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Original Research Article

Characterizing Chitin from *Dendrobranchiata* and *Caridea* Decapod Crustaceans

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ABSTRACT

Prawns and shrimps are marine crustaceans belonging to the decapoda family and are often distinguished in terms of their body structure and habitat. Both however, deliver nutritional values in similar amounts. In this study, the properties of a useful biopolymer – chitin chemically extracted from the exoskeletons of the two crustaceans were compared. Demineralization and deproteinization were carried out with the use of 2M HCl and NaOH respectively for complete chitin isolation. Morphology of both chitin particles appear as plate-like fibrils. The degree of acetylation (DA) of prawn chitin was 98.01% while that of shrimp was 98.03%. Activation energy Ea measured from thermogravimetric analysis (TGA) was 117.1 and 116.3 kJ/mol for prawn and shrimp chitin respectively. Results from Fourier Transform Infrared Spectroscopy (FTIR) showed that chitin from both sources contain similar functional groups which exist at similar wavenumbers. In addition, average hydrogen bond (E_H) calculated from FTIR result was 4.09 kCal for prawn chitin while that for shrimp chitin was 4.07 kCal. X-Ray diffraction results showed that the two biopolymers display similar diffraction patterns. The crystallinity (Crl) of prawn and shrimp chitin were 71.3 and 70.5% respectively. The comparable properties of chitin observed from these two sources suggest that either can be used for a specific application.

1. INTRODUCTION

Industrialization and urbanization are being noted as a product of human development in any modern society today. The various human activities in the modern society lead to the generation and disposal of wastes, which causes environmental pollution. The organic nature of waste in the food industry is reported to contain valuable components such as proteins, carbohydrates, fats and vitamins which can be recovered (Tarafdar and Biswas, 2013). The high biochemical oxygen demand (BOD) constituents of these organic wastes qualify them as high pollutants. In the mangrove/coastal regions, crustaceans such as shrimps and prawns which belong to the family *Decapoda*, are confirmed to be domiciled in different marine habitats (Ayoola *et*

al., 2009; Wahidah *et al*., 2017). The consumption of these marine invertebrates will foster the establishment of various shellfish industries and could contribute immensely to the economic growth of a country. Although, both are of the same family, they belong to different sub-orders which makes each possess dissimilar physical features which assist in distinguishing them. Shrimps are observed to be smaller than prawns. In terms of side plates, prawn shell is characterized with overlapping side plates which keep their bodies straight while the curly nature of shrimp is being attributed to the segmented side plates which are overlaid in front and behind the crustacean. Shrimp's natural habitat is salt water (Ayoola *et al*., 2009) while prawns are predominantly found in fresh water (Wahidah *et al*., 2017). In terms of nutritional values, both crustaceans deliver (in almost similar amount) lean protein, Omega-3 fatty acids, selenium (an antioxidant), vitamin B12, iron and phosphorus (Nesara and Paturi, 2018). They can therefore be consumed as recipes interchangeably. The edible tissues are either consumed or further processed by sea food industries while their exoskeletons are discarded as wastes. To curb environmental challenges initiated by these degrading shells, majority are been used as feeds in agriculture while some have served as source of proteins, fatty acids, crude fiber and carbohydrate (storage polysaccharide) as analyzed via proximate analysis (Ravichandran and Rameshkumar, 2009; Asaikkutti *et al*., 2016; Ali *et al*., 2017). According to Asaikkuttu *et al.* (2016), the amount of carbohydrate content in prawn shell is more than that present in the edible portion, while protein and lipid contents are more in the edible part compared to the shells. Comparing proximate analysis results of the fresh and frozen crustacean, they concluded that their essential constituents (nutrients) are not sustained when frozen.

