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ORIGINAL PAPER

Biopolymer (Chitin) from Various Marine Seashell Wastes: Isolation and Characterization

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Abstract Chitin has been produced from different sea waste sources including, molluscs (mussel and oyster shell), crustacean (prawn and crab) and fish scale (pang and silver scales) using deproteinization and demineralization as chemical methods. The conditions of chemical extraction process determine the quality of chitin. The obtained results revealed that, about 1 and 10% HCl and NaOH were adequate concentrations for deproteinization and demineralization process respectively. Chitin from oyster and crab shell waste had the highest yield of 69.65 and 60.00% while prawn, mussel shell, pang and silver scales had the lowest yield of 40.89, 35.03, 35.07 and 31.11% respectively. Chitin solubility is controlled by the quantity of protonated acetyl groups within the polymeric chain of the chitin backbone, thus on the percentage of acetylated and non-acetylated D-glucos-acetamide unit. Good solubility results were obtained in mussel, oyster and crab shells respectively. The chitin molecular weight characteristics and activity are controlled by the degree of acetylation (DA) and the distribution of acetyl group extending in the polymer chain. DA is determined by acid-base titration methods and molecular weight determined by Brookfield viscometry. Both methods are found to be effective.

Keywords Marine sea shell wastes · Chitin · Isolation · Characterization

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Introduction

Chitin and its main derivatives chitosan belong to the new families of biological macromolecules and their study are becoming interesting to many researchers in the domain of study. Chitin, also known as identified as poly 2-acetamido-2-deoxy-β-D-glucose firstly was identified in 1884 as pure polysaccharides and are available in large amount organic biopolymer material found in the physical world [1, 2]. Chitin is located next to cellulose, according to the amount produced annually by biosynthesis. This biopolymer shows excellent properties such as non-toxicity, ability to form film, biodegradability, biocompatibility, chelate metal ions and adsorption, which make it an attractive biopolymer to pharmaceutical, biochemical applications and in the industrial zone for the purification of water. More useful application of chitin and its derivatives chitosan have showed by many scholars in the literature study to be more than 200 [3]. This is due to the reason of its being second–most abundant natural biopolymer having high molecular weight and a versatile and environmental friendly polysaccharide [4]. Detail application of it this biopolymer is seen in the field of medicine, food, biotechnology, agricultural and cosmetic industry. This biopolymer can be sourced from the exoskeleton of domestic waste of crustaceans (crab, prawns, shrimps), molluscs (oyster, snails), fish scales (pang and silver), insert and in certain fungi [5]. Chitin is closely associated with component such as protein, inorganic materials which are mainly calcium carbonate and lipids. These components are selected from crustacean, which is made up of about 30–40% protein, about 30–50% of calcium carbonate and calcium phosphate, and 20–30% chitin [6]. Various methods such as deproteinization (treatment with sodium hydroxide) and demineralization (treatment with hydrochloric acid) have been adopted to purify these impurities from chitin shell waste, which have

been shown excellent removal according to many researchers [6, 7]. Physiochemical parameters for instance the degree of acetylation, solubility, intrinsic viscosity and molecular weight have shown excellent result in the purification of biopolymer chitin. Research have shown that chitin is an in relation to intractable polymer and despite its structural similarities to cellulose; it is insoluble in a typical solvent such as cuprammonium hydroxide, which is Schweizer's reagent, cupriethylenediamine and cadoxen. Despite that it is soluble in concentrated hydrochloric acid, sulphuric acid and phosphoric acid as well, but not in concentrated nitric acid, since it breakdown is not accompanies solution in these mineral acids that extend the backbone chain hydrolysis in phosphoric acid is considerable less than that in either hydrochloric or sulphuric acid [8]. Solubilities of chitin is successful in quantity of solvent ranges: carboxylic acid: formic acid, dichlo-acetic acid: trichloro-acetic acid [9]. Based on the processing methodology employed to purify chitin and the source, its degree of deacetylation may range from 30 to 95% [10]. In same line of idea, when characterizing chitin, molecular weight is one of the most important parameter to be considered. Molecular weight (MW) is one of the most fundamental parameters in characterizing a polymer. Molecular weight of chitin can be determined by different techniques. Gel permeation chromatography is known as a powerful technique to characterize the molecule weigh of chitin. One of the simplest and fast method used, is by the use of a viscometer, more precisely, intrinsic viscosity. Even though there is not an absolute method since it requires the determination of a constant. This intrinsic viscosity is denoted as seen in Eq. 1, where η, which function as the molecular weight, M, is represented by the Mark-Howwink Scakurade equation [η] versus log molecular weight which has been determined by an absolute methodology such as using a viscometer such as Brookfield viscometer.

$$\eta = KM^{\alpha} \quad (1)$$

where K and α are constants for a given polymer solvent temperature system. These constants are calculated by evaluation of a plot of log [η] against log molecular weight that has been determined by an absolute method such as using a viscometer such as Brookfield viscometer. These constant are determined by evaluating a plot of log [η] versus log molecular weight, where the molecular weight can be determined by an absolute method of a viscometer such as Brookfield viscometer.

