PHYSICOCHEMICAL ANALYSIS OF CHITIN BY EXTRACTION OF LEUCAENA LEUCOCEPHALA PODS WITH HCl

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***Abstract*— The objective of this study is to characterized chitin by extraction of wild Leucaena Leucocephala pods at different aging with 6M HCl by using Fourier Transform Infrared (FTIR). Leucaena Leucocephala is chosen to be used in this study because it is abundantly and can be found easily along the road as it is widely spread in Malaysia and available throughout the year. Leucaena Leucocephala is not fully utilized yet and it could be a new option and new resource for chitin. Results from FTIR shows that Amide I band in Leucaena leucocephala before and after extraction does not divided into two peaks which make it appear close to a β-chitin. Beside, Amide I band of Leucaena leucocephala before and after extraction is appearing wide (U-shaped) rather than sharp. From the results obtained, the chitin from Leucaena leucocephala is determined to be in the form of β-chitin.**

Keywords*— Chitin, Extraction, Fourier Transform Infrared (FTIR), Leucaena leucocephala.

I. INTRODUCTION

Chitin is the second most abundant biopolymer after cellulose. It is naturally abundant, biodegradable and renewable polymers. Chitin is usually found in diverse living organisms, for instance, shrimps, crabs, insects and tortoise. It is also found in the cell wall of fungi, internal structures of invertebrates and exoskeleton of arthropods [1].

In organisms that consist of chitin, chitinases has crucial role in normal life cycle purposes such as morphogenesis and cell division, whereas plants synthesis chitinases as part of their defence mechanism against fungal pathogens [2]. Chitinases are present in a wide range of organisms such as bacteria, fungi, yeasts, plants, arthropods and humans [3].

Chitinases (E.C 3.2.2.14) are hydrolytic enzymes that catalyse the hydrolysis of the β-1,4-N-acetyl-D-glucosaminidic linkages of the polysaccharide chitin [4]. Most of fungi and bacteria consists of chitinolytic enzymes to transform chitin into compounds that can perform as energy source [2]. Various fungi contain chitin as the main components in the cell wall. There is potential of plant chitinase target fungi cell wall components as substrate and has anti fungi function [5].

In this study, chitin is extracted from Leucaena Leucocephala pods. Leucaena Leucocephala in Fig. 1 is a perpetual non-climbing, non-spiky bush or tree. It also known as “petai belalang” locally in Malaysia. It is widely used in Malaysia as livestock forage, reforestation material and also furniture and construction timber [6]. Leucaena Leucocephala is not fully utilized yet and widely spread abundantly in Malaysia. It also can be easily found throughout the year.

Based on existing studies, the extraction of chitin was done by using HCl and NaOH in order to isolate chitin. In this study, the extraction of chitin from Leucaena Leucocephala will be done with 6M HCl only at different aging. Then it was characterized by using Fourier Transform Infrared (FTIR).

Fig. 1: Leucaena leucocephala

II. METHODOLOGY

A. Materials

The sample of Leucaena Leucocephala was collected from section 7, Shah Alam, Selangor. It grows along the road that widely spread in Malaysia. The sample collected according to their age. It was then cleaned and let to sun-dried to remove moisture content. After drying, they were kept at room temperature until used.

Fig. 2: Leucaena leucocephala according age (1, 2, 4, 7, 8 and 10 weeks) denoted as sample 1, 2, 3, 4, 5 and 6 respectively

The sample was grounded into small pieces by using a blender.

50g sample of Leucaena Leucocephala was stirred with 300ml of 6M HCl for 3 hours to extract the chitin. It was then filtered, a filtrate and together with its residue are obtained. The filtrate was stirred on a hot plate until a concentrated liquid obtained. While the residue were let to rest for about three days in the oven at 58°C to finish the process of drying.

B. . Fourier Transform Infrared (FTIR) spectroscopy

IR spectra of the chitin were measured using a FTIR spectroscopy Perkin Elmer. Samples were diluted 1:5 with KBr before acquisition.