Chitin, a structural polysaccharide is another useful component embedded in crustacean shells**.** Isolation is mostly carried out chemically with the use of acid and alkali solutions to remove calcium carbonate ($CaCO_3$) and protein respectively (Al Sagheer *et al*., 2009). The stronger the chitin- $CaCO_3$ interaction (which is also exemplified by the dominance of the mineral), the higher the energy required for the separation. This was observed in the works of Gbenebor *et al.* (2016) where chitin was isolated from shells of crab and shrimp. Crab shell contains more of $CaCO_3$ than that of shrimp and as a result, higher volumes of acid solution were consumed on interaction with crab shell. The properties of chitin sourced from these two crustacean wastes however differ. Physicochemical properties of chitin sourced from marine invertebrates such as Atlantic Krill, lobster, xiphosure, black coral, periwinkle and snail have been proven to be different) and this could be attributed to their variations in biological make ups (Wang et al., 2013; Juarez-de la Rosa *et al*., 2015; Gbenebor *et al*., 2017).

Apart from habitat, size and nutritional components as investigated by proximate analysis on prawn and shrimps, there is a need to explore the features of chitin that is being sourced from these two similar decapods. This work therefore entails comparative studies on physicochemical properties of chitin isolated from prawn and shrimp shells. Outcome of investigations informs whether both biopolymers from the two sources are different in features or if both can be used interchangeably in any preferred application.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Shrimps and prawns were collected from salt and fresh waters in Badagry, Lagos state. The edible portions were removed for consumption while their shells were thoroughly washed and sun dried at room temperature. Completely dried shells were ball milled to 200 µm particle sizes and treated with 2M HCl at room temperature to remove $CaCO_3$ (demineralization). The mixture was filtered and residues were washed with distilled water. Deproteinization was conducted at 100 ^oC as demineralized particles were refluxed in 2M NaOH for 1 hour. The residue obtained from the solution was washed and dried before depigmetization, which was carried out with the use of 1M H_2O_2 at room temperature. The depigmetized particles were

washed, filtered and dried for characterizations. This chemical method of chitin isolation was done as in Isa *et al.* (2012).

2.2. Sample Characterization

2.2.1. Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDS)

Samples were coated win Au to enhance proper electrical conductivity which was scanned using an ASPEX 3020 model variable pressure SEM. A Noran-Voyager energy dispersive spectroscope attached to the SEM machine was used for the EDS characterization where samples were analyzed at an accelerating voltage of 15 kV.

2.2.2. Thermogravimetric analysis (TGA)

Analysis of samples was carried out on TGA Q500 instrument where 2 mg of samples were heated to 750 ºC at 10 ºC/minute heating rate. In this test, the temperature at the onset of thermal decomposition, temperature at the end of decomposition and chitin content were deduced from the thermograms. The activation energy E_a was calculated using Broido method (Broido *et al.*, 1969) as shown in Equation 1.

$$\text{Ln} (- \text{Ln} (1- X)) = - Ea/RT + \text{Const.} \quad (1)$$

Ea is the activation energy of the degradation reaction (kJ/mol), R is the universal gas constant (8.314 J/mol·K) and T is the absolute temperature (K). The degree of decomposition X is given by:

$$X = [(W_o - Wi) / (W_o - W_f)] \quad (2)$$

Where W_o is the initial weight of the sample, W_i is the instantaneous weight of the sample at time t and W_f the final weight of the sample. The plot of Ln (- Ln (1- X)) against 1/T gives a straight line whose slope is – E/R.

2.2.3. Differential scanning calorimetry (DSC)

Samples were appropriately weighed and heated from 0 to 150 ºC at the rate of 10 ºC/min where flow of heat was plotted against temperature. This was done with use of a Mettler Toledo DSC equipment. Change in enthalpy values ((ΔH) were calculated by integrating the area under consideration using the expression shown in Equation 3.

$$\Delta H = \int Cp \, dT \quad (3)$$

Where Cp is the specific heat capacity (J/g) and T, is the temperature measured in degree Celsius. The Cp was obtained from the expression shown in Equation 4.

$$\frac{\text{Heat flow (mW/mg)}}{\text{Heating rate (10 ºC/min)}} \quad (4)$$

2.2.4. Fourier Transform Infrared spectroscopy (FTIR)

Functional groups were detected in the virgin shells and isolated chitin with the use of Nicolet 6700M spectrometer. Each sample of 10 mg was compressed after being dispersed in KBr Spectra measurement in absorbance mode was processed at a resolution of 4 cm^{-1} between 500–4000 cm^{-1}.

