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Chitin extraction from *Leucaena Leucocephala* pods

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***Abstract*—** In this work, chitin from *Leucaena leucocephala (LL)* pods were physicochemically characterized using acid hydrolysis process was investigated. This study was conducted to find out other sources of chitin rather than its common sources from aquatic organisms and also to ensure sufficient amount of chitin supply. A series of steps for the preparation of *LL* sample and the acid hydrolysis experiment were performed to obtain the chitin properties. The molarity of hydrochloric acid is varied between 1M to 3M were characterized by FTIR, XRD, Elemental Analyser ,TGA and DSC. Based from result obtained, the most suitable molarity of hydrochloric acid used in acid hydrolysis to extract chitin from LL was at 3 M concentration due to the good chitin characterization obtained throughout the study. However, characteristics of chitin are different from the chitin source.

Keywords—chitin; Leucaena leucocephala; extraction

I. INTRODUCTION

Chitin is the second most abundant biopolymer after cellulose [1] and it is also known that chitin and its derivatives are valuable due to their biological activities, industrial and biomedical applications [2]. Chitin is an *N*-acetyl-D-glucosamine (GlcNAc) linked by β-1,4 bonds, which is a homopolymer, can be found primary structural component in the cell walls of fungi, the exoskeleton of arthropods, shells of crustaceans, cuticle of insects and egg-shells of nematodes [3], [4], [5] . Physicochemical characteristics of chitin give impact on its functional properties such as solubility, chemical reactivity and biological activity [6]. Properties of chitin depended mostly on the factors of degree of acetylation, molecular weight, poly dispersity and crystallinity [7]**.** The degree of deacetylation also known as the molar fraction of the deacetylated units in the polymer chain and it is one of the most important factors that effects the properties of chitin such as the solubility and flexibility [8].

β-glucans, chitin and mannans is a carbohydryde polymers in fungal cell walls, where chitin apparently localizes near the plasma membrane [1],[2]. Therefore like to animals, plants also secure an innate immune system to alertness the occurrence of microbial invaders by means of receptors that identify microbial-derived molecules, which can be resistance against microbial infections. In the current study, the ability to *Leucaena leucocephala* (LL) as an alternative chitin source was investigated for the first time by means of determining the chitin characteristics in pods. The chitin is extracted from *LL* due to the factor that it is an abundant source and also a fast growing plant in Malaysia environment and weather. The present amount of chitin available is insufficient to meet the demand that is increasing for the applications in many industries such as medical and biomedical industries [3].

Leucaena leucocephala is from family Mimosaceae [4]. The genus of LL has more than 50 to ascribe to it [5] such as in Malaysia, LL tree is known as "Petai Belalang", and it traditionally known to cure high blood pressure. These wild trees were originally found in Central America and Mexico also spread entirely around the world including Asia and Malaysia [6]. The parts of the plant are edible to animals, including leaves, young stems, flowers, young and mature pods and seeds [7]. Chitin from *LL* plant has been investigated for the chitin activities and presents [8]. It found that the chitin exists in the *LL* seed but there is no further study been discussed on the development of chitin in *LL* as a biopolymer. It is recognized that chitin can be found in the cell wall of fungi and yeast [9]. The chitin content is found in leaf which is a waxy layer that is deposited on the plant surface, the plant cell wall [1], [10]. Chitin is a nitrogenous polysaccharide that has characteristics of tough, hard, inelastic, semitransparent and fibrous [11]**.** However, it is not possible for the chitin to be present in other parts of the *LL* plants as each of the other parts are also made up from plant cells that contain chitin act as the plant defense system again pathogen secreting chitinases.

In this study, the objective is to identify the suitable molarity of hydrochloric acid should be used in the chitin extraction from *LL* pod and the physicochemical properties of chitin from *LL* such as degree of acetylation, thermal analysis, nitrogen content using analytical instruments.

II. METHODS

A. Material

Leucaena Leucophala pods were collected from the local area in Shah Alam and it was dried in the oven dryer for 24 hours at the temperature of 60°C to remove its moisture content. After drying process, the skin pods wer grinded and sieved to obtain small powder.

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B. *Extraction of chitin*

The dried Leucaena leucocephala skin pods powders were soaked with ethanol to remove the chlorophyll and other residues present. Then the resulting samples were extracted using five different concentration; 1 M, 1.5 M, 2 M, 2.5 M and 3 M of hydrochloric acid (HCl) solution. 3 g of dried sample was refluxed with 300 mL HCl with different concentration for 1 hour then washed with distilled water until it reached its neutrality and being filtered. The residue was left dried until constant weight.

