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Chitin Preparation and charcterization from Chicken Bone

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ABSTRACT

Chitin is the second most ubiquitous natural biopolymer after cellulose on earth. Chitin is estimated to be produced almost annually as much as cellulose. Biopolymer have received recent attention in research because of their unique characteristics used in many applications and considered one of the important biopolymer. Different procedures have been adopted in extracting chitin. This work proposed a new approach using chicken bone in preparing chitin. All the bones of the chicken were used as chitin source in this work. The two chemical methods, deproteinization and demineralization were used in extracting chitin. The prepared chitin through this work was characterized by X-ray diffraction (XRD), X-ray fluorescence (XRF), and Fourier transforms infrared spectroscopy (FTIR). Many experiments were accomplished through this work, experiment E5 gave the best results, which is close to the standard result. The proposed work outperformed the state of art researches through the percentage of chitin extracted. The chitin extraction was 16.25% of the total weight of all chicken bone used in the experiment, compared to the state of art researches that used chicken feat only.

Key words : *Chitin extraction, Deproteinization, Demineralization, Fourier transform infrared spectroscopy, X-ray diffraction, and X-ray fluorescence*

Introduction

Chitin (β-(1-4)-poly-N-acetyl-D-glucosamine) (Figure 1) is the second most abundant polysaccharide and widely distributed in nature after cellulose. Chitin, is the major structural component in the exoskeletons of the crustaceans, such as crayfish, crab, shrimp, insects, and other organisms, such as fungus. Chitin requires long processing time, 17 –72h, that includes 1 – 24h of HCl processing and 16– 48h of NaOH (Ifuku, Nomura *et al.*, 2011; Azuma, Izumi *et al.*, 2015).

Chitin is a natural polymer that is also biodegradable and biocompatible, and have an advantage in biomedical and pharmaceutical applications. With respect to materials, chitin represent a good film forming properties and is valuable for packaging or other applications (Kabasci, 2014). Chitin is commercially important because it is an excellent

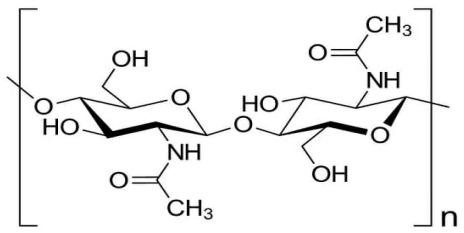

Fig 1. Chitin structure

biocompatibility, biodegradability, non-toxicity, chelating and adsorption power (Rinaudo, 2006, Ahmed, Ahmad *et al.,* 2014; Hamed, Özogul *et al.* 2016).

There are several methods of obtaining chitin, the biological processes, with the use of microorganisms and enzymes and chemical processes and even a combination of both to achieve an even more process proficient (Gortari and Hours, 2013).

This chemical process is deployed commercially, for isolation of chitin and chitosan (demineralization, deproteinization, and decolorization). Demineralization is achieved by removing the inorganic matter (calcium carbonate mainly) using HCl. Later deproteinization was used for protein extracted in an alkaline medium.

Temperature and alkali concentration are important for effective deproteinization. Further, treatment using 50% NaOH results in deacetylation of chitin forming chitosan (Kalut, 2008). Deproteinization, demineralization and deacetylation are the main processes for chitin and chitosan extraction, which are considered the most common methods to recover chitin from crab shell and the chitin obtained was characterized mechanically (Gadgey and Bahekar 2017). Chitin have been isolated from Antarctic krill, crab and shrimp (Wang, Chang *et al.,* 2013). An investigation on chitin and chitosan contents of insects compared with those of commercial chitin and chitosan has been accomplished. Their characteristics are similar to those of commercial chitin from crustaceans and other aquatic invertebrates (Zainol Abidin, Kormin *et al.,* 2020). Various methods of chitosan extraction will be compared; the importance of a new method of ecological extraction will be highlighted (El Knidri, Belaabed *et al.,* 2018). In this research, Chemical method were used, which is the most widely used method in both industrial and laboratory production. The purpose of any extraction process is to eliminate all organic and mineral content from the raw material. The chitin extraction process in done through two primordial steps: deproteinization and demineralization (Shimpi, 2017; Broquá, Zanin *et al.* 2018).