Hydrogen bond energy E_H (kCal) in chitin was calculated according to Ciolacu *et al.* (2010) using Equation 5.

$$E_H = [1/kx(V_o - V)/V_o] \quad (5)$$

Frequency attributed to free OH groups at 3600 cm^{-1} is represented by V_o. V is the frequency of the bonded OH groups and k = 1.68 x 10^{-2} $kcal^{-1}$.

2.2.5. X-Ray Diffraction (XRD)

A PAN analytical Empyrean was used for this study and samples were exposed to a monochromatic Cu Kα radiation (k = 1.5406), operating at 40 kV and 40 mA. The crystallinity, X_c was calculated from the height ratio in the diffractogram having crystalline (*Ic*) and amorphous (*Iamr*) peak intensities relationship using Equation 6 (Juarez-de la Rosa *et al*., 2012).

$$X_c\ (\%) = [I_c /(I_c + Ia)]\ x100 \quad (6)$$

Where I_c and I_a represent the intensities of the crystalline and amorphous regions respectively.

3. RESULTS AND DISCUSSION

3.1. Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM/EDS)

The particulate natures of both virgin shells as shown Figures 1a and b have been converted to plate-like fibrils (Figures 1c and d) after treatments with acid and alkali solutions. Morphologies of chitin particles show surface roughness indicating a low degree of deacetylation by NaOH solution. Gbenebor *et al*. (2017) reported that high degree of chitin deacetylation could lead to a smoother morphology as the acetyl group ($CH_3C=O$) is gradually removed. The EDS result (Table 1) shows that shells of both prawn and shrimp contain significant amount of $CaCO_3$, which is more in shrimp exoskeleton. Reduction of $CaCO_3$ content by 99.2 and 94.8.% in prawn and shrimp chitins respectively after treatment show significant removal of the mineral while there is an increase in the carbon content as compared to that observed in the virgin shells. This is an indication of the formation of α-chitin polymer chains. The N content in both shells is indicative of the presence of protein and chitin. This reduced to 6.4 and 5.8% in prawn and shrimp chitin respectively owing to process of deproteinization with NaOH solution. The degree of acetylation (DA) of chitin was calculated using the C/N ratio as shown in Equation 7 (Abdou *et al*., 2008).

$$DA = 100 - \frac{6.857 - C/N}{1.7143} \quad (7)$$

The DA of prawn chitin was 98.01% while that of shrimp chitin was 98.03%. Chitin is normally composed of *N*-acetyl-D-glucosamine with acetyl groups on the C-2. There are instances where free amino groups exist in the polysaccharide chain implying deacetylation which occurs during deproteinization. Chitin chain thus

comprises combination of N-acetyl and free amino groups except for complete acetylation where DA = 100%. From this study (using Equation (3)), the fractions of free amino groups are 1.99 and 1.97% for prawn and shrimp chitin respectively. This gives them room to have some degree of interactions with aqueous media. There are instances where chitin's DA > 100% and this was attributed to the presence of some inorganic components which were not completely removed during chitin isolation from their respective sources (cicada sloughs and bat guano) (Sajomsang and Gonil, 2010; Kaya *et al.*, 2014).

