Chitin Fiber from Mushroom as Reinforcement for Biobased Polymer

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Abstract. This project aimed to study the reinforcement effect of fungal chitin at different loading on chitin/PLA composite. The chitin nanofibers were extracted from three mushroom species (oyster mushroom Pleurotus ostreatus, shiitake mushroom Lentinula edodes, enoki mushroom Flammulina velutipes) and used as a reinforcement element in PLA. The chitin/PLA composite was fabricated using a solvent-casting method followed by the hot-compress molding method. In the solvent-casting method, the chitin nanofibers were dispersed in PLA/chloroform mixture and the mixture was left for solvent evaporation. The solvent-free chitin/PLA thin film was then filled in dog bone mold before proceeded with hot-compress molding at 190°C and 70 bar. The samples with different chitin loading were tested with tensile test to study the mechanical performance of nanocomposite. The chitin/PLA composite from oyster mushroom shows the optimum result (σ= 43 MPa, E= 12 MPa) at 5% chitin loading. The increment of the chitin loading leads to a decrease in both strength and strain. However, for the samples from enoki and shiitake mushrooms, the optimum chitin loading is 10% with 55 MPa and 56 MPa tensile strength, respectively. This study suggests the potential of fungal chitin as reinforcement in PLA.

1. Introduction
Chitin is a natural fiber that has been recently studied for its beneficial properties as reinforcing materials in biopolymers [1-3]. Its is abundantly found in crustacean shell, insect cuticle and cell wall of fungi. Fungal chitin has gained attention as the alternative to chitin source since it can provide continuous and consistent supply [4, 5]. Since fungal chitin has excellent properties such as biodegradability and biocompatibility, it can be used as a reinforcing element for biobased materials such as polylactic acid (PLA) [6].

PLA is the most promising biodegradable bioplastic which is considered as eco-friendly material to replace conventional plastics, especially in the packaging industry. However, its brittleness and poor stability limit its industrial applications. Therefore, modification of PLA with the addition of plasticizers, coupling and antistatic agents was done as a strategy to improve its performance so that it can compete with the conventional polymers used in the packaging industry [3]. Coltelli, et al. [7] discovered that incorporating PLA with chitin nanofibrils increased the elongation of the polymer by 180%. The chitin loading in PLA affects the mechanical properties of PLA composite [1, 8, 9]. Another study by Maskur, et al. [10] reported that the incorporation of 5 wt% crustacean chitin resulted in higher tensile strength and modulus. However, no study has been done yet on the effect of fungal chitin loading in PLA.
Therefore, this study demonstrates the effect of fungal chitin loading on the mechanical properties of chitin/PLA composite. The fungal chitin in this study was extracted from oyster (*Pleurotus ostreatus*), Shiitake (*Lentinula edodes*) and Enoki mushroom (*Flammulina velutipes*) before the chitin powder was mixed with PLA using chloroform. The mechanical properties of chitin/PLA composite were studied using a universal tensile machine.

2. **Materials and Methods**

2.1. **Materials**

Three different mushrooms which are oyster (*Pleurotus ostreatus*), Shiitake (*Lentinula edodes*) and Enoki mushroom (*Flammulina velutipes*) were used as fungal chitin sources in this study. The mushrooms were frozen at least overnight at -20°C. Sodium hydroxide (Merck, pellets) was used for chitin extraction process. The extracted chitin and PLA (Nature Works, Ingeo 3251D and Ingeo 3052D) were mixed using chloroform (Merck).

2.2. **Chitin Nanofiber Extraction**

The extraction was carried out according to a procedure performed by Nawawi, et al. [11]. The frozen mushrooms were thawed and washed with distilled water to remove any impurities before blending in a kitchen blender (Vita Mixer Innofood, SX766) for 5 minutes to break the cell wall. Distilled water was added to the blended mushrooms and the suspension was heated at 85°C for 1 hour to remove any water-soluble components. The water-soluble components were removed by filtration process using a cotton cloth. A vacuum pump (VALUE, VE 115N) was used to facilitate the filtration process. Then, the filter cake was soaked in 1 M NaOH (65°C, 3 hours) to remove protein, lipid and alkaline-soluble polysaccharides before neutralizing with distilled water until pH 7 was indicated by universal test paper. The neutralized chitin was freeze-dried to remove the excess moisture and ground into powder (150 µm) for further use.

