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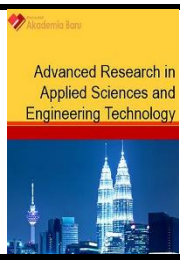

Characterization Of Chitin With Extraction Leucaena Leucephala Pods Using Hydrochloric Acid (HCl) By Fourier Transform Infrared

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ABSTRACT

The objective of this study is to characterized chitin from Leucaena leucephala pods at different aging stages with hydrochloric acid HCl by using Fourier Transform Infrared (FTIR). Leucaena leucephala 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 leucephala is not fully utilized yet and it could potential for new source for chitin. Results from FTIR shows that Amide I band in Leucaena leucephala before and after extraction range of 1650-1600cm-1 and is not divided into two peaks which make it appear close to a β-chitin. Beside, Amide I band of Leucaena leucephala before and after extraction is appearing wide (U-shaped) rather than sharp therefore the chitin from Leucaena leucephala is determined to be in the form of β-chitin.

1. 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 [1], crabs [2], insects [3] and tortoise [4]. It is also found in the cell wall of fungi, internal structures of invertebrates and exoskeleton of arthropods [5]. Chitin in nature structurally similar, however physicochemical properties of chitin are highly dependent on the source [6]. Chitin, is a linear polymer of N-acetylglucosamine units, which has three polymorphic crystalline forms; alpha, beta and gamma [7]. Chitin fibrils in insect together with proteins give insect cuticle mechanical strength and integrity [8]. Chitin in the inner surface of the insect gut gives mechanical strength and provide protection against reactive oxygen species and microbial invasion [9,10]. Conventionally, chemicals processing for demineralization and deproteinization have been applied to extraction of chitin from crustacean shells. Dilute hydrochloric acid was used to remove mineral salts such as calcium carbonate [11]. However, for in this research Leucaena leucephala pods as an alternative chitin source, therefore no calcium carbonate found in this plant. So, extraction method

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that is suitable is by hydrolysis of chitin using hydrochloric acid (HCl). In the current study, Leucaena leucephala pods as an alternative chitin source was investigated for determining the chitin characteristics. The Leucaena pods and seeds had higher ($P < 0.01$) crude protein (CP) contents [12], [13]. It also high proportions of reserve polysaccharides [14]. Leucaena leucephala is a perpetual nonclimbing, non-spiky bush or tree locally it is known as "petai belalang". It is widely used in Malaysia as livestock forage, reforestation material and also furniture and construction timber [15]. Leucaena leucephala is not fully utilized yet and widely spread abundantly in Malaysia. It also can be easily found throughout the year. This study was designed to investigate which physicochemical properties of chitin at aging stage of a Leucaena leucephala pods. Moreover, the chitin will be characterized by means of FTIR and their characteristic will be investigated.

2. Experimental

2.1 Materials

The sample of Leucaena leucephala was collected from section 7, Shah Alam, Selangor. The sample collected according to their age. The aging of Leucaena leucephala was determined as shown in Table 1 and Figure 1. Ages in between 2 to 10 weeks were selected based on growth of pods. It was then cleaned and let to sun-dried to remove moisture content. After drying, they were kept at room temperature until used.

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

Table 1
Aging of Leucaena leucephala

Week	Length (cm)	Width (cm)
1	8	0.4
2	18.8	0.7
3	24.5	1.8
4	25	2.3
5	25.5	2.5
6	25.7	2.7
7	26	2.8
8	26.3	3.0
9	26.5	3.1
10	26.8	3.1

2.2 Method

The samples were ground using a blender. 50g sample of (LL) was stirred with 300ml of 6M hydrochloric acid HCl for 3 hours in fume hood to extract the chitin which is chitin soluble in the hydrochloric acid [16]. The purpose of using HCl is to extract the chitin in LL. It was then filtered a filtrate and together with its residue is obtained. The residue was dried for three days at 58 °C to remove water until constant weight.