The research aimed to prepare chitin from different sea waste sources such as mollusk shell, crustacean shell and fish scales, using chemical treatment methods: deproteinization, demineralization and to characterize the obtained chitin using several physiochemical methods. The value of solubility, degree of acetylation and molecular weight of the different samples of chitin were estimated by Austin and Brine method (1981), acid-base titration method and Brookfield viscometer method respectively [5]. All methods were found to be effective. Recently several authors have devoted their attention in extracting chitin from some sea waste using different methods, for instance, Islam et al. [11] studied the structures, properties and application of chitin and chitosan in biomedical engineering, Younes and Rinaudo prepared chitin and chitosan from some marine sources and studied their structures and their possible applications [12]. Some other applications of chitin and chitosan were presented in [13, 14]. Using the so-called biological methods Arbia et al. [15] to extract chitin. Gortari and Hours [16] recovered chitin via biotechnological processes from crustacean shells, Younes and others extracted chitin and chitosan from shrimp shells using the so-called optimized enzymatic deproteinization [17]. A new trends in biological extraction was employed by Kaur and Dhillon to extract chitin from different marine shells [18–20]. Most of these results have been obtained using different methods and their properties differ. In this paper, we aim to perform extraction of chitin using a modified chemical method that consists on deproteinization and demineralization Molluscs (mussel and oyster), crustaceans (crab and prawns), and fish shells (silver and pang) shells were obtained from local restaurants in Bloemfontein, South Africa. It is important to note that there is no sign of extraction for some of these sea-waste that has not been reported in the literature for instance, there is no research that has been reported in which the chitin was extracted from molluscs as mussel and oyster, crustaceans as crab and prawns, and fish shells as silver and pang using both deproteinization and demineralization. In this work, we will attempt to extract chitin from mussel, oyster, crab, prawns, silver fish shell and pang using the deproteinization and demineralization and the method will be modified where needed.

Methodology

Materials and methods

Molluscs (mussel and oyster), crustaceans (crab and prawns), and fish shells (silver and pang) shells were obtained from local restaurants in Bloemfontein, South Africa (see Fig. 1). The shell wastes were cleaned with running warm water to get rid of soluble organic matters, others impurities and adherent proteins. Obtained cleaned shell wastes were dried in an oven at 35 °C (molluscs and fish shell) and 60 °C (crustaceans shell) for 12–24 h. The shells were later crushed using a laboratory blender and sieved to fine powder. Crushed powdered and flakes shell waste of the molluscs, crustaceans and fish scales were weighed, placed in an opaque glass and plastic containers and were

Fig. 1 Top (crustacean shell), middle (Molluscs shell) and bottom (fish scale) waste

stored in temperature surrounding the laboratory until were used. The extraction process of chitin is then summarised in Fig. 2 below. 100 g of each (molluscs, crustaceans and fish scales) sample were taken for extraction process. All reagents and solvents used were purchased from Sigma Aldrich and Merck chemical suppliers, South Africa.

Isolation and extraction of chitin

In the process of isolating chitin for the natural raw materials, we considered two steps: De-proteinization (DP) and demineralization (DM) [21].

