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Research paper

Extraction and Characterization of Chitin from Leucaena Leococephala

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Abstract

In this research, a new source of chitin was proposed. Chitin was extracted leucaena leucocephala pods using NaOH and HCl solutions for deproteination and demineralisation, respectively. The purposes of this research were to characterize chitin by extraction of LL pods at different length and aging with 6M HCL and 12M of NaOH by using TGA and elemental Analysis. Thermal property characterization of chitin from leucaena leucocephala pods (LL) extraction was obtained by using thermogravimetric analysis (TGA) and elemental analysis. The TGA and Elemental Analysis then tested the prepared samples for the thermal property. For TGA, the results show development stage at LL (Set 6) has the highest thermal stability. However, for elemental analysis, the results show that the degree of deacetylation is the highest for carbon-nitrogen ratio at young LL because of high protein content. Furthermore, it was found that the DA decreased with an increasing thermal property.

Keywords: *chitin; elemental analysis; leucaena leucocephala pods; TGA*

1. Introduction

Chitin is a long-chain polymer of an N-acetylglucosamine, a derived of glucose, and can be found in many places throughout the natural world. The structure of chitin is comparable to the polysaccharide cellulose, forming crystalline nano fibrils or whiskers. Chitin is the second most abundant polymer in nature, which is osmotic tensile strength and stability to countless rigid and exoskeletons. Usually physically appearance of chitin inelastic, white and hard nitrogenous polysaccharide [1]. Commercially, source of chitin such as agaricusbisporus mushroom [2] and fish bone [3]. Beside that chitin has showed versatile for several medicinal, industrial, and biotechnological purposes.

Different polymorphs of chitin are found in nature, the α-chitin being the most prevalent structure and resultant to a tightly compacted orthorhombic cell composed by alternated sets of parallel and antiparallel chains [4]. The β-chitin adopts a monoclinic unity cell where the polysaccharide chains are thrown out in parallel fashion and albeit the structure of γ-chitin has not be present consummately recognized, an preparation of one antiparallel and two parallel sheet has been suggested [5]. α-Chitin is usually insulated from the exoskeleton of crabs , crustaceans and concretely from shrimps and. β-Chitin can be detached from squid pens, and γ-chitin from yeast and fung.

Based on the earlier studies, most of the research focuses on the extraction of chitin from animal sources. In animal, chitin is a major constituent of the external skeleton, exoskeleton, of many arthropods such as, spiders, crustaceans and insects. There are studies on extraction of chitin from fish scales [6] , crab, shrimp, and insect. The process of extracting chitin from these animals includes demineralization and deproteinization of the raw materials with strong acids (HCl and bases (NaOH). However, limited potential for industrial acceptance such as the seasonal and limited supply of the raw material in the final product, thereby an alternative source need to cater for this problem. The use as an alternative nutritional source can favor earning a byproduct of value added.

Moreover, the chemical chitin purification is extremely hazardous, energy consuming and threatening the environment. The use of animal sources also require additional steps such as mechanical grinding, demineralization with acid and deproteination with alkali which can cause depolymerization that can affects the properties such as molecular weight, viscosity, and degree of acetylation of chitin[7]. While in plant, there are also studies on extraction of chitin. Chitin is a main constituent of fungal cell walls and has been expectable as a common elicitor of plant protection.

Leucaena leucocephala is a well-known tree since 1970s and early 1980s. Because of its worldwide success as a long lived tree which is highly in nutrition forage tree, it was known as miracle tree. Not only limited to that, Leucaena has its great variety of uses such as provide firewood, timber, human food, green manure, shade and erosion control (Brewbaker and Sorensson, 1990).Leucaena leucocephala is a perennial thornless, tropical plant, with a height of around 8 m that is natural to tropical America and belongs to family Fabaceae and subfamily Mimosoidea. Usually parts of Leucaena (seeds, leaves, pods and barks) have been used in for glycerol extraction [8], galactomannan extraction [9], mimosin extraction [10]

There are studies on extraction of chitin from root beet, grass and many more. Leucaena leucocephala pods (LL) is an alternative chitin source. At present, usually leucaena leucocephala pods can allocate timber, green manure, and erosion control [11]. Some

promoters tout leucaena leucocephala pods as a phytoremediator [12] and ruminant animals [13]. However, a study on extraction of chitin from LL has not been reported and yet it is believed that LL is a potentially plant because of it is an invasive species and now considered unwanted species, growing in arid, car parks, roadside areas, and abandoned land .