3. Results and Discussion

Figure 2 shows the FTIR spectrum data of grinded Leucaena leucephala 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 leucephala 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 1 to sample 6 has a band which indicates the stretching vibration of N-H. These bands consistent with the band reported by Al Sagheer [17]. The bands in this range is becomes wider with increasing age of the Leucaena leucephala. 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 [18] which reported that bands near to 2900cm-1 are representative bands for chitin. In the range of 1740 -1700 cm-1 and 1700-1500 cm-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 -1300 cm-1, which indicates to the CONH deformation and to the CH2 group (amide III), due to the formation of CO-NH group as reported by [19]. In the range of 1300 – 1000 cm-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 leucephala before extraction of chitin.

3.1 FTIR Measurement after Chitin Extraction (residue)

Figure 3 shows the FTIR spectrum data of Leucaena leucephala 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 Al Sagheer *et al*., [17].

There is no change in band in the range of 2950– 2850cm-1 from before extraction band which is in agreement with Ospina Álvarez [18] which reported that bands near to 2900cm-1 are representative bands for chitin. Similar 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 both range which attributes to C=O stretch and C=C bending respectively, similar to the band existed before chitin extraction. However, a new band existed in sample 1 to sample 6 which range in 1425 1475cm-1, attributes to stretching vibration of –CN according to Zaku *et al*., [20]. 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 Kaya *et al*., [20].

