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Fabrication of Cellulose Acetate Film From Oil Palm Empty Fruit Bunch (OP-EFB) and Cytotoxicity Evaluation

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ABSTRACT

The aims of this study were to fabricate cellulose acetate (CA) film from oil palm empty fruit bunch (OP-EPB), as well as to characterize and evaluate their biocompatibility. Several processes were carried out, and these included prehydrolysis-soda method, chlorine free bleaching method, including oxygen, ozone and peroxide, to produce the cellulose pulp. Then, a liquid phase acetylation method was applied through acetic acid-acetic anhydride-sulphuric acid. Triethyl citrate (TEC) ester was used as additive at different percentages of 10, 20, 30 and 40 wt%. The film produced was characterized by FTIR to identify the functional group of the CA film and their tensile properties were further characterized. Biocompatibility of the film was evaluated using cytotoxicity test. Stem cell derived from human deciduous teeth (SHED) was used with MTS assay. The results showed at 30% of TEC, the tensile strength and elongation of CA (OP-EFB) film was at the optimum and is therefore suitable to be used in dental application. The cytotoxicity evaluated showed that the fabricated CA (OP-EFB) films were non-toxic up to the concentration tested, and are thus compatible with SHED.

Keywords: Oil palm empty fruit bunch, cellulose acetate film, cytotoxicity

INTRODUCTION

In Malaysia, enormous volumes of agricultural wastes, empty fruit bunch (EFB) containing cellulosic fibres are generated annually (Suhaimi & Ong, 2001). Many of these wastes are allowed to rot away unutilized. These agricultural wastes can actually be maximized their utilization, as the focus of this study is, in the production of pulp for papermaking and conversion to cellulose derivatives, specifically cellulose acetate (CA). The application of CA is widely used in photography film, automotive coatings, selective filtration membranes in medicine, and also in dental field. The advantages of CA film, such as being tough, with good dimensional stability and optical properties, made it suitable to be used in dental field. Due to its flexible properties, CA film is usually used to assist dentists while performing tooth restoration. In the fabrication of CA, which is a thermoplastic material (also referred to as bioplastic), must be modified to make it suitable for matrix polymers for commercial composite application. Plastisizers are widely used in the plastic industry to improve their processibility, flexibility and ductility properties. Conventionally, cellulose ester plastics are plasticized with a petroleum–derived phthalate plasticizer, which is not environmental friendly. Mohanty *et al*. carried out a study on cellulose acetate plasticized with varying concentrations

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of an eco-friendly triethyl citrate (TEC) plasticizer (2003). The results were very promising and plasticized cellulose acetate was found to be processable at 170-180°C, i.e. approximately 50°C below the melting point of neat cellulose acetate.

The aims of this study were to fabricate cellulose acetate (CA) film from oil palm empty fruit bunches (OP-EPB) and investigate the effects of TEC plasticizer on the tensile properties of the resulting CA (OP-EPB) film. In addition, cytotoxicity was also evaluated. TEC was used as the plasticizer at different compositions. The CA films were characterized by Fourier Transform Infra-Red Spectroscopy (FTIR). Both the tensile strength and percentage elongation were also evaluated. Since the fabricated CA film is intended to be used in dental application and applied for oral purposes, the biocompatibility assessment is needed. One of the criteria for biocompatibility is that the material is not toxic to cells. Therefore, the fabricated CA films underwent a cytotoxicity test using MTS assay and the stem cell from human deciduous teeth (SHED) was also used. Vital staining was carried out as proposed by the National Guidelines for cytotoxicity test ISO 10993.

MATERIALS AND METHODS

Materials

Oil palm empty fruit bunches (OP-EFB) were collected from SABUTEK (M) Sdn. Bhd. in form of fibrous strands and used as raw material. TEC plasticizer was purchased from Merck Company.

Preparation of Cellulose Acetate Film

Preparation of the raw material

The raw material (OP-EFB) was cut into pieces and boiled with distilled water for 60 minutes at 170^0C, before the pulp underwent soda pulping using sodium hydroxide solution for 100 min at 160^0C to remove non-cellulosic materials. Later, it was washed with water and air dried.

Bleaching

The chlorine-free bleaching process studied here includes three sequential steps of oxygen, ozone and hydrogen peroxide bleaching. Oxygen (O_2) bleaching was carried out under alkaline conditions by addition of 1% NaOH (w/w) aqueous solution. Magnesium sulphate (0.1%) was also added as a protection reagent for cellulose. For ozone (O_3) bleaching, the ozone was produced from the supplied oxygen gas and was mixed with pulp. Total reaction time was 2.5 min with occasional mixing at 60 rpm. For hydrogen peroxide (H_2O_2) bleaching, the pulp was placed in a plastic bag and NaOH $MgSO_4$ was added according to the weight of the pulp (Tanaka *et al*., 2002). The reaction was then carried out at 60 ºC for 60 min in a water bath.

