Isolation and Characterization of Chitin and Chitosan from the Biomass of Nigerian Shrimp Shells and Conversion to Glucosamine

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Abstract: - The most important bio-polysaccharide for biochemical industries are chitin and chitosan. These chemical entities are nitrogenous polysaccharide polymers containing 2-acetamido-2-deoxyglucoside units. These products are useful in engineering, medicine, agriculture and other industrial uses. Glucosamine is a known drug for treating muscular skeletal problem and has been obtained from these two amino polysaccharides. The present study was aimed at isolation of chitin and chitosan from shrimp wastes by chemical methods involving, deproteinization, demineralization, depigmentation, filtration and deacetylation where necessary. These were simultaneously converted to glucosamine by acid hydrolysis using 37% HCl. The yield of chitin was 17% and deacetylation of chitin resulted in 56% chitosan. The proximate analysis of chitin isolated gave rise to the following result: moisture 2.9%, ash 4.54%, lipids 2.1% and protein 3.3%. These compounds were characterized using FTIR. In the infra-red spectrum, the shrimp chitin indicated amide (I) band at 1614cm^{-1} whereas chitosan and glucosamine showed no amide band but hydroxyl and amino groups between the ranges of 3110-3595 cm^{-1}. The results of these analyses including the proximate analysis compared well with literature values. There is paucity of information in literature about this type of study in Nigeria. This study has created awareness that these biomaterials from crustacean sources from Nigeria are technologically useful for conversion into useful products that can be used in Engineering, Medicine, Agriculture and other industrial products. This work provides route of controlling pollution menaces caused in fishing and canning sites. This is a case of creating Wealth from Wastes.

Key Words: — ***Chitin, Chitosan, Shrimp, Glucosamine, FTIR, Hydrolysis.***

I. INTRODUCTION

Man has been afflicted since time immemorial with different kinds of ailments and diseases such as Malaria, Cancers, Aids, and Arthritis, etc. In a bid to find cure to these illnesses, man has discovered many natural products from both plants and animals that can provide bioactive compounds and secondary metabolites that aid in their treatments (Sarker *et al*., 2006).

Natural products are products derived from natural resources such as plants, animals, and even microorganisms. Many of these extracts are used medicinally, in herbal medicine, and in the manufacture of pharmaceutical drugs. They are usually made up of novel chemical compounds that are so diverse and highly medicinal (Paul, 2006). Today millions of chemically and biologically active compounds exist in nature and when extracted can be used by man in combating diseases. Marine sources have been right avenues for producing bioactive compounds e.g. spongistatin. The waste products of marine crustaceans can be sourced to help generate a lot of these bioactive compounds such as glucosamine there by solving the problem of disease treatment and waste management. This research is on how chitin and chitosan can be sourced from marine crustacean wastes in order to derive value added product for treatment of musculoskeletal disorder. The study will be significant owing to the prevalence of ailments in the society and cost of therapy. The fact that discarded seafood can be utilized in a broad spectrum of health, medical and environmental field lead to a better solution for proper disposal of sea food waste and prevention of excessive buildup of seafood waste (felicity *et al*, 2007).

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A. Chitin

Chitin is a natural polysaccharide composed of β (1-4)-linked 2-acetamido-2-deoxy-β-D-glucose [Dutta *et al*., 2002]. Chitin is often considered as a cellulose derivative. Chitin and cellulose are structural polysaccharides; cellulose strengthens the cell wall of plant cells while chitin provides mechanical strength to fungal cell walls and exoskeleton of arthropods (Goody, 1990; Aslak, 2007).

Chitin naturally is a white, hard, inelastic, nitrogenous polysaccharide found in exoskeleton such as shrimp, crab, and crayfish shell as well as in the internal structure of invertebrates (Dutta *et al*., 2002).

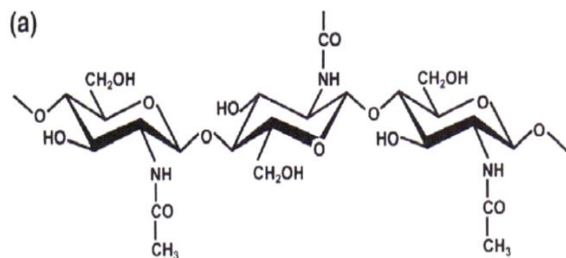

Fig.1. Chemical structure of chitin (Aslak, 2007)

B. Chitosan

Chitosan is a linear polymer of β-(1-4)-linked 2-amino-2-deoxy-B-D-glucopyranose. And is easily derived by N-deacetylation and is consequently a copolymer of N-acetyl glucosamine and glucosamine. When the degree of deacetylation of chitin reaches about 50%, it becomes soluble in aqueous acidic media and is called chitosan.