The limitation of the previous works was the amount of chitin production with respect to the total weight of bone. The maximum weight of chitin produced from previous works was with (Jalal, Risheed *et al.,* 2012), who produced an average of 10 g of chitin from 100 g of chicken feat bone, which is 10% of the total weight of chicken feat bone. Through this work, we were able to produce 13 g of chitin from 80 g of chicken bone which is 16.25% of the total weight of chicken bone.

The limitation of this work was time, which is the most challenging problem in the approach followed. Many steps through this approach need direct supervision followed.

Materials and Method

The materials and methods deployed in this work will be discussed in the following sections.

Materials and Preprocessing

All the chemicals (Acetic acid, oxalic acid, sodium hydroxide, and hydrochloric acid) used in this work were purchased from HIMEDIA (India). After extracting the chicken bone, and separating it from meat, it was cleaned completely using sharp tools, and left to dry under the sun light for one week (Figure 2a). To obtain the crude powder, the dried bone was grinded using a mill (IKA-A10B-Germany) (Fig. 2b).

Methodology

Chemical method used for extracting chitin from chicken bone follow a standard procedure (Aung, Win *et al.,* 2018). The standard procedure steps are shown in the following points (Fig. 2).

Step one: Demineralization chicken bone powder by treating with 1N hydrochloric acid at room temperature. After 38 hours the sample is filtered and rinsed with distilled water for many times to remove acid and calcium chloride (Fig. 2c and 2d).

Step two: the precipitate treated with 1.25 N sodium hydroxide at 90 °C for 24 hours (Fig. 2.e).

Step three: Filtering the crud chitin, and then treat it with 1% oxalic acid aqueous solution for 2h (Figure 2.f and Figure 2g).

Then chitin was collected and washed in distilled water, and then left to dry (Fig. 2 h).

The modification added through this work is shown in Figure 3.

Results and Discussion

Chitin extraction in the four experiments is summarized in Table 1. Five experiments were performed with different weights of crude powder to obtain the

(a) Chicken Bone

(b) Chicken Bone Powder

(c) Demineralization

(d) Filtration

(e) Deproteinization

(f) Washing

(g) Wet cake

(h) Crud Chitin

Fig. 2. Photographic illustration of Chitin Extraction.

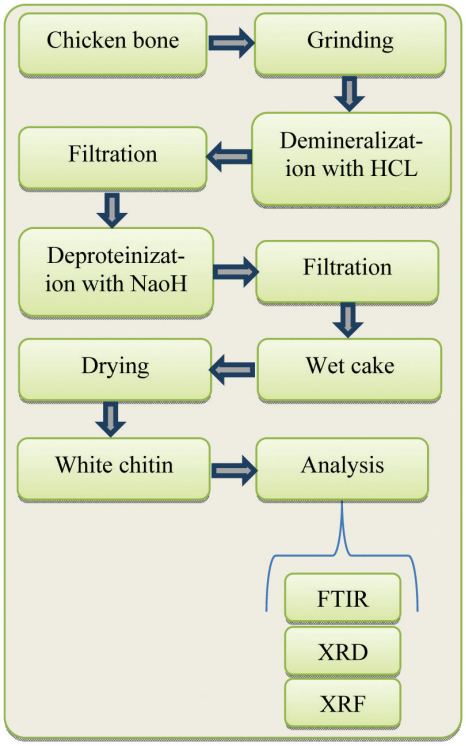

Fig 3. The process of extraction chitin from chicken bone

best results, the best result and best color obtained was in experiment five (E5) with crude powder of weight 80gm. In these experiments, all samples were treated with same concentration of NaOH solution in deproteinization at 90 °C and with same concentration of HCl solution in demineralization at room temperature.

Analysis of chitin extracted from chicken bone

The chemical compositions of chicken bone chitin were analysed by X-ray Fluorescence using (Spectro X-Labpro). The results in Table 2 show that the component of carbon and calcium oxide was the highest content of this composition in chitin powder of chicken bone.