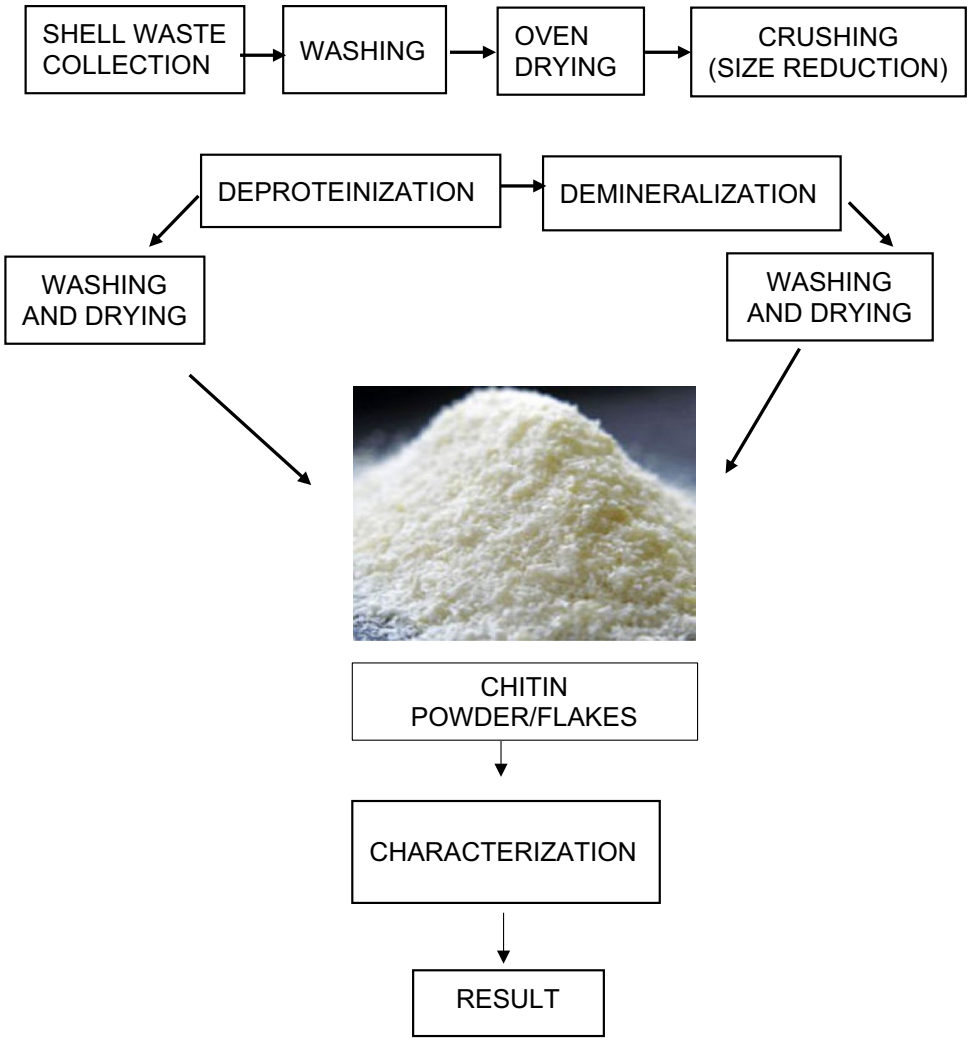

Fig. 2 Traditional isolation of chitin different from sea waste [21]

Deproteinization The chitin deproteinization was done employing 10% NaOH (1:10v/w) for (crustacean and molluscs shell), 1% NaOH (1:1 v/w) (fish scales) at ambient temperature (approximately 30 °C), to get rid of remaining proteins and other organic materials. The treatments with NaOH (10 and 1%) and their durations 18–24 h depend on the nature of species. The colorless indicated the absence of proteins. Then the solution was washed 5–6 times with distilled water to neutrality and the resulting solid product was dried to constant weight 35 °C to 60 °C for 24 h [21].

Demineralization The demineralization of the deproteinized shells was carried out by stirring in dilute HCl solution to remove acid and calcium chloride, calcium phosphate and water-soluble impurities. All species were treated with 10% HCl solution (1:10 w/v) (mollusks and crustacean) and 1% HCl (fish scales) at ambient temperature (approximately 30 °C). The treatments with HCl (10 and 3%) and their durations 16–72 h depend on the nature of species after treatment of the resulting solid fractional was with water cleaned 5–6 times with distilled water to neutrality and the product was dried to constant weight 35 °C to 60 °C for 24 h.

Physico-chemical parameters

Measurement of degree of acetylation

Employing the acid-base titration method suggested in [22] with modification, the degree of acetylation (DA) was measured. Briefly, chitin (0.25 g) was dissolved in 30 ml of HCl aqueous solution (0.1 mol/l) at room temperature. The solution was allowed to stir for about 50 min until complete dissolution of chitin. It was later cool down at room temperature and 5–6 drops of methyl orange were added. The red chitin solution was titrated with 0.1 mol/l of NaOH solution until it turned orange [22].

From the below formula, the DA was calculated

$$NaOH + HCl \rightarrow NaCl + H_2O$$

Molarity of HCl remains after reaction with chitin

$C_1 = V_2 \times C_2/V_1$

Conc of HCl that reacted with chitin

$C_{original} - C_{remaining} = C_{reacted}$ with chitin

No of moles of HCl that reacted with chitin

$C_1 \times V_1/1000$ ml,

Mass of chitin = no of moles chitin × molar mass of chitin

% DA chitin $= M_c/M_s \times 100$.

where C_1 is the concentration of standard HCl aqueous solution (mol/l), C_2 is standard NaOH solution (mol/l), V_1 is volume of the standard HCl aqueous solution used to dissolve chitosan (ml), V_2 is the volume of standard NaOH solution consumed during titration (ml),and M is the weight of chitin (g), Mc is the mass of chitin (g) and M_s = mass of sample (g).

Solubility of chitin

Mussels, oyster, prawns and crab shells, pang and fish scales chitin powder samples 0.1 g each were put within a centrifuge tube, dissolved with a 10 ml of 40% acetic acid for about 30 min employing an incubator shaker which was running at 240 rpm and at 25 °C. The obtained result was submerged within a boiling water bath for about 10 min, and then cooled down to room temperature at (25 °C), thus centrifuged at 10,000 rpm for about 10 min and the supernatant was decanted. Particles that was not dissolved were washed in 25 ml of distilled water and further centrifuged a 10,000 rpm. The supernatant was taken away and non-dissolved pellets dried at 60 °C for about 24 h. Lastly the particles were weighted and the percentage of solubility determined, this was followed by calculation employing the below formula to determine the solubility of chitin [23]:

$$\%\ \text{of solubility} = \frac{(\text{initial weight of tube} + \text{chitosan}) - (\text{final weight of tube} + \text{chitosan})}{(\text{initial weight of tube} + \text{chitosan}) - (\text{initial weight of tube})} \times 100 \quad (2)$$

Intrinsic viscosity

The viscosity of chitin samples were employing a Brookfield viscometer were determined. In 1% of acetic acid at 1% concentration on dried basic, chitin solution was prepared. The measurement was done in duplication via a No.5 spindle at 50 rpm on 25 °C solution with reported values in centipoises (cPs) and percentage (%) units [24].

Average viscosity of molecular weight

The average viscosity of molecular weight (Dalton), the intrinsic viscosity (η) of the polymer were employed. Using the mark-Houwink mathematical equation suggested in the work by [24] the molecular weight was calculated.

$$[\eta] = KM^{\alpha} \quad (3)$$

In the above formula, the average molecular weight is M, the constants are α and K and their values are function of polymer type and the selected solvent. Chitin and solvent, these values are 1.82×10^{-3} and 0.93 are the respective values and are not function of deacetylation degree [25].

