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SYNTHESIS AND CHARACTERIZATION OF CHITOSAN FROM LOCUST

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ABSTRACT
Chitin was isolated from locust using a standard procedure as described in literature with slight modifications. Chitosan was then synthesized from the isolated chitin by treatment with 50% concentration of NaOH, the degree of the deacetylation obtained was 64.4% using Potentiometer Titration method. Both chitin and chitosan ash content and moisture content were then analyzed. They were then characterized using FTIR, TGA, DSC and XRD and the results compared with chitin and chitosan obtained from other sources and they thus show similar physicochemical and chemical structure. Thus locust is a promising alternative source for both chitin and chitosan.

Keywords: *chitin, chitosan, locust, isolation, characterization, FTIR, TGA, DSC, XRD.*

1.0 Introduction

Chitin found in arthropods (insects, crustaceans, arachnids and myriapods), is the second most abundant biopolymer after cellulose (Rudall and Kenchington, 1973). Structurally it is a derivative of cellulose with only an acetamido group replacement at position C – 2, i.e., β- (1,4)- 2 – acetamido- 2- deoxy- D- glucose. Chitin is insoluble in most solvent because of its compact structure. Therefore, chemical modifications of chitin are performed to obtain a soluble analogs, among which, chitosan derived by partial N- deacetylation of chitin, is the most common of such derivatives (Shahidi and Abuzaytoun, 2005; Khor *et al.*, 2003).

Chitosan and its derivatives are examples of value-added materials. They are produced from chitin, which is a natural carbohydrate polymer found in the skeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes (Shahidi and Abuzaytoun, 2005). Insects, such as butterflies and ladybugs, also have chitin in their wings and the cell walls of yeast, mushrooms and other fungi also contain this substance (Tharanathan and Kittur, 2003). Chitin a homopolymer of 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) residues linked by ß-(1-4) bonds is a common constituent of insect exoskeletons, shells of crustaceans and fungal cell walls (San-Lang *et al*, 2006). Chitosan a polymer obtained from deacetylation of chitin, is a cationic polysaccharide with linear chain consisting of β-(1, 4)-linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β- D-glucopyranose (Mathur and Narang., 1990).

Chitin and its derivatives have several applications in many field.These include, biomaterials, food, waste water, biocatalysts, emulsifying agents, agriculture, paper and textile industry (Freier *et al.,* 2005; Kalut, 2008). It has been reported that isolation of chitin from different sources is affected by the nature of the source and also the percentage of chitin where it is found varies according to the origin of the source (Muzzareli, 1997; Abdon *et al*., 2008).

The objective of the present research is to investigate the potential of locust as an alternative source for the production of chitin and chitosan.

2.0 Materials and Method

2.1 Brief History on the Life Cycle of Locust

Locusts are insects that are related closely to grasshoppers. All species of locust undergo three main life stages: egg, nymph and adult locust (Ashall and Ellis. 1962). Though all locusts go through the phases, the amount of time spent in each stage varies according to the specific species of locust. The male locust locates a female locust and inserts his semen into a sperm sac located on the female locust's abdomen. The female then releases eggs that mix with the male's semen and become fertilized. Female locusts lay their eggs in the ground, most commonly in hard, firm soil (Cressman, 1996). The eggs are deposited into the ground in groups of approximately 50. These groups are known as "pods" and they can be deposited up to 4 inches underground. A female locust will lay anywhere between one and three egg pods at one time. The locust eggs generally hatch about two weeks after they were laid. These baby locusts are referred to as "hoppers" or "nymphs." Over the next month to two months after hatching, the nymph locusts go through five molting stages called "instars." After the fifth instar, the locust's wings are fully developed (Roessingh, and Simpson. 1994). After the fifth molt, the locusts are called "fledglings." The fledglings cannot fly yet. Their bodies take approximately seven days to harden and become capable of flight. During this early stage of adult life, the locust must continually feed on vegetation in order to store up the energy necessary for reproduction and flying. It takes approximately two weeks for the fledgling locust to reach sexual maturity. Adults often group together into swarms containing thousands of locusts. Adult locusts typically live about 10 weeks. During that time, they mate and the females lay eggs (Greathead, 1996).

