Asian Journal of Fisheries and Aquatic Research

Volume 26, Issue 8, Page 95-114, 2024; Article no.AJFAR.120695
ISSN: 2582-3760

Extraction and Utilization of Chitin and Chitosan from Waste Yields of Economically Important Crustaceans and Molluscs for Improved Fish Production

Joseph Abiodun Olugbojo [a*], Adeolu Akanji Akinyemi [b], Samuel Olubodun Obasa [b] and Enock Olugbenga Dare [c]

[a] Department of Biological Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.
[b] Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria.
[C] Department of Chemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration with all authors. Author AAA originally formulated the idea and revised the manuscript. Author SOO reviewed and edited the manuscript. Author EOD reviewed, edited, and revised the manuscript. Author JAO developed the methodology, sourced the materials used, and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajfar/2024/v26i8799

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120695

Review Article

Received: 01/06/2024
Accepted: 02/08/2024
Published: 12/08/2024

ABSTRACT

Background: Every year, about eight million tons of different species of crustacean shells are produced, with several million tons of various mollusc shells world-wide. These shell wastes are usually disposed into the water body or mainland, littered everywhere, with significant impact on the environment.

**Corresponding author: Email: josephgbojo2012@gmail.com*

***Cite as:** Olugbojo, Joseph Abiodun, Adeolu Akanji Akinyemi, Samuel Olubodun Obasa, and Enock Olugbenga Dare. 2024. "Extraction and Utilization of Chitin and Chitosan from Waste Yields of Economically Important Crustaceans and Molluscs for Improved Fish Production". Asian Journal of Fisheries and Aquatic Research 26 (8):95-114. https://doi.org/10.9734/ajfar/2024/v26i8799.*

Aim: To provide a profitable way of reducing and possibly removing shell wastes, and harness them for a better purpose through an eco-friendly and sustainable technological approach.
Main Body of the Abstract: This review underscores a broad list of economically important crustaceans and molluscs, harvestable in Nigeria coastal waters whose shells usually ended up as wastes. Chitin, being the primary component of these wastes can be extracted and deacetylated into chitosan, or synthesized into nanoparticles for better applications in various aspects of aquaculture. Both chemical and biological methods of chitin extraction were discussed, however, bioextraction method was recommended due to its eco-friendliness, biocompatibility, and non-hazardousness. Various ways by which these shell wastes can be processed and utilized for improve aquaculture practices were also discussed. These include fish growth enhancement, pond water treatment, as antioxidant, as immuno-stimulant, as antimicrobial, as drug carrier, and several other purposes.
Conclusion: There are much hidden wealth in these fisheries by-products (Crustacean and Mollusc shells) if properly valorized. It has a great potential to transform aquaculture industry, and bring significant, and sustainable economic development in Nigeria.

Keywords: Aquaculture; bioextraction; chitinous shell wastes; shell wastes valorization; economic development; nanotechnology.

1. INTRODUCTION

The global aquaculture output depicted about 50% expansion between the years 2000 to 2019, which tends towards almost 180 million tons in the year 2019, showing a tremendous growth of about 55 million tons from the year 2000. Out of the overall output, molluscs represent 13%, while crustaceans were 9% [1]. Every year, about 8 million tons of crustacean shell wastes are produced, of which 60% represents crab mass [2]. Also, more than 10 million tons of mollusc shells are produced yearly; and oyster, clam, scallop and mussel shells represent 70% of this [3]. Of the total weight of molluscs, shells constitute 65-90%; and this also depends on species [4]. Mussels produce and contribute more shells compared to other species of shellfish [5]. Abalone and other gastropod also contribute a significant ratio of their body mass as shells [6]. Therefore, Shells should be seen as a major by-product, which should not be regarded as waste, but treated as a new raw material to be highly maximized. Unfortunately, Nigeria happens to be among those nations whose coastal marine resources are being over-exploited with mass wastage of shellfish exoskeleton [7]. Moreover, uncontrolled disposal of shell into the sea are usually sent back to the land through upwelling and ocean cleansing which usually brought about a negative impact on the soil and water [8]. As a result of this, piles of shells were found in virtually everywhere, causing environmental pollution and damage due to the foul odors that are being released from the decomposition of residual tissues attached to them [6]. From field observations, many of these shells could be found either as harvest wastes or processing wastes from individuals or processing industries. The ways by which they are consumed are different from one place to another. They are either served after the removal of the shell or processed with the shell and served.

1.1 Historical Utilization of Shell Wastes

Much value has always been attached to shells over the ages. Traditionally, this has been established and passed from one generation to another so that its economic and social value can be retained. From the archeological point of view, it was discovered that since Paleolithic periods shell has been in use as a valuable resources [9, 10] and have been used to make tools or ornaments. In several cultural setting, it has also been used as a legal tender, and in exchange for goods and services, such as cowries shells and shell beads [11,12], even till date in some places [13]. This shows that value also determines the type of standard that can be put in place about any material, which also determines how long such standard can remain. Till date, some species of mollusc shells are still much in use because of their aesthetic value. Currently, over 5000 different species of molluscs are sold because of their ornamental worth, especially gastropods. Unfortunately, there is no adequate record on this [14,15]. In addition, there are several failed attempt to reintroduce the effective use of shells, especially as marine ornamental ventures which involved millions of gastropods as clean up animals [16]. In several places, efforts have been made on sustainable use of these shell wastes as ornamental items, but this has not gone far because there are more important ways by which these shell wastes can be used which were not discovered until recent times when many industries begin to consider a new and more profitable enterprise with respect to shell valorization.