III. RESULTS AND DISCUSSION

A. FTIR measurement before extraction

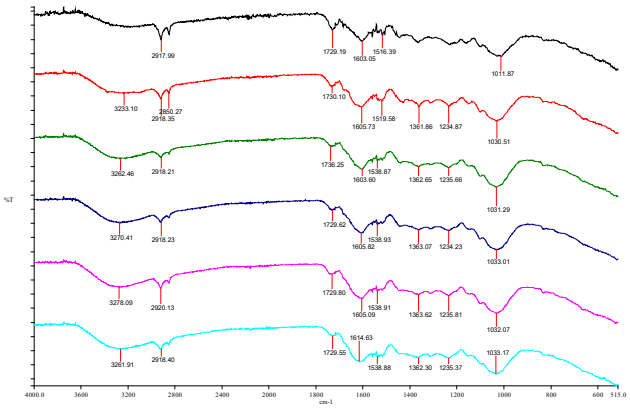

Fig. 3: FTIR spectrum data of Leucaena leucoephala efore chitin extraction

Fig. 2 above shows the FTIR spectrum data of grinded Leucaena leucocephala before isolation of chitin. The sample noted as sample 1, 2, 3, 4, 5 and 6 from above to bottom. From a reference made on a Leucaena leucocephala tree for about 14 weeks, the age of the sample is recognized as 1, 2, 4, 7, 8 and 10 weeks respectively. From the FTIR spectrum data above, it can be seen that in the range of 3550 – 3200 cm^{-1}, sample 2 to sample 6 has a band which indicates the stretching vibration of N-H. These bands consistent with the band reported by [7] . The bands in this range is becomes wider with increasing age of the Leucaena leucocephala. Sample 1 shows no band in this range, as it is a very young Leucaena leucocephala pods.

Each sample shows a band in the range of 2950 – 2850cm^{-1}, which represents the alkyl C-H stretch. This band is in agreement with [8] which reported that bands near to 2900cm^{-1} are representative bands for chitin. In the range of 1740-1700cm^{-1} and 1700-1500cm^{-1}, each sample has band in that both range which attributes to C=O stretch and C=C bending respectively. Sample 2 to sample 6 has bands in the range of 1400-1300cm^{-1}, which indicates to the CO-NH deformation and to the CH_2 group (amide III), due to the formation of CO-NH group as reported by (Paulino et al., 2006). In the range of 1300 – 1000cm^{-1}, sample 1 to 6 show bands which represent the C-O-C stretching. Table 1 shows the summary of FTIR bands assignment of Leucaena Leucocephala before extraction of chitin.

Table 1: FTIR bands assignments of Leucaena leucocephala before chitin extraction

Sample	FTIR Peak (cm^{-1})	Assignments
1	2918	Alkyl C-H stretch
	1729	C=O stretch
	1603	C=C bending
	1516	C=C bending
	1012	C-O-C stretch
2	3233	N-H stretch [7]
	2918	Alkyl C-H stretch
	2850	Alkyl C-H stretch
	1730	C=O stretch
	1606	C=C bending
	1520	C=C bending
	1362	Amide III
	1235	C-O-C stretch
	1031	C-O-C stretch
3	3262	N-H stretch [7]
	2918	Alkyl C-H stretch
	1736	C=O stretch
	1604	C=C bending
	1539	C=C bending
	1363	Amide III
	1236	C-O-C stretch
	1031	C-O-C stretch
4	3270	N-H stretch [7]
	2918	Alkyl C-H stretch
	1730	C=O stretch
	1606	C=C bending
	1539	C=C bending
	1363	Amide III
	1234	C-O-C stretch
	1033	C-O-C stretch
5	3278	N-H stretch [7]
	2920	Alkyl C-H stretch
	1730	C=O stretch
	1605	C=C bending
	1539	C=C bending
	1364	Amide III
	1236	C-O-C stretch
	1032	C-O-C stretch
6	3262	N-H stretch [7]
	2918	Alkyl C-H stretch
	1730	C=O stretch
	1615	C=C bending
	1539	C=C bending
	1362	Amide III
	1235	C-O-C stretch
	1033	C-O-C stretch