Table 1: Elemental constituents of samples obtained from EDS

	Elements (wt. %)				
	Ca	C	N	O	Si
Prawn shell	11.9	7.0	7.8	73.3	-
Shrimp shell	15.4	4.2	7.9	72.1	0.4
Prawn chitin	0.1	22.1	6.4	71.4	-
Shrimp chitin	0.8	20.2	5.8	73.2	-

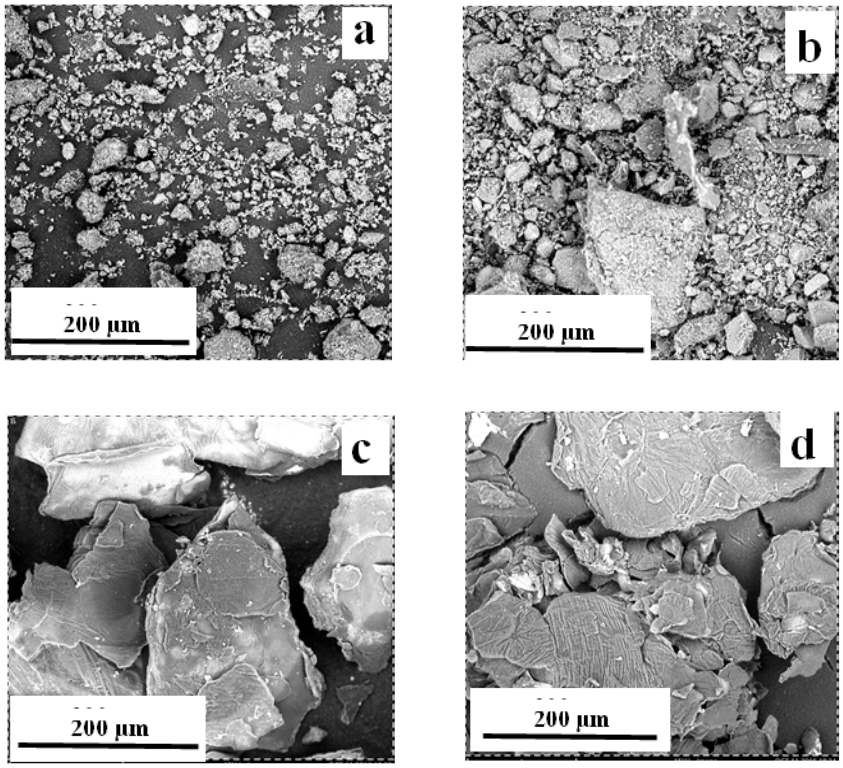

Figure 1: SEM of (a) prawn shell (b) shrimp shell (c) prawn chitin (d) shrimp chitin

3.2. Thermogravimetric Analysis (TGA)

Thermogravimetric results (Figure 2) show that the amount of biopolymer contained in prawn exoskeleton (37.1%) is more than that present in shrimp shell (33.7%). This is illustrated in the second stage of decomposition which ranges between 250- 400^{o}C for the two biopolymers The Figure also reveals a

stronger polysaccharide-$CaCO_3$ interaction in shrimp shell evidenced by 18.9% calcite which is more than that observed in prawn chitin (8.2%) as indicated in the third decomposition stage. The E_a shows that a stronger thermal energy of 163 kJ/mol will be needed to decompose shrimp $CaCO_3$ while a lesser energy of 89.6 kJ/mol decomposes prawn $CaCO_3$. Reagent treatment has thus improved the energy to thermally decompose chitin. This increases from 56 kJ/mol for embedded chitin in shrimp shell to 116.3 kJ/mol for fully extracted chitin. In the prawn shell, Ea of 58.6 and 117.1 kJ/mol were recorded for embedded and fully extracted chitin. There is also an increase in chitin content as 65.4% is realized from prawn shell while 53.5% is obtained from shrimp shell.

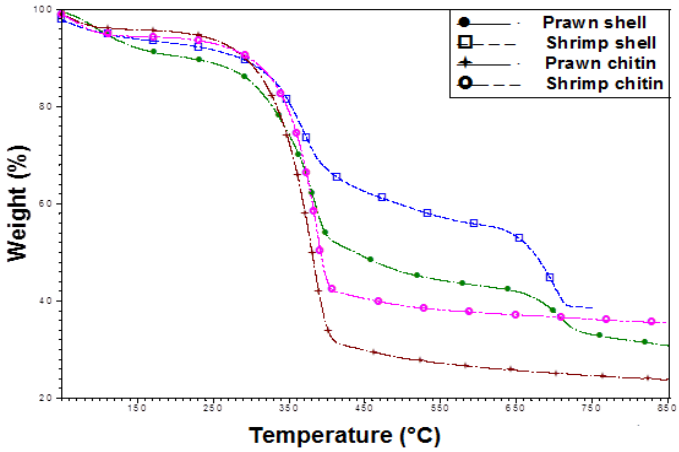

Figure 2: TGA of virgin prawn shell, virgin shrimp shell, prawn and shrimp chitin