The primary aim of this article is therefore to provide a broad picture of shell waste re-utilization or utilization possibilities apart from traditional and inadequate current uses. This paper also aims at finding solution to reduce wastes, harnessing them for better purposes through an eco-friendly and sustainable technological (Nanotechnology) approach in order to enhance economic growth, and free the environment from the current burden of this avoidable waste (Shell wastes) in Nigeria and to serve as a template for global application.

2. METHODOLOGY

Data were sought from research articles, books, book chapters, review papers, yearbooks, booklets, and conference proceedings from high-impact factor journals in Scopus, schimago, google, and semantic scholars' database. Moreover, articles from 1990 to 2023 were used. Articles, starting from the year 1990 to 2000 are just 6.2 %, from 2001 till 2009, 15.38 %, from 2010 till 2014, 22.31 %, while publications dating from year 2015 till January 2023 are 51.54 %. Thus, about 74 % of all articles used were published within a short period of about 12 years. Some articles were added after cross-citation analysis of the selected materials. The key terms or phrases used for internet search include: shell waste, chitinous exoskeleton, shell waste re-utilization, mollusk waste, crustacean shell waste etc. This were used without adding values in between Boolean searching tools. The results were streamlined based on titles and abstracts, in order to ensure appropriate selection. During the search, various fields in Applied Sciences were focused on, such as Fisheries, Aquaculture, Agriculture, Food Sciences, etc. while Fisheries and Aquaculture application was the centre focus. However, so many recent literature discussed more on biomedical, general agriculture, and cosmetics applications while few discussed Fisheries and Aquaculture applications, which is the main focus of this paper, with the aim of making relevant documents available to the researcher, academics, and industrialists on the current state of chitinous wastes (a byproduct of fisheries and aquaculture), and thus provide modern economic solution most especially to improve fisheries and aquaculture practice, training and research.

3. ECONOMICALLY IMPORTANT CRUSTACEANS AND MOLLUSCS IN NIGERIA COASTAL WATERS

3.1 Crustaceans

Crustaceans are a large and diverse group of invertebrates, which fall under the Arthropod phylum, with about 45,000 species. One of the defining features of crustaceans is their hard exoskeleton, made primarily of chitin, which protects their body and provides structural support. Crustaceans can be aquatic, semi-aquatic, or terrestrial. They include well-known animals such as crabs, lobsters, shrimps, crayfish, barnacles, and woodlice. As they grow, crustaceans undergo a process called molting, where they shed their old exoskeleton and form a new one. Crustaceans typically have segmented bodies that are bilaterally symmetrical, with each segment potentially carrying a pair of appendages [17,18].

3.2 Molluscs

Molluscs are the second largest phylum of invertebrate after Arthropod. Members are known as molluscs or mollusks. Around 76,000 extant species of molluscs are recognized. Molluscs are any soft – bodied invertebrates of the phylum Mollusca, usually wholly or partly enclosed in a calcium carbonate shell secreted by a soft mantle covering the body and live in aquatic or damp habitats. They are grouped into three major classes such as gastropods; which includes slugs and snails; bivalves such as oysters, clam, scallops, seas mussels; and cephalopods such as squids, cuttle fish and octopus [17].

3.3 Coastal Waters

Coastal waters are those parts of the ocean closest to the land. They start where the breakers hit the beach and then stretch seaward as far as 100 nautical miles [19]. Coastal biotopes include lagoons, coastal wetlands, delta, coral reefs, mangrove forests, kelp forests, and upwelling areas. Any bays, inlets, covers, or harbours in this range are also considered part of the coastal waters. [20,21].

Nigeria has a coastline of 853 km, a maritime area of 46,500 km^2, and an exclusive economic zone (EEZ) of 210,900 km^2. This territorial waters lies from latitude 4^0 $10'$ to 6^0 $20'$ N and longitude 2^0 $45'$ to 8^0 $35'$ E. It is characterized with high temperatures during the day, and up to 34^0C in the dry season. It could also be highly humid, especially in the evening periods, or during high rainfall. The mean annual rainfall is about 1200 mm which occurs mostly between late March to late October, and sometimes till early November. Sometimes it may rain for a continuous period of about 24 hrs which could result in floods [22,18]. Fig. 1 represents the Map of Nigeria showing the coastal areas. The blue colour symbolizes water body.

The predominant mean of livelihood in the coastal communities is capture fisheries in which different types of nets were used for fishing. The fishing activities are usually carried out in the creeks, offshore, inshore, mudflats and lagoon. Commercial fishing are also engaged with government license, using ocean going fishing vessels such as trawlers.

