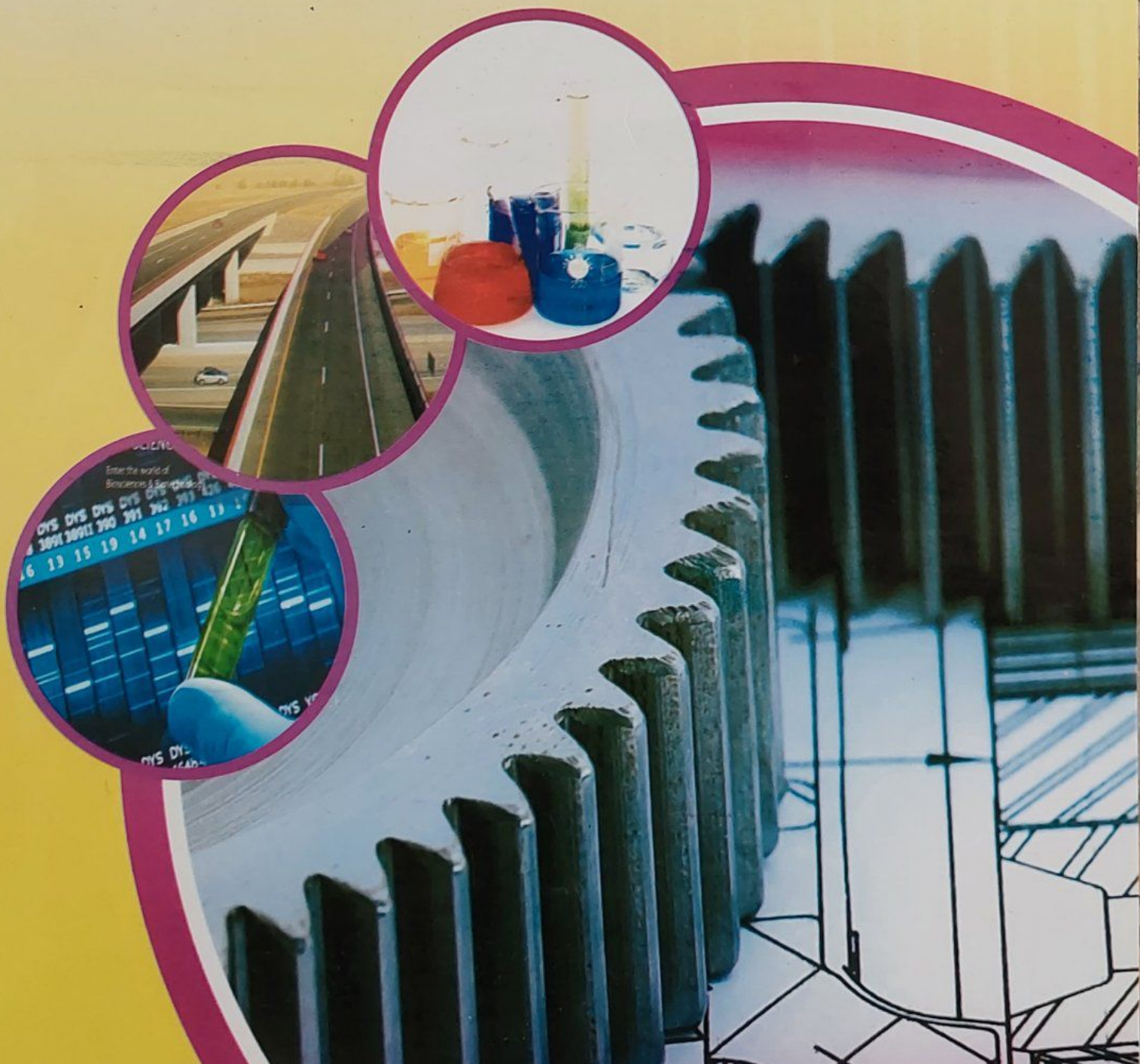




# NIGERIA JOURNAL OF ENGINEERING AND APPLIED SCIENCES (NJEAS)

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## **NIGERIA JOURNAL OF ENGINEERING AND APPLIED SCIENCES (NJEAS)**

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## Proximate Analysis and Characterization of Some Aquatic Wastes as Potential Feedstock for Chitin/Chitosan Production