Result and discussion

The synthesis method for the extraction of chitin was formed accordingly to the procedure reported by [21]. This method was slightly modified where needed, as will be seen in the result and discussion that follows. Chitin extraction was formed in two stages: first removal of proteins (DP), followed by the removal of minerals (DM) to form chitin. The deproteinization is usually done via method of extraction with dilute sodium hydroxide solution 1 to 10% at high temperature ranging from 65 to 100 °C for 1 to 6 h, see the work in [26, 27], they extracted protein from shrimp shells with 3% NaOH at 100 °C for an hour and also they treated crawfish shell waste with 3.5% NaOH at 65 °C for 2 h. In a similar way, the process of demineralization requests removal of minerals, primarily calcium carbonate and is achieved via acid treatment employing HCOOH, CH_3COOH, HCl, HNO_3 and H_2SO_4. This process is easily achieved due to the involvement of decomposition of calcium carbonate within the water-soluble calcium salts with the release of carbon dioxide as presented. This process is achieved easily due to the involvement of decomposition of calcium carbonate into the water-soluble calcium salts with the release of carbon dioxide as presented in the following equation [26, 27]:

$$HCl\ CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2$$

Deproteinization of shells

In a 500 ml beaker, a heap a head spoon spatula of mollusks (oyster and mussels shell), crustaceans (prawns and crab shell) and fish shells (pang and silver scales) where added gradually to 10% NaOH solution (mollusks, crustaceans) and 3% (fish shell). Foam appeared and flooded above the

surface of the beaker and the solution color changes. After continuous stirring for about 12 to 24 h, depending on the marine waste sample, the reaction was completed and the solution color changes to dark white and the resultant product was cleaned with distilled water four to five time until the pH of the water 7 and obtained results was dried within a vacuum oven at constant weight and the yield were recorded in Table 1.

Demineralization of shells

Minerals were removed from the de-proteinized product by gradual addition of 10% HCl (mollusks and crustacean) and 3% HCl (fish scales) after every minute and the solution cooler changes slightly to brown. The temperature of the demineralized product was increase to 50 °C and later to 70 °C to 80 °C depending on the chitin sample. After continuous stirring for about 18 to 72 h, the reaction was completed and the shells were a little bit squashy and the solution color changes to brown and the sample result was thus washed consecutively with water until the solution was near neutral. Additionally the sample was with distilled water washed and dried within a vacuum oven at constant weight and thus yields were obtained as indicated in the below Table 1.

Table 1 Percentage composition of the four different chitin samples from marine waste

Waste source	% Protein	% Chitin
Oyster shell	98.85	69.65
Mussel shell	86.42	35.03
Crab	63.73	60.00
Prawn shell	58.80	40.89
Pang scale	44.36	35.07
Silver	40.22	31.11

Decoloration (DC)

Decoloration step in the formation of chitin was omitted in this research work, the reason being that, decoloration of chitin is not really necessary since it just involve bleaching to remove the color of the final product chitin. It also decreases the viscosity of the final chitin sample, a work done in [28] suggested that it is not acceptable to use bleach for material at any state as bleaching considerable lessen the viscosity of final chitin product [28].

Yield of chitin

The calculation of yields was done for dry weight ranged from 13.70 to 30.27 g for crustacean, mollusks and fish shell powder. Chitin yield ranged from 31.11 to 69.65%. The highest yields were obtained from oyster and crab shell.

The results of chitin composition from various marine sea waste depicted in Table 1 above. The quantity of protein was lowest in prawn shell as 40.89% and highest in oyster shell to be 98.85%. This highest protein content indicates that the shell contained more organics matter than the other samples. Mussel contain a high protein contents of 86.73% than crab shell and pang scales which were reported to be 63.73% and 44.36 respectively. Yield has been calculated for mussel, oyster, crab, prawn shells, pang and silver scales waste chitin (Table 2). Removal of organic matter ($CaCO_3$ content), oyster shell had the highest inorganic matter of chitin as 69.65% and the lowest was observed in silver scales to be 31.11%. Crab shell had a higher inorganic chitin content of 60.00% than mussel and prawns which were reported to be 35.03% and 40.89% respectively.

Table 2 Composition distribution of the chitin sea waste samples in terms of percentage, mass and solubility on dry basis at 25 °C

Raw materials	Mass (g)	Base (NaOH) concentration %(w/v)	Appear-ance (De-mineraliza-tion Product)	Acid (HCl) concen-tration (%)	Weight of chitin (g)	% of chitin	Product appear-ance	Solubility in acetic acid
Mussel	100	1:0	Ash	10	30.27	35.05	Greyish brown white	Almost com-pletely dis-solved
Oyster	100	1:10	White powder	10	68.85	69.65	White	Almost com-pletely dis-solved
Prawns	100	1:10	Light pink	10	36.11	40.89	Orange pink	Slightly dissolved
Crab	100	1:10	Slightly brown-ish	10	38.24	60.00	White (slightly brown)	Slightly dissolved
Silver scales	100	1:100	Light brown	1	13.70	31.11	Super white	Almost com-pletely dis-solved
Pang scales	100	1:100	Light brown	1	18.08	35.07	Super white	Slightly dissolved