Moreover, the coastal biotope is blessed with a variety of fisheries resources. A total of 46 economically important species of crustaceans, belonging to 33 genera (Table 1) found in the Nigeria coastal water include decapods (Crabs, Lobsters, and shrimps), isopods (land-based crustaceans), amphipods (sandhoppers), stomatopods (mantis shrimps) and euphausiids (krill). Different species of Prawn, shrimp and crabs were also included in high abundance, and dominate most of the catches. They are found in the lagoons and the adjacent rivers. The most economically important family of crab in the Nigeria coastal water is Portunidae (*Callinectes* sp. and *Portunus* sp.). [21,6]. Table 1 shows the various classifications of crustaceans available in Nigeria coastal waters.

In Nigeria, molluscs can be located in various substrata, within the fresh/brackish water, and estuaries/marine environments. The followings are the list of economically important molluscs in a taxonomic form: Bivalves (Table 2a), Gastropods (Table 2b) and Cephalopods (Table 2c).

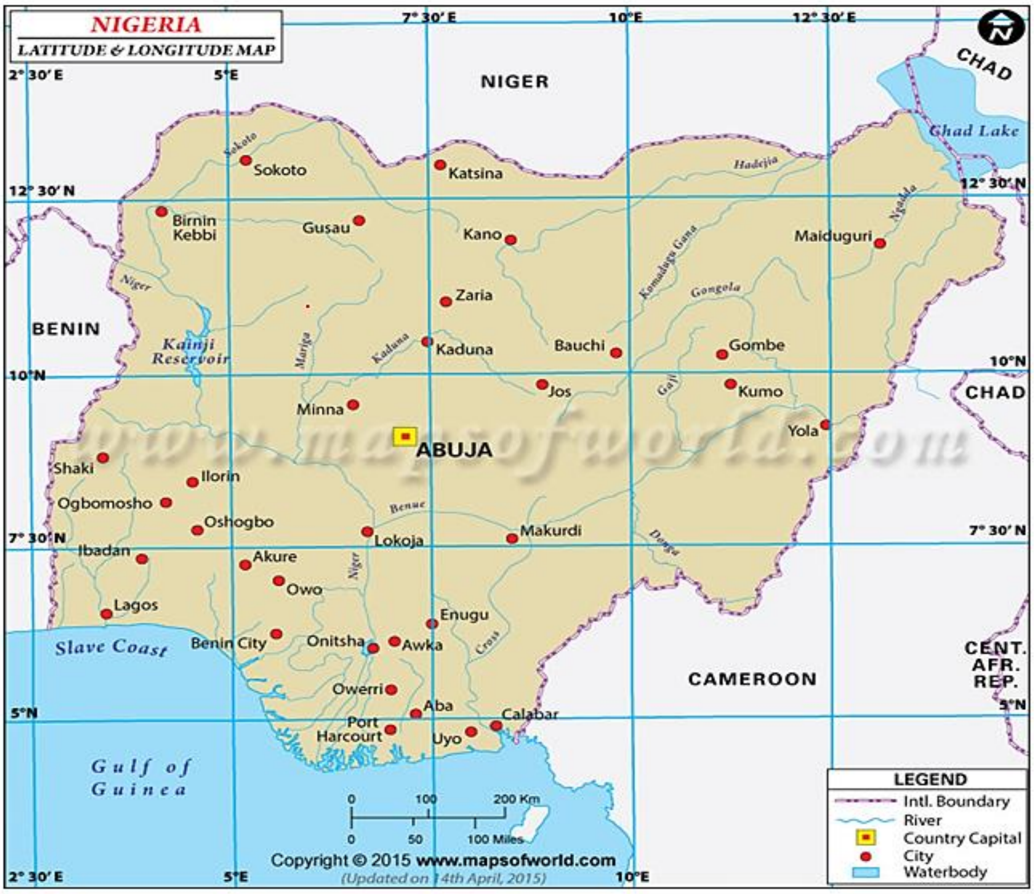

Fig. 1. Nigeria Map showing the coastal area. Ateme [20]

Table 1. List of economically important Crustaceans in Nigeria coastal Waters

S/N	Family	General	Species
1	Hippolytidae	1	1
2	Nematocarcinidae	1	1
3	Palaemonidae	1	7
4	Pandalidae	3	3
5	Pasiphaeidae	1	1
6	Palinuridae	1	1
7	Scyllaridae	1	1
8	Aristeidae	2	2
9	Penaeidae	3	4
10	Sicyonidae	1	1
11	Solenoceridae	1	1
12	Calappidae	1	3
13	Gecarcinidae	1	1
14	Geryonidae	1	1
15	Graspsidae	4	4
16	Homolidae	1	1
17	Majidae	1	1
18	Ocypodidae	2	3
19	Portunidae	4	7
20	Xanthidae	2	2
	Total	**33**	**46**

Table 2a. List of economically important Bivalves (Molluscs) in Nigeria's coastal Waters