3.3. Differential Scanning Calorimetry (DSC)

Similar DSC plot is displayed by prawn and shrimp chitin (Figure 3) but with different intensities and temperatures. Peaks showing temperature at 101 and 110 ^oC for shrimp and prawn chitin respectively indicate water loss, which best explains the hydrophilic nature of the structural polysaccharide from both sources (Juarez-de la Rosa *et al*., 2015). At 101 ^oC, prawn chitin gets dehydrated while shrimp chitin loses its water molecules at 106 ^oC. Difference in their water retention strength is further evidenced in the enthalpies related to the biopolymers which are calculated to be 138.5 and 115.3 J/g for prawn and shrimp chitin respectively. Complete decomposition of macromolecules is illustrated in the second thermal peaks at 383 ^oC (ΔH= 23.3 J/g) and 365 ^oC (ΔH = 21.4 J/g) for prawn and shrimp chitin respectively.

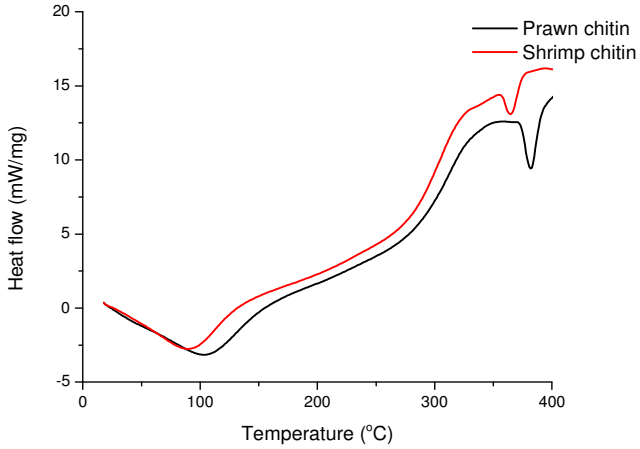

Figure 3: DSC of prawn and shrimp chitin

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra between 3700- 3000 cm^{-1} as shown in Figure 4 illustrates the existence of OH stretching of the water molecule at 3438 cm^{-1} and NH groups (3274 and 3112 cm^{-1}) respectively for prawn shell (Figure 4a). Comparing this with that of shrimp shell, the absorption spectrum shows similar functional groups with water molecule OH stretching occurring at the same wavenumber (3438 cm^{-1}) while absorbance at 3274 and 3112 cm^{-1} represent the NH stretching. Spectrum within this wavelength for prawn shell occupies a broader area than that of shrimp shell. Complete chitin isolation from these shells show similar spectra within this range with four distinct peaks compared to their corresponding shells. Two OH (3485 and 3440 cm^{-1}) and NH (3260 and 3104) stretching are observed with the prawn chitin while similar groups are formed on 3467 cm^{-1}, 3440 cm^{-1} (OH groups), 3261 cm^{-1} and 3104 cm^{-1} (NH groups), which are of comparable wavenumbers.