B. FTIR measurement after chitin extraction (residue)

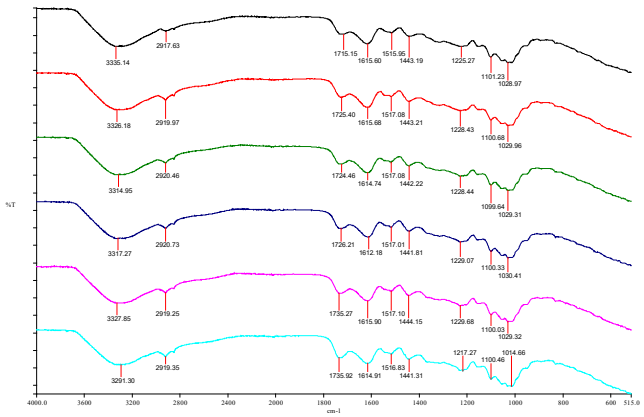

Fig. 4: FTIR spectrum data of Leucaena leucocephala after chitin extraction (residue)

Fig. 3 above shows the FTIR spectrum data of Leucaena leucocephala residue after chitin extraction by using 6M HCl for about 3h. From the spectrum data above, in the range of 3550-

3200cm^{-1} sample 1 to 6 shows a band respectively which indicates the stretching vibration of aliphatic O-H, and also represents N-H stretch which near to bands reported by [7].

There is no change in band in the range of 2950–2850cm^{-1} from before extraction band which is in agreement with [8] which reported that bands near to 2900cm^{-1} are representative bands for chitin. Same with the FTIR result from before extraction; the band in that range represents the alkyl C-H stretch. In the range of 1700-1500cm^{-1}, each sample has band in that both range which attributes to C=O stretch and C=C bending respectively, same with band ranged in that range that exist before chitin extraction. However, a new band exist in sample 1 to sample 6 which range in 1425 - 1475cm^{-1}, attributes to stretching vibration of –CN according to (Zaku et al., 2011). In the range of 1300 – 1000cm^{-1}, sample 1 to 6 show bands which represent the C-O-C stretching, near with band as reported by (Zaku et al., 2011). Table 2 shows the summary of FTIR bands assignments of Leucaena Leucocephala residue after chitin extraction

Table 2: FTIR bands assignment of Leucaena leucocephala after chitin extraction (residue)

Sample	FTIR Peak (cm^{-1})	Assignments
1	3335	Alcohol/phenol O-H stretch, Amine N-H stretch
	2918	Alkyl C-H stretch
	1715	C=O stretch
	1616	C=C bending
	1516	C=C bending
	1443	-CN stretch [10]
	1225	C-O-C stretch
	1101	C-O-C stretch
	1029	C-O-C stretch
2	3326	Alcohol/phenol O-H stretch, Amine N-H stretch
	2920	Alkyl C-H stretch
	1725	C=O stretch
	1616	C=C bending
	1517	C=C bending
	1443	-CN stretch [10]
	1228	C-O-C stretch
	1101	C-O-C stretch
	1030	C-O-C stretch
3	3315	Alcohol/phenol O-H stretch, Amine N-H stretch
	2920	Alkyl C-H stretch
	1724	C=O stretch
	1615	C=C bending
	1517	C=C bending
	1442	-CN stretch [10]
	1228	C-O-C stretch
	1100	C-O-C stretch
	1029	C-O-C stretch
4	3317	Alcohol/phenol O-H stretch, Amine N-H stretch
	2921	Alkyl C-H stretch
	1726	C=O stretch
	1612	C=C bending
	1517	C=C bending
	1442	-CN [10]
	1229	C-O-C stretch
	1100	C-O-C stretch
	1030	C-O-C stretch
5	3328	Alcohol/phenol O-H stretch, Amine N-H stretch
	2919	Alkyl C-H stretch
	1735	C=O stretch
	1616	C=C bending
	1517	C=C bending
	1444	-CN [10]
	1230	C-O-C stretch
	1100	C-O-C stretch
	1029	C-O-C stretch
6	3291	Alcohol/phenol O-H stretch
	2919	Alkyl C-H stretch
	1736	C=O stretch
	1615	C=C bending
	1517	C=C bending
	1441	-CN [10]
	1217	C-O-C stretch
	1100	C-O-C stretch
	1015	C-O-C stretch