S/N	Family	General	species
1	Arcidae	4	4
2	Aloididae	4	4
3	Cardiidae	1	3
4	Carditidae	1	2
5	Chamidae	1	2
6	Donacidae	3	7
7	Dreissenidae	1	1
8	Ericinidae	1	1
9	Garidae	1	1
10	Glycymeridae	1	1
11	Hiatellidae	1	1
12	Mactridae	2	5
13	Mytilidae	4	7
14	Ostreidae	1	3
15	Pectinidae	2	2
16	Petricolidae	1	1
17	Pholadidae	3	3
18	Pinnidae	2	2
19	Pteriidae	1	3
20	Saxicavidae	1	1
21	Solecurtidae	2	2
22	Solenidae	3	3
23	Tellinidae	2	4
24	Teredinidae	1	1
25	Veneridae	4	6
26	Vulsellidae	1	1
	Total	**49**	**71**

Table 2b. List of economically important Gastropods (Molluscs) in Nigeria coastal Waters

S/N	Family	Genera	Species
1	Haliotidae	1	1
2	Fissurellidae	2	3
3	Patallidae	1	1
4	Neritidae	1	2
5	Littorinidae	2	4
6	Turritella	1	4
7	Architectoma	1	1
8	Vermetidae	2	3
9	Melanidae	1	2
10	Potamididae	1	2
11	Cerithidae	2	3
12	Calyptraeidae	1	2
13	Xenophoridae	1	1
14	Strombidae	1	1
15	Naticidae	1	8
16	Cypraeidae	1	4
17	Cassididae	1	3
18	Doliidae	1	1
19	Muricacea	4	15
20	Columbellidae	1	1
21	Buccinidae	1	2
22	Galeoidae	1	1
23	Nassidae	4	7
24	Olividae	1	3
25	Mitridae	1	1
26	Harpidae	1	1
27	Volutidae	1	5
28	Turridae	3	6
29	Conidae	1	5
30	Terebridae	1	2
	Total	**42**	**94**

Table 2c. List of economically important Cephalopods (Molluscs) in Nigeria coastal Waters

S/N	Family	Genera	Species
1	Sepiidae	2	6
2	Loliginidae	2	2
3	Ommastephidae	8	9
4	Octopodidae	1	2
	Total	**13**	**19**

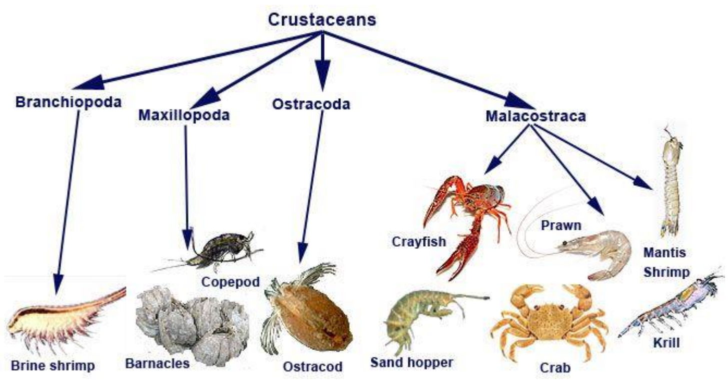

Fig. 2. Examples of common crustaceans found in Nigeria coastal waters [23]

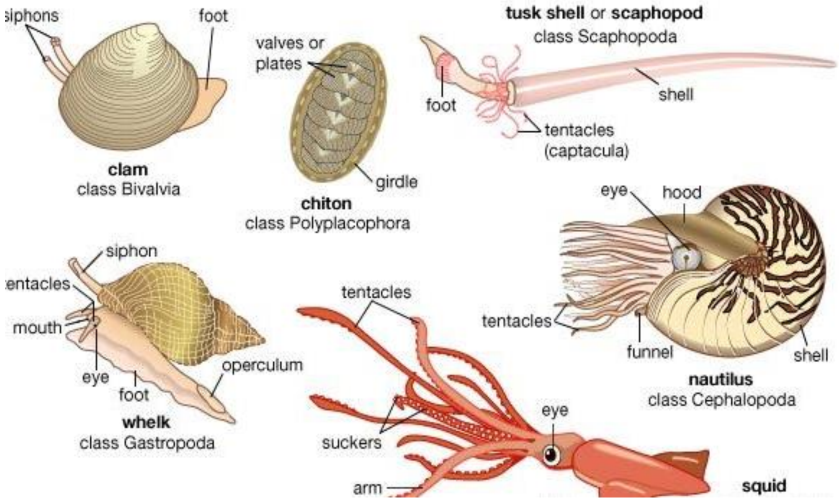

Fig. 3. Examples of common mollusks found in Nigeria coastal waters [24]

4. UTILIZATION OF SHELL WASTE OF CRUSTACEANS AND MOLLUSCS OF NIGERIA COASTAL WATERS FOR SUSTAINABLE ECONOMIC GROWTH

4.1 Cleaning of Shells

For ease of processing towards reutilization, shells need to first be cleaned. However, cleaning can be undertaken through blanching with hot water to remove dirt and some organic materials clinging to them and then thoroughly wash. Moreover, since the target is to produce chitin and chitosan, for onward utilization, there are standard procedures that are required to obtain pure chitin and chitosan. These polymeric substances (chitin and chitosan) are the focus of this discourse, how they can be obtained from shell wastes, and be channeled towards aquaculture development (in Nigeria). After cleaning, chitin will then be extracted, and to get chitosan, further step involving de-acetylation will be required. These procedures are necessary prior to their utilization. Apart from chitin, these shells can also serve as sources of calcium and phosphorous (Minerals) for animal feeds [4,6].