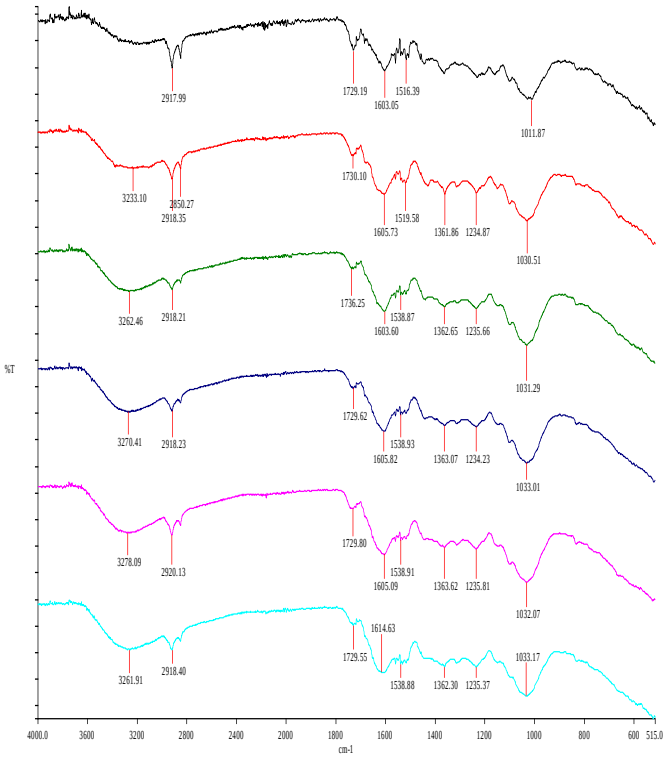

Fig. 2. FTIR spectrum data of Leucaena leucoephala before chitin extraction

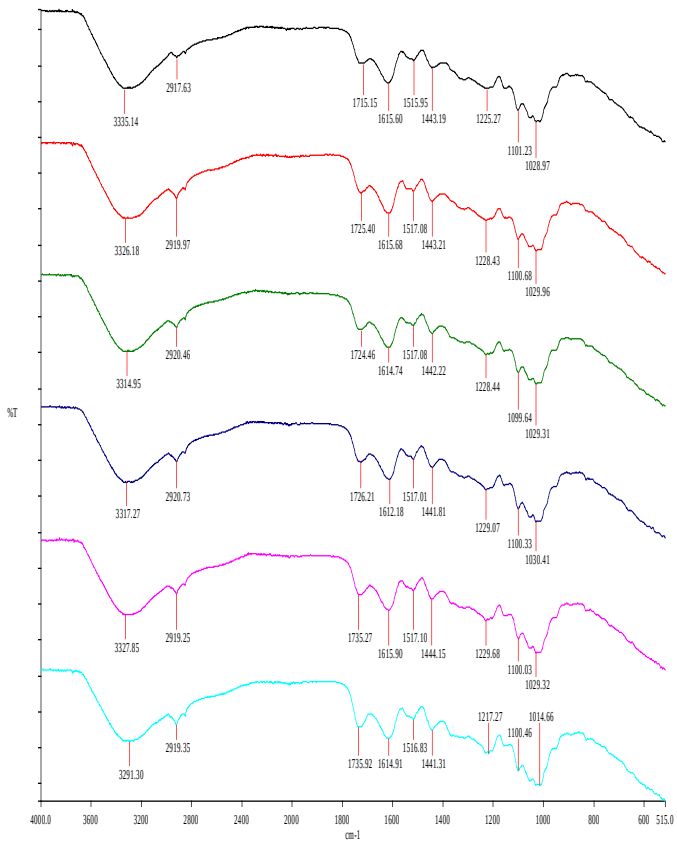

Fig. 3. FTIR spectrum data of Leucaena leucocephala after chitin extraction (residue)

Figure 4 shows the FTIR spectrum data of the extracted chitin from Leucaena leucephala 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 Al Sagheer [17]. 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

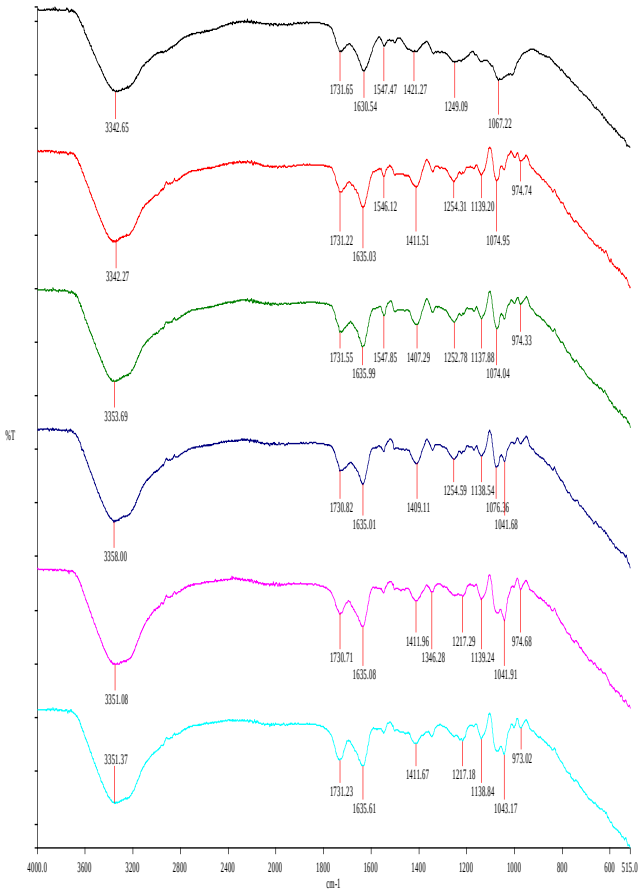

Fig. 4. FTIR spectrum data of Leucaena leucocephala after chitin extraction (filtrate)

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*., [21]. The band indicates the existing of protein since de-proteinization exist due to no treatment with NaOH to removed protein or a process called de-proteinization was not carried out during the process. The sharpness of this band increase in sample 2,3, and 4 and widened 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 Zaku *et al*., [20]. There is also band near 974cm-1 in this FTIR spectrum data which represents Amide III according to

Prabu and Natarajan [22]. Table 3 shows the summary of FTIR bands assignment of extracted chitin from Leucaena leucephala for sample 1 to 6.