C. FTIR measurement after chitin extraction (filtrate)

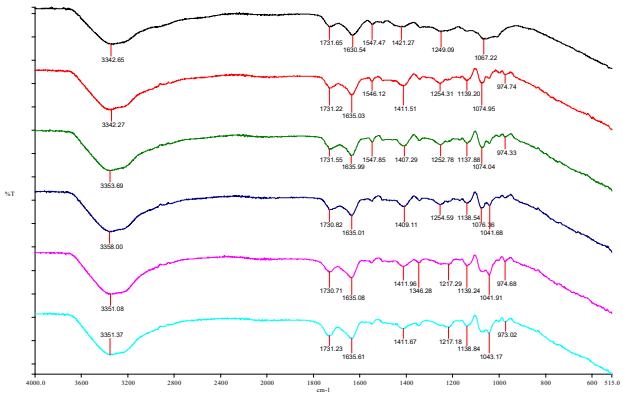

Fig. 5: FTIR spectrum data of Leucaena leucocephala after chitin extraction (filtrate)

Fig. 4 above shows the FTIR spectrum data of the extracted chitin from Leucaena leucocephala pods by using 6M of HCl. Same with the FTIR spectrum data of the residue before this, in the range of 3550-3200cm^{-1} sample 1 to 6 shows band which indicates the stretching vibration of aliphatic O-H, and also represents N-H stretch which near to bands reported by [7]. However, the band in the range of 2950 – 2850cm^{-1} which represents the alkyl C-H stretch, which is exists in the FTIR spectrum data before chitin extraction, has disappeared in this spectrum data. The bond that indicates alkyl C-H stretch may break during the chitin extraction process.

There is no change in number of bands that exist in the range of 1700-1500cm^{-1}, which each sample has band in that both range which attributes to C=O stretch and C=C bending respectively as before the chitin extraction, except for the sample 1, 2 and 3 which has two bands for C=C bending. It is also can be seen that the absorption band near the 1420cm^{-1} for each sample, which indicates to protein as reported by (Kaya et al., 2015). The band indicates to protein exist due to no treatment with NaOH to removed protein or a process called deproteinization is not done. From the FTIR spectrum, it can be seen that this band that represents protein is not sharp in sample 1, but gradually obvious in sample 2,3, and 4, and becomes wide in sample 5 and 6. This means that protein is highly contained in sample 2,3 and 4 but low in sample 1, 5 and 6. From this result, it can be seen that a very young pods which age of 1 week and oldest pods which age 8-10 weeks is low in protein compared to pods that age between 2-7 weeks. Same with the band exist in the range of 1300 – 1000cm^{-1} before extraction, sample 1 to 6 show bands which represent the C-O-C stretching, near with band as reported by [10]. There is also band near 974cm^{-1} in this FTIR spectrum data which represents Amide III according to [12]. Table 3 shows the summary of FTIR bands assignment of extracted chitin from Leucaena Leucocephala for sample 1 to 6.

