoskeletons of anthropoids and cell walls of yeast and fungi. It is also found in the shells of crabs, lobsters and shrimps, in insect cuticles and squid pens and in krill. Chitin has an important role in the morphogenesis of these species as it provides scaffold for surrounding protein, a biological process which develops an organism's shape [4]. Chitin is greatly used to immobilise enzymes and whole cells, meaning that it has applications in the food industry and as biosensors. It is also used in wound dressing [2]. Crustacean shells are the commercial source of chitin due to its high chitin content and the possibility of extracting pure chitin. However, crustacean shells are difficult to obtain due to seasonal and geographical aspects. It also has an inconsistent quality due to water pollution and is a major allergen. Also, due to the high calcium carbonate content in crustacean shells, the source needs to go through a demineralisation process. On the other hand, isolation of chitin from mushrooms do not require demineralisation due to the negligible amount of minerals. Extraction of fungal chitin require fewer steps, less costly, shorter time and uses less harsh solvents and conditions. Moreover, fungi is more easily cultured than prawn or crabs. The main aim of this study is to extract chitin from waste Pleurotus ostreatus (oyster mushroom) using hot water extraction and deproteinisation process with NaOH, forming chitin thin films using a filtration method and mechanically testing the effect of H2O2 as a decolourising agent using a tensile machine. Finding the optimum concentration, time and temperature for best mechanical properties of chitin thin film is the expectation of this study.

II. MATERIALS

The raw material used iswaste oyster mushroom purchased from Johor, Malaysia. The NaOH used was from Merck, EMSURE® ISO in the form of white pellets and the H2O2 was 30% Analytical Reagent from R&M Chemicals. The glassware used include beakers (2000 mL, 500 mL), buchner funnels, falcon tubes (15 mL), and conical flasks (2000 mL). The consumable items used include petri dishes, disposable pipettes, filter papers and microplate (96 wells). The

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equipment used are as listed in Table 1.

Table 1: List of equipment used.

No.	Equipment	Brand	Model
1	High speed blender	Vita Mixer Innofood	SX 766
2	Hot plate	IKA®	G-MAG HS 7
3	Vacuum pump	VALUE®	VE 115N
4	Universal pH meter	Macherey-Nagel	REF 921 10
5	Oven	Faber	Forno 36
6	Tensile machine	Shimadzu	AG-X
7	Spectrophotometer	Thermo Science	Mutiscan™ GO

III. METHODOLOGY

In this study, chitin nanofibres were isolated from waste *Pleurotus ostreatus*. The chitin nanofibres were obtained from a sequence of physical and chemical treatments as illustrated in Figure 1. The procedure in achieving the aims of this study is elaborated and divided into sections: chitin extraction, preparation of chitin thin film and decolouration and mechanical testing. The purpose of the first step was to remove proteins, inorganic materials and glucans, the next step to make thin films so that the chitin can be mechanically tested using a tensile machine.

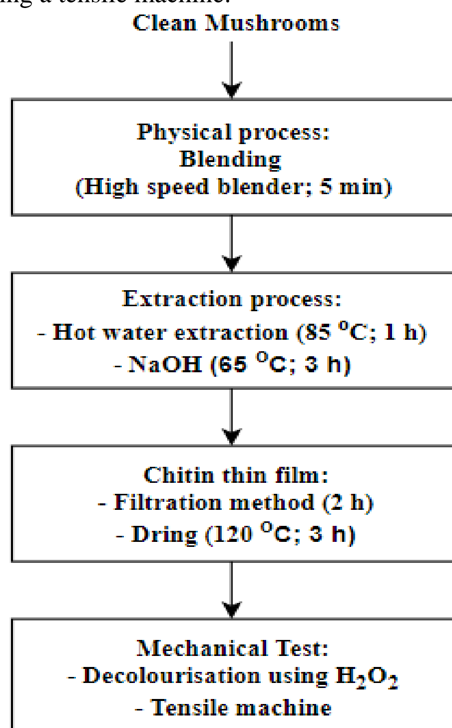

Fig. 1: Process flow for current study

3.1 Chitin Extraction

One kilogram of waste mushroomswas rinsed with distilled water to remove any inorganic materials on the mushroom surface. This was important because these materials may be contaminants or inhibit the extraction process. The cleaned mushroom was placed in a blender and distilled water was added to the 1 L mark and then subjected to high speed blending to attain the nanofibres. The total duration of blending with breaks was 7 min using the following procedure: 2 min blending → rest for 1 min→ 2 min blending → rest for 1 min → 1 min blending.