4.2 Extraction and deacetylation of chitin from shell wastes using chemical method

Animal exoskeletons are made up of three primary components. These are proteins, chitin and calcium carbonates ($CaCO_3$) with pigment and salts in small quantities [25]. Extraction of chitin can be carried out in three sequential steps. The first two are fundamental, the third are mainly for shell obtained from crustaceans.

1. Removal of mineral/Decalcification using hydrochloric acid (HCl);
2. Removal of Protein/Deprotenization at high temperature. It is performed using sodium hydroxide (NaOH).
3. Removal of pigments and salts using hydrogen peroxide or oxalic acid in order to obtain a pure white powder. This is done only for crustacean shell whose colour is usually a pale–pink. Furthermore, to convert chitin to Chitosan, whose uses has greater advantage than chitin, it has to be subjected to a process known as Deacetylation, which is the removal of the acetyl group from chitin [26].

The schematic diagram in Fig. 4a summarizes the chemical process of chitin extraction and its deacetylation to give rise to chitosan [27].

However, the use of chemical method of chitin extraction could be hazardous, due to high acid and alkali concentrations, coupled with high temperatures. Moreover, chemical synthesis of these biopolymers presents a lot of limitations such as non- environment friendly, requires much chemicals, non-sustainability, much is spent on energy, and much waste is produced whose products, including proteins and pigments, are difficult to recover [28,29,30]. Also, several issues involving the best desirable control of reactions such as anomerization, altering the properties of chitin to reduce molecular weight, and increasing entropy to enforce depolymerization etc are difficult. The discovery of bioextraction of chitin as a newly green procedure, is a promising alternative to chemical processes with better and more effective results and less hazardous to biological system.

4.3 Biological extraction of chitin

The biological extraction method can be undertaken in two ways: the use of proteases (protein digesting enzymes), or microbial fermentation [28,31,32].

4.3.1 Enzyme- mediated deproteinization and demineralization of chitin

One of the suggested biological method for the removal of protein from shells is the use of proteases isolated from plants, animals, fungi or other microbes. This approach does not involve the use of sodium hydroxide and the likes. The result does not lead to much wastes as in the chemical approach and the yield also give rise to a nutritious protein derivatives (hydrolysates). Depending on the starting waste, the protease can lead to various deproteinization yields according to the conditions tested. It is important that removal of calcium carbonate (decalcification) be carried out first, this help to allow more penetration into the tissue and reduce enzymes inhibition which may hinder effective penetration of protein removing enzymes such as trypsin, pepsin, and pancreatin among others. Eventually, the resulting chitin has a better advantage in terms of physical and chemical properties than the one produced chemically. One of the best result was obtained by Mhamdi *et al*. [33] using heat stable serine alkaline protease from actinomycetes strain (*Micromonospora chaiyaphumensis* S103) to extract chitin from shrimp (*Penaeus kerathurus*) shell waste powder which prove better than other extraction methods. After 3 hours of enzymatic extraction (hydrolysis) at 45^{0}C and pH 8, with enzymes to substrate ratio (E/S) of 20U/mg, the percentage deproteinization was 93%. A similar high percentage deproteinization was obtained from the use of alkaline proteases obtained from *Portunus segnis* viscera and used to deproteinize blue crab (P. segnis- 85%) and shrimp (P. kerathuru- 91%) shells with enzymes to substrate (E/S) ratio of 5U/mg of protein, after incubated for 3 hrs at 50^{0}C [34,35]