The high percentage of organic matter of chitin obtained can be explained to be caused by the less concentration of HCl and this could not therefore remove minerals from different shell waste samples and thus increase the yield of chitin. The work done by [29] pink shrimp, crab and crayfish shells were reported to have $CaCO_3$ content of 42.26 and 63.94% respectively. Also indicate in Table 2, when acid and base concentrations are increase in deproteinization and demineralization or in demineralization and deproteinization steps respectively, chitin production slightly decrease due to the extensive deproteinization and demineralization (see Table 2). This research was done to obtain more deproteinization and demineralization end products which will lead to loss of weight from the different marine sea waste. Due to the challenge to get rid of all minerals because of heterogeneity of the solid, a wider volume leading to more concentration of acid solution can also be utilized. After the process of demineralization chitin was accounted for the shell. The remainder of the product could be attributed mainly to protein, the shell retained its slightly brown color, and so it is unlikely that pigments were removed by this treatment. It is important to note that, the withdrawal of more protein and other inorganic acid bring a more-white colour end product. Thus the obtained results contains more chitin thus more white in colour however tittle brownish could be explained by a lower grade of the final product that possess lowest chitin content because of the incomplete deproteinization and demineralization steps (see Table 2). According to colour and weight loss of the end product, it is possible for one to identify the chemical (HCl and NaOH) concentration produces the good chitins end product. Acid treatment using 10% concentration of HCl, the products were brown and brownish white which indicate that the pigments were present in chitin. Using 10% alkaline (NaOH) treatment, the products were dark white and whitish and of good quality. These concentrations are economic and safe to the environment as it leaves less residual acid and bases to the soil. From these results, it was concluded that for chitin production, the best alkali (NaOH) acid and (HCl) concentration used is 10% (Table 2), since chitin produces whiter products of oyster, crab shells and pang scales having 69.65, 63.73 and 44.36% respectively. The final products of the samples of chitin are almost completely and slightly soluble in acetic acid. It is pointed out that, the product (chitin) of good quality. These products also indications that pigments are present in these products.

Analysis of degree of *N*-acetylation

We shall note that the degree of acetylation of chitin product has influence on all the physiochemical properties (molecular weight, viscosity, solubility and so on), this implies it is one of the most important parameters. The NaOH concentration has great influence on the degree of acetylation. The acetyl group bound in chitin is not obvious to remove, thus needs high temperature and concentration of NaOH. The percentages the degree of N-acetylation results obtained in this work are shown in Table 3 below. Employing the acid-base titration method, the degree of acetylation was measured, the volume (v) of the end point of the titration correspond to neutralization of HCl acid consume where indicated by a color change from (red to orange solution) and was used to calculate for the six chitin samples the degree of acetylation. Based on titration result of chitin solution, a linear relationship between percent DA (Table 3) versus volume of NaOH was obtained (Fig. 3).

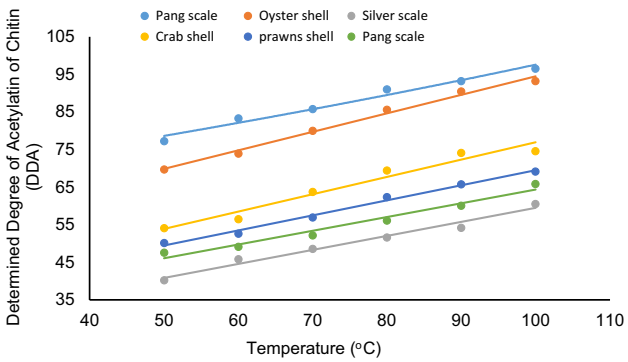

Fig. 3 Variation of the degree of acetylation (DA) values determined for different chitin samples dissolved by heating at varied temperature: 50, 60, 70, 80, 90 and 100 °C for 60 min

Table 3 Influence on the physiochemical characteristics of chitin from sea shell waste (0.1 M NaOH and CH_3COOH, T = 80 °C)

Sample of chitin (shell and scales waste)	Chitin (%)	Degree of acetylation (DA, %)	Average viscosity (η) (Cps)	Molecular weight, Mw (Da)
Mussel	6f 9.65	91.00	4500	7.53×10^6
Oyster	35.03	85.62	3500	5.75×10^6
Prawn	60.00	51.61	2300	3.66×10^6
crab	40.89	69.40	1500	2.31×10^6
Pang scale	31.11	62.35	1000	1.50×10^6
Silver scale	35.07	56.12	600	0.86×10^6

Table 4 Degree of acetylation (DA) (%) of chitin samples from sea waste at varied temperature (°C)

Samples	50	60	70	80	90	100
Mussel shell	77.21	83.31	85.75	91	93.22	96.51
Oyster shell	69.68	73.98	80	85.62	90.44	93.27
Prawns shell	40.17	45.78	48.59	51.61	54.22	60.56
Crab shell	54.1	56.49	63.7	69.4	74.16	74.57
Pang scale	50.11	52.58	56.89	62.35	65.77	69.12
Silver scale	47.59	49.16	52.18	56.12	60.1	65.85

Table 5 Solubility of chitin from different sources of crustaceans, molluscs and fish scales, expressed in percentage