Chitosan is the only pseudo natural cationic polymer, and thus, it finds many applications that follow from its unique character (flocculants for protein recovery, de-pollution, etc.) (Rinaudo, 2006).

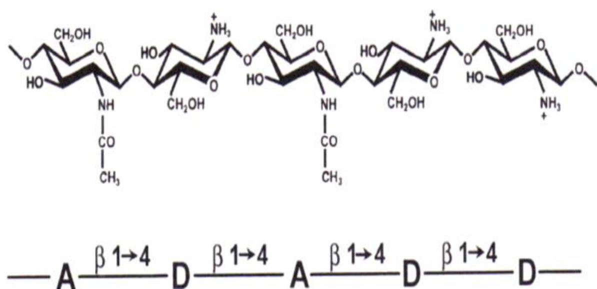

Fig.2. Chemical structure of a partially N-deacetylated chitosan (Aslak, 2007)

Applications of chitin and chitosan:

Chitin and chitosan have uses or applications in many areas of life. Medical applications, cosmetology, water engineering, paper industry, textile industry, food processing, agriculture, etc.

C. Glucosamine

Glucosamine is 2-amino-2-deoxy-D-glucose.one of the building blocks of chitin and chitosan. Glucosamine is commercially produced from chitin, a process that is mainly performed in concentrated acid (Novikov *et al.*, 1997). Glucosamine is believed to be a good pain reliever especially in the treatment of osteoarthritis (Anderson *et al.*, 2005). It has been approved in developed countries as a dietary supplement with little side effects.

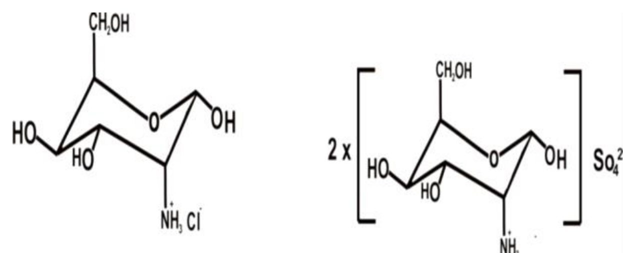

Fig.3. Glucosamine

D. Procurement of samples: (Shrimp)

Shrimp shells were collected from a local fish market (Town market) at Port-Harcourt in Rivers State and were brought to the laboratory in a sterile container filled with ice in order to avoid decay and offensive odor. The shells of shrimp were scraped of loose tissue. It was washed under running water and dried under shade and ground to pass through a 250 micro meter sieve. Chitin was extracted from the reduced shell of the crustaceans. All the reagents were of analytical grade. Fourier transform Infrared Spectroscopic analysis was conducted on Buck Scientific Spectrophotometer using Nujol oil.

II. Proximate Characteristics Of Dry Weight Shell Chitin, Chitosan And Glucosamine From Shrimp

The proximate characteristics includes moisture, fat, ash, degree of deacetylation, solubility, pH and protein contents of shrimp dry weight, chitin, chitosan and glucosamine was determined according to the AOAC (2007)

A. Ash Content

Ash content of the extracted chitin was determined according to the method of AOAC (2007). Two grams of shrimp chitin was weighed into crucible, heated in a moisture extraction oven for 3 hours at 100 °C before being transferred into a muffle furnace at 550 °C, until it turned white and free of carbon. The sample was then removed from the furnace, cooled in a desiccator to a room temperature and reweighed immediately. The weight of the residual ash was then calculated as:

Percentage ash = Weight of ash/Weight of original sample x 100

B. Moisture Content Determination (AOAC Method, 2007)

Two grams of the sample was weighed into dried weighed crucible. The samples were put into a moisture extraction oven at 105 °C and heated for 3 hrs. The dried samples were put into desiccators and allowed to cool and reweighed. The process was repeated until constant weight was obtained. The difference in weight was calculated as a percentage of the original sample.

