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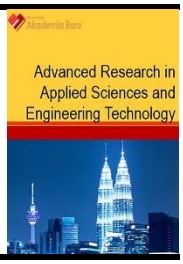

Elemental Analysis of Chitin from Extraction of Different Ages of Leucaena Leucephala with Hydrochloric Acid

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Noor Harliza Abd Razak[1,*], Nur Wahida Mohd Shukori[1], Ku Halim Ku Hamid[1]

[1] Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam Selangor, Malaysia

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ABSTRACT

Chitin is a biopolymer that forms the exoskeleton of arthropods, and found in the shells of crustacea and in the cell walls of certain fungi and algae. Commercially, chitin is obtained from processing the outer skeleton of crustacea such as shrimp, crab, prawn, and crayfish. Extraction of chitin was carried out using various chemical procedures. The study aim is to examine characteristic of chitin for different aging of Leucaena leucephala pods using hydrochloric acid (HCl). Different aging of the raw materials was used to study their effect of nitrogen content in the pods. In this study, chitin in Leucaena leucephala was extracted using chemical methods by using hydrochloric acid (HCl). The extracted chitin was then characterized by using elemental analyzer. The results obtained revealed that the percentage of nitrogen and carbon content in the samples was significantly reduced after extraction. Elemental analysis, the N% value in younger pods is closer to the theoretical value than adult pods. The purity of chitin in younger pods is higher than chitin in the adult pods.

1. Introduction

Chitin is the most available biopolymer after cellulose. It is sourced mainly from exoskeleton of crustaceans such as shrimp [1], crab [2], fish scale [3,4] and also available from other sources such as fungi and some other insect's wing. Chitin, a β (1,4) linked homopolymer of N-acetylglucosamine, is a simple polysaccharide that is present in the cell walls of all fungi studied to date [1,2]. Chitin is an integral part of insect peritrophic matrices, which function as a permeability wall between the food bolus and the midgut epithelium, boost digestive process, and defend the brush border from mechanical disruption as well as from attack by toxins and pathogens [5].

Chitin is useful as antimicrobial emulsifying, thickening and stabilizing agents in the food industry. They also show notable bioactivity in biomedical fields, including wound-healing promotion. In animal, chitin is usually isolated from the exoskeleton of crustaceans and more particularly from shrimp and crabs. It is the most important constituent of invertebrate. Extraction of chitin in animal goes through a lot of processes. In extraction of chitin from Leucaena leuchephala, we will minimize the process that can reduce the cost. In animal, calcium carbonate (CaCO3) can be eliminated with

* *Corresponding author.*
E-mail address: harliza8566@salam.uitm.edu.my (Noor Harliza Abd Razak)

HCl [6]. Recently, there has been a need for new source of chitin for new usage areas for chitin and its derivatives for biotechnology and extreme biomimetics area [7]. There are no recent studies of chitins in the Leucaena leucephala pods. In the current study, the ability to use Leucaena Leucephala pods as an alternative chitin source was investigated with different aging. Leucaena leucephala (Lam.) de Wit grows naturally in most tropical areas globally. It is neglected and underutilized. Pods are 11–19 cm long, and 15–21 mm wide, 5–25 per flower head, linear- oblong, acute or rounded at apex, flat, 8–18 seeded, mid- to orange-brown, glabrous, and slightly lustrous or densely covered in white velvety hairs and papery [8]. The aim of this study is to examine the characteristic of chitin for different aging of Leucaena Leucephala pods, which were analyzed using an elemental analyzer.

2. Methodology

2.1 Materials

Leucaena leucephala pods were collected in Shah Alam. A Leucaena leucephala pod was distinguished according to different developmental stages at different week. The samples of Leucaena leucephala in six stages of growth as shown in Figure 1 were collected, dried and ground.