2.1.1 Material

The Locust used for this work was purchased from a market in Maiduguri, Borno State, Nigeria. This was then washed with water severally to get rid of dirt and other unwanted materials and dried in open air for 72h. This was then followed by grinding into fine powder.

2.2 Methods

2.2.1 Demineralization

The powdered locust (400 g) was treated with 1 M HCl solution (1000 mL) at 100 °C for 30 min to remove minerals and catechols. The demineralization treatment was followed by rinsing with distilled water until neutral to litmus paper (Johnson and Peniston, 1982).

2.2.2 Deproteination and Decolourization

Deproteination was performed using alkaline treatment with 1 M NaOH (2000 mL) solution at 80 °C for 24 h (Johnson and Peniston, 1982), and the product was washed with distilled water until the pH became neutral. For the purpose of decolourization, the product was further treated with 1% potassium permanganate solution (1000 mL) for 1 h. Finally, a lightly brown chitin was obtained which was washed with distilled water and dried in an open air for 72 h.

2.2.3 Deacetylation (Production of Chitosan)

This was achieved by treating chitin produced above with 50 % Sodium hydroxide (NaOH) solution (2000 ml) for 5h at 100 ºC. This was followed by thorough washing with distilled water until a neutral point is reached (using litmus paper). The deacetylated and washed chitosan were finally dried in an oven at 50 ºC.

2.3 Characterization

2.3.1Moisture Content

Moisture content of the prepared chitosan was determined by the gravimetric method. The water mass was obtained by drying the sample to constant weight and measuring the sample before and after drying. The water mass (or weight) was the difference between the weights of the wet and oven dry samples. The analysis was done in triplicate:

$$M\ (\%) = \frac{W - W_0}{W} \times 100 \quad (1)$$

M = % moisture content,

W = wet weight in grams,

W_o = dry weight in grams

2.3.2 Ash Content

To determine the ash value of both chitin and chitosan, 2.0 g each of chitin and chitosan sample was separately placed into previously ignited, cooled, and tarred crucibles. The samples were heated in a muffle furnace preheated to 650 ºC for 4 h. The crucibles were allowed to cool in the furnace to less than 200 ºC and then placed into desiccators with a vented top. The analysis was done in triplicate.

$$Ash\ (\%) = \frac{W}{W_0} \times 100 \quad (2)$$

W, weight of residue in grams; W_0 weight of sample in grams

2.3.3 Degree of Deacetylation (Potentiometer Titration)

The Chitosan, 0.125 g was dissolved in 25 cm^3 aqueous solution of 0.1 $mol.dm^{-3}$ hydrochloric acid; this was stirred for about 30 mins until the chitosan was totally dissolved. The solution was titrated against 0.1 $mol.dm^{-3}$ NaOH. The degree of deacetylation was calculated as follows:

$$DD\ (\%) = \frac{NH_2\%}{9.94\%} \quad (3)$$

$$NH_2(\%) = \frac{(C_1V_1 - C_2V_2)}{G(100 - W)} \times 100 \quad (4)$$

Where:NH_2 % is the calculated amount of amino group percentage present in the chitosan, 9.94 % is the theoretical amount of NH_2 percentage present in 1mole of chitosan, C_1 is HCl concentration in $mol.dm^{-3}$, C_2 is NaOH concentration in $mol.dm^{-3}$, V_1 volume of HCl solution in cm^3, V_2 is volume of NaOH solution in cm^3, and 0.016 is the molecular weight of NH_2 in $1cm^3$ of 0.1 $mol.dm^{-3}$ HCl in g, G is the sample weight in g, W is the water percentage of the sample in %.

2.3.4 Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR of chitin and chitosan was recorded on SHIMADZU, FTIR-8400S infrared spectrophotometer in the range of 4000-400 cm^{-1}.

2.3.5 Thermo Gravimetric Analysis

Thermogravimetric analysis was conducted to measure the thermal weight loss of the chitin and chitosan on a TGA Q500 V20.13 Build 39 instrument at a heating rate of 100 °C per minute in nitrogen atmosphere. The weight losses at different stages were analyzed.