Percentage moisture = W2−W1/W2−W3 x 100 where,

W1 is initial weight of empty crucible

W2 is weight of crucible + undried sample

W3 is weight of crucible + dried sample.

C. Crude Protein Determination of Chitin (AOAC, 2007)

The micro method described by AOAC (2007) was used. Two grams of the samples was mixed with 10ml of concentrated sulphuric acid in a heating tube. One spatula of selenium catalyst was added to the tube and mixture heated inside a fume cupboard until the solution turned colorless. The digest was transferred into distilled water with intermittent shaking and cooling until the addition of water generated heat. It was transferred quantitatively into a100 ml standard flask and made up to the volume with a 10ml pipette, solution was transferred to the reaction chamber of the Micro-Kjeldhal distillation apparatus where ten ml portion of the digest was mixed with equal volume of 40% sodium hydroxide were added to the chamber. Distillation was done for 5 minutes and ammonia liberated was absorbed into 4% boric acid containing 3 drops of methyl red indicator. A total of 50 ml distillate was collected and titrated as well. The amount of ammonia liberated was determined by titration with 0.01N standard hydrochloric acid.

The Nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content. This is given as

Percentage Nitrogen = (100 x N x 14 xVf) T/100 x Va

Where,

N is normality of the titrate (0.1 N)

Vf is total volume of the digest = 100 ml T is titer value

Va is aliquot volume distilled.

D. Fat Content Determination (AOAC, 2007)

Two gm of the sample was loosely wrapped with a filter paper and put into the thimble which was fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120 ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5 hrs. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was received as mass of fat and is expressed in percentage of the sample.

The percentage oil content is the percentage fat, = W2−W1/W3 x 100 where,

W1 is weight of the empty extraction flask

W2 is weight of the flask and oil extracted

W3 is weight of the sample.

E. Degree of Deacetylation (DD)

The DD of chitin / chitosan is the most important parameter that influences their various properties including biological, physicochemical, mechanical effectiveness, behaviors of chitin/chitosan and its derivatives and functionality has been found to be dependent on the DD. The expansion and stiffness of the macromolecular chain conformation and the tendency of the macromolecule chains to aggregate depend strongly on the DD. The determination of the DD for the polymers is essential for studying their chemical structures, properties, and structure-properties relationships. Knowledge on the DD is very important to maximize chitin applications. If the DD is known many properties and applications can be predicated. Degree of deacetylation depends on raw material and method used for deproteinization, source of chitin, time, and alkali concentration. High degree of deacetylation is due to lesser amount of protein and acetyl group. The degree of deacetylation must be at least 85% to achieve desired solubility, reactivity and biodegradability. Higher yield of chitosan is achieved due to repeating process of demineralization and deproteinization which resulted in removing protein and minerals at higher concentration of alkali and acid giving rise to depolymerized and deacetylated residue and excessive loss of sample during washing, the yield of glucosamine also increases with increasing degree of de acetylation.

IR technique was used for determining the DD. Chitosan sample was made into KBr discs and the FTIR spectra obtained with a BUCK FTIR- spectrophotometer. It was determined by comparing the absorbance of the measured peak to that of the reference peak at. Therefore, for chitosan the DA was calculated from the absorbance (A) ratios according to the following equation used by (Baxter *et al.,* 1992)

$$DA=(A1625/A3467) \times 115$$

On the other hand, the DD for the crab chitosan sample was determined using

The absorbance ratio A1276/A1418

F. Solubility of Shrimp Chitosan and Glucosamine

Solubility of the extracted chitosan and glucosamine were determined according to the method of (Kim., 2004). The solubility of chitosan was demonstrated in various solutions like distilled water, methanol, ethanol, acetic acid and lactic acid. Chitosan powder samples (0.1 g) was placed into a centrifuge tube then dissolved in 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 10,000 rpm for 30min at 25ºC.. The supernatant was decanted and discarded. The undissolved particles were washed in distilled water (25ml) then centrifuged at 6,000 rpm. The supernatant was removed and undissolved pellets were dried at 60ºC for 24hr in an oven. Finally, the particles were weighed and the solubility percentage was calculated as follows:

Solubility (%) = (initial weight of tube + sample) – (Final weight of tube + sample) * 100

(Initial weight of tube + sample – (initial weight of tube)

The solubility of chitosan greatly limits its application though several methods have been adopted for chemical modification and also improve its water solubility.