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### Abstract

The aim of this study is to evaluate the proximate and mineral characteristic of Fish scale (FS), Shrimps shell (SS) and Crab shell (CS) as potential raw materials in the production of Chitin/Chitosan. The analyses carried out include proximate analysis, mineral composition and characterization of samples of these aquatic wastes using known and established standard procedures. It was observed that proximate and mineral composition analyses revealed that samples of Shrimp shell, Crab shell and Fish scale contain 7.09, 5.24 and 6.31 % moisture, 51.67, 59.31 and 42.73 % ash content, 7.19, 6.75 and 4.88 % lipid, 4.10, 2.52, and 5.43% nitrogen, 25.67, 15.75 and 37.17% crude protein, 14.0, 7.30, 5.7% crude fiber, 85.92, 93.02, 86.56 % Ca and 0.5796, 0.5869, 0.8115 % K respectively. The functional groups of the aquatic wastes (FS, SS, CS) was established by Fourier-transform infrared (FTIR) spectroscopy. The mineral compositions and thermal stability tests were assessed by X-ray fluorescence (XRF) and thermogravimetric analyses (TGA) respectively. The performance of these aquatic waste samples as potential raw materials for different industrial applications, were ascertained. Also, various results of the analyses disclosed that proximate and mineral compositions of aquatic wastes are fundamental parameters for different chitin/chitosan production.

**Keywords:** Proximate, Mineral, Aquatic wastes, Characterization and chitin/chitosan

### Introduction

Aquatic resources have been reported as important food commodities in the international trade and that they constitute an important food component for a large section of world population with widely acceptable sources of protein and other elements necessary for the maintenance of healthy body (Adebayo-Tayo *et al.*, 2012; Nunoo *et al.*, 2013). Utilization of these resources have increased because of their availability, palatability and affordability in nature (Foran *et al.*, 2005; Gokoglu, and Yerlikaya, 2015). Also, aquatic food sources play an important role in food security and poverty alleviation in some parts of the world. Although, aquatic animals are extremely liable to deterioration immediately after harvest if there is no provision for adequate storage techniques

and this could bring about possibly economic losses and different environmental problem such as aquatic wastes (Hei and Sarojnalini, 2012; Adejonwo, 2016).

Huge amount of these aquatic wastes is generated from the production and processing of these aquatic resources such as fish, crabs and shrimps which constitute another source of water pollution since most of them are littered on river banks thereby restricting tourist activities (Mejia-Saules *et al.*, 2006; Abubakar *et al.*, 2017). However, effective utilization of generated waste materials from aquatic resources has been identified as another way towards achieving environmental pollution control and sustainable environmental development since it has long been known that these wastes are rich sources of proteins and

mineral elements (Mridha *et al.*, 2005; De Holanda and Netto, 2006; Gortari and Hours, 2013; Divya *et al.*, 2014). Therefore, this study plans to evaluate the proximate and mineral composition, as well as the characteristics properties of fish scale (*Oreochromis niloticus*), shrimps shell (*Penaeus notialis*) and crab shell (*Callinectes pallidus*) as potential raw materials of products (like chitin, chitosan etc.) for various industrial applications.

## Materials and Methods

### Material selection

The major materials used in this research work include samples of waste scales and shells of Africa Arowana fish scale, White Shrimp shells and Gladiator Swim crab shells. Major reagents used during the experimental work include Hydrogen sulphate ( $H_2SO_4$ ), Copper sulphate ( $CuSO_4$ ), Hydrogen chloride (HCl), Sodium hydroxide (NaOH), Ammonium acetate ( $C_2H_7NO_2$ ), Calcein indicator ( $C_{30}H_{26}N_2O_{13}$ ), Ethylenediaminetetraacetic acid -EDTA ( $C_{10}H_{16}N_2O_8$ ) and Petroleum ether, all of which were of analytical grades manufactured by Analar BDH. Major apparatus and instruments used were X-ray fluorescence (EA1200VX), oven (SWT320), Thermogravimetric Analyser (TGA 4000), Distillation Apparatus (Z250325), Digital weighing balance (Scout pro SPU), Muffle furnace (FSE-621-010L) and Fourier Transform Infrared spectrometer (Parkin elmer).