2.3.6 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements were carried out on a DSC Q2000V24.10 Build 122.

2.3.7 X-ray Diffraction Studies (XRD)

X-ray Diffraction Studies was carried out on the samples to determine the pattern of crystalline and amorphous region on XPER-PRO PWO3050/60 Diffractometer system.

The crystalline index (CrI) was determined by the following equation:

$$CrI110 = \frac{[I110 - Iam]}{I110} \times 100 \quad (5)$$

Where I110 is the maximum intensity at 2θ ≅ 20° and Iam is the intensity of amorphous diffraction at 2θ ≅ 16°.

3.0 Results and Discussions

3.1 Moisture Content

The moisture content of chitin was found to be 10 .1% ± 0.5 as can be seen in Table1.0 which is lower than that of chitosan and this was expected because chitin has a high compact and crystalline structure which makes it insoluble in most solvents. The absence of extensive hydrogen bonding that promotes the semi-crystalline nature of chitin as well as presence of cohesive energy makes chitin solubility limited in all of the typical solvents including aqueous and most organic solvents (Pillai *et al*, 2009; Dutta *et al*., 2004). The moisture content of the synthesized chitosan from locust was found to be 15.1 %. It can be observed that there was an increase in moisture content of chitosan when compared to that of chitin and this can be attributed to the fact that the compact structure of chitin has been loosen by deacetylation and it can be related to the fact that chitosan has a semi-crystalline structure. The increase in moisture content with DD is probably due to increased hygroscopic nature of chitosan where more hydrogen bonds are made available (Roberts, 1992; Chandit *et al*., 1998). This significant high moisture content indicates that chitin cannot be preserved for a reasonably long period of time without the risk of microbial deterioration and spoilage (Ahmad *et al*., 2013).

3.2 Degree of Deacetylation

The degree of deacetylation (DD) was calculated by titrating aqueous solution of the prepared chitosan against a standard solution of NaOH. Depending on the source and preparation procedure, DD may range from 30 % to 95 % (Martino *et al*., 2005). This study revealed that, DD of the prepared chitosan is 64.4 % (Table 1.0). It is rare to produce chitosan with 100% degree of deacetylation as it would lead to degradation and denaturing of the product (Martino *et al*., 2005). Therefore, commercial chitosan with various degree of deacetylation in the range of 75–85 % is commonly found (Martino *et al*., 2005).

The degree of deacetylation (DD) influences the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristics, biodegradability, self-aggregation, sorption properties, and the ability to chelate metal ions. In addition, the degree of deacetylation, which determines the content of free amino groups in the polysaccharide (Li *et al*., 1992), can be employed to differentiate between chitin and chitosan. Higher degree of deacetylation resulted in higher swelling due to the presence of more amine groups generated during deacetylation processes (Hussain *et al*, 2013). Increase in temperature, alkali concentration and time of deacetylation reduce viscometric molecular weight and increase DD (Hussain *et al*, 2013).

3.4 Ash Content

The ash content of chitin is indicative of the effectiveness of the method used for removal of inorganic materials. From Table 1.0 the ash content of chitin was calculated to be 10.1 % ±0.08 while the ash content of chitosan was calculated to be 8.5 %. It was reported by Wang and Kinsella, (1976) that commercial chitosan contains ash about 1.18 %.Ash content for lobsters and crabs are normally higher than in shrimps and reducing their levels is important for ensuring good quality chitosan. Ash contents as high as 63 % have been reported in crayfish (No *et al*., 1989), lobsters *Linuparus trigonus* 54.7% (Ahn and Lee, 1992), crab *Callinectes sapidus* 50 % (Muralidhara and Maggin, 1985), prawn *Paenaeus monodon* 29 % (Benjankul and Sophanodora, 1993). The initial ash content for the crab *Sylla cerrata,* the lobster Panulirus ornatus and prawn *Paenaeus indicus* were calculated to be 45 %, 35 % and 27 % respectively (Oduor-Odote *et al*., 2005). The reduction in ash is achieved by increasing the acid concentration though there could be a risk of molecular weight (M_V) reduction due to hydrolysis of glycosidic bonds (Roberts, 1992). The relatively high ash content of chitosan could be attributed to the fact that locust is highly rich in mineral content and may require further treatment to reduce the quantity of inorganic matter. The high ash content can be reduced by increasing the concentration of acid during demineralization but this could lead to a reduction in molecular weight as a result of hydrolysis of glycosidic bonds (Oduor-Odote *et al*., 2005).

