Physicochemical Properties of Chitosan Extracted from Leucaena Leucocephala Pods Using Deprotenization and Decolorization Steps

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Abstract. Chitosan was extracted from Leucaena leucocephala (LL) pods collected from three locations in west coast Malaysia. The primary objectives of this study were to extract and characterize the extracted chitosan pectin from LL pods. Besides that, the physicochemical properties of extracted chitosan were determined and compared with the commercial chitosan. Chitosan A extracted from LL pods gave a higher percentage of yield compared to chitosan B, 70.9% and 67.6%, respectively. Besides that, the color analysis and whiteness index of both extracted chitosan were evaluated. From the data obtained, chitosan B has higher whiteness index, which is 61.6% and chitosan A was only 48.5%. Based on this study, it can be concluded that decolorization treatment with method B produced chitosan with a higher percentage of whiteness index compared to decolorization treatment with method A.

1. Introduction
Chitosan is a polycationic polymer that contains more than 5000 glucosamine units [1]. It is obtained from shrimp and crab shell chitin by alkaline deacetylation. However, this source of chitosan may become unfeasible since they are limited and seasonal [2]. Thus, finding an alternative source for this polymer is needed. A recent study by Ref.[3], [4] had shown that Leucaena leucocephala (LL) pods have the potential to be an alternative source for chitin. Therefore, this study aimed to extract the chitosan from LL pods by using deproteinization and decolorization steps. The extracted chitosan was characterized, and the physicochemical properties were determined and compared with the commercial chitosan.

2. Material and methods

2.1. Sample preparation and chitin extraction
Young Leucaena leucocephala (Lam.) de wit pods (voucher no. MFI 0079/19) were collected from the three different locations in west coast Malaysia. The size of the pods was range between 14 to 18 cm long and was green. After that, the pods were cleaned using tap water and then dried in an oven (Memmert Universal Oven, German) for 24 h at 60°C before ground into powder using a miller machine (Laboratory Mill 120, Perten, Sweden).

The powder of LL pods was treated according to the method of Ref.[5] with slight modification. The powder was treated with 0.5 M sodium hydroxide (NaOH) at the ratio of 1:10 (w/v) at 100°C for 3 h. This step was aimed to remove a protein in LL powder, and obtained chitin. The mixture was then filtered and washed with distilled water until pH 7 was obtained. The crude chitin was obtained as the precipitate. Next, the extracted chitin was then dried in an oven at 80°C.

2.2. Deacetylation of chitin to produce chitosan
The deacetylation of chitin to obtain chitosan was done with 50% of NaOH. For chitosan A, the ratio of crude chitin to NaOH used was 1:30 (w/v), and the solution was then heated at 100°C for 60 min. Then, the extracted chitosan was washed with distilled water until pH 7 and dried overnight at 80°C. For chitosan B, and the precipitate was decolored with 1% sodium hypochlorite three times, each for 1 h [5]. Following decolorization, the extracted chitosan was washed with distilled water until pH 7 was obtained and dried in the oven at 60°C for 24 h.

2.3. Fourier Transform Infrared Spectroscopy (FTIR) analysis
Infrared spectroscopy was used to determine the chitosan structure. The Fourier Transform Infra-Red FTIR spectra of LL chitosan and commercial chitosan were recorded using FTIR spectrophotometer (ThermoFisher Scientific, United States), equipped with an attenuated total reflectance (ATR) element. The OMNIC software version 8.0 was used for data analysis. The FTIR spectra were then collected at the mid-infrared region (400 - 4000 cm-1) using 32 scans and at a resolution of 4 cm-1 and were recorded in triplicates.

2.4. Physicochemical properties of extracted chitosan
The reflective surface color of powder of LL powder and chitins A and B were measured using a Color Measurement system (Colorimeter Konica) by using RSEX (Reflectance Specular Excluded) mode type, and the area view was 0.190 in. The value of L*, a*, and b* were recorded, and each sample was individually measured in triplicate. Data obtained was then used to calculate the whiteness index (WI) based on the following equation [6]:

\[ \text{Whiteness index (WI)} = 100 \cdot \sqrt{(100 - L)^2 + a^2 + b^2} / 2 \]  
(Eq. 1)

3. Results and discussion
3.1. Yield percentage of chitin and chitosan
The yield percentage of chitin and chitosan extracted from LL pods was showed in Table 1. Chitin A showed a higher yield percentage compared to chitin B, with 19.2% to 12.8%. A slight increase trend was observed to the previous study [5]. They found that the extraction yield of chitin from shiitake stipes was between 25.08 to 36.72%. However, Ref.[2] found that chitin extracted from house cricket showed a decrease in yield percentage, which only between 4.3 to 7.1%.

On the other hand, the yield percentage of chitosan extracted from LL pods were presented in Table 2. Chitosan A showed higher yield (70.9%) compared to chitosan B (67.6%). Also, a similar yield was observed from the previous study [7] The chitosan obtained from Nigerian mushrooms (H.ernaecius and P.tuberagium) was 61.11% and 52.15%, respectively. Moreover, Ref.[8] found that the yield of chitosan from the super worm was 75.5%.
Table 1. Yield percentage of chitin extracted from LL pods.

| Sample     | Chitin A | Chitin B |
|------------|----------|----------|
| Yield (%)  | 19.2±4.2 | 12.8±5.6 |

*Chitin A; without decolorization treatment. Chitin B: with decolorization treatment.