### Sample collection and labelling

Three different fresh waste samples each of Africa Arowana (*Oreochromis niloticus*), White Shrimp shells (*Penaeus notialis*) and Gladiator Swim crab shells (*Callinectes pallidus*) were obtained during the rainy season, from Mobil and Makoko

fish market, in Chanchaga and Yaba Local Government Areas of Niger and Lagos States, Nigeria respectively. The samples were washed and placed in an insulated ice bag for preparation and analysis (Ibrahim *et al.*, 2013; Abubakar *et al.*, 2017)

### Sample preparation

The samples of aquatic wastes (FS, SS and CS) were thoroughly washed, oven dried at 60 °C for 24 h and sieve at 300  $\mu$ m. The crushed samples were stored inside a desiccator for further analysis (Hossain and Iqbal, 2014; Ruchira *et al.*, 2015; Abubakar *et al.*, 2017).

## Method

The modified method reported by Abdou *et al.* (2008), Zaku *et al.*, (2011), Muhammed *et al.*, (2012), Abdulwadud *et al.* (2013) and Abubakar *et al.* (2017) were used in carrying out proximate and mineral content analyses of the prepared samples (fish scale, crab and shrimp shells). The proximate analysis consists of percentage moisture content, percentage ash content, crude fiber, lipid, nitrogen and crude protein.

### Ash content

The determination of ash content was carried out by weighing an empty cleaned gooch crucible ( $W_1$ ) and about 2 g of each of the samples was added to the crucible and then weighed ( $W_2$ ). Thereafter, both were placed in Muffle furnace (FSE-621-010 L) for ashing at 550 °C for 6 h. After ashing, the crucibles containing each sample were cooled in desiccator and weighed again ( $W_3$ ). Percentage ash content (PAC) was calculated using equation (1) (AOAC, 2005; Fawole *et al.*, 2007; Kucukgulmez *et al.*, 2006; Musaiger and Souza, 2008; Ali and Kumars, 2010).

$$PAC = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)} \quad (1)$$

#### Lipid content

The lipid content was determined by either extraction method using Soxhlet apparatus as reported by Musaiger and Rumaidh (2005), Kucukgulmez *et al.* (2006), Musaiger and Souza, (2008) and Abdou *et al.* (2008). Filter paper was weighed, ( $W_1$ ) and 1 g of moisture free sample was added ( $W_2$ ) which were introduced in to the extraction tube. The Soxhlet apparatus was assembled and allowed refluxing for 6 h. Extract obtained was placed on clean glass dish and transferred into an oven at  $110^{\circ}\text{C}$  for 45 min, cooled in desiccators and weighed again ( $W_3$ ). The percentage lipid content (PLC) was determined by using equation (2).

$$PLC = \frac{(W_2 - W_3) \times 100}{(W_2 - W_1)} \quad (2)$$

#### Protein content

The protein content of the sample was determined using kjeldahl method, where 0.5 g of dried samples was taken into digestion flask that contains 15 ml of concentrated  $\text{H}_2\text{SO}_4$  and 0.5 g cupric sulphate. 10 ml of digest was introduced into the distillation tube. Thereafter, 10 ml of 40 % NaOH was gradually added. The 50 ml of collected distillate was then titrated against standard 0.1 M HCl solution till the appearance of pink colour. Equation (3) and (4) were used to calculate Percentage crude protein content of each sample (Kucukgulmez *et al.*, 2006).

$$\% \text{ Nitrogen} = \frac{S \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V} \quad (3)$$

$$\% \text{ crude protein} = 6.25 \times \%N \quad (4)$$

where, S = Sample titration value (ml); N = Normality of HCl; V = Volume taken for

distillation (ml); D = Dilution of sample after digestion (ml); 0.014 = Milli equivalent weight of Nitrogen

#### Mineral composition analyses of selected aquatic wastes

The mineral compositions were carried out with the aid of versinate titration method. For the determination of calcium, 20 ml of filtrate was transferred into conical flask with 20 ml of 20 % KOH. About 50 ml of distilled water and 0.5 g of calcein indicator was added to each sample, and then titrated with 0.1 M EDTA until there was a colour change (AOAC, 2005; Kucukgulmez *et al.*, 2006; Musaiger and Souza, 2008; Ali, and Kumars, 2010; Abubakar *et al.*, 2017).

#### Characterization of Samples

The surface areas and functional groups of each samples were carried out with the help of Gas Sorption Analyzer (GSA) and Fourier Transform Infrared spectrometer (FTIR). Also, Mineral composition of each samples were carried out with the aid of X-ray fluorescence (XRF) and thermal analysis with the aid of Thermogravimetric Analyzer(TGA) (Prashanth, 2003; Muhammed *et al.*, 2012).