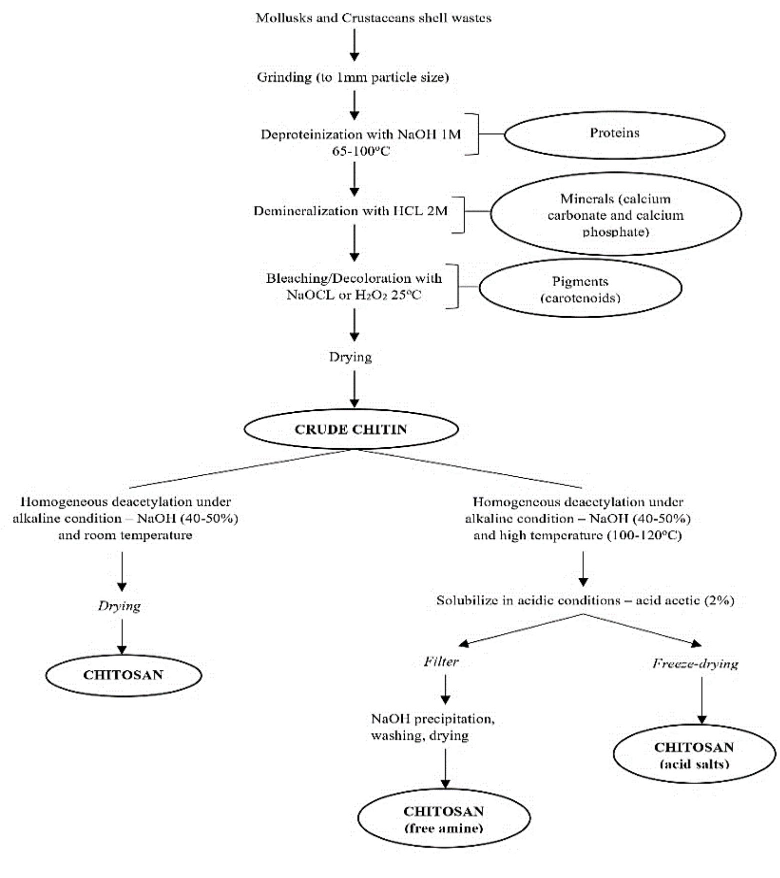

Fig. 4a. Schematic diagram of chemical preparation of Chitin and Chitosan Casadidio *et al*. [27]

4.3.2 Chitin bacteria fermentation (Bacteria mediated chitin extraction)

The use of lactic or non-lactic acid bacteria is another cheaper method of extracting chitin from shell wastes. This Fermentation technique involves the addition of selected microorganisms' strains which follows one-stage, and two-stage fermentation; auto-fermentation and co-fermentation.

4.3.2.1 The use of lactic acid bacteria

Fermentation involving Lactic Acid Bacteria (LAB) is a novel approach for chitin extraction, combining the use of acid and alkali at a very low quantity [32]. Another advantage of using LAB is that, it leads to the production of lactic and acetic acids. Production of pure proteins, minerals, and pigment from the liquid by-products of LAB-mediated chitin extract from the waste shell is easier with bioextraction of chitin than the chemical extraction method. The low pH produced during the extraction, especially when lactic acid is used to precipitate chitin helps to activate the release of proteases. This approach has also been employed to recover carotenoids from silage prawn waste. Different *Lactobacillus* spp strains have been used for fermentation. In recent times, purified chitin has been extracted from *Allopetrolisthes punctatus* (crab) with the use of *Lactobacillus plantarum* sp., a highly rich lactic acid producing gram-positive bacteria, isolated from Coho salmon [36]. The percentage decalcification was 85% while deproteination was 95.3%. This was undertaken within 60 h fermentation at 10% inoculum, 85% crab biomass, and 15% sucrose) [36, 27]

4.3.2.2 Non-lactic acid bacteria fermentation

Ghorbel-Bellaaj et al. [37] carried out an experiment to isolate a protease bacterium known as *Pseudomonas aeruginosa* A2 among other bacteria, which were regarded as inoculum source to prove that protease present in them can also be used for the recovery of chitin. The result obtained was similar to that of chemically extracted one which were used for commercial purposes. This result highlighted the ability of these enzymes to remove protein from shrimp shell wastes to recover chitin, and thus avoid the challenge of the chemical method [37]. He also tried to optimize various variables such as concentration of shrimp shell wastes (50g/L), glucose concentration (50g/L), period of incubation, and size of inoculum to prove and confirm the efficacy of this bacteria. It was exciting to discover that the percentage of protein removal was 89% and that of decalcification was 96% [37]. Since *Bacillus* sp is the common bacteria used to produce protease, research was conducted using six different strains of *Bacillus* sp to extract chitin from waste crab shell and supernatant of a fermented crab. The result shows that fermented crab supernatant has very effective antioxidant and antimicrobial properties [38]. Concerning fungi as a source of proteolytic enzymes, *Aspergillus niger* strains 0576, 0307, and 0474, were selected by Teng et al. [39] and used to confirm the microbial hydrolysis of chitin. The study focus was to determine two different chitin origin as he added fermented shrimp shell to mushroom directly. Eventually, the proteolitic enzymes that was produced by fungi during the protein removal and decalcification of shell wastes, lead to the production of amino acids, which is a source of nitrogen and was also useful in enhancing the fungal growth [39].

The schematic diagram in Fig. 4b summarizes the biological method of chitin extraction and its deacetylation to produce chitosan [27].

4.3.3 Enzymatic deacetylation of chitin

Although chemical deacetylation could help in mass or industrial production, its toxicity, non-eco-friendliness, high energy consumption, and less biocompatibility is a major drawback. However, the advent of enzymatic deacetylation of chitin is an innovative approach that make use of enzymes known as chitin deacetylase, which presents an excellent alternative that does not exhibit this limitation. Research was conducted using viral, fungal, and bacterial chitin deacetylase enzymes, isolated from each of these microbial sources. After the experiment, 14 partially acetylated chitosan tetramers with a well-defined degree of deacetylation, and some traces of acetylation, which only require further purification to achieve an excellent deacetylation were produced [40].