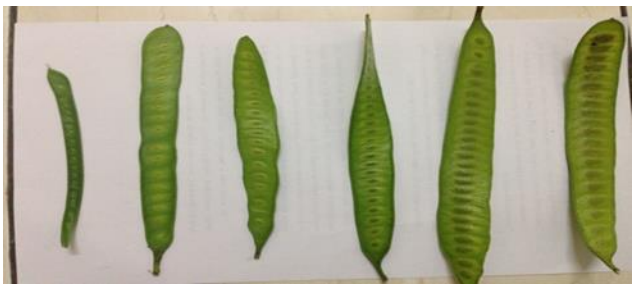

Fig.1. Different age of *Leucaena leucephala* pods (week 1 to week 6)

2.2 Chitin Extraction

For the isolation of chitin from Leucaena leucephala pods, 50g was dispersed in 6M HCl solution. The mixture was stirred at 60°C for 3 h and later was cooled to room temperature under flow of air. The mixture was filtered to separate solid and liquid.

2.3 Degree of Acetylation (DA)

A FlashEA-1112 CHNS-O Elemental Analyser was used to determine the carbon, hydrogen and nitrogen contents in the Leucaena leucephala pod. According to Kaya *et al*., [9] the degree of acetylation (DA) of the chitin samples is calculated according to the following formula where C/N is the ratio of carbon to nitrogen:

$$DA = \left[\frac{\frac{C}{N} - 5.14}{1.72} \right] \times 100 \quad (1)$$

3. Results and Discussion

3.1 Elemental Analysis

An elemental analysis (Table 1 and 2) was conducted to provide a comparison between the elemental compositions of before and after extraction. Elemental analysis of chitins from Leucaena leucephala before extraction, including the carbon, nitrogen, and hydrogen contents and C/N ratio was recorded in Table 1. In different developmental stages the nitrogen, carbon and hydrogen contents as well as C/N ratio also increased (Table 1). The percentages of nitrogen before extraction were recorded as 4.8006, 4.2812, 3.8567, 3.7966, 3.7101, and 3.8065 for sample 1 (week1) to 6 (week6) respectively.

Table 1
Elemental analysis before extraction

Samples	Contents %			
	N	C	H	C/N
1	4.8006	37.4912	5.8561	7.8097
2	4.2812	40.7104	6.6728	9.5091
3	3.8567	40.3612	6.7647	10.4652
4	3.7966	40.1590	6.8448	10.5776
5	3.7101	41.9792	7.0001	11.3148
6	3.8065	42.1055	6.9807	11.0615

The nitrogen content of Leucaena leucephala pods was mainly distributed in protein and chitin [2,10]. After extraction, the chitin was removed as shown in Table 2. The percentage was reduced to 1.4647 for sample 1, 2.4360 for sample 2, 0.3710 for sample 3, 0.2544 for sample 4, 2.2160 for sample 5 and 2.2529 for sample 6 respectively.

The percentage of nitrogen and carbon content in the samples was significantly reduced after extraction. Sample 1 contains 4.8006 of nitrogen before extraction and reduced to 1.4647 after extraction. The theoretical N content value for chitin is 6.89% [13]. In the present study, N content observed deviates from the theoretical value indicating the minimum amount of protein left [11]. Similar results regarding the purity of chitin have been reported by other authors [12-14], and [11]. According to Liu *et al*., [15], the low levels of ash and nitrogen contents are indicative of the effectiveness of the chitin extraction method. Therefore, the low levels of nitrogen after extraction indicates higher chitin.

Table 2
Elemental analyses after extraction

Samples	Contents %			
	N	C	H	C/N
1	1.4647	22.5309	7.2791	15.3826
2	2.4360	26.6489	7.1925	10.9396
3	0.3710	16.1619	2.6977	43.5631
4	0.2544	19.5325	3.4593	76.7787
5	2.2160	28.8888	7.2294	13.0365
6	2.2529	34.1418	7.0754	15.1546

The percentage of carbon content also decreased from 37.49 to 22.53 before and after extraction. According to Table 1 and 2, other samples also reduced in percentage of nitrogen and carbon content. Similar effectiveness of chitin extraction was reported in Liu *et al*., [15]. The nitrogen

content decreased after extraction which is compared before extraction. According to Kaya *et al*., [9,16], theoretical percentage for nitrogen content of chitin is 6.89% which was not consistent with our results. In the present study, nitrogen content observed was different from the theoretical value because the chitin extracted in this study was from a plant sample which differs from animal chitin. This proved that as the Leucaena leucephala become adult, the chitin content in the pods was reduced.