Table 2. Chemical compositions of chitin powder from chicken bone

Element	E3	E4	E1	E5
C	62.63	74.3	86.34	98.7
MnO	13.46	9.02	3.02	-
Mg	0.182	0.11	0.09	0.06
P	1.658	1.28	0.76	0.33
CaO	22.42	16.34	9.07	0.448

X-ray diffraction (XRD) Results

The resultant samples from this experiment were analysed and characterized by X-ray diffraction (XRD), XRD analysis was applied to detect the crystallinity of the isolated chitin according to XRD result, the structure of chitin is crystalline (Table 3)(Figure 4). The following were noticed:

In XRD results, the chitin of E1 has sharp peak intensity, at two-theta (degree) is 780.

The chitin of E3 has peak intensity at two-theta (degree) is 561, chitin of E4 has sharp peak intensity at two-theta (degree) is 689, chitin of E5 has sharp peak intensity at two-theta (degree) is 1023. So, it indicates that all samples were formed as chitin.

Fourier transform infrared spectroscopy (FTIR)

The chitin obtained in four experiment were analysed by FTIR as shown in Table 4 and the best result for experiment five (E5) as shown in Figure 5.

IR spectra were measured by a KBr- over the frequency range 4000–400 cm-1 at a resolution of 4 cm-1 using a model of 2000 Perkins-Elmer spectrophotometer. The sample was thoroughly mixed with KBr; the dried mixture was then pressed to result in a homogeneous sample/KBr disc

Table 1. Five experiment for extraction process

Experiment No.	Weight of powder	Demineralizer by treated with 1N HCL (powder/HCL)	Deproteinizer By treated with 1.25N (Powder/ NaoH)	Treated with oxalic acid	Weight after drying	Appearance
E1	10g	(10g/180 ml)	10:120 ml	0.1	0.5	Dark
E2	20g	(20g/360 ml)	20:240 ml	0.2	No result	No result
E3	30g	(30g/540 ml)	30:360 ml	0.3	4g	Light brown
E4	60g	(60g/1080 ml)	60:720 ml	0.6	9g	Light brown
E5	80g	(80g/1440 ml)	80:960 ml	0.8	13g	Supper white

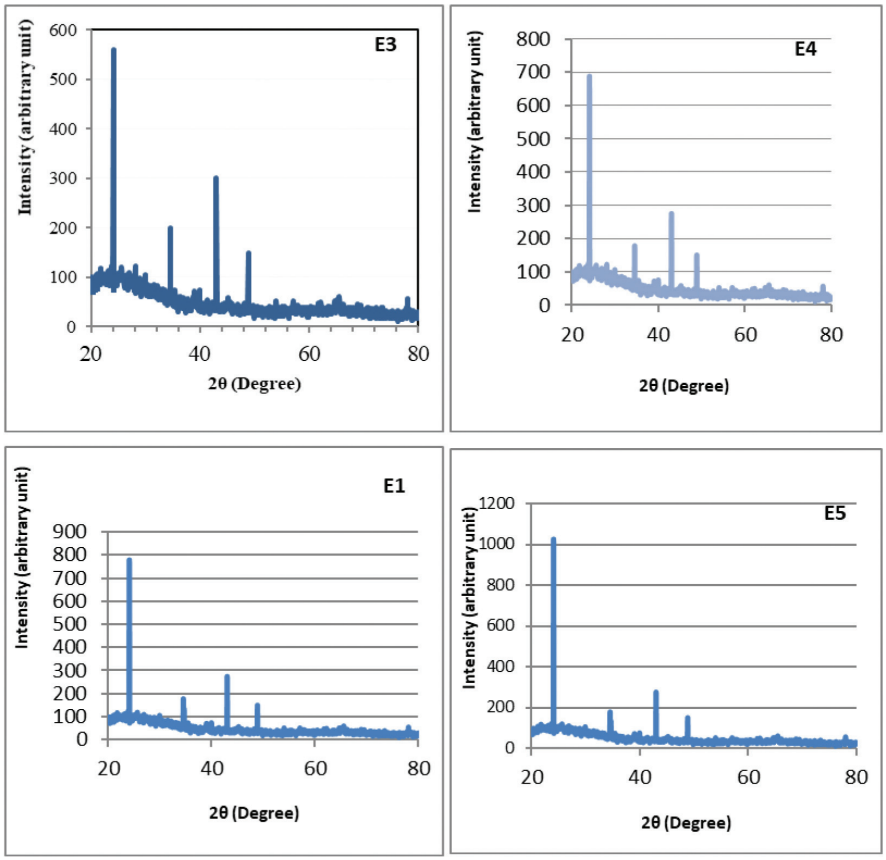

Fig. 4. XRD for chitin in four experiments.

