Extraction and Characterization of Chitin from Leucaena Leucocephala

Shareena Shahira Binti Tajul Arus, Noor Harliza Binti Abd Razak

Faculty of Chemical Engineering, Universiti Teknologi Mara

***Abstract*—Thermal property characterization of chitin from Leucaena Leucocephala (LL) extraction was obtained by using Thermogravimetric analysis (TGA) and Elemental Analysis. 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. By using the instruments, the thermal property is determined. The samples are prepared by chemical process, which are demineralization and deproteinization. The prepared samples then were tested for the thermal property by the TGA and Elemental Analysis. For TGA the results showed at old LL (Set 6) has the highest thermal stability. However, for Elemental Analysis, the results showed that the degree of deacetylation for carbon-nitrogen ratio at young LL was the highest because of high protein content.**

I. INTRODUCTION

Chitin is a long-chain polymer of an N-acetylglucosamine, a derivative of glucose, and is 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, providing the osmotic stability and tensile strength to countless cell walls and rigid exoskeletons. In terms of function, it may be compared to the protein keratin. Chitin has proved 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 corresponding to a tightly compacted orthorhombic cell composed by alternated sets of parallel and antiparallel chains. 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 been consummately identified, an arrangement of two parallel and one antiparallel sheet has been proposed. α-Chitin is by far the most abundant and is conventionally isolated from the exoskeleton of crustaceans, concretely from shrimps and crabs. β-Chitin can be extracted from squid pens, and γ-chitin from fungi and yeast.

From present study, most of the research focus on the extraction of chitin is from animal sources. In animal, chitin is a major constituent of the exoskeleton, or external skeleton, of many arthropods such as insects, spiders, and crustaceans. There are studies on extraction of chitin from fish scales, crab, shrimp, and insect. The process of extracting chitin from these animals include demineralization and deproteinization of the raw materials with strong acids (hydrocloric acid) and bases (sodium hydroxide).

While in plant, there are also study on extraction of chitin. Chitin is a major component of fungal cell walls and has been recognized as a general elicitor of plant defense. There are studies on extraction of chitin from root beet, grass and many more. Leucaena leucocephala (LL) is an alternative chitin source. However, the study on extraction of chitin from LL have not be done yet it is the most suitable plant because of it is an invasive species and now considered unwanted species, growing in arid, roadside areas, car parks, and abandoned land.

LL known as "Petai Belalang" is a small, fast-growing mimosoid tree native northern Central America and southern Mexico. LL is used for a variety 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 as a source of quality animal feed, but also for residual use for firewood or charcoal production. LL has been considered for biomass production. LL showed high chitinase activity relative to other tropical plant.

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.

II. METHODOLOGY

A. Materials

Leucaena Leucocephala was collected from Batu Pahat, Johor region, south coast of Malaysia and the seeds were separated from the pods. The pods were dried by using oven for 2 days to remove the moisture content. The pods have been divided into 6 sets of samples depending on the length of the pods.

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

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.

B. Extraction of chitin

The prepared samples were 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 deproteinization, 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.

C. Characterization of chitin

Thermogravimetric analysis (TGA)

The dynamic degradation studies for the samples were carried out by nitrogen gas in a TGA/DTA. 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. The measuring accuracy of sample temperature was checked by the onset fusion temperatures.

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 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. 10-15 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) 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:

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

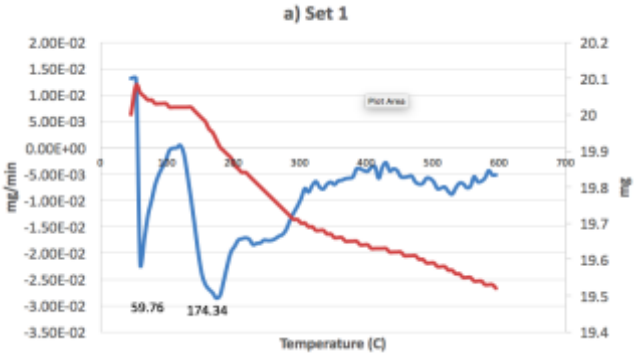
III. RESULTS AND DISCUSSION

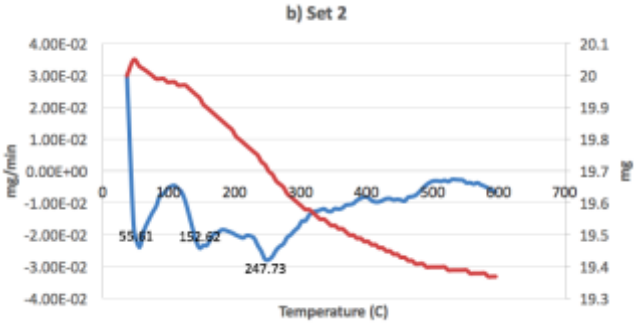
A. Thermogravimetric analysis (TGA)