#### Moisture content and sample stability test

Moisture content and stability of the samples were determined using thermal analyzer (TGA 4000 Perkin-Elmer) to automatically measure the weight loss of each sample as a function of temperature and time. The samples were subjected to pyrolysis under  $\text{N}_2$  flow (35 ml/min) with heating rate of  $20^{\circ}\text{C}/\text{min}$  (Hu *et al.*, 2008; Basta *et al.*, 2009; Liou, 2010; Dai *et al.*, 2011; Abubakar *et al.*, 2017).

### Mineral composition of the selected aquatic wastes

The mineral composition of the selected aquatic wastes was determined with the use of EA1200VX X-ray fluorescence (XRF) spectrometer. The samples were loaded into the primary x – ray beam. Samples were transferred into crystal chamber where diffracted secondary x – ray occurs with the aid of collimator and the percentage composition were obtained (Ameh *et al.*, 2013).

### Surface functional group analysis

The surface functional groups of the samples were determined by Fourier Transform Infrared (FTIR) spectroscopy using a spectrometer (Parkin Elmer -200). About 2 mg of the sample was weighed and mixed with 100 mg of the binder potassium bromide (KBr). The spectra of these aquatic waste samples were obtained within ranges of  $\lambda = 400 - 4000 \text{ cm}^{-1}$  at each of temperature setting. The data obtained were plotted with the aid of essential Fourier Transform Infrared (eFTIR) software (Paulino *et al.*, 2006; Dai *et al.*, 2011; Liu *et al.*, 2011).

## Results and Discussion

The study of proximate analyses and mineralogical composition of the waste samples from aquatic species like shrimp, crab shells and fish scales are essential in estimating their quality and potential to serve as possible raw material for utilization in different technological processes (Fawole *et al.*, 2007; Hei and Sarojnalini, 2012; Abubakar *et al.*, 2017). However, the proximate analysis and mineral compositions of aquatic wastes were determined to gain the knowledge of the

cost associated with further preparation toward chitin/chitosan synthesis and its application. The results of proximate and mineral composition of the aquatic wastes; shrimp shell (SS), crab shell (CS) and fish scale (FS) were presented in Table 1 and 2.

**Table 1:** Proximate composition of the shrimp shell (SS), crab shell (CS), fish scale (FS)

Parameter	SS (%)	CS (%)	FS (%)
Moisture	7.09	5.24	6.31
Ash	51.67	59.31	42.73
Lipid	7.19	6.75	4.88
Nitrogen	4.10	2.52	5.43
Crude Protein	25.67	15.75	37.17
Crude Fiber	14.00	7.30	5.70

**Table 2:** Mineral composition of the shrimp shell (SS), crab shell (CS), fish scale (FS)

Element	SS (%)	CS (%)	FS (%)
S	2.0387	0.4024	1.4891
K	0.5796	0.5869	0.8115
Ca	85.9217	93.0236	86.5558
Sc	0.2731	0.1791	0.4159
Mn	LOD	0.0237	LOD
Fe	0.3860	0.0259	0.4086
Zn	0.0297	0.0015	0.0183
As	LOD	0.0007	LOD
Rb	0.0098	0.0040	0.0018
Sr	0.7571	0.7507	0.2968
Zr	0.0032	LOD	LOD
Mo	LOD	LOD	0.0014
Hg	LOD	0.0015	LOD
Th	0.0011	LOD	0.0008

LOD: Low Detection

Proximate analysis obtained for the samples from Shrimp shell (SS), Crab shell (CS) and Fish scale (FS) were as presented in Table 1. It can be seen that sample of Shrimp shell, Crab shell and Fish scale contains 7.09, 5.24 and 6.31 % moisture lower than 8.27 % reported by Elegbede and Fashina (2013), these differences could be due to

techniques of moisture content removal and the nature of species. Abubakar *et al.* (2017) reported that the amount of moisture absorbed by different raw samples prior to further synthesis may result to high quality of chitin/chitosan production.