LL known as "Petai Belalang" is a fast-growing, small, mimosoid tree innate in northern Central America and southern Mexico. LL is used for a variability of purposes, such as fiber, firewood and livestock fodder. For its multiple uses, it was promoted as a "miracle tree". It has also been described as a "conflict tree" in that it is both promoted for forage production and spreads like a weed in some places. Most importantly, it does not only offer as a source of quality animal feed, but also for residual use for firewood or charcoal production (note: incomplete sentence). LL has been considered for biomass production. LL showed high chitinase activity relative to other tropical plants.

In this study, the objective is to characterize chitin by extraction of LL pods at different aging with 6M HCL by using thermagravimetric analysis (TGA) and elemental analysis.

2. Materials and Method

2.1. Materials

Leucaena leucocephala pods was obtained from Batu Pahat, Johor region, south coast of Malaysia and the seeds were separated from the pods. The samples were dried by using oven for 2 days to resulting in the moisture content 4.2 wt% that is. The pods have been divided into 6 sets of samples depending on the length of the pods as shown in Table 1 and Fig. 1. The samples were naturally dried in the atmosphere (25 ◦C) for five days resulting in the moisture content that is presented in Section 3.1. The samples were later ground with a milling machine to fine powder with size less than 125 micrometers. To get the size, the samples were sieved by using siever at size of 125 micrometers. The chemicals used in this pretreatment study were of analytical grade purchased from Sigma–Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Table 1: Different size of the samples

Samples	Length of the pod (cm)
Set 1	7-13.5
Set 2	14-14.5
Set 3	15-15.5
Set 4	16-16.5
Set 5	17-17.5
Set 6	18-20

Fig. 1: Six different ages of *Leucaena Leucochephala* pods

2.2. Extraction of chitin

Leucaena leucocephala pod was extracted with 6M of HCl with ratio of 1:10 (3g of Leucaena Leucocephala: 15ml of 6M HCl). The samples were extracted for 48 hours and stirred by magnetic stirrer and hot plate at room temperature to extract chitin from LL. The mixture was filtered to get clear solution. Then the samples were treated with 12M of NaOH drop by drop until pH 7. After de-proteinization, the chitin was dried for 2 days at temperature of 50°C by the oven. It was then blended by using blender and kept for use.

2.3. Characterization of chitin

2.3.1. Thermogravimetric analysis (TGA)

The thermogravimetric analyses were carried out by nitrogen gas in a TGA-851 Metler Toledo TGA analyzer. The samples were heated from 50°C to 600°C at the desired heating rate β (10°C/min). The loss of weight was monitored, allowing the calculation of the extension of conversion as a function of the reaction time. The DTG curves were used to determine the rates of degradation of chitin and chitosan versus the extent of conversion α.

2.3.2. Elemental analysis

C/N is the ratio carbon/nitrogen as determined by elemental analysis. Elemental analysis was recorded using LL samples from 30°C to 800°C. Differential scanning calorimetry was conducted using Metler Toledo DSC. 10-15 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.

The degree of acetylation (DA) [14] 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, the values of DA are calculated:

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

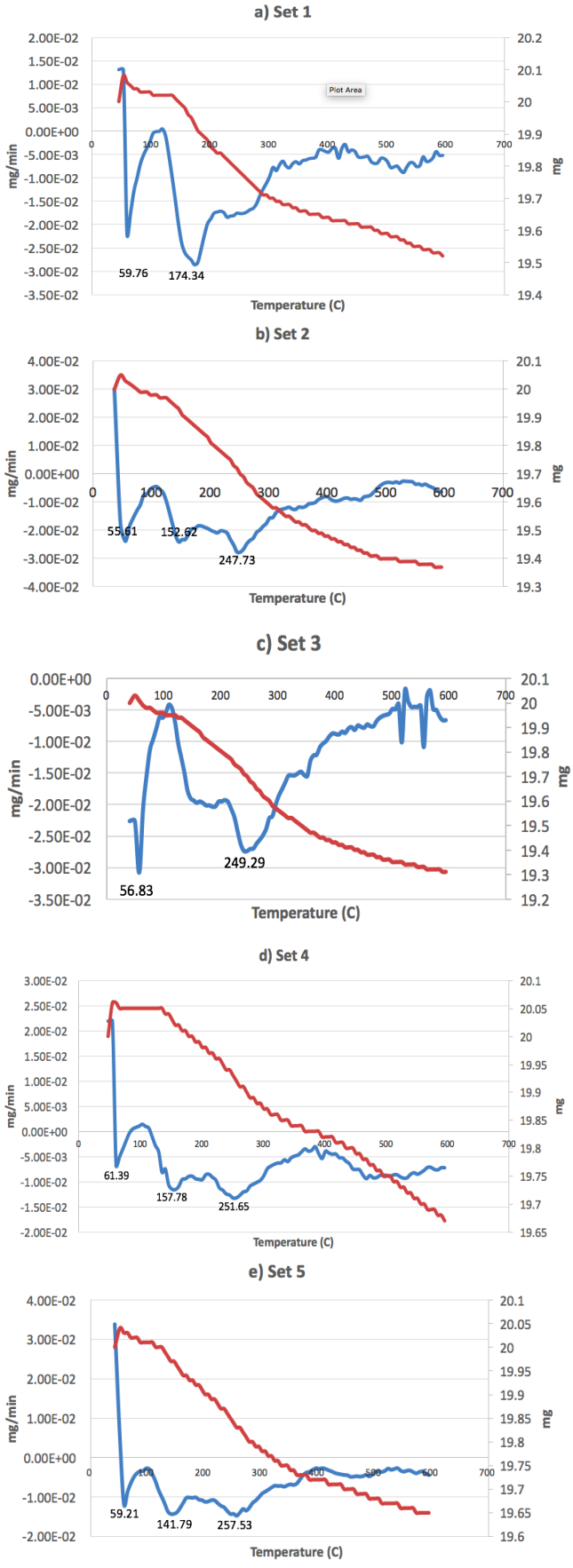
3. Results and Discussion

The thermogravimetric analyses were carried out by nitrogen gas in a TGA-851 Metler Toledo TGA analyzer and the comparison is shown in Fig. 2. The samples were heated from 50°C to 600°C at the desired heating rate β (10°C/min). The loss of weight was monitored, allowing the calculation of the extension of conversion as a function of the reaction time. The DTG curves were used to determine the rates of degradation of chitin and chitosan versus the extent of conversion α.

Moreover, the maximum temperature degradation (TGAmax) of LL occurred was at 496.56°C [15]. For Set 6 showed the highest thermal stability while Set 1 showed the lowest thermal stability since it started at 174.34°C, because of the stable structure of the chitin of set 6 represent old LL compared to Set 1 represent young LL. In earlier studies, the TGAmax value for chitin is between 300-450°C generally. Therefore, only Set 6 suited the criteria for chitin.

In previous studies, the TGAmax value of isolated chitin from shrimp shells was examined around the first one (44–102°C) with 10-15% weight loss, corresponds to the evaporation of physically adsorbed and strongly hydrogen bonded water to chitin and chitosan [15]. The second weight losses, occurring in the range 250–400°C, were 65 % and 50% for chitin, respectively, and were caused by depolymerisation/decomposition of polymer chains through deacetylation and cleavage of glycosidic linkages. The last stage, for temperature more than 500°C which is (10 and 15% weight loss) due to the breakdown of the residual carbon in thermal destruction of pyranose ring and. The quantitative determination of the degradation products by TGA in the temperature range 250–450°C, which assigned the total weight loss, where the amino groups on the glucosamine structure of chitin can be divided in two different methods through ammonia release and heteroaro-

matic rings formation, which defined the deprivation of the bi-opolymer in breake down of C-O-C skeletal bonds [16].



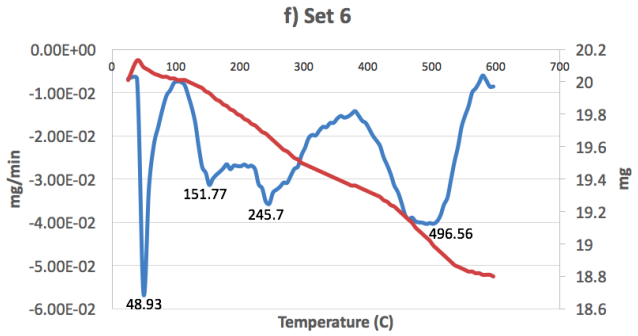


Fig. 2: Comparison of TGA analysis for chitin and Leucaena leucocephala LL for different length

The peak temperature and height varied with the heating rate according to the kinetic parameters. The shifting profile of the peak temperature with the heating rate was related to the activation energy, while that of the peak height was cramped by the reaction order together with the activation energy [5].

Elemental analysis of chitins from LL comprising the nitrogen, carbon and hydrogen contents and C/N ratio are shown in Table 2. Theoretically, the DA and N values of chitin 100% and 6.89% respectively [17]. In the present study, set 6 were observed closer to the theoretical value. It is known that the molecular the DA value is more than 100% there are mineral residues in the structure [17]. These results revealed that there are mineral residues that could not be totally removed in the Leucaena leucocephala pods. After purification with HCl and NaOH LL1 to LL6 were high in their nitrogen, carbon and hydrogen contents, as well as N/C ratios.