Table 1.0 moisture content, Ash content and Degree of Deacetylation Results

Sample	Moisture Content	Ash content	Degree of Deacetylation
Chitin	10.1 % ±0.5	10.1 % ±0.08	-
Chitosan	15.1 % ±0.5	8.5 % ±0.2	64.4 % ±2

3.5 FTIR Analysis

The FTIR spectrum of chitin from locust is shown in the Figure1 (a). This spectrum is quite similar and can be compared to those of α- chitin isolated from other sources as recorded in the literatures (Majtan *et al*., 2007; Sagheer *et al*., 2009) The spectra were characterized by three important amide bands at 1647, 1435.09, and 1187.23 cm^{-1}. These band correspond to the amide I stretching of C=O, the amide II of the N-H and amide III of the C-N respectively.

Figure1 (b) is the FTIR spectral of chitosan which gives a characteristic band at 3407.37 cm^{-1} which could be attributed to $–NH_2$ and –OH groups stretching vibration and the band for the amide I at 1638.58cm^{-1} is seen as the infrared spectrum of chitosan obtained from hosts of other sources that have been previously worked on (Majtan *et al*., 2007; Sagheer *et al*., 200).

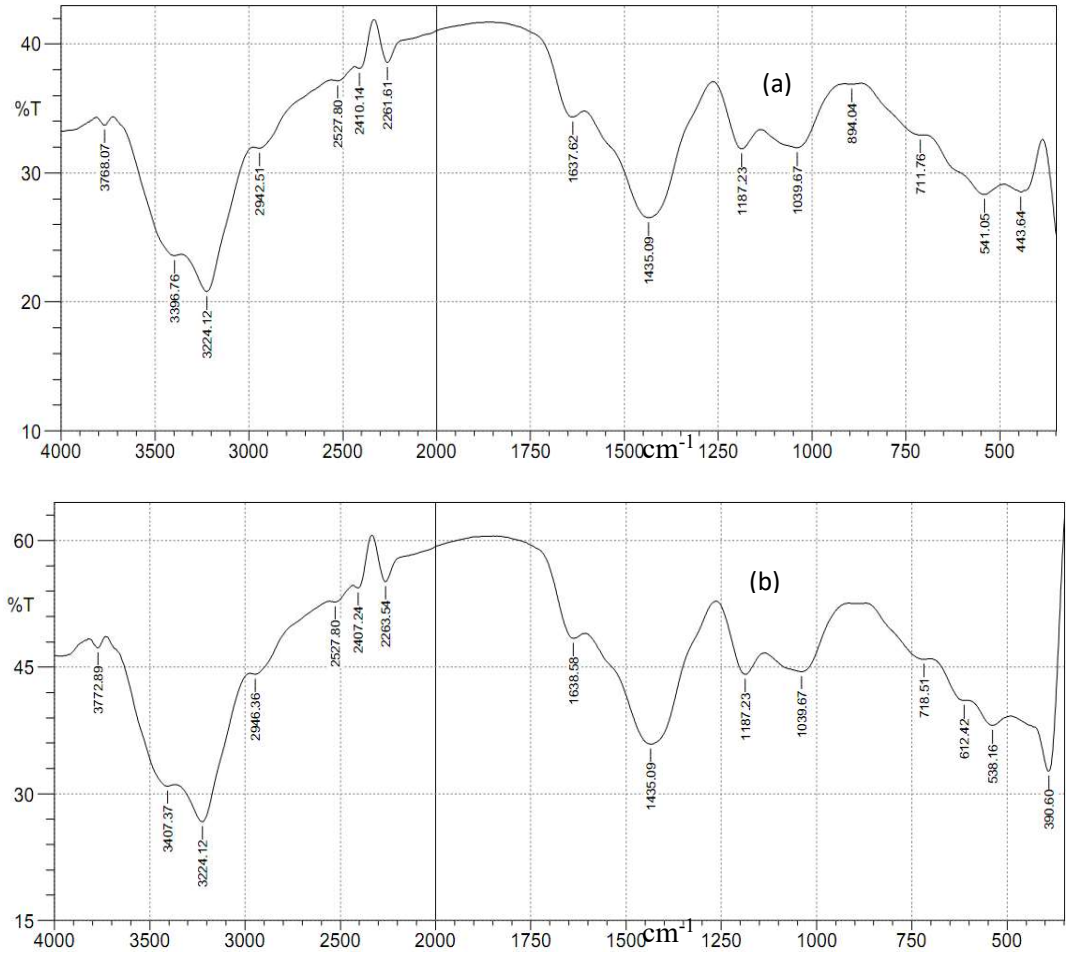

Figure1.0 FTIR Spectrum of Chitin (a) FTIR Spectrum of Chitosan (b)

3.6 TGA Analysis

The TGA of chitin is shown in Figure 2(a). The TGA of chitin shows a weight loss at prominent three stages. The first stage is at 199.13 °C, this shows about 10 % loss in weight. This may be associated to the loss of adsorbed and bound water (Shanmugapriya *et al.,* 2011). The second stage of weight loss occurs at about 313.15 °C which correspond to about 50 % of weight loss due to the decomposition when the derivative weight curve is considered. The last stage of decomposition occurs at 358.30 °C.

Figure 2(b) which is the TGA of chitosan equally shows a weight loss at three stages. The first stage is at 260.35 °C corresponding to about 10 % loss in weight. This may be attributed to the loss of adsorbed and bound water (Shanmugapriya *et al*., 2011). The second stage of weight loss occurs 346.05 °C while the last stage of decomposition occurs at 382.78 °C. A close observation of these values reveals that deacetylation of chitin has enhanced its thermal stability. From the results obtained chitosan is therefore more thermally stable than chitin.

3.7 DSC Analysis

Figure 3(a) Show the DSC thermogram of chitin. For chitin the first exothermic peak was recorded at 61.08 °C and the second exothermic transition temperature was recorded at 277 °C.

Figure 3(b) Shows the first exothermic peak of chitosan at 62.39 °C which correspond to the glass transition temperature (Tg) of chitosan. The second exothermic transition of chitosan is at 263.92 °C, which is lowered when you compare with that of chitin and this may correspond to the decomposition of the polysaccharide. This analysis is in agreement with what Prerna and Ramanand (2012), Sakurai *et al* (2000) Yanming *et al* (2004) have obtained.

(a)