Ash content is a measure of the mineral (Ca, K and Mg) content of raw sample. Table 1 revealed the ash content of 51.67, 59.31 and 42.73% for shrimp shell, crab shell and fish scale which is higher than 46.01 % reported by Elegbede and Fashina (2013). The concentration of minerals composition that contribute to the total ash content can be influenced by some factors such as seasonal, particle size, food source and nature of the water where the aquatic animals were caught (Adejonwo, 2016). The observed value of ash content indicated that all the species of the selected aquatic wastes contain minerals such as calcium, potassium and magnesium. This suggests that, demineralization will not be omitted during the process of chitin/ chitosan synthesis for various industrial applications. This study has revealed the percentage lipid content of shrimp shell, crab shell and fish scale as 7.19, 6.75 and 4.88 % lower than 11.70 % reported by Obaroh *et al.* (2015). The Lipid content of all aquatic wastes were relatively lower, it may be as a result of the environment and the type of diet they consumed (Obaroh *et al.*, 2015). The results of crude fiber for shrimp shell, crab shell and fish scale were 14.0, 7.30 and 5.7 % respectively, which is lower and higher than 53.56 and 1.81 % reported by Elegbede and Fashina (2013) and Adejonwo, (2016). The nitrogen content calculation is linked with crude protein. The values of nitrogen content are 4.10, 2.52, and 5.43 % for shrimp shell, crab shell and fish scale, while that of crude protein were 25.67, 15.75 and 37.17 % respectively. This observation is in

line with the findings of 13.41, 21.21, and 38.40 % reported by Elegbede and Fashina, (2013), Adejonwo, (2016) and Fawole, (2007) respectively. The differences in the nitrogen and crude protein content could be as a result of different varieties of species used and the high protein content of their diets. This also suggests that, deproteination will not be omitted during the process of chitin/ chitosan synthesis for various industrial application.

Table 2 shows results of mineral composition of the samples under study. It can be seen that Shrimp shell (SS), Crab shell (CS), and Fish scale (FS) contain 85.95, 93.02 and 86.56 % elemental Ca respectively. The crab shell had a relatively higher content of Ca when compared to others. The % compositions of Ca were found to be higher but with lower K values in this study as against the reported values in the work of Ameh *et al.* (2013). This observation may be attributed to different environment and species used. However, the mineral elemental content of each aquatic wastes is a function of the availability of these elements in the water body where they live (Adejonwo, 2016). The mineral composition content indicates that the species are good sources of macro elements such as calcium, potassium, and sulphur.

#### *Thermogravimetric Analysis (TGA)*

Thermogravimetric analysis (TGA) examine the thermal degradation and phase transition of raw materials, the thermal analysis in which changes in physical and chemical properties of materials were measured as a function of increasing temperature. The results of Thermogravimetric Analysis (TGA) and Derivative Thermogravimetric (DTG) of the



aquatics wastes are shown in Figs 1– 3. The Figs show the graphical representation of TGA/DTG analyses of Fish scale, Shrimp shell and Crab shell samples respectively.

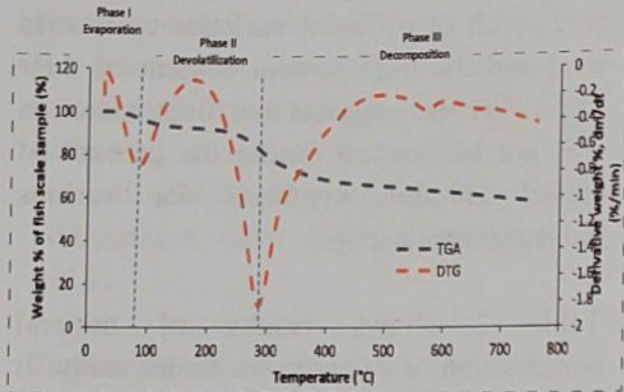


Figure 1: Thermogravimetric Analysis (TGA) and Derivative Thermogravimetric (DTG) of Fish scale

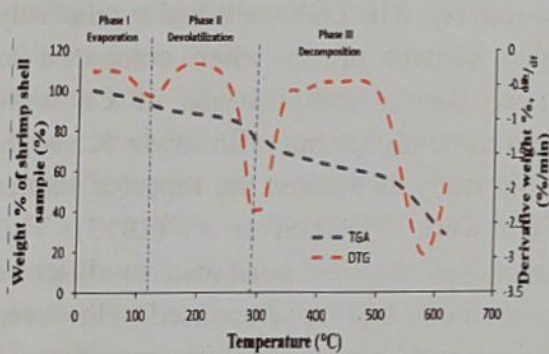


Figure 2: Thermogravimetric Analysis (TGA) and Derivative Thermogravimetric (DTG) of Shrimp shell

Analysis of the graphs (Figs 1-3) show mainly two major weight losses, the first one was recorded at 90, 110 and 150 °C respectively.