3.2 Type of Chitin

It can be seen Table 1 and from the FTIR spectrum before and after extraction of chitin, sample 1 to sample 6 shows only one band in the range of 16501600cm-1 and is not divided into two peaks. According to a Greven *et al*., [23], 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 Kumirska *et al*., [24] 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 βchitin which only has one component or band. Besides, Wang *et al*., [25] 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 in chemical structure of chitin and suggested to be α-chitin. In addition, Al Sagheer *et al*., [17] 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). Greven *et al*., [23] reported that α-chitin has a higher degree of crystallinity compared to β-chitin. According to Greven *et al*., [23], 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. Based on these statements from Greven *et al*., [23], Kumirska *et al*., [24], Wang *et al*., [25] and Al Sagheer *et al*., [17], it is clearly suggested that chitin from Leucaena leucephala is a β-chitin. The presence of acid which is can break the bonding between chitin and cellulose in cell wall, therefore polymorphic crystalline is forms (alpha, beta and gamma) from extraction [26].

Table 2
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 (Vshaped)	Amide I band is wide (U-shaped)

4. Conclusion

Results from FTIR showed that Amide I band in Leucaena leucephala 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 leucephala before and after extraction is appearing wide (U-shaped) rather than sharp. From the results obtained, the chitin from Leucaena leucephala is determined to be in the form of βchitin.

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 leucephala. 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

remove protein or a process called deproteinization. Besides, extraction also can be done with using different molar of HCl.

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References

[1] Yen, Ming-Tsung, Joan-Hwa Yang, and Jeng-Leun Mau. "Physicochemical characterization of chitin and chitosan from crab shells." *Carbohydrate Polymers* 75, no. 1 (2009): 15-21.
[2] Hamdi, Marwa, Amal Hammami, Sawssen Hajji, Mourad Jridi, Moncef Nasri, and Rim Nasri. "Chitin extraction from blue crab (Portunus segnis) and shrimp (Penaeus kerathurus) shells using digestive alkaline proteases from P. segnis viscera." *International journal of biological macromolecules* 101 (2017): 455-463.
[3] Kittur, F. S., KV Harish Prashanth, K. Udaya Sankar, and R. N. Tharanathan. "Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry." *Carbohydrate polymers* 49, no. 2 (2002): 185-193.
[4] Pasteels, Jacques M., Olivier Deparis, Sébastien R. Mouchet, Donald M. Windsor, and Johan Billen. "Structural and physical evidence for an endocuticular gold reflector in the tortoise beetle, Charidotella ambita." *Arthropod structure & development* 45, no. 6 (2016): 509-518.
[5] Usman, Ali, Khalid Mahmood Zia, Mohammad Zuber, Shazia Tabasum, Saima Rehman, and Fatima Zia. "Chitin and chitosan based polyurethanes: A review of recent advances and prospective biomedical applications." *International journal of biological macromolecules* 86 (2016): 630-645.
[6] Kaya, Murat, Karwan Sofi, Idris Sargin, and Muhammad Mujtaba. "Changes in physicochemical properties of chitin at developmental stages (larvae, pupa and adult) of Vespa crabro (wasp)." *Carbohydrate polymers* 145 (2016): 64-70.
[7] Imai, Tomoya, Takeshi Watanabe, Toshifumi Yui, and Junji Sugiyama. "The directionality of chitin biosynthesis: a revisit." *Biochemical Journal* 374, no. Pt 3 (2003): 755.
[8] Ehrlich, Hermann. "Chitin and collagen as universal and alternative templates in biomineralization." *International Geology Review* 52, no. 7-8 (2010): 661-699.
[9] Barbehenn, Raymond V., and Jasmine Stannard. "Antioxidant defense of the midgut epithelium by the peritrophic envelope in caterpillars." *Journal of Insect Physiology* 50, no. 9 (2004): 783-790.
[10] Merzendorfer, Hans. "Insect chitin synthases: a review." *Journal of Comparative Physiology B* 176, no. 1 (2006): 1-15
[11] Younes, Islem, Sawssen Hajji, Marguerite Rinaudo, Moncef Chaabouni, Kemel Jellouli, and Moncef Nasri. "Optimization of proteins and minerals removal from shrimp shells to produce highly acetylated chitin." *International journal of biological macromolecules* 84 (2016): 246-253.
[12] Ngwa, A. T., I. V. Nsahlai, and M. L. K. Bonsi. "The potential of legume pods as supplements to low quality roughages." *South African Journal of Animal Science* 30, no. 4 (2000): 107-108.
[13] Dhamothiran, L., M. Paramathma, C. Surendran, and P. Chandrasekharan. "Leucaena. A source of protein and concentrate." *Leucaena Research Reports* 12 (1991): 29.
[14] Torres, Luis G., Sandra Carpinteyro-Urban, and Luis J. Corzo-Rios. "Use of Annona diversifolia and A. muricata seeds as source of natural coagulant-flocculant aids for the treatment of wastewaters." *European Journal of Biotechnology and Bioscience* 1, no. 2 (2013): 16-22.
[15] Wan-Mohd-Nazri, W. A. R., and Nor Yuziah. "Strand properties of Leucaena leucocephala (Lam.) de wit wood." *African Journal of Agricultural Research* 6, no. 22 (2011): 5181-5191.
[16] Rupley, J. A. "The hydrolysis of chitin by concentrated hydrochloric acid, and the preparation of low-molecular-weight substrate for lysozyme." *Biochimica et Biophysica Acta (BBA)-Specialized Section on Mucoproteins and Mucopolysaccharides* 83, no. 3 (1964): 245-255.
[17] Al Sagheer, F. A., M. A. Al-Sughayer, S. Muslim, and M. Z. Elsabee. "Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf." *Carbohydrate polymers* 77, no. 2 (2009): 410-419.
[18] Ospina Álvarez, Sandra Patricia, David Alexander Ramírez Cadavid, Diana Marcela Escobar Sierra, Claudia Patricia Ossa Orozco, Diego Fernando Rojas Vahos, Paola Zapata Ocampo, and Lucía Atehortúa. "Comparison of extraction methods of chitin from Ganoderma lucidum mushroom obtained in submerged culture." *BioMed research international* 2014 (2014).