Fig. 5a depicts the chemical structures of pure chitin before deacetylation, while Fig. 5b shows the chemical structure of chitosan; a deacetylated form of chitin with one of the acetyl group already removed, and replaced with amine [41]

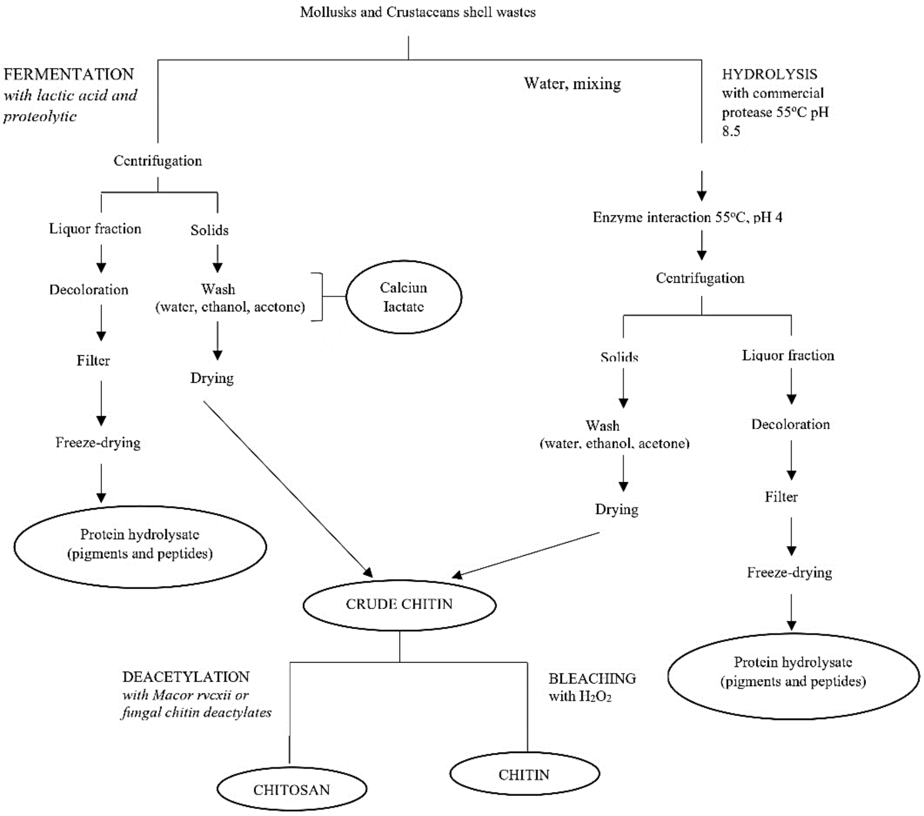

Fig. 4b. Schematic diagram of enzymatic and microbial preparation of chitin and chitosan Casadidio *et al.* [27]

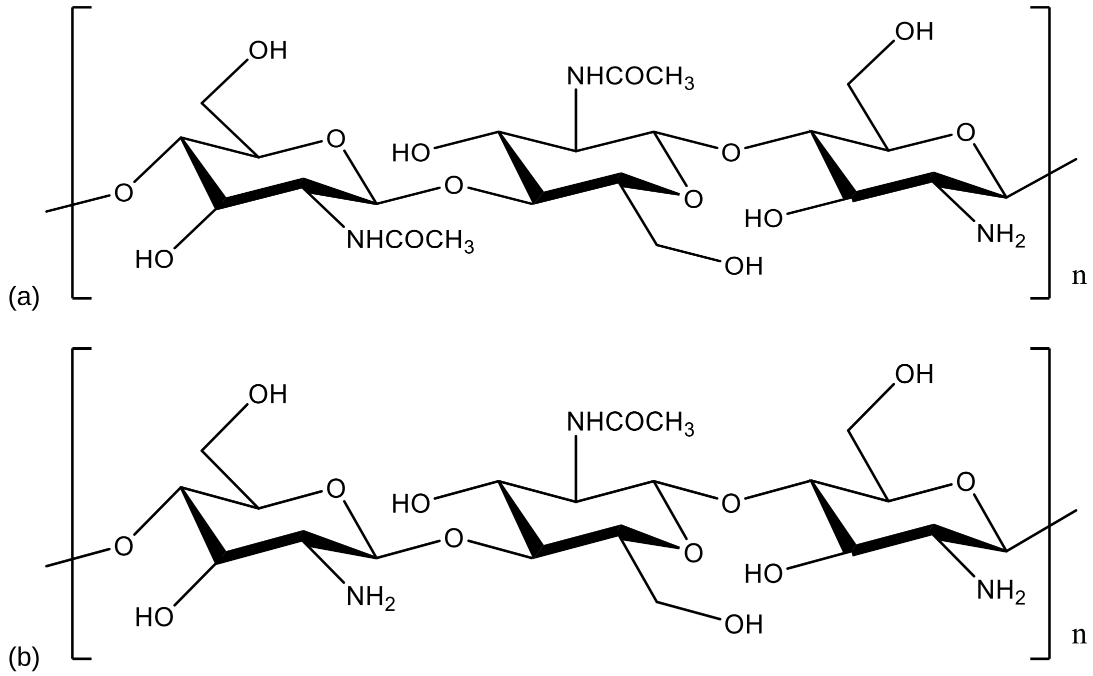

Fig. 5. Chemical structures of chitin (a) and Chitosan (b). El-Naggar *et al*. [41]