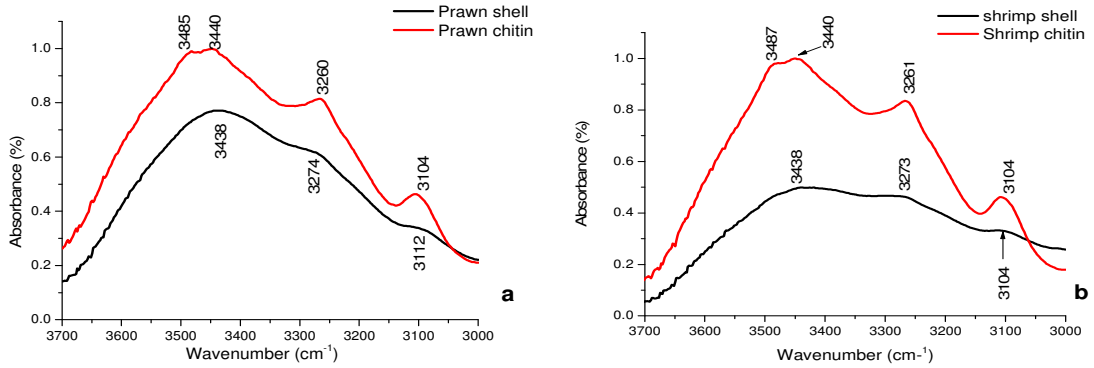

Figure 4: FTIR spectra of (a) prawn shell and prawn chitin (b) shrimp shell and shrimp chitin between 3700 – 3000 cm^{-1}

The existence of dominant mineral – $CaCO_3$ is represented by the spectrum at 1423 cm^{-1} for prawn shell and 1422 cm^{-1} for shrimp shell (Figures 5a and b). Apart from been existing on a comparable band, the $CaCO_3$ spectra are similar and this suggests similar extraction process and with implication that shell from either source will exhibit similar characteristics, A single peak of C=O secondary amide stretch (Amide I) exists in both shells at 1656 cm^{-1} for prawn shell and 1658 cm^{-1} for shell of shrimp, implying that there is chitin

embedded in the exoskeleton. Complete chitin extraction via the chemical process further attenuates the existence of Amide I band to form two peaks 1661 cm^{-1} and 1628 cm^{-1} in prawn chitin while this is formed on 1664 cm^{-1} and 1625 cm^{-1} in shrimp chitin. This implies that chitin generated from these two sources is in the ∝- form. Other amide peaks that characterize chitin, Amides II (NH bend and CH stretch) and III (CH_2 wagging) are revealed on 1559 cm^{-1} and 1313 cm^{-1} respectively on prawn chitin (Figure 5a). These peaks, which also exist in the shell, are amplified after extraction. This is replicated in shrimp chitin where Amides II and III are formed at 1559 cm^{-1} and 1309 cm^{-1} respectively.

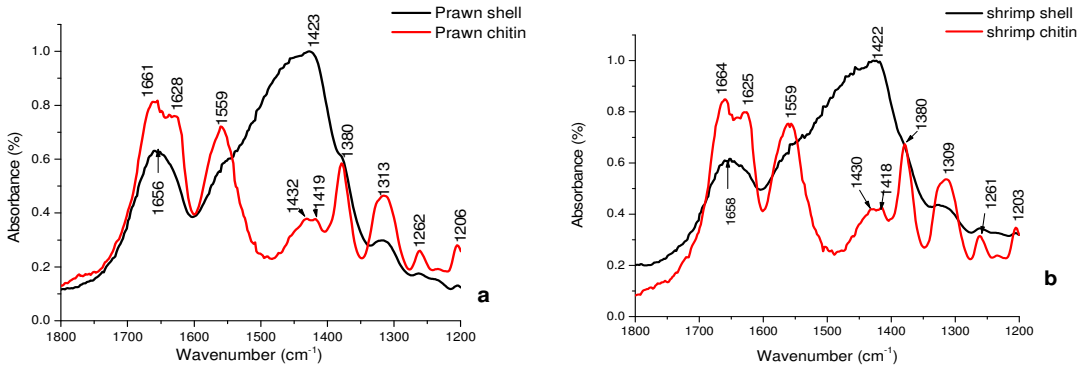

Figure 5: FTIR spectra of (a) prawn shell and prawn chitin (b) shrimp shell and shrimp chitin between 1800 – 1200 cm^{-1}