IR result, the wavelength pattern of all the functional groups show that they are close to the standard results, except for the E3 in the amide I and NH stretching in E3 and E4.

The range of wavelength pattern in four experiment in comparison with standard chitin indicates that the resultant product can be determined as chitin, but the best result in experiment number five in comparison with the standard.

The results gained in this work show the success of the approach followed. The main idea in this work is to extract chitin from all bones in chicken in the same time. Chitin extraction from chickenbones was followed by two researchers, (Zaeeda, Issaa *et al.*, 2017) and (Jalal, Risheed *et al.*, 2012). Both worked on bone of chicken feet, but no research was

Table 3. The result of XRD of chitin in E5.

Exp. No.	2è (Deg.)	FWHM (Deg.)	dhkl Exp.(Å)	dhkl Std.(Å)	hkl
E1	28.0881	0.1712	3.1742983	3.1698	113
	29.9479	0.1511	2.98126961	2.9877	230
	75.4085	0.1	1.25951271	1.2655	311
E3	28.0881	0.1712	3.1742983	3.1698	113
	29.9479	0.1511	2.9812696	2.9877	230
	75.4085	0.1	1.2595127	1.2655	311
E4	28.0881	0.1712	3.1742983	3.1698	113
	29.9479	0.1511	2.9812696	2.9877	230
	75.4085	0.1	1.2595127	1.2655	311
E5	28.0881	0.1712	3.174298304	3.1698	113
	29.9479	0.1511	2.981269605	2.9877	230
	75.4085	0.1	1.259512712	1.2655	311

Table 4. The FTIR result of main band of chitin chicken bone for four experiment in comparison with standard chitin.

Band type	Standard chitin	E1	E3	E4	E5
C-O stretching	1030.08	1039.74	1041.08	1154.21	1250.87
Amide III	1315.04	1319.10	1307.77	1335.20	1331.44
Amide II	1558.84	1620.62	1620.17	1623.09	1588.42
Amide I	1653.83	1670.62	-	1730.65	1620.73
CH bending	1387.67	1363.82	1466.97	838.28	1455.35
Symmetric CH3 stretching	2923.48	2919.42	2923.47	2920.49	2980.41
NH stretching	3113.46	3330.00	-	-	3200.81
OH stretching	3439.58	3431.53	3400.92	2849.84	3431.04
CH stretching	2888.65	2837.21	2809.64	2822.54	2885.11

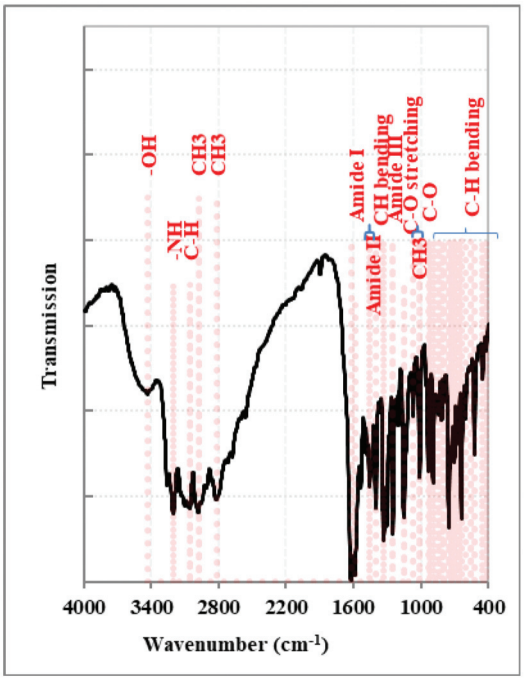

Fig. 5. Chitin FTIR spectra in E5.

found that worked on all bones of chicken, as in this work.

Conclusion

Chitin are most abundant and renewable polysaccharide, and had more attention by many researchers in last year, using as biopolymer in many applications, in this study waste recycling of chicken bone was used as inexpensive source for extraction chitin with good quantities compared to previous research.

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