Table 3: FTIR bands assignment of Leucaena leucocephala after chitin extraction (filtrate)

Sample	FTIR Peak (cm^{-1})	Assignments
1	3343	Alcohol/phenol O-H stretch, Amine N-H stretch
	1732	C=O stretch
	1631	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1547	C=C bending
	1421	Protein [11]
	1249	C-O-C stretch
	1067	C-O-C stretch
2	3342	Alcohol/phenol O-H stretch, Amine N-H stretch
	1731	C=O stretch
	1635	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1546	C=C bending
	1412	Protein [11]
	1254	C-O-C stretch
	1139	C-O-C stretch
	1075	C-O-C stretch
	975	Amide III [12]
3	3354	Alcohol/phenol O-H stretch, Amine N-H stretch
	1732	C=O stretch
	1636	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1548	C=C bending
	1407	Protein [11]
	1253	C-O-C stretch
	1138	C-O-C stretch
	1074	C-O-C stretch
	974	Amide III [12]
4	3358	Alcohol/phenol O-H stretch, Amine N-H stretch
	1731	C=O stretch
	1635	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1409	Protein [11]
	1255	C-O-C stretch
	1139	C-O-C stretch
	1076	C-O-C stretch
	1042	C-O-C stretch
5	3351	Alcohol/phenol O-H stretch, Amine N-H stretch
	1731	C=O stretch
	1635	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1412	Protein [11]
	1346	Methyl C-H stretch, Amide III
	1217	C-O-C stretch

	1139	C-O-C stretch
	1042	C-O-C stretch
	974	Amide III [12]
6	3351	Alcohol/phenol O-H stretch, Amine N-H stretch
	1731	C=O stretch
	1636	Amide C=O stretch, Alkenyl C=C stretch, Aromatic C=C bending
	1412	Protein [11]
	1217	C-O-C stretch
	1139	C-O-C stretch
	1043	C-O-C stretch
	973	Amide III [12]

D. Type of chitin

It can be seen from the FTIR spectrum before and after extraction of chitin, sample 1 to sample 6 shows only one band in the range of 1650-1600cm^{-1} and is not divided into two peaks. According to a [13], the divided amide I band into two peaks is assigned to α-chitin, while a single amide I band is assigned to β-chitin. This is also supported by [14] which reported that for α-chitin, the amide I band is split into two components due to the influence of hydrogen bonding or the presence of an enol form of the amide moiety compared to β-chitin which only has one component or band. Besides, [15] which analyse on Antarctic krill chitin results two splitting band of amide I which depended on the hydrogen binding between the C=O groups and one type of the O6-H groups from the glucoseamine units and suggested to be α-chitin.

In addition, [7] reported that a single band of amide I is observed in the case of β-chitin which is commonly attributes to the stretching of the CO group hydrogen bonded to amide group of the neighbouring intra-sheet chain.

Amide I band in sample 1 to sample 6 before and after extraction is not appearing to be a sharper, but wide (U-shape). [13] reported that α-chitin has a higher degree of crystallinity compared to β-chitin. According to [13], amide I band of α-chitin is appearing to be sharper (V-shaped) compared to β-chitin (U-shaped). Table 4 shows the summary on type of chitin observed.

Table 4: Summary on type of chitin

Criteria	α	β
Band	Amide I band splits into two components or peaks	Amide I band has only one components or peak
Shape	Amide I band is sharp (V-shaped)	Amide I band is wide (U-shaped)

Based on these statements from [13], [14], [15] and [7], it is clearly suggested that chitin from Leucaena leucocephala is a β-chitin.

IV. Conclusion

Results from FTIR shows that Amide I band in Leucaena leucocephala before and after extraction does not divided into two peaks which make it appear close to a β-chitin. Beside, Amide I band of Leucaena leucocephala before and after extraction is appearing wide (U-shaped) rather than sharp. From the results obtained, the chitin from Leucaena leucocephala is determined to be in the form of β-chitin.

From the results attained, the bands in the range of 3550 – 3200 cm^{-1} that represents stretching vibration of N-H is becomes wider with increasing age of the Leucaena leucocephala. This result shows that stretching vibration is weaker with increasing age of the pods.