In hot water extraction, the mushroom slurry was poured into a beaker and distilled water was added until the 1.5 L mark.The process took place for 1 h at 85°C. Upon completion, the mushroom slurry was filtered using a vacuum pump and cloth for 30 min to remove excess water.Remaining water was pressed out of the mushroom cake. The purpose of this step was to remove any water soluble glucans. One molar NaOH was prepared by mixing 60 g of NaOH pellets with distilled water. The The mushroom cake was poured into a beaker and 1 M NaOH added until the 1.5 L mark. The process took place for 3 h at65°C. The purpose of this step was to remove proteins. Upon completion, the suspension was filtered and neutralised by adding distilled water until neutral pH was reached using a vacuum pump as in the previous filtration step. The chitin cake was kept in a container sealed using parafilm and stored in a refrigerator to avoid any contamination.

3.2 Preparation of Chitin Thin Film

Since the chitin cake was semi-solid, dilution was imperative to ease filtration process and preparation of chitin thin film. First, the chitin dry weight for every stock was measured. The chitin stock was diluted to 0.7 % (w/w) by blending it with distilled water for 30 s. Refer to Eq. (1) for method of dilution.

$$Volume\ of\ chitin\ added\ (L) = \frac{0.7\% \times 1.5\ L}{dry\ weight\ of\ chitin\ in\ stock} \quad (1)$$

The desired dimensions of chitin thin film for this reserach was 110 mm diameter and 80 gsm. The dry weight of diluted chitin was calculated to find how much dilute chitin was required for filtration to attain desired dimensions. Filtration of diluted chitin took2 h using 2 sheets of filter paper (Sartorius 1288) and a vacuum pump. The chitin cake was lifted from the filter paper and placedbetween 8 layers of POS thermal papers, two metal plates with another 5 kg weight placed on top such as in Figure 2. The chitin cake was dried for 1 h at 120 °C before the POS thermal papers were replaced with new ones and then dried again for another 2 h. Refer to Eq. (2) to find the amount of diluted chitin required for preparation of chitin thin film.

$$Mass\ of\ dilute\ chitin = \frac{(100\%)\left(80\frac{g}{m^2} \times \frac{\pi 0.11^2}{4}\right)}{(0.7\%)} \quad (2)$$

Fig. 2: Drying process of chitin cake in the oven at 120 °C

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3.3 Decolourisation and Mechanical Testing

Chitin films were cut into rectangular pieces of 60 mm by 10 mm. For every decolourisation process, one chitin strip was placed in a falcon tube and filled up with a mixture of H_2O_2 and distilled water accordingly, to a total volume of 10 mL. Mapping was done at different decolourisation concentrations (20% – 100%) for different process durations (3 h – 30 h) at room temperature (25 °C). Using a microplate spectrophotometer, the chitin film transmittance was measurede the duration with the highest trasmittance selected Five replications of chitin strips were prepared once the duration was chosen for H_2O_2 concentrations: 20%, 40%, 50%, 60%, 80% and 100% at that specific time at room temperature. Note that H_2O_2 used is 30% and this was considered as 100% hence 80% in actuality is 24% and so on. These concentrations were prepared by diluting it with distilled water. After decolourisation, the chitin strips were washed with distilled water and dried under weight to keep it flat and avoid any crinkling. Once dry, the strips were tabbed with mounting board (1 mm thick) using epoxy adhesive glue (Araldite® Standard) to prevent sample damage at grip point and slippage during tensile testing. Tensile test was carried out as in Figure 3 with sample grip pressure of 4 bar. The specimen gage length and test speed was set to 30 mm and 1 mm/min, respectively. The concentration which gave the highest tensile strength was chosen for the next OFAT whereby concentration and temperature were kept constant and process duration ranged from 3 h to 24 h.The best time was found and then time and concentration were kept constant for the last and final OFAT to find the optimum temperatute. Temperatures were ranged from 25 °C to 70 °C. At the end of this three step process, the optimum decolourisation concentration, time and temperature for chitin thin film was obtained.

Fig. 3: Testing the mechanical strength of chitin strip

IV. RESULTS AND DISCUSSION

The dry weight of oyster mushroom was calculated to be 8.1 ± 0.38 % (w/w) and the chitin 4.5 ± 0.77 % (w/w). This means that 91.9% of oyster mushroom is water, 4.5% is chitin while 3.6% is a mixture of protein and glucans. According to Kurita (2005), the source which have the highest chitin content is shrimp cuticle in the range of 30 – 40% [5]. This is perhaps why extraction from crustacean shells, despite the presence of calcium carbonate, is preferred over mushrooms. Nonetheless, different mushroom species have different chitin content and hence it is possible that other species have much higher chitin content.