Sample	Solubility (%)
Mussel	85.71
Oyster	77.78
Crab	70.67
Pang	68.00
Silver	67.74
Prawns	58.33

(Solvent: acetic acid)

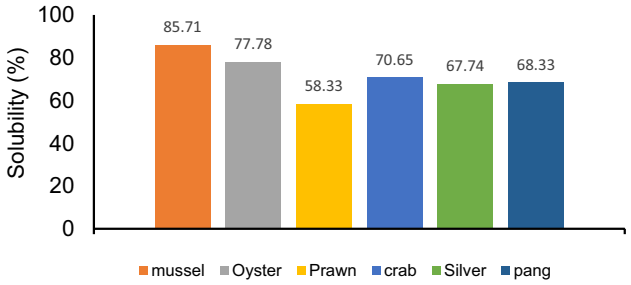

Fig. 4 Solubility of chitin: molluscs, crustacean and fish shell waste express in percentage

From the results in Table 3 above, it is shown that mussel and oyster shell had the highest degree of acetylation of 91.00 and 85.62% followed by crab shell and pang scales with a DA of 69.40 and 62.65% respectively. Lowest degree of acetylation was observed in prawn shell and silver scales which are 51.61% and 56.12% respectively. For this situation, the possible increase in NaOH concentration leads to the decreased of enhancement the degree of acetylation grade where highest acetylation grade of 91% from mussel shell. The determination degree of N-acetylation was also perfume at different temperature of 50–100 °C (see Table 4).

From Table 4, one can see that DA of chitin from the six samples were determined at temperature ranges from 50 to 100 °C for 60 min each (to help dissolve the chitin) using the acid-base titration method earlier discussed above. Based on the result in Table 4, it is shown that a rise in temperature from 50 to 100 °C resulted in a striking increase in % DA of mussel, oyster prawn, crab shell, silver and pang scales chitin (Fig. 3). The temperature increases the degree of acetylation of chitin samples markedly and confirmed that reaction temperature plays a dominant role in achieving higher DA of chitin. Extreme high temperature may cause depolymerization of chitin polymerization of the chitin samples.

Solubility

The trichloroacetic acid (TCA), dichloroacetic acid (DCA) as strong polar protic solvents were with properties to dissolve chitin see that work done in [30]. This study, Chitin from various marine seashell waste were treated with 40% acetic acid to determine the solubility and results were reported in Table 5.

Based on the results (Table 5), one can see that; all the three chitin samples: mussel, oyster and Crab demonstrate excellent and good solubility results ranges from 70.67 to 85.71% with little or no significant difference while silver and pang scales showed slightly lower solubility's values ranges from 67.74 to 68.00%. Prawns shell had the lowest solubility value of 58.33% (see Table 5). The main character of this method is the reaction with the acetyl group, the protein contaminants remaining in the sample in the course of the analysis process may adversely react with the results. Mussel chitin sample posed the highest N-residue of 85.71% as indicated in Table 5, thus the deproteinization process for the six samples have been almost completed however, prawns still had some remaining or other impurities.

The poor solubility of prawns shell, silver and pang fish scales chitin samples, occur as a product packing of chains with strong inter and intramolecular bonds within the hydroxyl and acetamide group [31]. Figure 4 below shows a bar chart for the solubility of chitin from various marine shell waste. The percentage of solubility increases drastically from molluscs shell (mussels and oyster shell), then a rapid drop to prawn shell, then later rise up again for crab shell, and finally a little drop for silver and pang scales shell waste.

According to the work done by Austin, who investigated the use of co-solvents like 2-chloroethanol or dichloromethane in conjunction with formic acid, while the co-solvent is being added to the solution of chitin in HCOOH to lessen the solution viscosity, this can be confirmed in the works [10, 32]. The author with name Austin is the first to report

on the utilisation of DCA and TCA as solvents for chitin see in [21]. Base on his result of the two acids he used, DCA is more suitable for use being a liquid at room temperature, nonetheless it is less efficient solvent and provides viscous solutions at relatively lw temperatures concentration of chitin.

Even though TCA is more appreciable solvent for chitin, but is solid at room temperature thus one will require the presence of a co-solvent and solutions containing 20–50 wt% to be used. Another researcher; Brine and Austin, noted lower solubility solubility's values during their research work, which suggest incomplete removal of protein, when dissolving chitin in trichloro-acetic (TAC) acetic acid as solvent.

Following the process of pulverization using two parts with weight of chitin and addition to 87 parts by weight of a solvent solution containing 40% TCA, chloral hydrate 40% and dichloromethane 20% (DCM) see in [33, 34]. Another research has been done trying to dissolve chitin in TCA containing chlorinated hydrocarbon like MC and 1,1,2-trichloroethane see in [30, 35]. Similar patents have been reported for which a solution of water and DCA and a solution of TCA/CH/DCM or TCA/DCM/MC solvent system have been employed in [36–39]. The DCA and TCA are known to be very corrosive, very high concentration of solvents to break down polymer of chitin thereby lessening the molecular weight to the level for which the strength of the fibres will be affected.