The percentage of nitrogen content after extraction was decreased compared to the nitrogen content before extraction. Dramatic increase in nitrogen removal between sample 3 and 4 might be caused by higher protein content in both sample which is protein, is binding with nitrogen. Removal of protein with HCl could affect results of nitrogen content. According to Younes and Rinaudo [17], HCl that has been used was to reduce residual proteins and mineral in the samples. However, removal nitrogen content for sample 5 and 6 decreased after extraction which is might be due to the decreasing of protein content.

3.2 Degree of Acetylation

The data from the elemental analysis allowed the determination of the average degree of acetylation of the chitin samples as shown in Figure 2 which was calculated by using equation 1. Degree of acetylation was the important factor used for differentiating chitin in each sample.

Degree of acetylation for each sample was significantly increased after extraction. DA value for each sample was more than 100%. In this study the value observed is greater than 100%, which established that there were mineral residues in the structure of chitin [9,19]. This might be due to there were mineral residues that could not be totally removed from the pods of Leucaena leucephala [20] which is why the mineral is left with powder and the high content of mineral. It seems that acid treatment was an effective method for extraction which is the chitin (filtrate liquid in filtration) was removed from powder which is left over during filtration.

According to the results of elemental analysis, the N% value in younger pods was closer to the theoretical value than adult pods. Similarly, the degree of acetylation value of younger pods is also closer to 100% than adult pods. This proved that the purity of chitin in younger pods was higher than chitin in the adult pods. The degree of acetylation values of the pods was slightly higher after extraction compared to before extraction. This indicated that the chitin has been extracted by using HCl and the level of chitin content in the samples after extraction was decreased in filtered powder (powder form which is separated from liquid during filtration). Earlier reports on DA values calculated from elemental analysis are as follows; 118% 152% for crude crab chitin [11], 237%for shrimp chitin [13], 151.5 % for crab shell chitin [2], larvae, pupa and adult chitins were calculated as 109.1%, 127.5% and 108.8% [14].

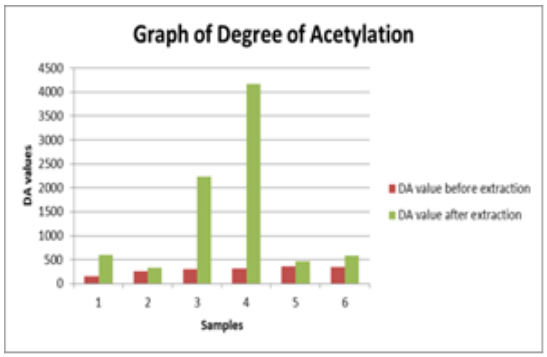

Fig. 2 Graph of Degree of Acetylation

4. Conclusion

The evaluation of the extraction of Leucaena leuchephala pods demonstrated the roles that the hydrochloric acid on the nitrogen removal. The extraction with HCl showed its effectiveness for removing protein and mineral residues in the samples. The extraction rate for adult pods was lower compared to the younger pods due to the bonding in the structure was difficult to break. This study presents showed the optimal removal of nitrogen content for development stage at week 3 and week 4 of Leucaena leuchephala pods. For further studies, the extracted liquid that has been filtrated must be used for analysis to determine the chitin that has been extracted for more accurate analysis. Additional and comprehensive research is crucial to remove seeds in the pods to make sure the protein and mineral in the seeds would not affect the results of the analysis

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