2.3. **Chitin/PLA Composite Fabrication**

Prior to chitin/PLA composite fabrication, the PLA was dried overnight at 70°C to remove moisture content. Then, the fabrication process started by first dispersing the chitin powder (5%, 10%, 15% and 20%) in chloroform before dispersing in PLA/chloroform mixture. The mixture was then stirred at high speed using the magnetic hot plate (IKA®, G-MAG HS 7) until the chitin was homogenously dispersed within the mixture. The mixture was poured into glass petri dish covered with aluminium foil and left in the fume hood until the chloroform was fully evaporated, leaving behind chitin/PLA film composite. The film composite was ground and filled into the dog bone mold cavities (Type IV, ASTM D638) that were coated with a releasing agent (Loctite 770-NC Frekote) to ease the removal process. The step was proceeded with compression molding by hot press (SUN RUN, SPH-900) at 190°C and 70 bar for 15 minutes. As a control, neat PLA sample was prepared by adding the PLA granule until it fully occupied the dog bone mold cavities.

3. **Results and Discussion**

3.1. **Chitin/PLA Composite Fabrication**

The agglomeration of chitin during composite fabrication was minimized by grinding freeze-dried chitin into powder form. The smaller size of chitin powder increases the contact area with PLA/chloroform mixture which contributes to a homogenous dispersion within PLA polymeric matric. Stirring was required to promote a homogenous dispersion of chitin since the chitin is not easily solubilized in a common solvent, such as chloroform [12]. During the solvent casting method, the chloroform was slowly evaporated by covering the glass petri dish with aluminium foil. This step was important to prevent bubble formation in the film which would affect the preparation of tensile test specimens via compression molding. Figure 1 shows the chitin/PLA film with different chitin loading. The accumulation spots of chitin become more visible as higher chitin loading is introduced. This formation
describes the properties of chitin that attracted and crystallized when less solvent was present. The higher chitin loading in PLA induces more interaction between chitin nanocrystals and the solvent.

![Chitin/PLA composite](image1)

**Figure 1.** Chitin/PLA composite with (a) 5 wt%, (b) 10 wt%, (c) 15 wt% and (d) 20 wt%.

Figure 2 shows the tensile specimen of neat PLA. No bubble formation was observed within the tensile specimen. This was achieved by pre-dried PLA and grinding the film before tensile specimen preparation. The formation of bubbles in the samples acts as a weak point for crack propagation to initiate. Thus, will affect the mechanical properties.

![Tensile specimen of neat PLA](image2)

**Figure 2.** Tensile specimen of neat PLA.

### 3.2. Mechanical Properties of Chitin/PLA composite

The mechanical properties of oyster chitin/PLA composite were compared to neat PLA (Ingeo 3052D). While for shitake and enoki chitin/PLA composite, PLA (Ingeo 3251D) was used since we are unable to purchase the same product due to technical limitations. However, the effect of fungal chitin loading
in PLA still could be discussed except that we could not compare the result for oyster with shiitake and enoki chitin/PLA composite. Table 1 shows the mechanical properties of oyster chitin/PLA at different chitin loading.

Table 1. Mechanical properties of oyster chitin/PLA at different chitin loading.

| Sample  | Tensile strength (MPa) | Young modulus (MPa) | Tensile strain (%) | Tensile toughness (MJ/m²) |
|---------|------------------------|---------------------|--------------------|--------------------------|
| NP1     | 17.76 ± 7.83           | 12.86 ± 0.5         | 1.56 ± 0.73        | 0.42 ± 0.36              |
| CP1O5   | 43.04 ± 2.45           | 12.15 ± 0.14        | 4.35 ± 0.45        | 0.91 ± 0.08              |
| CP1O10  | 41.52 ± 0.54           | 19.15 ± 9.24        | 3.16 ± 1.13        | 0.73 ± 0.26              |
| CP1O15  | 30.37 ± 0.86           | 12.07 ± 0.51        | 2.77 ± 0.05        | 0.44 ± 0.01              |
| CP1O20  | 34.75 ± 4.10           | 12.54 ± 0.3         | 3.10 ± 0.40        | 0.59 ± 0.15              |

*The numbers represent the chitin loading percentage in PLA.