C. *Characterization of chitin*

Infrared spectra of *LL* sample were measured with the frequency range 4000-515 cm^{-1} using Spectrum One Fourier Transformed infrared (FT-IR) from Perkin-Elmer. X-ray Diffraction measurements were carried out at 2θ = 5°-45° using Shimadzu X-Ray Diffractometer that was operated with receiving slits at 40 kV and 30 mA. The degree of acetylation (DA) of chitin samples was determined using the data of elemental analysis that was done using the Thermo Fischer Scientific Flash Elemental Analyzer equipment. By following the equation [12], [13] the values of DA are calculated:

$$DA = \frac{\left(\left(C/N\right) - 5.14\right)}{1.72} \qquad (1)$$

where C/N is the ratio carbon/nitrogen as determined by elemental analysis. In addition elemental analysis was recorded using *LL* samples that have been hydrolyzed with HCl from 30°C to 800°C at heating rate of 10°C/min using TGA-851 Metler Toledo TGA analyzer under Proximate analysis.

Differential scanning calorimetry was conducted using Metler Toledo DSC. 10 mg of sample was located into stainless crucible closed by a sample encapsulating press. Samples were heated from 40 to 400°C at 10°C/min.

III. RESULTS AND DISCUSSION

A. *Fourier transform infrared spectroscopy (FTIR)*

The Fourier transform infrared spectroscopy (FTIR) spectra of chitin, bands at 3450, 3262, 3114, 2960, 2930, 2888, 1658, 1628, 1560, 1418, 1382, 1318, 1260, 1204, 1158, 1118, 1074, 1026, 952 and 896 cm^{-1}. The structure is consistence to α chitin [14]. However, Fig. 1.(a) shows only 2960 cm^{-1} is appear for 1M to 3M LL. 1418 cm^{-1} for all LL, 1260 cm^{-1} for 3M very weak peak, 1074 cm^{-1} for 2M and for 1204 cm^{-1} for all LL. However, there are two spectra band that were observed at all samples situated at 2919 cm^{-1} correspond to asymmetric and symmetric of C-H stretching where this indicates the present of chitin in all samples [15] . Fig. 1. (b) 1M LL, 1.5M LL, 2M and chitin displays strong IR spectra indicating strong carbonyl (C=O) at 1737 cm^{-1} while 2.5 M and 3M shows the weak peaks. While all LL shows existence of C-O at 1217 cm^{-1} however chitin shows strong peaks. Based on these bands, it indicates the process of acetylation of chitin extracted from *LL* [16]. From the results it can conclude that there is 3M show the similarity to the chitins' chemical composition and bonding types.

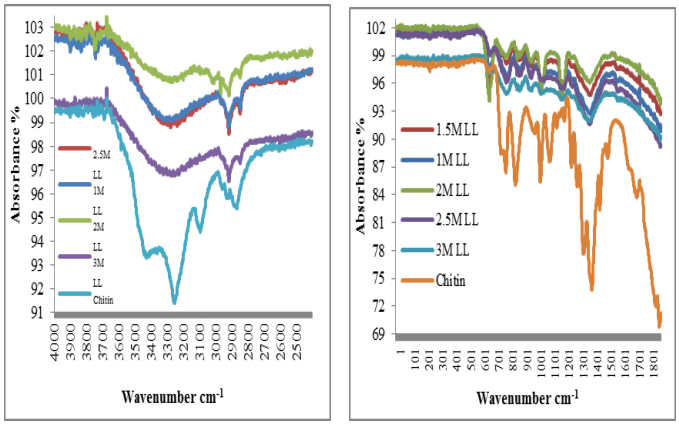

Fig. 1. Wavelength of LL and chitin from (a) 4000 cm^{-1} to 2450 cm^{-1} (b) 1 cm^{-1} to 1831 cm^{-1}

B. *X-ray diffraction (XRD)*

XRD analysis was applied to detect the crystallinity of the isolated chitin in the LL sample. In this study, the crystallinity structure of samples extracted using different concentration of acid; 1, 1.5, 2, 2.5 and 3 M with fixed volume and duration of time was investigated. Depending on the concentration of the hydrochloric acid used in the extraction process, different XRD patterns were observed. The result for LL and chitin shows in Fig 2 was obvious peaks 2θ at 9◦ and 19◦ in their crystallinity structure. [17] also found that XRD pattern of purified crab chitin showed two crystalline peaks at 9◦ and 19◦. These peaks are observed of α-chitin[18] . This is also proven from the other study where it has been identified that the α chitin has 4 peaks situated at 9.6°, 19.6°, 21.1° and 23.7° [18]. In addition, [19] also state that the peaks of XRD of α chitin were not only situated at the 4 peaks but also at the range of 12-13° and also at the 26°.