G. Determination of pH

pH of glucosamine from shrimp samples was determined according to the APHA (1998). About 1 g of the sample was dissolved in 100 ml distilled water and the pH was recorded using a digital pH meter.

III. Extraction Of Chitin From Shrimp Shell

A. Deproteinization

Extraction steps of chitin from shrimp shell were according to (Synowiecki., 1997) method. The process involves deproteinization with 1M sodium hydroxide solution (10:1 v/w)then stirring for 2hr to remove protein, separation of alkali-insoluble fraction by centrifugation (4000 rpm, 15min) then washing of alkali-insoluble fraction with distilled water, then drying at 40 ºC overnight (Rekso., 2004).

B. Demineralization of Shrimp Shell

The removal of mineral was carried out at a temperature of 25–30°C using 100ml of 1MHCl solution 1:10 (g/ml), and then stirred for 24 hrs, filtered to take precipitate. The precipitate was washed with distilled water until neutral pH, then filtered and dried at a temperature of 60-70°C.

C. Depigmentation of Shrimp Shell

Shrimp depigmentation was carried out by bleaching with 0.315% NaOCl (w/v) for 5 minutes at room temperature, at 1:10 (w/v), and then it was washed with distilled water until neutral pH, then was filtered and residue was dried , this residue is known as shrimp chitin.

Proximate characteristics of chitin from shrimp (AOAC.,2007)

Statistical analysis:

SPSS 18.0 was used in this study to analyze the data. One-way of variance analysis was carried out using Duncan's test with a confidence level as $p < 0.05$.

IV. Results And Discussion

The physical component of the shrimp shell includes protein, calcium and pigment. The result of the physiochemical properties of dry exoskeleton shell from shrimp composition are presented in the tables below. The result of the analysis showed that yield has been calculated from shrimp shell.

Table.1. Chemical composition of shrimp shell after being dried and milled

Parameter (%)	Experimental	References Value
		Muhammed *et al.*,2012
MINERAL	70.63±.12	76.80
PROTEIN	20.57±.06	15.05

Mean ± Standard deviation of triplicate determination.

The results showed that shrimp shell contained 70.63% mineral, 20.57 % protein on a dry weight basis. Generally, the chemical composition of shrimp shells varies with species, seasons, and many other factors. Muhammed *et al.,*2012 reported that shrimp shells contained 15.05 protein, 76.80 mineral based on dry weight which was slightly different from that of the protein and mineral content of shrimp shell (Table 1)

A. Proximate composition of the chitin from shrimp (AOAC 2007)

Chitin, mineral, and protein present in crustacean vary depending on the specie. The result of the physiochemical properties of chitin from shrimp composition are presented in

the tables below. The result of the analysis showed that yield has been calculated for the extracted chitin from shrimp.

Table.2. Result of proximate analysis of chitin from shrimp

Parameter (%)	**Experimental**	**References Value**	
		Muhammed *et al.*,2012	Darmanto,2002
MOISTURE	2.90±.12	8.70	3.98
ASH	4.54±.14	5.60	4.48
PROTEIN	3.30±.06	4.16	
LIPID	2.10±.06	1.30	
CHITIN YIELD	17.10±.17		
CHITOSAN	56.00		

Mean ± Standard deviation of triplicate determination.

Shrimp chitin contained ash in the range of 4.46 while commercial chitin from shrimp contains 4.48% ash (Darmanto., 2002). High value of ash content indicates high mineral content after demineralization while a low value of ash is an indicator for effective demineralization. (Muhammed *et al.,* 2012.) reported ash value to be 5.60 This may be due to the extent of demineralization. Chitin from shrimp showed efficient demineralization. The result of this work showed that chitin from shrimp contained moisture in the range of 2.83. There were slight differences between values reported for Nigeria sourced shrimp may be due to variation in composition and different treatment applied on the chitin. The moisture content of shrimp from Nigeria according (Muhammed *et al.,* 2012) is 8.70%. This may be due to small amount of moisture absorbed by the samples after preparation. (Ibitoye.,2018), reported moisture in the range of 4.48%. After deprotenization, the shrimp chitin contained protein in the range of 3.27. The protein content of shrimp from Nigeria according (Muhammed *et al* 2012) is 4.16%. Protein content of each sample is an indication of the amount of organics (nitrogenous substance) in the sample after deproteinization. The result of the proximate composition indicates low protein content. The lipid content of chitin from shrimp was determined. The lipid may be good substitute to synthetic source. After dilapidation, the shrimp chitin contain lipid within the range of 2.07%. The lipid content of shrimp from Nigeria according (Muhammed *et al* 2012) is 1.30%.