The strength and toughness of PLA were improved when oyster chitin was added. The composite containing 5 wt% of oyster chitin recorded the highest tensile strength and toughness with the value of 43 MPa and 0.91 MJ/m², respectively. The result was in agreement with a study by Maskur, et al. [10] when they investigate the effect of crustacean chitin loading in PLA. A lower chitin loading promotes a better homogenous dispersion within PLÁ matrix. Since chitin is less hydrophilic, lower chitin loading makes it more compatible with the hydrophobic PLA matrix [8]. As the result, the affinity of chitin and PLA matrix contributes to the properties of composite [2]. Figure 3 shows the trends of tensile strength when more chitin loading was introduced. When the chitin loading was increased to 10, 15 and 20 wt%, the tensile strength and strain were decreased. At higher chitin loading, the compatibility of chitin with PLA is weaker and the chitin tends to agglomerate. Therefore, the bigger chitin particle gives a negative impact on the strength of the composite. However, there is no reduction of strain to failure observed, proposing that no damage on fibers occurred during the extraction process.

![Figure 3. Tensile strength and strain of chitin/PLA nanocomposite for oyster mushroom.](image)

Table 2 presents the mechanical properties of enoki and shiitake chitin/PLA composite. For both enoki and shiitake chitin/PLA composite samples, the highest tensile strength and toughness were
exhibited when 10 wt% chitin was added into PLA. The result showed that PLA (3251D) promotes better chitin compatibility up to 10 wt% chitin loading compared to PLA (3052D) which only up to 5 wt% chitin loading. An increase in fiber content in a composite system theoretically would increase the tensile strength \([9, 13, 14]\). However, the compatibility between the fiber with PLA matrix also contributes to the tensile strength. Therefore, if better compatibility at higher fiber content could be maintained, the tensile strength probably would be improved further.

### Table 2. The mechanical properties on chitin/PLA nanocomposite of Enokii and Shiitake mushroom.

| Sample   | Tensile strength (MPa) | Young modulus (MPa) | Tensile strain (%) | Tensile toughness (MJ/m²) |
|----------|------------------------|---------------------|--------------------|--------------------------|
| NP2      | 48.63 ± 11.83          | 12.22 ± 0.42        | 5.97 ± 2.22        | 1.90 ± 1.08              |
| CP2E5    | 53.18 ± 4.98           | 12.37 ± 0.20        | 6.06 ± 0.87        | 1.91 ± 0.45              |
| CP2E10   | 55.87 ± 0.56           | 10.27 ± 4.16        | 7.21 ± 0.34        | 2.33 ± 0.10              |
| CP2E15   | 53.97 ± 0.71           | 9.78 ± 0.48         | 7.27 ± 0.59        | 2.22 ± 0.24              |
| CP2E20   | 48.77 ± 0.22           | 13.68 ± 0.04        | 4.68 ± 0.07        | 0.68 ± 0.67              |
| CP2S5    | 52.05 ± 1.19           | 12.13 ± 0.21        | 5.35 ± 0.19        | 1.58 ± 0.10              |
| CP2S10   | 56.85 ± 2.01           | 11.85 ± 0.45        | 6.46 ± 0.50        | 2.17 ± 0.25              |
| CP2S15   | 51.33 ± 4.47           | 13.02 ± 0.20        | 5.15 ± 0.52        | 1.53 ± 0.29              |
| CP2S20   | 47.27 ± 4.78           | 13.86 ± 0.36        | 4.37 ± 0.66        | 1.20 ± 0.33              |

*CP2E and CP2S indicate the samples from chitin enoki and shiitake respectively. The numbers represent the chitin loading percentage in PLA.

The highest modulus was recorded at 20 wt% chitin loading for both enoki and shiitake chitin/PLA samples. According to Rizvi, et al. \([9]\), the modulus is expected to increase when the nanofiller content increases up to some limit. Differ from tensile strength, the modulus is probably not affected by the compatibility of chitin and PLA (3251D) since the result showed a different pattern. Nevertheless, the tensile strain of enoki chitin/PLA composite showed a slight increase up to 15 wt% of chitin before it plummeted at 20 wt% (Figure 4). The tensile strain for enoki chitin/PLA at 15 wt% showed no significant difference with the one at 10 wt% chitin loading. Therefore, we can claim that the tensile strain pattern for both enoki and shiitake chitin/PLA composite was quite similar.

![Figure 4. Tensile strain of enoki and shiitake chitin/PLA nanocomposite at different chitin loading.](image-url)
4. Conclusions
The use of fungal chitin nanofiber as a reinforcement element in PLA has a positive effect to enhance its mechanical properties. The chitin/PLA (3052D) composite has optimum tensile strength and toughness when 5 wt% oyster chitin was incorporated into the PLA. However, for enoki and shitake chitin/PLA (3251D) composite, the optimum tensile strength and toughness were recorded at 10 wt% chitin loading. The overall result suggested the potential of fungal chitin nanofiber as a reinforcement element in the development of sustainable material.

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