Acetylation of Cellulose

The cellulose acetate is usually produced by treating cellulose with acetic acid first as activation phase. To 5 parts of pulp, 90 ml of acetic acid, and 0.5 ml of sulphuric acid were added and stirred vigorously. After one min, 25 ml of acetic anhydride was added and the stirring was continued. Later, an equal volume of water was added into the reaction mixture to precipitate CA. The degree of acetylation of cellulose was determined using a standard method based on ASTM D-871-61T. The degree of acetylation found was 37.41%, and thus, the degree of substitution of CA was 2.2.

Preparation of Films

CA (OP-EFB) film was made using a casting method. First, a mixture solution of CA in acetone at 16 % (w/v) was prepared. Plasticizer material, TEC, was then dissolved in the solvent mixture at different compositions (namely CA1, CA2, CA3, and CA4) as in Table 1 and stirred for 48 h. Next, the mixtures were centrifuged at 3000 rpm for 1 h. The CA solution of 12 ml was slowly and evenly poured into a petry dish of 8 cm in diameter and was dried in a dessicator for 24 h. The thickness of the CA film obtained was approximately 0.1 mm.

TABLE 1
CA at different compositions of plasticizer

Specimen	CA/Acetone solution (ml) (w/v%)	Plasticizer (ml) Composition of TEC (wt%)
CA1	16	10
CA2	16	20
CA3	16	30
CA4	16	40

Characterization

FTIR Spectroscopy

The IR spectra of various specimens of the CA films were taken using Nicolet Impact 400 Fourier Transform Infrared spectrophotometer using KBr pellet. For each CA at different compositions of plasticizer, three specimens (n=3) were used for the FTIR evaluation. The peaks of C=O, C=C and C-O were taken and compared with a standard CA powder and also experimental CA (OP-EFB) powders.

Tensile Strength and Elongation

The tests were carried out using a Hounsfield TX0201 Tensile Testing System (H10KS model) according to the ASTM D 882. The thickness of the specimens for the tensile evaluation was maintained at approximately 0.1 mm and the length between the grips was set at 30mm. The measurements of CA films were made at three specimens, (n=3) for each CA at different compositions of plasticizer.

Cytotoxicity Evaluation

The national guidelines for cytotoxicity test ISO /EN 10993-5 are followed for the biocompatibility evaluation. The MTS assay of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] was used for the cytotoxicity evaluation with the CA(OP-EFB) extracts on the stem cell derived from human deciduas teeth (SHED). This cytotocixity test was carried out according to the ISO 10993-5 standard (ASTM Standard D882). The SHED were initially cultured at cell density 5 x 10^3cells/cm for 24 hours at 37 °C in 96 well plates. The medium was replaced with the sample extract. However, only one representative sample was used for this cytotoxicity evaluation. The plates were incubated in a CO_2 incubator for 72 hours. Next, 5mg MTS powder was measured and mixed thoroughly with 1 ml dulbecco's phosphate buffered saline

(DPBS) in a small sterile universal tube. After being thoroughly mixed, the MTS solution was filtered. 10µl of the MTS solution was added into all the 96 wells after 72 hours of incubation. The plates were further incubated for 2-4 hours in 5% CO_2 incubator. After that, the culture medium and excessive MTS solution were removed by inversion and blotted carefully on tissue paper. Next, 100µl of dimethylsulfoxide (DMSO) was added into each well and gently shaken for 5 minutes to achieve complete dissolution. Finally, an Elisa reader (TECAN) was used to read the absorbance at the reference and test wavelengths of 600nm and 570nm. The cell viability percentages were calculated according to the following equation:

$$\%\text{Cell viability} = \frac{OD_{sample}}{OD_{control}} \times 100\%$$

where, OD= Optical density.

RESULTS AND DISCUSSION

The degree of the substitution of the CA found was 2.2. In general, those cellulose acetates with acetyl substitution numbers of 2.2 or less are biodegradable in soil and marine environment and are therefore suitable for composting compared with higher substitution numbers from 2.2 to 3.0. Hence, the CA (OP-EFB) film produced was confirmed as biodegradable.

The CA (OP-EFB) films of all the different percentages of TEC plastisizers were confirmed using the FTIR evaluations (Table 2). The present of the functional group C=O, C=C, and C-O indicates that the structure of CA was confirmed, as compared to the commercial CA which was done only on the powder form (Table 3). The acetate groups were found in each sample; however, the peak value was different from the control group since they were in the powder form. Nonetheless, there was not much difference between the peaks in the powder form of the commercial CA and (OP-EFB) CA, as shown in *Fig. 1*.