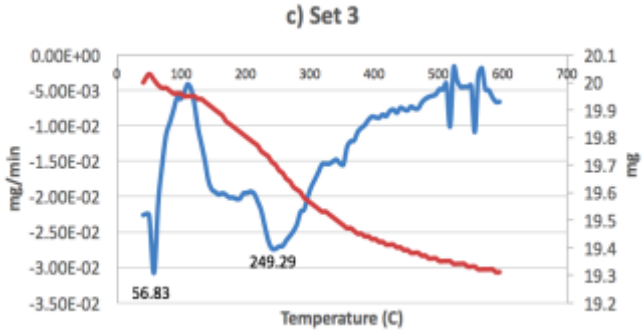
TGA can be used to evaluate the thermal stability of a material. In a desired temperature range, if a species is thermally stable, there will be no observed mass change. Negligible mass loss corresponds to little or no slope in the TGA trace. TGA also gives the upper use temperature of a material. Beyond this temperature, the material will begin to degrade.

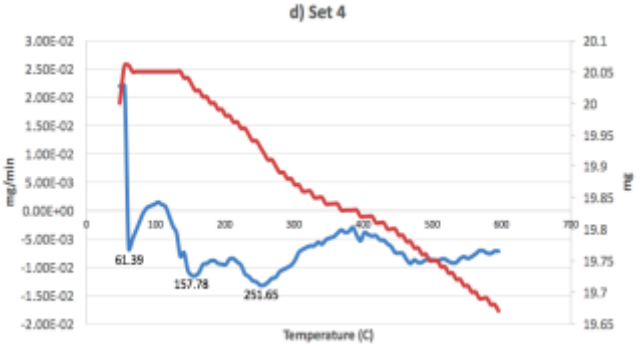
Thermal stability, which can be analyzed using the TGA techniques is a critical factor for determining the potential applications of chitin and its derivatives. For dissociation of structure chitin needs high thermal energy. Polysaccharides is easily to hydrate due to amorphous structure in solid state 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 graph, shows decomposition steps. However, for Set 1 and Set 3 showed two major peaks, which presented peaks on the TGA. The first Peak is recognized to water evaporation. The second peaks created at later contributed to cleavage of glyosidic amine units by dehydration or deamination. The length of Set 2, Set 4 and Set 5 have similar results which were three major peaks. But for Set 6 showed four major peaks. The third and fourth peaks corresponds to the thermal destruction of pyranose ring and the decomposition of the residual carbon.









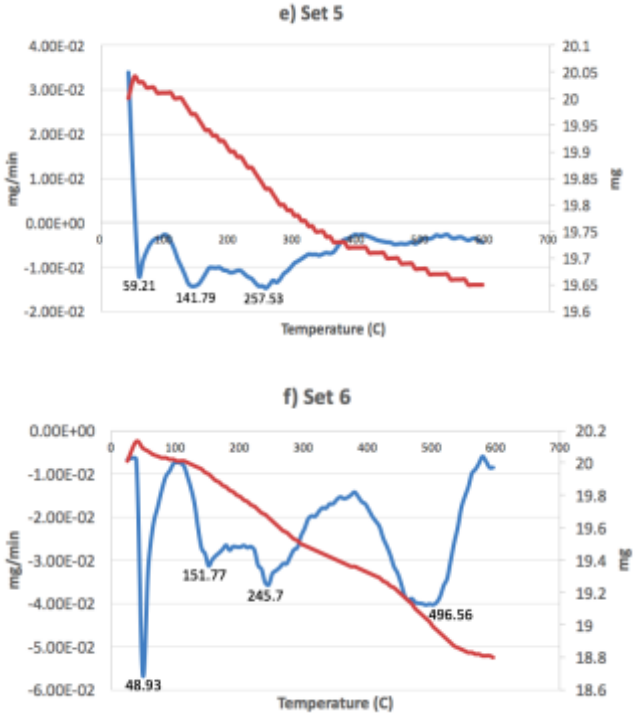


Figure 1. Comparison of TGA analysis for chitin and Leucaena leucocephala LL for different length