Immediately after deproteinization, the oily nature of the sample was visibly observable.

The chitin yield of shrimp is 17.10%. Shadidi and Synoweecki reported chitin yield in shrimp to between 17-32.2%. Abdou *et al* reported that the yield of chitin in shrimp is 23.72. The result of the yield of chitin corresponds with that of those reported in the literature. The yield of chitin extracted from shrimp shows that this could be good source of chitin for industrial production According to SPSS, ANOVA reveal that the values are highly significant at $p<0.05$ level.

Table.3. the FTIR (CM^{-1}) band of chitin isolated from shrimp

Vibration Mode	**Kaya *et al*;2015**	**Current study**	**Ibitoye *et al*., 2018**
Ring stretching	846	896	952
OH stretching	3476	3463	3431
N-H stretching	3179	3190	3103-3259
C-H stretching	2797	2833	2889
C-O Amide I stretch	1618	1614	1622-1653

The FTIR spectrum of shrimp chitin showed N-H and O-H in the range of 3463-3190cm^{-1} and carbonyl stretching of amide 1 in the range of 1614cm^{-1}. Similar study by Kaya *et al*., 2015 reported O-H and N-H in the range of 3476-3176 cm^{-1} and carbonyl stretching of amide 1 in the range of 1618cm^{-1}. The FTIR spectrum of chitin from shrimp is shown in figure.4. below.

Table.4. the FTIR (CM^{-1}) band of chitosan isolated from shrimp

Vibration mode standard	**Current Study**	**(Mourya *et al*., 2010)**
C=O Amide stretch (amide I)	1616	1654
N-H bond which overlaps with N-H Bend	-	1580
N-H stretching	3408	3420
C-Hstretching	2704	2921
OH stretching	3110	3420

The FTIR spectrum of chitosan showed N-H and O-H in the range of 3408-3110cm^{-1} and carbonyl stretching of amide 1 in the range of 1616cm^{-1}. Similar study by Mourya *et al*., 2010 reported O-H and N-H in the range of 3420 cm^{-1} and carbonyl stretching of amide 1 in the range of 1654cm^{-1}. The FTIR chitosan from shrimp is shown in figure.5.

Table.5. the FTIR band CM^{-1} of glucosamine isolated from shrimp

Diego *et al*., 2018 Glucosamine	**Glucosamine (Boots shop)**	**Extracted Glucosamine**	**Vibration mode**
3346-3031	3358-3085	3395	N-H stretching
-	3551	3508	OH stretch
2841	2885	2984-2809	CH stretch

The FTIR spectrum of glucosamine extracted from shrimp showed N-H and O-H in the range of 3508-3395cm^{-1} and absence of carbonyl stretching[1]. Similar study by Diego *et al.*, 2018 reported O-H and N-H in the range of 3346-3031 cm^{-1} . The FTIR spectrum confirms complete deacetylation of glucosamine. The spectrum of glucosamine from shrimp is shown in figure.6.