C. *Elemental analysis*

The value for degree of acetylation (DA) of the LL samples was determined from the data of elemental analysis and is shown as in the Table 2. Generally, in protein and chitin has nitrogen content [20]. Proteins are bound by covalent bonds to the chitin through aspartyl or histidyl residues or both forming stable complexes such as glycoproteins. The nitrogen content decrease from 3.7950% to 3.3420 % for 3M to 1M due to removal of protein content therefor is recognized to the increase in nitrogen percent which approve the appearance of chitin [21]. The nutrigen content of chitin in other research was reported between 2.96 and 6.8% [22], [23] . As this value is close to 6.89%, it exhibits the chitin's purity [24]. LL for

3M shows the highest nitrogen content however it far from the commercial chitin which is at 6.2635%. Due to this factor, it is important to determine the nitrogen content in the LL samples being used in this study to verify the present of chitin thus proving the objective of this study.

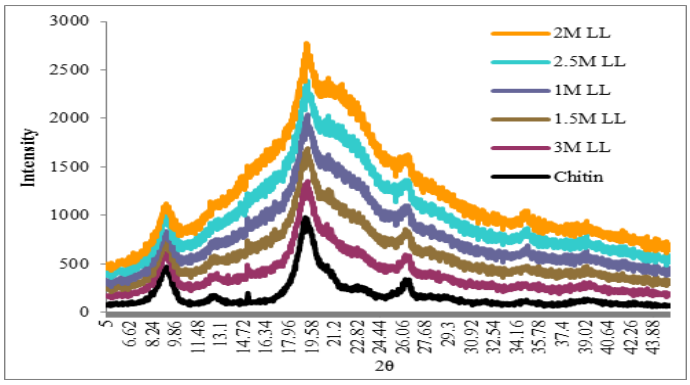

Fig. 2. XRD patterns of a-chitin and *Leucaena leucocephala* (LL) with different molarity

The LL sample being extracted using 3.0 M of hydrochloric acid shows the furthest value when compared to the DA value of reference sample of chitin. The typical degree of acetylation of chitin 0.90 [11]. The pure chitin if the DA value was assumed as 1[25]. If the DA value is more than 1, this indicates that there are mineral residues in the structure and removal of some inorganic is incomplete [20]. By considering this reason, there are mineral residues that could not be removed completely in chitin structure from LL undergo acid hydrolysis. In this study, the degree of acetylation of chitin obtained is between 1.7 until 3.4 of the different samples. This may be due to the reason that the typical sample of chitin is originated from the vertebrates whereas in this study, plant originated chitin samples are used and other mineral residues exist.

TABLE I
THE ELEMENTAL ANALYSIS AND THE CORRESPONDING OF DEGREE OF DEACETYLATION

Sample	Content (%)			C/N	DA
	N	C	H		
Chitin	6.2635	42.9154	6.9395	6.8516	0.9951
1M LL	3.3420	27.0727	5.5964	8.100	1.7213
1.5M LL	3.3517	29.1015	6.0849	8.6826	2.0596
2M LL	3.3570	30.0133	6.2952	8.9405	2.2096
2.5M LL	3.4619	34.3373	6.3952	9.9186	2.7782
3M LL	3.7950	41.9522	6.4945	11.0546	3.4387

D. Thermal analysis

Thermal stability, which can be analyzed using the TGA techniques is a critical factor for determining the potential applications of chitin and its derivatives [26]. For dissociation of its crystalline structure chitin needs high thermal energy [27]. Polysaccharides is easily to hydrate due to amorphous structure in solid state [28]. [29] proved that hydration properties of polysaccharide macromolecules could reflect its polysaccharide composition and crystalline structure. The hydration behavior of chitin could contribute to its thermal properties and were evaluated using the TGA techniques.

In the LL thermogram , Fig. 3. shows three decomposition steps. However, for chitin showed two major peaks, which presented peaks on the DTG, peaks at 80ºC and 389.5ºC. The chitin and *LL* have similar results which is the interval between 0 and 100 C, the peak is recognized to water evaporation [30]. The second peaks created at shows range from 335 to 340 °C. contributed to cleavage of glyosidic amine units by dehydration or deamination [21].