Table 2. Yield percentage of chitosan extracted from LL pods.

| Sample     | Chitosan A | Chitosan B |
|------------|------------|------------|
| Yield (%)  | 70.9±4.1   | 67.6±1.7   |

*Chitosan A; without decolorization treatment. Chitosan B: with decolorization treatment.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a convenient method to determine the chemical structure of chitin [9]. Besides that, the spectrum can be used as a fingerprint. In this study, FTIR spectra were used to compare the differences in the spectral patterns of extracted chitosan samples, as shown in Figure 1. The assignments of chitosan were according to previous literature [10], [11]. The O-H and N-H stretching was assigned as broad bands at 3477 and 3258 cm\(^{-1}\), respectively. The bands ranging from 3109 to 2880 cm\(^{-1}\) represent CH, CH\(_3\) symmetric stretching, and CH\(_2\) asymmetric stretching. The three significant amide bands at ~1620 cm\(^{-1}\) are assigned to Amide I band (two types of hydrogen bonds in a CO group with the NH group of the adjacent chain and the OH group of the inter-chain), Amide II band (in-plane N-H bending and C-N stretching mode) and Amide III band (in-plane mode of the CONH group) at 1552 and 1331 cm\(^{-1}\), respectively [9]. Besides that, it was also compared with commercial chitosan, purchased from Sigma Aldrich.

![Commercial chitosan](image1.png)

Figure 1. FTIR spectrum for chitosan A and B extracted from LL pods.

According to Figure 1, the spectrum of chitosan A and B, obtained from LL pods were all almost similar to commercial chitosan. The three significant amide bands were showed, and for chitosan A and chitosan B, Amide I band was observed at 1651 cm\(^{-1}\), Amide II was observed at 1508 cm\(^{-1}\), and Amide III was observed at 1264 cm\(^{-1}\) for chitosan A, but for chitosan B, only amide I and III were present. The spectra of chitosan from LL pods exhibited a characteristic band at 3567cm\(^{-1}\) was attributed to -NH stretching and -OH groups stretching vibration at 3630 cm\(^{-1}\). Then, the band at 2901 cm\(^{-1}\) was an aliphatic C-H stretching that converges to OH stretching with N-H. From the spectrum, it can be
observed that chitosan B showed a higher percentage of transmittance compared to those of chitosan A and commercial chitosan.

### Table 2. The FT-IR bands (cm\(^{-1}\)) of chitosan isolated from LL pods

| Functional group and vibration modes | Chitosan A | Chitosan B | Commercial chitosan |
|--------------------------------------|------------|------------|---------------------|
| (NH\(_2\)) associated with primary amines and OH associated with pyranose ring | 3404 | 3381 | 3388 |
| (CH\(_3\)) in CH\(_2\)OH group | 2922 | 2913 | 2923 |
| (C\(=\)O) in pyranose ring | 2323 | 2324 | 2323 |
| (C\(=\)O) in NHCOCH\(_3\) group (amide I band) (1700-1600) | 1650 | 1650 | 1650 |
| (NH\(_2\)) in NHCOCH\(_3\) group (amide II band) (1600-1500) | 1508 | - | 1570 |
| (CH\(_3\)) in CH\(_2\)OH group | 1457 | - | 1419 |
| (NH\(_2\)) in NHCOCH\(_3\) group | 1368 | 1368 | 1377 |
| Complex vibrations of NHCO group (amide III band) (1350-1200) | 1264 | 1224 | 1230 |
| (C\(=\)O\(=\)C) (glycosidic linkage) | 1058 | 1060 | 1069 |
| (C\(=\)O) in primary OH group | 897 | 897 | 896 |
| Pyranose ring skeletal vibrations | 811 | 802 | 800 |

### 3.3. Color analysis and whiteness index (%)

One of the characteristics of good quality of chitosan is the color. The right color would increase the preference and acceptability of consumers. The product quality can be improved by the decolorization process following chemical treatment. Therefore, for chitosan B, a decolorization process was applied. The decolorization treatment was done using 1% of sodium hypochlorite. The color analysis of chitosan extracted from LL pods was presented in Figure 2. The percentage of whiteness index (WI) for both samples was 48.5% and 61.6%, respectively. These values were slightly lower compared to that of commercial chitosan that had 78.5% of WI. However, chitosan produced from LL pods showed a similar value of WI compared to the previous study by Sarbon et al., (2014). The WI of chitosan extracted from mud crab was 62%. Besides that, Ref.[12] found that the WI of extracted chitosan from crawfish had slightly lower values (ranging between 24 to 47%).
4. Conclusion
In conclusion, chitosan was successfully being extracted from LL pods by using deproteinization and deacetylation method with sodium hydroxide. Analysis of FTIR showed a similar pattern of the spectrum was obtained between extracted chitosan (chitosan A and B) with the commercial one. Besides that, yield percentage of chitosan A was higher compared to chitosan B. However, analysis of color and whiteness index (WI) showed that chitosan B have higher WI% (61.6%) compared to chitosan A with only 48.5%. Thus, chitosan B that under decolorization treatment before deacetylation process produced better chitosan in terms of color and whiteness index, thus this treatment could be adapted to prepare chitin and chitosan from LL pods for further application.

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