Besides, extracted chitin shows protein is highly contained in sample 2,3 and 4 but low in sample 1, 5 and 6. From this result, it can be seen that a very young pods which age of 1 week and oldest pods which age 8-10 weeks is low in protein compared to pods that age between 2-7 weeks.

For future studies, it is recommended to further the process of extraction with treatment using NaOH after treatment with HCl in order to removed protein or a process called deproteinization. Besides, extraction also can be done with using different molar of HCl.

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References

[1] A. Usman, K. M. Zia, M. Zuber, S. Tabasum, S. Rehman, and F. Zia, “Chitin and chitosan based polyurethanes: A review of recent advances and prospective biomedical applications,” *Int. J. Biol. Macromol.*, vol. 86, pp. 630–645, 2016.

[2] M. B. Brurberg;, B. Synstad;, S. S. Klemsdal;, D. M. F. van Aalten;, L. Sundheim;, and V. G. H. Eijsink, “Chitinases from Serratia marcescens Nima,” *Biotechnol. Lett.*, vol. 27, no. 9, pp. 649–653, 2005.

[3] R. Hamid *et al.*, “Chitinases: An update,” *J. Pharm. Bioallied Sci.*, vol. 5, no. 1, pp. 21–29, Nov. 2013.

[4] D. Wit, M. Kaomek, K. Mizuno, T. Funmura, P. Sriyotha, and J. R. K. Cairnsi, “JSist,” 2003.

[5] E. Saboki, K. Usha, and B. Singh, “Pathogenesis Related (PR) Proteins in Plant Defense Mechanism Age-Related Pathogen Resistance,” *Curr. Res. Technol. Adv.*, pp. 1043–1054, 2011.

[6] N. Yuziah, “Strand properties of Leucaena leucocephala (Lam .) de wit wood,” vol. 6, no. 22, pp. 5181–5191, 2011.

[7] F. A. Al Sagheer, M. A. Al-sughayer, S. Muslim, and M. Z. Elsabee, “Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf,” *Carbohydr. Polym.*, vol. 77, no. 2, pp. 410–419, 2009.

[8] S. Patricia *et al.*, “Comparison of Extraction Methods of Chitin from Ganoderma lucidum Mushroom Obtained in Submerged Culture,” vol. 2014, 2014.

[9] A. T. Paulino, J. I. Simionato, J. C. Garcia, and J. Nozaki, “Characterization of chitosan and chitin produced from silkworm crysalides,” *Carbohydr. Polym.*, vol. 64, no. 1, pp. 98–103, 2006.

[10] S. G. Zaku, S. a E. O. C. Aguzue, and S. a Thomas, “Extraction and characterization of chitin ; a functional biopolymer obtained from scales of common carp fish (Cyprinus carpio l .): A lesser known source,” *African J. Food Sci.*, vol. 5, no. 8, pp. 478–483, 2011.

[11] M. Kaya, B. Bitim, M. Mujtaba, and T. Koyuncu, “Surface morphology of chitin highly related with the isolated body part of butterfly (Argynnis pandora),” *Int. J. Biol. Macromol.*, vol. 81, no. August, pp. 443–449, 2015.

[12] K. P. and E. Natarajan, “Isolation and FTIR spectroscopy characterization of chitin from local sources,” *Adv. Appl. Sci. Res.*, vol. 3, no. 2, pp. 1870–1875, 2012.

[13] H. Greven, M. Kaya, and T. Baran, “The presence of α-chitin in Tardigrada with comments on chitin in the Ecdysozoa,” *Zool. Anz.*, vol. 264, pp. 11–16, 2016.

[14] J. Kumirska, Z. Kaczy, and A. Bychowska, “Application of Spectroscopic Methods for Structural Analysis of Chitin and Chitosan,” pp. 1567–1636, 2010.

[15] Y. Wang *et al.*, “Crystalline structure and thermal property characterization of chitin from Antarctic krill (Euphausia superba),” *Carbohydr. Polym.*, vol. 92, no. 1, pp. 90–97, 2013.