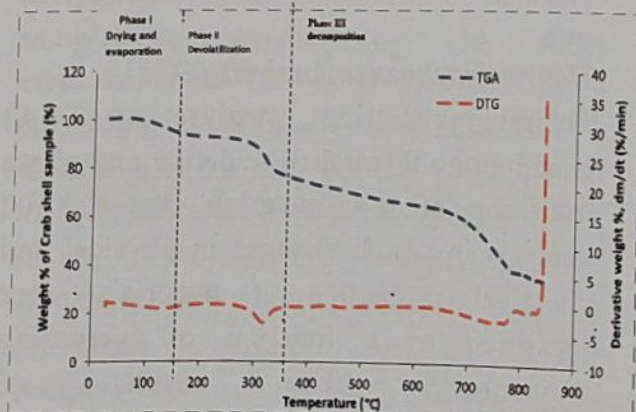


Figure 3: Thermogravimetric Analysis (TGA) and Derivative Thermogravimetric (DTG) of Crab shell

The second losses recorded before 290, 290 and 320 °C respectively. These are within the values of 30 – 100 and 210 – 450 °C for first and second stages as reported by Saravanan *et al.* (2011). The first one could be attributed to the elimination of water molecules from the corresponding samples with moisture contents of 6.31, 7.09 and 5.24 % (as shown in Table 1). These observations were found to be in line with the values obtained by Obaroh *et al.* (2015). The second observed weight losses could be attributed to the decomposition of volatile products (Vijayalakshmi *et al.*, 2016). Maximum temperature occurred at 303, 288 and 282 °C for Crab shell, shrimp shell and fish scale respectively which is in line with the values reported by Vijayalakshmi *et al.* (2016).

#### Fourier transform infrared (FT-IR)

The surface functional groups of these aquatic wastes were carried out by FT-IR. The results obtained were presented in Fig. 4.

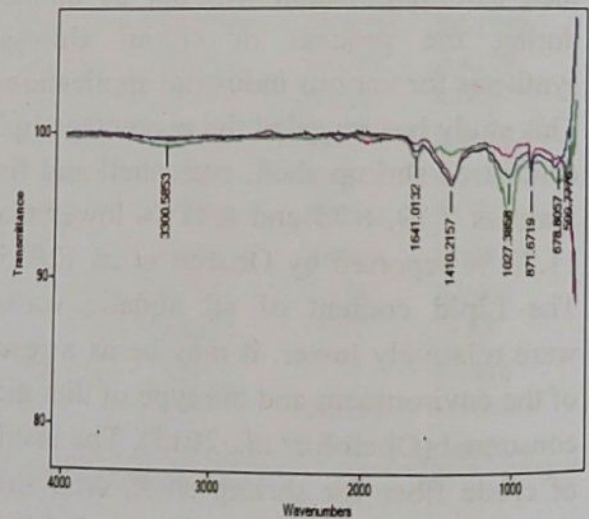


Figure 4: FTIR vibration of aquatic wastes - SS, CS and FS.

Fig. 4 shows the FTIR vibration of aquatic wastes used (SS, CS and FS). Based on this result, it can be seen that the FTIR spectra of the various aquatic wastes sample analyzed were related to each other, although there were slight differences in the absorption intensities and fall within

standard value as indicated in Figure 4. The vibration peak which represents the -C-O stretching and primary alcoholic group (O - H) were observed at 1027.39, 1025.05, 1240.02 and 2834.74, 2485.65, 3297.70  $\text{cm}^{-1}$  for SS, CS and FS respectively. While Saravanan *et al.* (2011) reported values of -C-O stretching and primary alcoholic group (O - H) of 1030 and 3434  $\text{cm}^{-1}$ . Also, the main vibration values were compared with standard such as N-H stretching, N - H bending, C=O stretching in secondary amide (amide I) and C-N- stretching in secondary amide (amide II). The FT-IR spectral analysis of aquatic wastes confirm the functional properties of polymeric matter such as chitin and its derivatives (Tamimi and Herdyastuti, 2013)

### Conclusion

The evaluation of the proximate, mineral and physiochemical characteristic of Fish scale (FS), Shrimps shell (SS) and Crab shell (CS) as potential feedstock in the production of Chitin/Chitosan was achieved. The results suggest that the raw materials play vital roles in chitin/chitosan production as outline in this research work and can avert the environmental problems associated with aquatic wastes disposal. The FT-IR spectral of aquatic wastes confirm the functional groups of biopolymeric matter like chitin and chitosan.

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