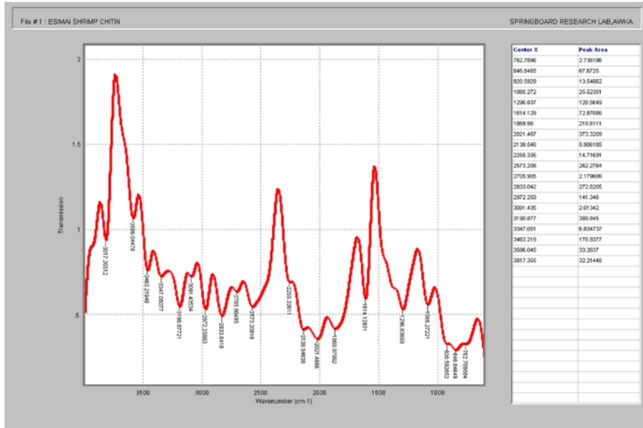

Fig.4. the FTIR Spectrum of Chitin Extracted From Shrimp Shell

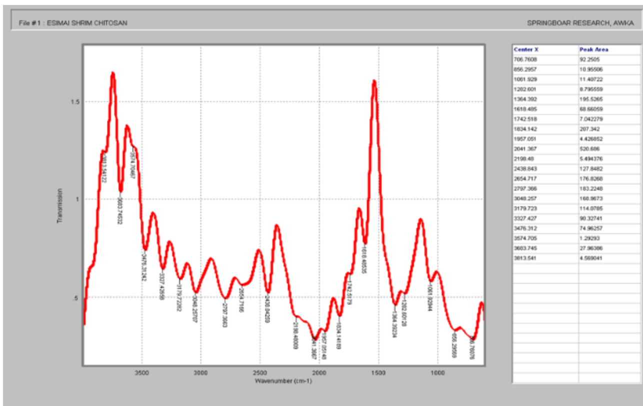

Fig.5. *FTIR* Spectrum of Chitosan Extracted From Shrimp Chitin

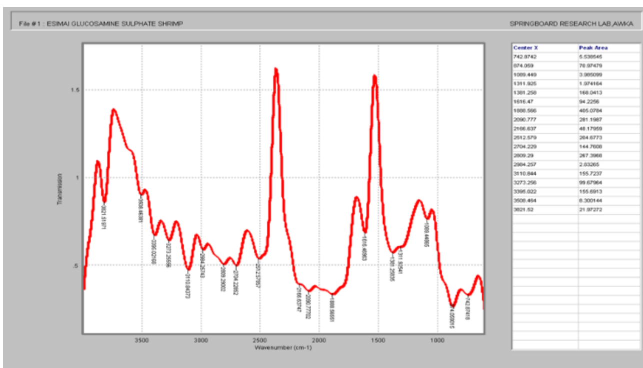

Fig.6. FTIR Spectrum of Glucosamine Extracted From Shrimp Chitin

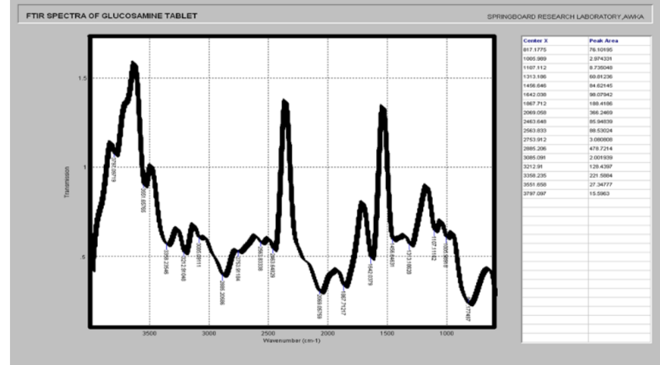

Fig.7. FTIR Spectrum of Glucosamine from Boot Shop, London

V. Conclusion

The present work is aimed at extraction, characterization of chitin, chitosan, and glucosamine from Nigerian shrimp. From this research work, it has been confirmed that that waste from crustacean can be converted to a value-added product. The shell of crustacean has considerable amount of protein, lipids and phosphate (Zamain et al., 2010). These impurities must be removed before hydrolyzing the chitin and chitosan portion to get glucosamine via hot alkali treatment (Yaghobi; 2012, Zamani; 2010). The FTIR confirm the production of chitin, chitosan and glucosamine. Chitin was transformed to chitosan and glucosamine to improve its solubility.

Crystal of glucosamine was purified and recovered by recrystallization in methanol. It was dried at 550C. Finally, the consistency of the FTIR spectrum of the experimental glucosamine with that of the glucosamine from Boot shop and literature work by Diego et al., 2018 also confirms its purity. This work has succeeded in converting waste to wealth, reducing pollution and saving foreign exchange for importing them.

References

[1]. Abdou, E.S., Nagy, K.S.A. and Elsabee, M.Z. (2008). Extraction and characterization of chitin and chitosan from local sources. Biores. Technol., 99: 1359-1367.