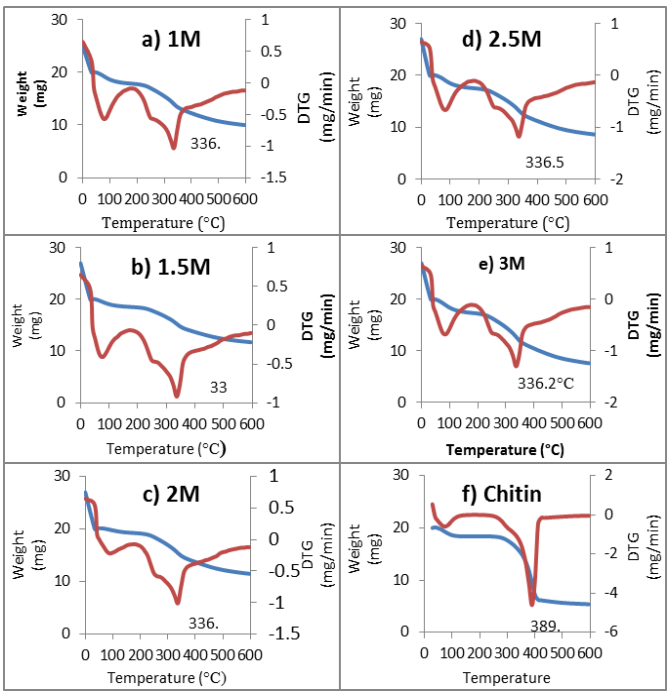

Fig. 3. Comparison of TGA analysis for chitin and *Leucaena leucocephala* LL for 1M to 3M

The temperature at which the maximum degradation (DTGmax) of LL occurred was at 336.5ºC. Chitin from *Leucaena leucocephala* (LL) showed the lower thermal stability since it started at 336.5ºC. This is due to *Leucaena leucocephala* (LL) is a plant based, therefore the thermal stability is lower than chitin from animal based. DTG max for *Ganoderma* lucidum fungus showed has lower thermal stability since the degradation starts at 313.57 ºC [15].

In previous studies, the DTGmax value of isolated chitin from shrimp shells was examined around to 330ºC [21] were quite similar to another. However, for this present study commercial chitin the thermal degradation temperature is quite high which is at 389.5 ºC. The DTGmax value was recorded

from various source recorded between 350 and 390 °C[[24],[12], [31] [18]]. However for the black coral's value of DTG disintegration temperature of α chitin was approximately 300°C and chitin used in this study as reference showed DTG value at 398.5°C. Hence, this verified that the DTG disintegration temperature of α chitin structures extracted from different organisms are different. The uses of chitin can be determined based on the disintegration temperature of chitin [32]. It can be observed that above 500ºC the percentage of residual mass remained constant and no weight loss.

The degradation temperature of chitin molecules extracted from LL shows the chitin extracted from plants are degraded in lower temperature compared to the chitin sample used as reference that originated from the aquatic organisms. The disintegration temperature of chitin from aquatic organisms based on other studies also displayed a higher range of temperature [33]. This may be happen due to reason that the acetylated polymer units of chitin molecules originated from aquatic organisms are durable compare to the chitin originated from plant. The thermal stability of the extracted chitin from LL samples fell in the order 3 M > 2.5 M > 1.5 M >2 M > 1M of LL. We observed that that all extracted chitin structures from LL are thermally stable.

IV. CONCLUSION

In conclusion, it was found that *Leucaena leucocephala* contain the biopolymer called chitin after undergo extraction via acid hydrolysis of hydrochloric acid. The degree of acetylation, thermal analysis, nitrogen present was being studied as the physicochemical characteristics of chitin. While the suitable molarity of hydrochloric acid used in acid hydrolysis were also been identified and it was found that the most suitable molarity of hydrochloric acid to be used in the acid hydrolysis to extract chitin from LL in this study is at 3 M where it displays most of the physicochemical characteristics of chitin in LL.

Analytical instruments such as FTIR, TGA, XRD and elemental analyzer were successfully used to investigate the physicochemical characteristics of chitin extracted from LL samples.

Although there are several papers which are the chitin from different sources however in the literature consulted, nothing about chitin obtained. This paper presents research results currently innovative, because all the technique used for processing and material manufacturing chitin match to chitin extracted from animal based.

Hence, chitin originated from LL can be used as biopolymer, an alternative of synthetic polymer that are more environmental friendly, less polluted and able to be used in many applications. In addition, it may also meet the demand of supplying chitin thus ensuring sufficient amount of chitin needed.

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