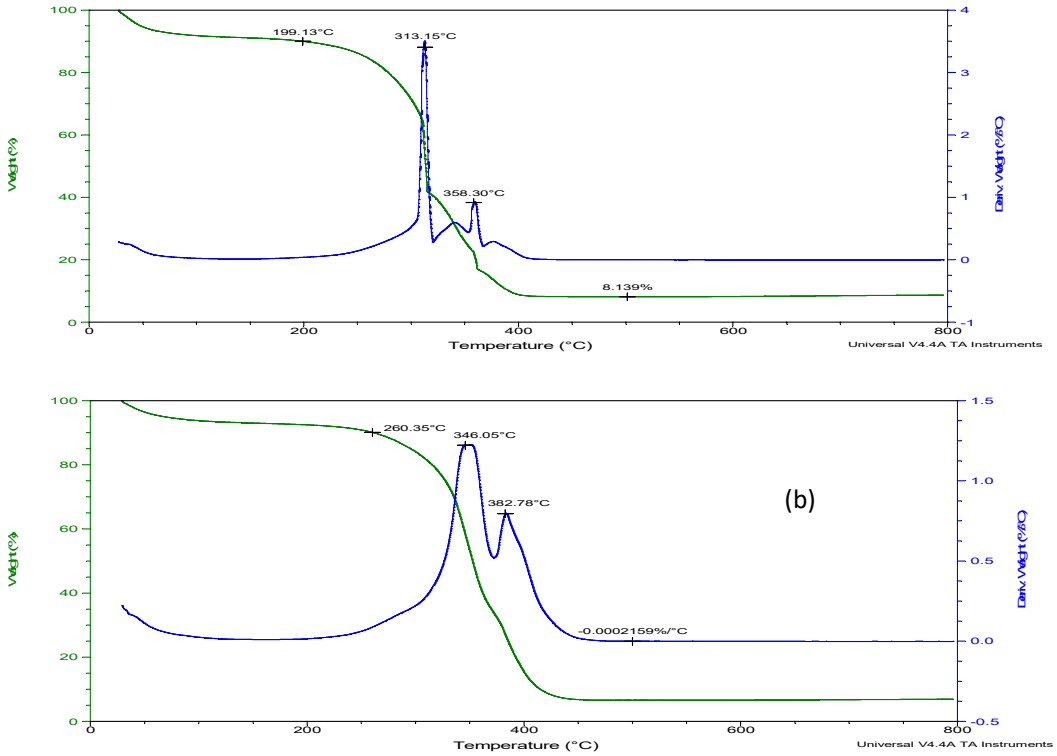

Figure2: TGA of chitin (a) and TGA of chitosan (b)

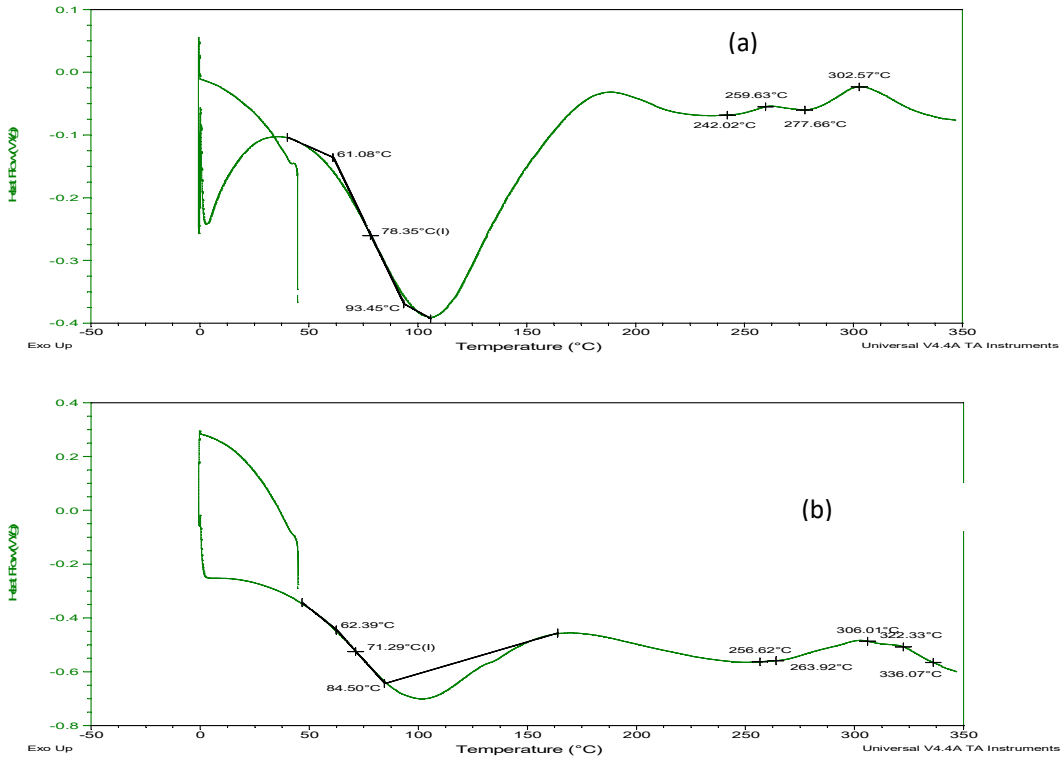

Figure 3: DSC of Chitin (a) and DSC of Chitosan (b)

3.8 X-Ray Diffraction Studies

Figure 4(a) represents the XRD pattern of chitin extracted from locust as it can be seen from the result obtained on the graph, there are two strong reflections at 2θ angles of 9.1 and 19.15° and other minor reflections at 12.5, 22.9 and 26.4°. When this was compared to chitin extracted from beetle species like cicada sloughs which was also reported, followed a similar pattern with

reflections at 2θ 9.20 and 19.18, while its own minor reflections appeared at 12.60, 20.68, 23.30 and 26.48° respectively (Sajomsang and Gonil, 2010), one will agree that there is a great similarities in their diffraction patterns. The crystallinity of this chitin was calculated to be 88.9% using the crystalline index (CrI) equation as stated earlier.