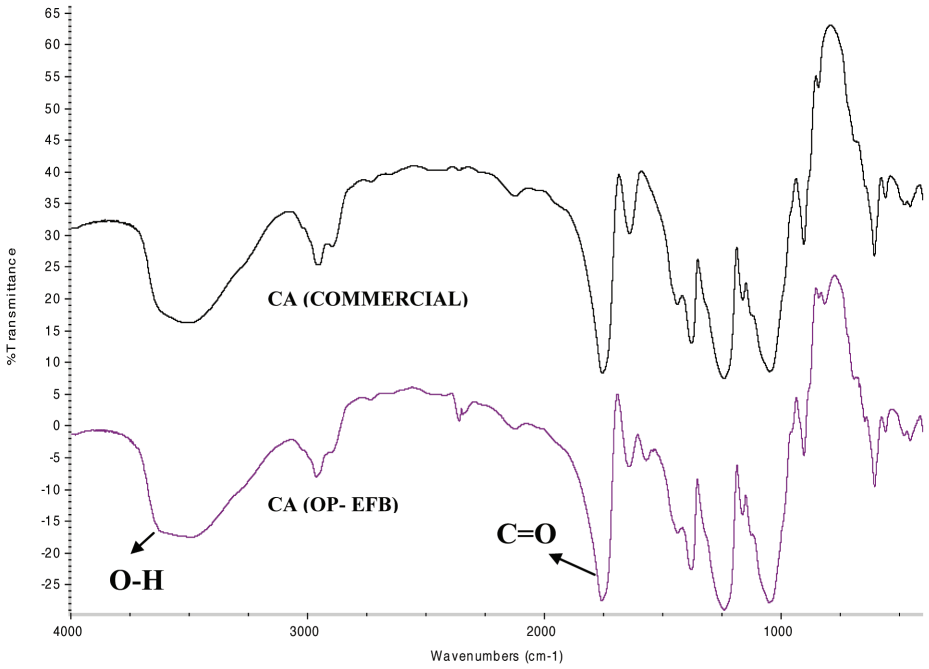

Fig. 1: The FTIR peaks of CA (OP-EFB) films and CA commercial

TABLE 2
The FTIR peak results of the CA (OP-EFB) films

Specimen	C=O (cm^{-1})	C=C (cm^{-1})	C-O (acetate) (cm^{-1})
CA1	1711.51 (0.85)	1637.41 (0.20)	1071.73 (15.45)
CA2	1736.75 (22.75)	1636.71 (1.26)	1071.69 (2.17)
CA3	1765.42 (1.13)	1636.15 (0.33)	1087.22 (34.00)
CA4	1753.85 (16.10)	1636.27 (0.13)	1152.63 (94.75)

Note: The reported are mean values with their standard deviation in brackets.

The FTIR analysis identified all the important peaks present in the sample CA (OP-EFB), which were nearly similar to the peaks that appeared in the CA commercial. A summary of the peak wave number for all the functional groups C=O, C=C, and C-O present in CA commercial and CA (OP-EFB) is tabulated in Table 3.

TABLE 3
The FTIR peak results of CA in powder form

Specimen	C=O (cm^{-1})	C=C (cm^{-1})	C-O (acetate) (cm^{-1})
CA (Standard commercial)	1754.56	1638.57	1239.53
CA (OP-EFB)	1754.51	1643.01	1243.31

In the tensile properties evaluation, the results presented in Table 4 show an increasing trend of the tensile strength of CA (OP-EFB) films with a decreasing composition of TEC plasticizer. Although 10% of TEC has the highest value of tensile strength, the CA1 film produced was brittle, not very flexible, and it tended to wrinkle. This is shown by having the lowest elongation of 6.51%. At 20% of TEC, CA2 film also has the same features as those of CA1, but with improved elongation properties. On the other hand, CA3 gave a tensile strength of about 15 MPa but the film was very flexible and it showed the highest elongation. An optimum balance between the tensile strength and elongation is depending on the CA application. As the fabricated CA was intended to be used in dental application, which is supposed to bend easily around a tooth, the CA3 composition is therefore suitable. For CA4 film, the tensile strength was the lowest as compared to the other composition of plasticizer. The result found is in agreement with the finding by Mohanty *et al*. (2003).

TABLE 4
The effect of different compositions of TEC on the tensile strength and elongation of CA (OP-EFB) films

Specimen	CA/Acetone (w/v%)	Composition of TEC (wt%)	Tensile strength (MPa)	Elongation (%)
CA1	16	10	27.04 (1.98)	6.51
CA2	16	20	21.11 (0.22)	10.67
CA3	16	30	15.71 (1.13)	10.76
CA4	16	40	7.48 (0.40)	10.61

Note: Reported are mean values with their standard deviation in brackets.