The main aim in this study was to analyse the effect of decolourisation on the mechanical properties of chitin thin film. According to the decolourisation mapping for 600nm at room temperature, chitin with treated for 24 h give the highest transmittance, as shown in Figure 4a. This specific wavelength was chosen because the chitin film is light brown and the human eyes is most sensitive to this wave range.

Chitin strips treated with different H_2O_2 concentrations was prepared at room temperature for 24 hfor the first OFAT. Figure 4b shows that 60% H_2O_2 gives the highest mechanical strength, with an average tensile strength, modulus and toughness of 33.9 MPa, 18.2 GPa and 0.57 MJm^{-3} respectively. The concentration was kept at 60% while the process duration was varied from 3 h to 24 h in the second OFAT. The most optimum duration as shown from Figure 4c is 18 h with an average tensile strength, modulus and toughness of 33.6 MPa, 17.4 GPa and 0.9 MJm^{-3} respectively. The last and final OFAT was done to obtain the optimum temperature. The concentration and time was kept constant at 60% and 18 h while the temperature was varied from room temperature to 70 °C. The result is as illustrated in Figure 4d. It was found that 40 °C gives the best mechanical properties with tensile strength, modulus and toughness of 25.7 MPa, 10.2 GPa and 1.08 MJm^{-3}. Figure 5 compares optimised chitin film with raw chitin film, plastic and decolourisation at 70 °C

Comparing the results of the first, second and third OFAT revealed that the first OFAT gave the highest tensile strength and modulus while the third OFAT gave the highest toughness. This depicts great inconsistency as OFAT 3 should theoretically give the most optimum conditions, meaning that the highest mechanical properties should finally be achieved at OFAT 3. Possible reasons for this strange results include inconsistent chitin film thickness, low accuracy in dimensions when calculated to the nearest second decimal place and positioning of chitin strips on the tensile machine. It is also imperative to point out that the chitin extracted from oyster mushroom has a lot of debris and these debris result in inconsistent thickness and consequently weaker strength or inconsistent mechanical properties. In order for results of chitin extraction from oyster mushroom to be reliable, the error range should be at a minimum which is not true for the results of this study. For instance, the error range of toughness for OFAT 3 is 0.59 MJm^{-3}. Therefore, it is advised to either repeat the experiment, prioritising accuracy of measurements or to use mushroom source with lower to zero amount of debris. According to Mushi (2014), nanopaper treated with water CPF-Wat has the highest mechanical properties [6]. This chitin nanopaper is produced from crab exoskeleton. Assuming that mechanical testing results are reliable,

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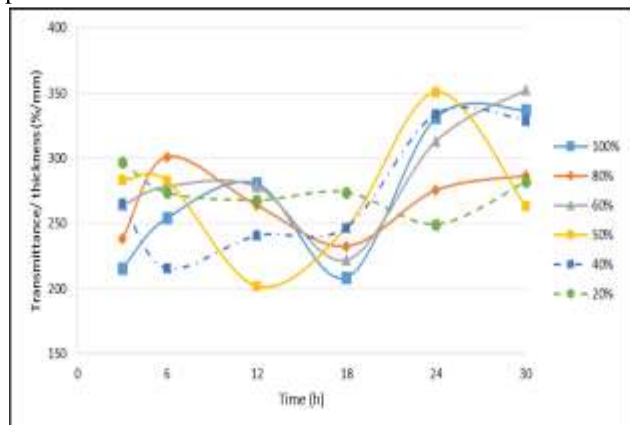
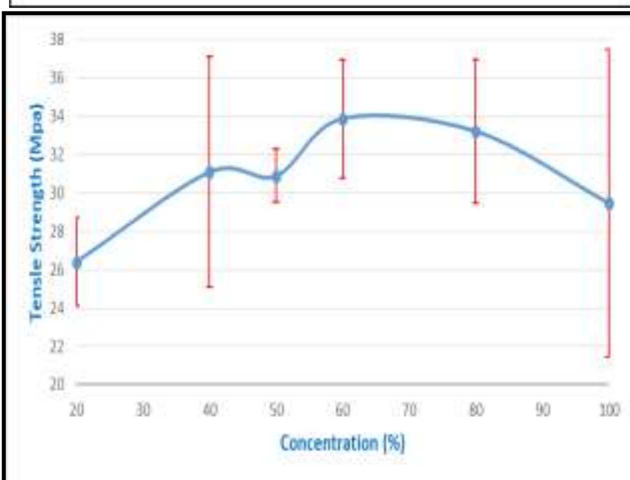
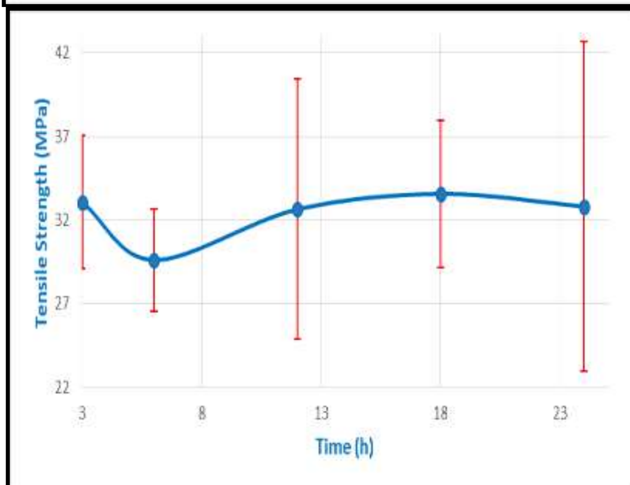
although the tensile strength and modulus of this CPF nanopaper is higher, the decolorized chitin thin film under optimum condition from oyster mushroom requires more work to fracture, it is tougher. While the CPF nanopaper has toughness of 0.77 MJm-3, the decolourised chitin thin film under optimum condition has a toughness of 1.08 MJm-3. This shows that chitin originated fromoyster mushroom is tougher than crab shell and can be made into stronger biocomposites. Figure 5 shows the appearance of raw and optimized decolourised chitin.