The OH (6)…OC, OH (3)…O (5), CO…HN and OH…OC intra and intermolecular hydrogen bond positions of isolated chitin is shown in Figure 6. Intra molecular OC, OH (3) bond is the most prevalent (in terms of occupancy) where 57.2% is occupied by this bond in prawn chitin and 49.9% for shrimp (Table 2). This is followed by 30.5% and 39.2% occupied by inter molecular CO…HN bonds in prawn and shrimp chitin respectively. The energy of the hydrogen bond, E_H is high for inter molecular bonds compared to their intra molecular counterparts as the wavenumber approaches 3000 cm^{-1}. This is possible because as the hydrogen bond length increases, the energy increases as well. The average E_H has comparable values of 4.09 and 4.07 kCal for prawn and shrimp chitin respectively.

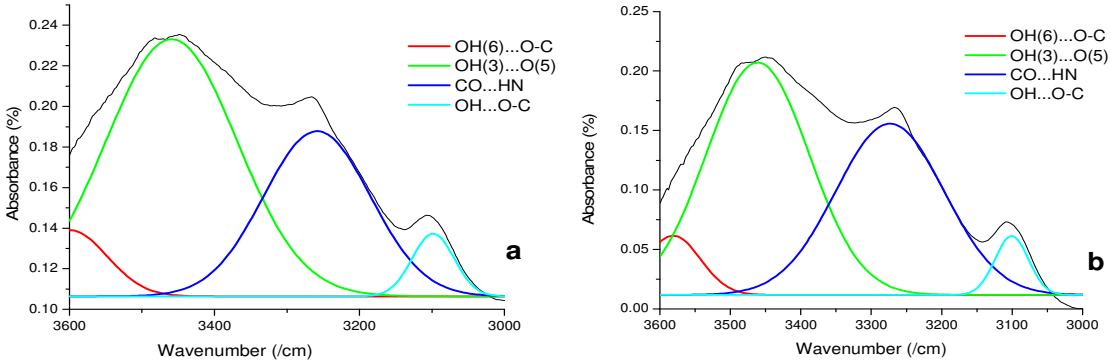

Figure 6: FTIR spectra of (a) prawn chitin and (b) shrimp chitin between 3600 – 3000 cm^{-1} showing inter and intra molecular hydrogen bond positions

Table 2: FTIR absorption band assignment to the OH band (3600-3000 cm^{-1}) for shrimp chitin

Chitin source	OH(6)…OC			OH(3)---O(5)			CO…HN			OH…OC			
	cm^{-1}	Amount (%)	E_H (kCal)	cm^{-1}	Amount (%)	E_H (kCal)	cm^{-1}	Amount (%)	E_H (kCal)	cm^{-1}	Amount (%)	E_H (kCal)	Average E_H (kCal)
Prawn	3599	8.3	0.02	3459	57.2	2.33	3258	30.5	5.65	3098	4.0	8.34	4.09
Shrimp	3581	6.8	0.31	3461	49.9	2.30	3273	39.2	5.43	3101	4.2	8.25	4.07

3.5. X-Ray Diffraction (XRD)

Presence of calcite and chitin in shells of these crustaceans are made visible by XRD patterns illustrated in Figures 7a and b. Diffractions at 2θ = 19.7 and 26.1° in prawn shell XRD represent the existence of chitin embedded in the shell while the calcite peaks are diffracted on 2θ = 10.9,16.1 and 29.1°. Comparing the amount of calcite in prawn shell with that of shrimp, more of this dominant mineral is present in the latter as diffractions occur at 2θ = 28, 40.7 and 48.2^0 while the rest represent the presence of chitin in the shell. Diffraction pattern of extracted chitin from both sources look similar with more chitin peaks existing in the prawn chitin (Figures 7c and d), indicating presence of more chitin in the crustacean.