However, to achieve the tensile strength of CA (OP-EFB) that is similar to the commercial CA films, more extensive research is needed. Their tensile strength is about 100–140 MPa (Zugenmaier & Peter, 2004). Many factors may contribute to their high tensile strength, such as different origins of cellulose, molecular weight, purity, and different methods of film preparation.

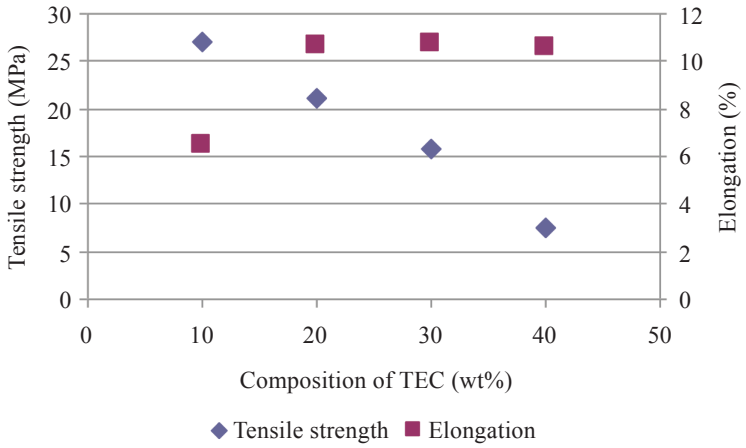

Fig. 2: The effect of TEC plasticizer on the tensile strength and elongation of the CA films

In this study, CA (OP-EFB) was found to be not fully dissolved in acetone, which resulted in the obtained supernatant after centrifuged had thinner film compared to the commercial CA. Hence, different types of solvent need to be further explored. TEC composition is also important in the final product of CA film. The final application of the CA film is dependent upon the plasticizer composition. Other factors, such as drying method, have also been reported to affect the quality of the film. Meanwhile, the drying process plays an important role in affecting the wrinkles of the fabricated film.

Fig. 3 presents the cell viability of the CA films in function of extract concentration. IC_{50} (50 % Inhibitory Concentration) endpoint was used to evaluate the cytotoxicity effects of the materials at different concentrations applied. From the figure, the percentages of cell viability decrease when the CA extraction concentration is high. However, at the concentrations of 150mg/ml onwards, the curve line becomes a plateau at slightly above 50%. Therefore, the fabricated CA can be considered as non-toxic. However, further test on biocompatibility is still needed, such as genetoxicity and Ames test.

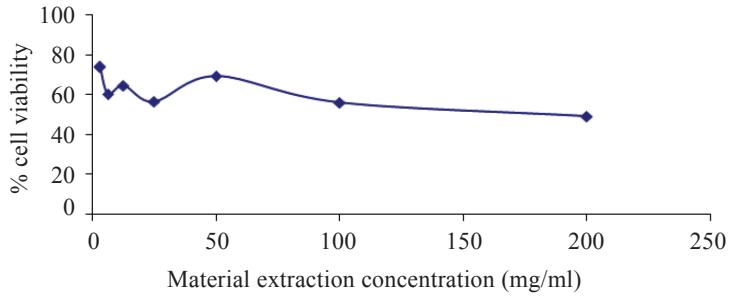

Fig. 3: The percentage of the cell viability of CA film extraction on SHED

CONCLUSIONS

In this study, the CA produced from OP-EFB obtained a DS of 2.2. Therefore, it is possible to use TEC as it is eco-friendly and promoting green technology. However, increasing the amount of TEC plasticizer significantly reduced the tensile strength. The optimum balance of strength and stiffness of the CA film at 30 (wt%) plasticizer is found to be suitable for dental application. The cytotoxicity evaluation provides evidence which indicates that CA (OP-EFB) is non-toxic to the SHED, up to the concentration tested.

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   Swan and Kanwal (2007) reported that …
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     - Tan, S.G., Omar, M.Y., Mahani, K.W., Rahani, M., & Selvaraj, O.S. (1994). Biochemical genetic studies on wild populations of three species of green leafhoppers *Nephotettix* from Peninsular Malaysia. *Biochemical Genetics*, *32,* 415 - 422.

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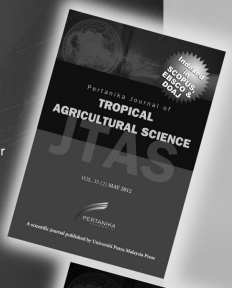

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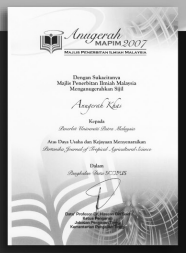

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