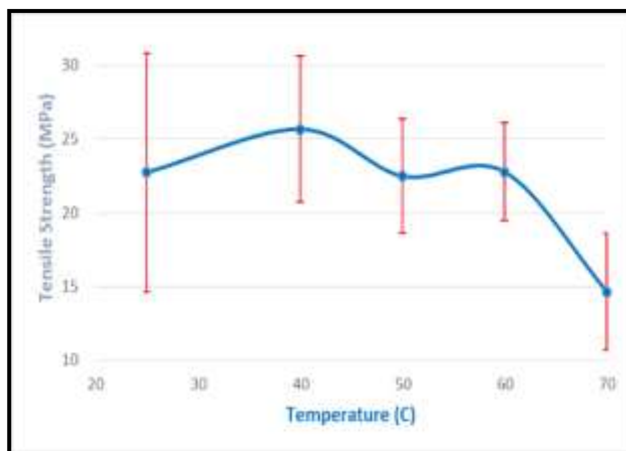


Fig. 4: (a) Transmittance of chitin films for different H2O2 concentrations, time at room temperature (b) OFAT 1: Varied concentrations at room temperatute for 24 h (c) OFAT 2: Varied time for 60% H2O2at room temperature (d) OFAT 3: Varied temperature for 60% H2O2and 18 h.

Fig. 5: (from left to right) raw chitin film, optimized decolourised chitin film, decolourised chitin film at 70 °C, plastic.

V. CONCLUSION

The main problem with commercial sources of chitin is its limited availability due to seasonal and geographical aspects as well as it being a major allergen. Thus, more research needs to be done on alternative sources of chitin to improve convenience and safety. This study uses waste fungal chitin which is easily cultured and is much safer in terms of minimal allergen as well as minimal requirement for harsh solvents and conditions.

The objectives of this research project are to extract chitin nanofibre from waste oyster mushrooms, Pleurotus ostreatus, form and test the strength of chitin thin films using a filtration method and tensile machine respectively. The parameters chosen for this study were concentrations of H2O2, process duration and reaction temperature with the following response: transmittance, tensile strength, young's modulus and toughness. It was found that at a decolourisation process done in room temperature that the highest transmittance was achieved when the process was done for 24 h. The optimum decolourisation condition was 60% H2O2 at 40 °C for 18 h. The value of tensile strength, young's modulus and toughness for optimized conditions were 25.7 MPa, 10.2 MPa and 1.1 MJm-3 respectively.

It was also found that chitin film formed from oyster mushroom have a much higher toughness than that fabricated from crab shells. This means that chitin nanofibre from

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mushroom have very high potential for the future of biocomposites, nanomaterial and tissue engineering.

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LIST OF ABBREVIATIONS

OFAT	One factor at a time
NaOH	Sodium hydroxide
H_2O_2	Hydrogen peroxide
°C	Degree celcius

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