The temperature at which the maximum degradation (TGAmax) of LL occurred was at 496.56°C. 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. 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. 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 higher than 500°C (10 and 15% weight loss), corresponds to the thermal destruction of pyranose ring and the decomposition of the residual carbon. On their quantitative determination of the degradation products by TGA in the temperature range 250–450°C, they assigned the total weight loss, where the amino groups on the glucosamine structure of chitin can be released in two different ways via ammonia release and via heteroaromatic rings formation, confirming that the degradation of the biopolymer can take place by random breaking of C-O-C skeletal bonds (R. M. Abdel-Rahman, 2015).

The functional relationship between the peak temperature and height was closely related to the activation energy and reaction order, but rarely affected by the pre-exponential factor and heating rate. Determining peak temperature and height at a heating rate was enough to characterize a thermal reaction of first-order kinetics or known reaction order, but was insufficient to specify a reaction without knowing the shifting pattern of either peak temperature or peak height with heating rate. The peak temperature and height varied with the heating rate according to the kinetic parameters. The shifting pattern of the peak temperature with the heating rate was related to the activation energy, while that of the peak height was confined by the reaction order together with the activation energy (Seungdo Kim, 1995).

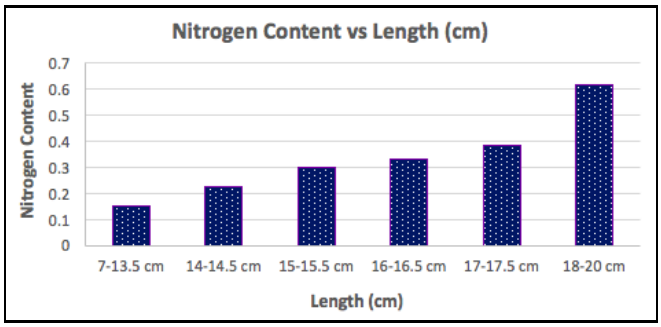
B. Elemental Analysis

Elemental analysis of chitins from LL including the carbon, nitrogen and hydrogen contents and C/N ratio are shown in Table 4.1 From chitin, the nitrogen contents of the sample come mainly from protein and chitin. Chitin, indicating the minimum amount of protein left. Proteins are bound by covalent bonds to the chitin through aspartyl or histidyl residues or both forming stable complexes such as glycoproteins. 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

	Content (%)				
Length (cm)	Nitrogen	Carbon	Hydrogen	C/N	DA
7-13.5	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

The pure chitin of the DA value was assumed as 1 by the 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. The nearest DA value to 1 is Set 6 which is 1.1720. That indicates the chitin content in Set 6 is at most compared Set 1 which is at least. 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.



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.

IV. 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 by 6M HCl and neutralization by 12M NaOH. This process involved HCl and NaOH of very high molarity. Polymer degradation might have occurred if the process take places too long or at very high temperature. Therefore, all the samples need to be monitored closely.

The degree of acetylation, thermal analysis, nitrogen present was being studied. The suitable length of pods was also being identified and it was found that the most suitable length of pods to extract chitin from LL in this study is Set 6 (18-20 cm) at where it characterized most of the characteristics of chitin in LL.

Further research can be done by Characterizing with different temperature use for the process, different time taken for the extraction process and with different ratio of LL (g) to HCl (ml) of the extraction.

ACKNOWLEDGMENT

Thank you to my supervisor, Madam Noor Harliza Abd Razak for the guidance and support throughout my project. Thank you to Universiti Teknologi Mara for the supplying me all the chemicals and equipments.

References

[1] Tang, WJ; Fernandez, JG; Sohn, JJ; Amemiya, CT. "Chitin is endogenously produced in vertebrates". *Curr Biol*. 25: 897–900.