From chitin, the nitrogen contents of the sample come mainly from protein and chitin. Proteins closely associated with chitin. In the recovery of chitin from shrimp waste, associated proteins and minerals should be removed [19].

After alkaline treatment, the protein was removed [18]. Proteins are bound with covalent bonds to the chitin through aspartyl [5] or histidyl [19] residues or both forming stable complexes such as glycoproteins [20]. The carbon-nitrogen analysis with degree of deacetylation may be attributed to the nature of the raw material used, its immediate environment, and also the methods applied during the processes.

Table 2: Elemental Analysis of Degree of Deacetylation

Length (cm)	Content (%)			C/N	DA
	Nitrogen	Carbon	Hydrogen		
7 – 3.5	0.1823	3.2807	0.3619	17/9962	7.4745
14 – 14.5	0.2237	3.4931	0.4096	15.6151	6.0902
15 – 15.5	0.3012	3.1064	0.4451	10.3134	3.0078
16 – 16.5	0.3310	3.5457	0.5380	10.7121	3.2396
17 – 17.5	0.3849	4.2101	0.5300	10.9382	3.3710
18 – 20	0.6181	4.4230	0.6181	7.1558	1.1720

From Table 2, it was found that the DA% decreases as the LL1 to LL6. It seems that development stage has effect to DA value DA value of pure chitin was assumed as 1 based on previous studies. 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 [15]. The nearest DA value to 1 is in Set 6 which is 1.1720. This indicates that the chitin content in Set 6 is at the most compared to Set 1 found to be having the least. Due to this factor, the nitrogen content in the LL samples being used in this study was determined and represented in Fig. 3. The purpose is to verify the presence of chitin and thus meeting the objective of this study. It seems that the chitin from leucaena leucocephala pods is relatively lower than the other forms of chitin.

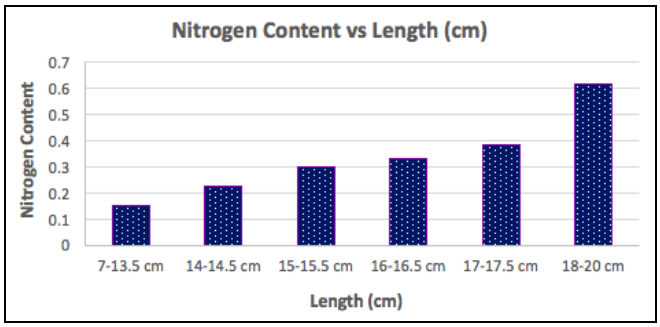


Fig 3: Nitrogen content for chitin and Leucaena leucocephala LL for different length

The highest DA of the compound is 7.4745. However, these higher DA values are the consequence of the high amount of protein, yielding high quality of chitin suitable for the pharmaceutical application. DA affects the chemical, physical, and biological properties of chitosan, such as adsorption, covalent linking, encapsulation.

3.1. Chromameter test

Table 3 shows the coordinates of the sample obtained from chromameter test. From the coordinates obtained, it can be concluded that the different aging of the sample gives distinct colour characteristic. As the age is increase, the lightness of the sample will decrease because aging cause the surface of the samples become darker in colour. Red/ green coordinates, a* shows a descending value due to the green colour of samples decrease respect to aging. As for the yellow/blue colour, the coordinates show an increasing value due to the samples become more yellowish colour as the sample aging.

Table 3: Coordinates of the samples in Chromameter test.

Sample	L*(lightness)	a*(red/green)	b*(yellow/ blue)
A	L*=60.23	a*=-4.71	b*=7.16
B	L*=52.47	a*=-11.49	b*=17.59
C	L*=51.39	a*=-16.83	b*=32.59
D	L*=55.21	a*=-19.61	b*=36.01
E	L*=52.77	a*=-13.28	b*=30.39
F	L*=35.53	a*=11.87	b*=11.52

4. Conclusion

In conclusion, chitin can be extracted from both animals and plants. But the difference is on the chitin content and the mineral residue. Extraction of chitin from LL at different pods length is by extraction (6M HCl) and neutralization (12M NaOH). This process involved HCl and NaOH of very high molarity. Polymer degradation might have occurred if the process takes places too long or at very high temperature. Therefore, all the samples need to be monitored closely. It was also that the most suitable length of pods to extract chitin from LL in this study is associated with Set 6 (18-20 cm) (at where it characterized most of the characteristics of chitin in LL, note: rephrase; not clear). Further research can be done by varying some parameters for the characterization process such as temperature, time taken for the extraction process, and ratio of LL (g) to HCl (ml) of the extraction.

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