The XRD pattern of chitosan is illustrated in Figure 4(b). The XRD pattern of chitosan exhibits broad diffraction peaks at 2θ angles of 9.8º and 21º which are typical finger prints of semi-crystalline chitosan. The crystallinity of chitosan was calculated to be 76 %. When compared to what Bangyekan *et al,* (2006) have also reported. Yen and Mau (2007) found that fungal chitosan also showed two crystalline reflections at 9.7º and 19.9º. This also supports what has been obtained this research. Remember that chitin is highly crystalline with its molecules closely packed which impede its aqueous solubility therefore to reduce the crystallinity chemical modification in the form of deacetylation has to be performed which is what has been carried out and the result from XRD patterns confirms this result(Shahidi and Abuzaytoun, 2005; Khor *et al*., 2003). From the result of the crystallinity calculated it is evident that chitin with 88.9 % is more crystalline than chitosan with 76 % reason been that chitosan is a semi-crystalline polymer.

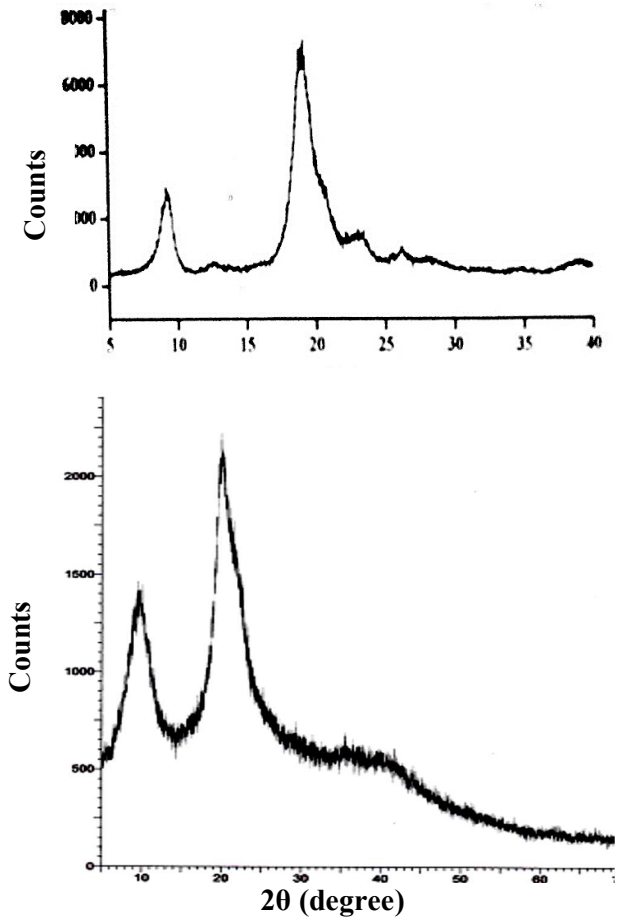

Figure 4: XRD of Chitin (a) and XRD of Chitosan (b)

4.0 CONLUSION

Chitin was extracted from locust using a standard procedure as obtained from literatures with a slight modification of the process. The chitin extracted was successfully converted into chitosan with a degree of deacetylation of 64.4 % which was determined using Potentiometer Titration. The moisture and Ash content of both chitin and chitosan was determined. FTIR, TGA, DSC and XRD were used to furnish the structure of chitin and chitosan and this was compared to those obtained from other sources and it was evident that there were similarities between them. Evidently from the result obtained locust is a potential alternative source for the extraction of chitin and synthesis of chitosan.

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