[19] Paulino, Alexandre T., Julliana I. Simionato, Juliana C. Garcia, and Jorge Nozaki. "Characterization of chitosan and chitin produced from silkworm crysalides." *Carbohydrate Polymers* 64, no. 1 (2006): 98-103.
[20] Zaku, S. G., SA Emmanuel OC 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 Journal of Food Science* 5, no. 8 (2011): 478-483.
[21] Kaya, Murat, Betül Bitim, Muhammad Mujtaba, and Turgay Koyuncu. "Surface morphology of chitin highly related with the isolated body part of butterfly (Argynnis pandora)." *International journal of biological macromolecules* 81 (2015): 443-449.
[22] Prabu, K., and E. Natarajan. "Isolation and FTIR spectroscopy characterization of chitin from local sources." *Adv. Appl. Sci. Res* 3 (2012): 1870-1875.
[23] Greven, Hartmut, Murat Kaya, and Talat Baran. "The presence of α-chitin in Tardigrada with comments on chitin in the Ecdysozoa." *Zoologischer Anzeiger-A Journal of Comparative Zoology* 264 (2016): 11-16.
[24] Kumirska, Jolanta, Małgorzata Czerwicka, Zbigniew Kaczyński, Anna Bychowska, Krzysztof Brzozowski, Jorg Thöming, and Piotr Stepnowski. "Application of spectroscopic methods for structural analysis of chitin and chitosan." *Marine drugs* 8, no. 5 (2010): 1567-1636.
[25] Wang, Yanchao, Yaoguang Chang, Long Yu, Cuiyu Zhang, Xiaoqi Xu, Yong Xue, Zhaojie Li, and Changhu Xue. "Crystalline structure and thermal property characterization of chitin from Antarctic krill (Euphausia superba)." *Carbohydrate polymers* 92, no. 1 (2013): 90-97.
[26] Suenaga, Shin, Kazuhide Totani, Yoshihiro Nomura, Kazuhiko Yamashita, Iori Shimada, Hiroshi Fukunaga, Nobuhide Takahashi, and Mitsumasa Osada. "Effect of acidity on the physicochemical properties of α-and β-chitin nanofibers." *International journal of biological macromolecules* 102 (2017): 358-366.