[2]. Anderson J. W., Nicolosi R. J. and Borzelleca J. F. (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food and Chemical Toxicology 43(2): 187-20.

[3]. AOAC, (2007). Official Methods of Analysis of the Association of Official Analytical Chemistry (A.O.A.C.) International, 18th ed., Gaithersburg, Maryland, USA.

[4]. APHA (1998). Standard methods for the examination of water and waste-water, 20th edition, Washington, DC, American Public Health Association. 4500.

[5]. Aslak E., (2007). Characterization of chitin and a study of its acid–catalyzed hydrolysis. A thesis work submitted in partial fulfillment for the award of Ph.D., Dept. Of Biotech. Fac. Of Natural Sci. and Tech., Norwegian Univ. of Science and Tech. Trondheim; Pp 5-15.

[6]. Darmanto, (2002). The effect of chitin and chitosan of crab shell on water sorption of isotherm and denaturation of myofibrils during dehydration.

[7]. Diego, L.B, Tiago B.B., Naylil, M.R.C., Catia, O. (2018). General Protocol to Obtain D-Glucosamine from the Biomass Residue: Shrimp shell, Cicada Slough and Cockroaches. 0046.

[8]. Dutta P. K., Ravikumar M N V, and Dutta J., (2002). Chitin and chitosan for versatile applications, JMS Polym Rev, 42:307.

[9]. Felicity B., Clifford L., Abazinge M., Oghenekowe O., (2007). Extraction and evaluation of chitosan from crab exoskeleton as seed fungicide and plant growth Enhancer. American-Eurasian Journal of Agric. And environmental Science 2 (2) 103-111.

[10]. Gooday G. W. (1990). The Ecology of Chitin Degradation. Advances in Microbial Ecology 11: 387-430.

[11]. Ibitoye, E B., I.H Lokman, M.N.M Hezmee, Y.M Goh, A.B., Z., Zukiand A.A Jimoh (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket Biomed. Mater. (13): 025009.

[12]. Kaya M, Mujtaba M, Bulut, E., Akyuz, B., Zelencova, L., and Sofi, K., (2015). Fluctuation in Physicochemical Properties of Chitins Extracted from different body parts of Honeybee Carbohydr. Polym.132: 9–16.

[13]. Kim C. H., Chun H. J., and Choi K. S., (1997). Synthesis of chitosan derivatives with quaternary ammonium salt and their antibacterial activity, Polym Bull, 38:387.

[14]. Mourya V. Ka, Nazma N.I and Ashutosh T (2010).Carboxymethyl chitosan and its applications VBRI press 1(1), 11-33.

[15]. Muhammed, .LT, Alewo O.A., Joseph, O.G and Kenneth, K.A., (2012) Extraction and Characterization of Chitin from Nigerian Source (21):73-81.

[16]. Novikov V.Y., Ivanov A.L. (1997). Synthesis of D (+)-glucosamine hydrochloride. Russian Journal of Applied Chemistry 70 (9): 1467-1470.

[17]. Paul M., (2006). Medicinal Natural Products: A biosynthetic approach. Derwick John Wiley &sons; pp 1-40.

[18]. Rekso, G.T. (2004). Graft Copolymerization in Chitin by Irradiation Techniques as Metal Ion Chelating Materials. Bandung Press.

[19]. Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. Prog.Polym. Sci. ,31: 603–632.

[20]. Sarker S. D., Latif Z., Gray A. I., (2006). Methods in Biotechnology-Natural Products Isolation, 2nd Ed. Humana Press, New Jersey; pp 1-47, 269-273.

[21]. Synowiecki, J. (1997). Application of chitin derivatives for animal and human healing (in Polish). Medycyna Wet., 53:647–649.

[22]. Synowiecki, J. and Shahidi, F. (1991). Isolation and characterization of nutrients and Value added products from Snow Crab and Shrimp processing discards. Journal of Agricultural and Food Chemistry.39; pp 1527-1532.

[23]. Y Kangde, L Junjie, Y Fanglian and Y Yuji., (2012). From Chitin to Chitosan CRC Press, Newyork.

[24]. Zamani, A. and Taherzadeh, M.J. (2010). Production of Low Molecular weight Chitosan by hot dilute sulfuric acid. Biores.,5: 1554-1564.