Determination of intrinsic viscosity

Intrinsic viscosity is an important factor in the conventional determination of the molecular weight of chitin. A large molecular weight of chitin usually gives highly viscous solutions but not necessary for commercial use. The chitin viscosity in acetic acid seems to increase while the pH decrease, nevertheless it reduces with decreasing of pH in HCl see [28], leading to the definition "intrinsic viscosity". Intrinsic viscosity of chitin is connected to degree of ionization also to ion strength see [40]. In this research work, Intrinsic viscosity of six different marine chitin samples obtained were demonstrated at different temperature using Brookfield viscometer, and results were obtained in centipoises (CPs) and percentage (%) as shown in Figs. 5 and 6.

Based on the results in Figs. 5 and 6, It is showed that the intrinsic viscosity dropped dramatically when the chitin solution was heated and measure using brook-field viscometer at 0 °C to 40 °C. The intrinsic viscosity reduced from 45,000 to 2500 CPs for mussel, 25,500 CPs to 1500 CPs for oyster, 11,000 CPs to 800 CPs for prawn shell, 8000 CPs to 600 CPs for crab shell, 6000 CPs to 200 CPs for pang scales and 400 CPs to 130 CPs for silver scales chitin. Later, the intrinsic viscosity further decreases slowly from 900 CPs to

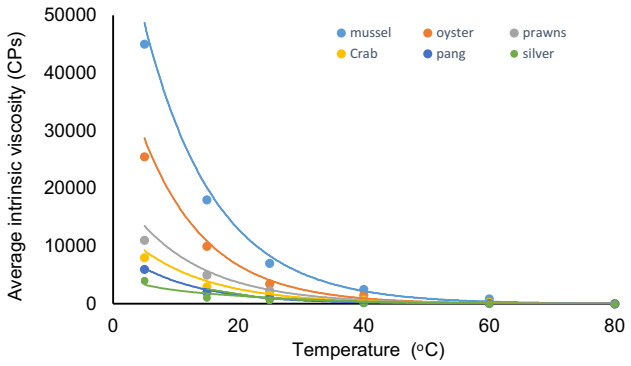

Fig. 5 Changes of intrinsic viscosity (centiposes) as function of different Temperature changes (5, 15, 25, 40, 50 and 80 °C)

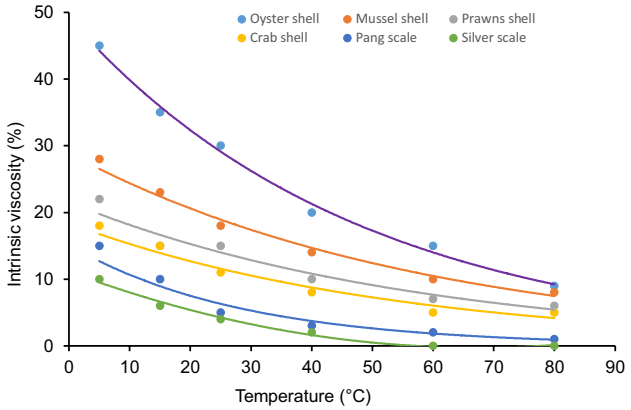

Fig. 6 Changes of intrinsic viscosity (%) of various marine sea waste as function of different Temperature changes (5, 15, 25, 40, 50 and 80 °C)

34 CPs for mussel, 170 CPs to 14 CPs for oyster shell, 300 CPs to PCS for prawn shell, 270 CPs to 60 CPs for crab, 100 CPs to 3 CPs for pang and 40 CPs − 1 CPs for silver scales chitin, a temperature range from 60 to 80 °C.

Determination of the molecular weight

Chitin is known as high molecular weight biopolymer and changes with the sources and the methodology of preparation [41]. It was suggested that the molecular weight of original chitin commonly larger than 1 million Daltons see [42]. The viscosity-average molecular weight was obtained using employing Eq. 1 from the obtained intrinsic viscosity in our study. The average molecular weight viscosity were measured at different speed shear rate. A reduction of intrinsic viscosity was followed by decreasing in viscosity-average molecular weight of chitin samples. (Fig. 7).

From the result in Fig. 7, A drastic drop of speed (shear rate) was observed from 1 to 4 s (shear rate) for mussel

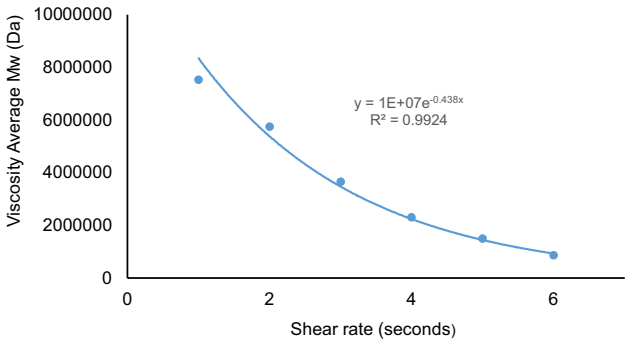

Fig. 7 Change of viscosity-average molecular weight (VAMW) of the various marine sea waste mussel, oyster, prawns and crab shell, pang and silver scales as a function of shear rate speed (at 1,2,3,4,5 and 6 s)

shell to silver scales where the molecular weight reduces from 7.53×10^6, 5.75×10^6, 3.66×10^6 and 2.31×10^6 Da respectively.