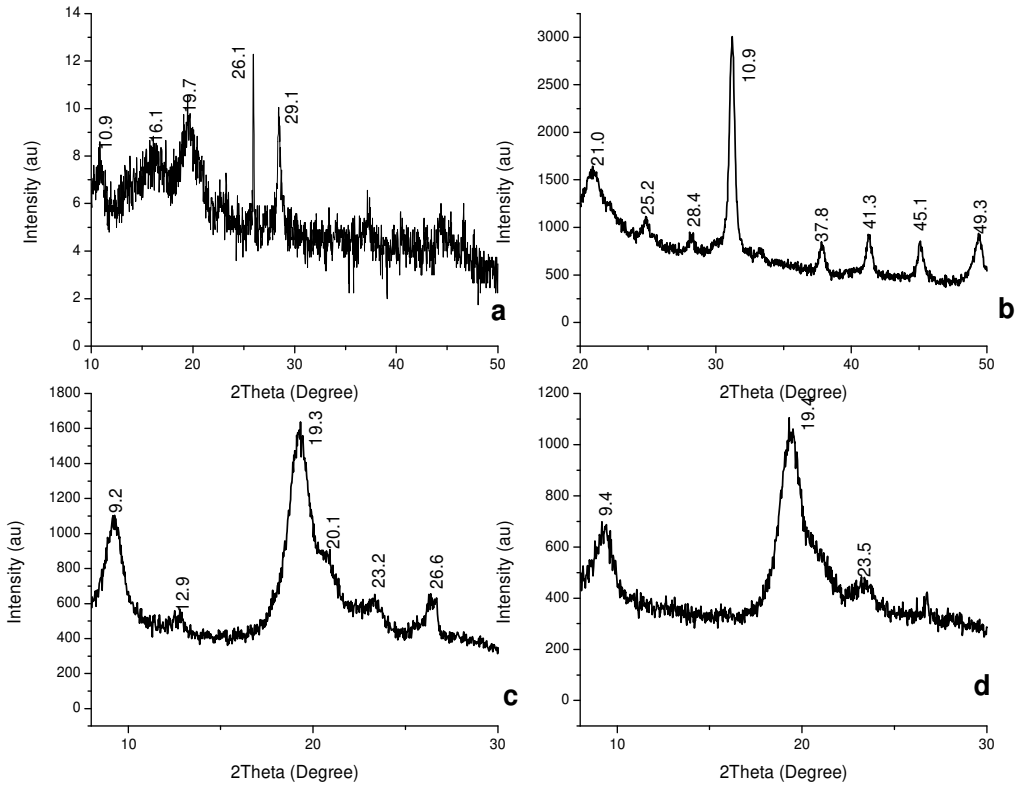

Figure 7: X- Ray patterns of (a) prawn shell (b) shrimp shell (c) prawn chitin and (d) shrimp

Two strong peaks are diffracted at 2θ = 9.2, and 19.3^0 for prawn chitin while similar peaks are diffracted on 2θ = 9.4 and 19.4^0 for shrimp chitin. Both XRD are typical of α-chitin where the strongest peak is usually represented by (110) while the next to this is taken to be (020). Prawn chitin is further characterized with weak chitin peaks at 2θ = 12.9 (021), 20.1 (120), 23.2 (130) and 26.6° (013) while the only weak peak in

shrimp chitin is at $2\theta = 23.5^{o}$ (130). Crystallinity of prawn chitin is 71.3% while that of shrimp chitin is 70.5%. Chitin's Crl from these two sources look comparable despite more peaks found from prawn. The two main peaks at (020) and (110) may have been responsible for this observation.

4. CONCLUSION

Chitin particles extracted from two crustaceans of similar family has been investigated. Shells of shrimp contain more of $CaCO_3$ than that of prawn while chitin embedded in these shells are more in the latter than the former. Isolated chitin from both sources appears as plate-like fibrils with similar functional groups and 0.02% difference in DA. Prawn and shrimp chitin will have comparable thermal stability as there exists a difference of 0.68% in their Ea. Diffraction patterns of the two biopolymers are similar but with more crystallographic planes present in the prawn chitin whose Crl is 71.3% compared to 70.5% possessed by its shrimp equivalent. Aside the fact that more chitin can be sourced from prawn shell, their comparable properties suggests that both will behave in a similar manner and thus either can be used for a specific application.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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