[2] Hofmann (1929) "Über den enzymatischen Abbau des Chitins und Chitosans" (On the enzymatic degradation of chitin and chitosan), Ph.D. thesis, University of Zurich (Zurich, Switzerland).

[3] P. Karrer and A. Hofmann (1929) "Polysaccharide XXXIX. Über den enzymatischen Abbau von Chitin and Chitosan I," *Helvetica Chimica Acta*, 12 (1): 616-637. Nathaniel S. Finney and Jay S. Siegel (2008) "In Memorian: Albert Hofmann (1906-2008)," *Chimia*, 62 (5): 444-447.

[4] Campbell, N. A. (1996) *Biology* (4th edition) Benjamin Cummings, New Work.

[5] A. Acosta, C. Junenez, V. Borau, A. Heras (1993). Extraction and characterization of chitin from crustaceans. Biomass and Bioenergy, 5 (2) (1993), pp. 145–153.

[6] [6] Paulino et al., 2006 Alexandre T. Paulino, Julliana I. Simionato, Juliana C. Garcia, Jorge Nozak Characterization of chitosan and chitin produced from silkworm crysalides Carbohydrate Polymers, 64 (2006), pp. 98–103.

[7] Brugnerottoa et al., 2001 J. Brugnerottoa, J. Lizardib, F.M. Goycooleab, W. ArguÈelles-Monalc, J. DesbrieÁresa, M. Rinaudo. An infrared investigation in relation with chitin and chitosan characterization Polymer, 42 (2001), pp. 3569–3580.

[8] Arora, Naresh, Bindiya Sachdev, Rani Gupta, Y. Vimala, and Raj K. Bhatnagar. 2013. "Characterization of a Chitin-Binding Protein from Bacillus Thuringiensis HD-1" edited by R. Subramanyam.

[9] Bueter, Chelsea L., Charles A. Specht, and Stuart M. Levitz. 2013. "Innate Sensing of Chitin and Chitosan" edited by J. Heitman.

[10] Dutta, Pradip Kumar, Joydeep Duta, and V. S. Tripathi. 2004. "Chitin and Chitosan: Chemistry, Properties and Applications." *Journal of Scientific and Industrial Research* 63(1):20–31.

[11] Ekpenyong, T. E. 1986. "Nutrient and Amino Acid Composition of Leucaena Leucocephala (Lam.) de Wit." *Animal Feed Science and Technology* 15(3):183–87.

[12] Kumari, Suneeta and Pradip Kumar Rath. 2014. "Extraction and Characterization of Chitin and Chitosan from (Labeo Rohit) Fish Scales." *Procedia Materials Science* 6:482–89.

[13] Liu, Shaofang et al. 2012. "Extraction and Characterization of Chitin from the Beetle Holotrichia Parallela Motschulsky." *Molecules* 17(4):4604–11.

[14] Meena Devi, V. N., V. N. Ariharan, and P. Nagendra Prasad. 2013. "Nutritive Value and Potential Uses of Leucaena Leucocephala as Biofuel - A Mini Review." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4(1):515–21.

[15] Mittal, Neeraj, Pooja Mattu, and Gurpreet Kaur. 2016. "Extraction and Derivatization of Leucaena Leucocephala (Lam.) Galactomannan: Optimization and Characterization." *International Journal of Biological Macromolecules* 92:831–41.

[16] Nehdi, Imededdine Arbi, Hassen Sbihi, Chin Ping Tan, and Saud Ibrahim Al-Resayes. 2014. "Leucaena Leucocephala (Lam.) de Wit Seed Oil: Characterization and Uses." *Industrial Crops and Products* 52:582–87.

[17] Nexo, Ebba et al. 2002. "Holo-Transcobalamin Is an Early Marker of Changes in Cobalamin Homeostasis. A Randomized Placebo-Controlled Study." *Clinical Chemistry* 48(10):1768–71.

[18] R. M. Abdel-Rahman, R. Hrdina, A. M. Abdel-Mohsen, M. M. G. Fouda, A. Y. Soliman, F. K. Mohamed, K. Mohsin, and T. D. Pinto, "Chitin and chitosan from Brazilian Atlantic Coast: Isolation, characterization and antibacterial activity.," Int. J. Biol. Macromol., vol. 80, pp. 107–20, Sep. 2015.