The average viscosity molar mass drop drastically from 1 to 4 shear rate of mussel shell to silver scales, which were 7.53×10^6, 5.75×10^6, 3.66×10^6, 2.31×10^6, 1.50×10^6 and 0.86×10^6 Da respectively. After 4 s, the average molecular weight of chitin samples from 1.50×106 to 0.86×106 at speed of 5–6 s. Several factors while producing chitin includes high temperature, reaction time and concentration of alkali, particle size, acid concentration, shear stress or rate may influence the molecular weight of chitin [12]. Our six sea marine chitin waste samples were likely undergoing some polymerization stages, which resulted in some of the chitin product to have low molecular weight compared to those in the literature. The reduction was because of the chain scission of chitin backbone, where degradation process took place.

Remark 1

During the extraction of chitin from Mollusks shell waste (oyster and mussels shell), on their dry basis, HCl acid was used to examine the effect of demineralization. During these treatment process, demineralization method was treated before deproteinization method since these shell waste was having a faint hard cover, it was necessary to be removed the minerals first since more of the minerals were easier to be removed during these process which leads to the loss of weight of these shell waste, which indicates that proteins were lost. Same procedure was applied to the fish scales.

On the other hand, extraction of crustacean shell (prawns and crab shell) deproteinization was done before demineralization because the shell having a thick light cover. Therefore it was necessary to remove the proteins first before the minerals since the protein were easier to be removed, which leads to the loss of weight of these shell waste, which indicates that protein were removed.

This result shows that acid and base concentrations are increasing in demineralization and deproteinization step respectively, chitin protein slightly decreases due to the extensive deproteinization and demineralization.

The demineralization and deproteinization products leads to the weight loss of the shell waste. The removal of more proteins and inorganic minerals bring a whiter colour final product. The end product which contains more chitin, most be whiter in colour and the little brownish will be less chitin end product and full brownish contain the lowest quantity of chitin end product due to the incomplete removal of demineralization and deproteinization.

We shall note that, the deacetylation (DA) is an important parameter that has great influence on physicochemical characters for instance molecular weight as discussed in [43]. This parameter is also useful for the elongation at break see the work done in [43] and more importantly the tensile strength this aspect was discussed in detail in [43, 44]. The parameter also has a great impact on biological properties, let us name few, the work done in [44, 45] proved that DA influences the biodegradation by lysozyme, more impressively, it was reported in [46] the influence of DA to the wound-healing properties and the osteogenesis enhancement was presented in [47].

Remark 2

The temperature of 35 °C was used for mussels and fish scales. Due to their hard thin shell cover, it was necessary to dry them between 35 and 50 °C.

The temperature of 60 °C was used for crustacean shell waste (oyster and prawns). Due to their think light shell cover, it was necessary to dry them between 50 and 65 °C.

Chemical composition of raw materials

In this section, we present in detail the chemical composition of each raw material studied in this work as has been reported in several works in the literatures. We shall start with mussel. In 1988, Nielsen reported the following chemicals measured in the mussel [9]. He reported some trace of metals including: Arsenic, nickel, mercury, selenium, copper, lead and zing [9]. The following organic compounds were also reported namely, dichlorodiphenyltrichloroethane (DDT), chlordane, dieldrin, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and butyltin [9]. The work done by Yoon and others reported the following chemical composition of oyster-shell including $CaCO_3$, SiO_2, MgO, Al_2O_3, SrO, P_2O_5, Na_2O and SO_3 [48, 49]. Kucukgulnez and others have studied approximate composition and mineral

contents of blue crab and reported that, blue crab is made up of protein, fat, ash and moisture however the protein were more dominant in percentage [50]. In 2000, a study of prawn/shrimp was carried out in [51] in which they studied the composition of shrimp shell and reported the following chemical components: Ca, Na, Mg, Sr, Ba, Cu, Ni, Co and Fe see table of of [51]. Nakano and other have reported presence of Uronic acid, sialic acid and nitrogen in pang shell [52].

Conclusion

Chitin extracted from mussel, oyster, prawns, crab shell, silver and prawn shells waste by chemical methods; deproteinization and demineralization methods have been found successful in isolation and characterization during our study. Within the list of treatments methods employed in this work, 1 and 10% have been successful to extract chitin from the different marine sea waste sources. These concentrations are economic and safe to the environment as it leaves less residual acid and bases to the soil. From these results, it was concluded that for chitin production, the best alkali (NaOH) acid and (HCl) concentration used is 10% (Table 2), since chitin produces whiter products of oyster, crab shells and pang scales having 69.65, 63.73 and 44.36%% respectively. The final products of the samples of chitin are almost completely and slightly dissolvable within acetic acid. In our study, the products (chitin) are good quality. These products also indications that pigments are present in these products. Furthermore, chitin scission or degradation took place when chitin samples were measured at different temperature and shear rate for 50 rpm. Both intrinsic viscosity and viscosity-average molecular weight were reduced drastically from 15 to 40 °C and later slowly to 80 °C. This shows that, intrinsic molecular and average molecular weight viscosity (AMWI) for chitin from different sources of sea waste can be determined by Brookfield viscometer. Furthermore, it is then concluded that the shell waste of crustacean, molluscs and fish scales contain chitin which was successfully in the elimination of proteins and mineral during preparation, and was successful analysis using the various physiochemical parameters of the chitin products, giving good average and also low yields.

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