4.4 Molecular Weight (Mw)

The Mw of commercial chitosan is between 100 and 1200 kDa [42, 43]. The molecular weight of chitin and chitosan differs based on the source and the extraction methods used. When the molecular weight is high, the solubility of chitosan in a solvent will be very low which also affects its viscosity (high viscosity) and thus limits its availability for aquaculture and agricultural use. When the molecular weight is lower, such as in chitosan extracted from shrimp and conus shells, their antibacterial activity will be very high [44]. Chitosan with a moderate molecular weight has anti-cholesterol capacity. High-performance liquid chromatography (HPLC) and the Viscometry approach are usually employed to determine the molecular weight of chitosan and chitin [45]. Chitin has different molecular weights based on their sources, which can be tapped into, for various uses. Antiseptic and anticancer agents require low molecular weight which can also be found in most crustaceans and some mollusks, and thus makes them excellent candidates in drug production.

4.5 Degree of Deacetylation (DD)

Deacetylation degree (DD) is a parameter that is used to know the percentage or ratio of glucosamine ($C_6H_{13}NO_5$) monomer units in un-deacetylated or partially deacetylated chitin structure compared to a fully deacetylated one (100% chitosan). The solubility of chitin depends largely on its degree of deacetylation. At least, with a 50% deacetylation degree, solubility in aqueous acetic acid solution can be achieved [46]. Various spectrometric and chromatographic approaches can be used to determine the degrees of deacetylation of chitin or chitosan. This includes Proton and Carbon Nuclear Magnetic Resonance Spectroscopy (PCNMR), High-Performance Liquid Chromatography (HPLC), Ultraviolet (UV) Spectroscopy, and Infra-red (IR) Spectroscopy, [47,48].

Depending on the extraction technique, the degree of deacetylation plays a major role in the biological, physico-chemical and mechanical properties of chitin and chitosan. Studies shows that at higher deacetylation degree, chitin can be used in scaffolds and implantations in the biomedical engineering, which can find good applications in human and veterinary medicine; this is a significant development as regards the application of the degree of deacetylation [43,49].

Moreover, according to El-Naggar et al [41], an optimum deacetylation degree was realized in 6 hours when treated chitin with sodium hydroxide. It was also noted that there was an increase in the DD of chitosan when increased the concentration of sodium hydroxide and the temperature. Thus, a conclusion was drawn on the fact that the highest DD took place at 107ºC when treated with 60% NaOH [41,50].

5. APPLICATION OF CHITOSAN AND CHITOSAN NANOPARTICLES IN AQUACULTURE

The advent of Nanotechnology in the 21st century is a landmark discovery. Although still emerging, it has significantly influenced material science at a nanoscale (1-100 nm), with a tremendous impact on the physical and chemical properties of materials, and thus optimized its potential through increased surface area compared to volume. This discovery has made it possible to use nanoparticles in different applications which ordinarily would not have been possible outside the nanoscale [51]. Chitosan nanoparticles are easy to prepare at low cost, non-toxic, highly biocompatible, highly stable, and eco-friendly. It has found good applications in veterinary medicine and plant pathology. It has been adjudged as an effective feed inclusion for therapeutic and prophylactic treatment in fish [52]. Moreover, its effectiveness was due to its minute size (Nano-scale size), shape, and crystallinity. These are properties that determine the effectiveness of nanoparticles against any target cell [53]. The low side effects of Chitosan and Chitosan nanoparticles made them applicable in a variety of uses, coupled with their ability to enhance fish growth [54, 55, 56]. Additionally, chitosan and chitosan nanoparticles can be encapsulated to carry drugs [57]. Likewise, they can be used for the treatment of pond water owing to their chelating ability [58, 59]. Given these, chitosan and chitosan nanoparticles are good products and are applicable in water quality enhancement [60].

5.1 As Calcium Supplement

Waste shell have been considered as raw materials in form of natural shells, calcined shells and several biopolymers and their derivatives which includes chitin and chitosan. Animal shells has always been a source of calcium and has been used to produce food supplements, likewise chitin and chitosan has find good application in food and pharmaceutical industries. They have also been used as biopackaging materials [46,8]. Calcium extracted from shell has been used for strong teeth and bone, livestock blood circulation, high quality milk and egg-shells [6]. It is a cheaper source of $CaCO_3$, performing equally to limestone in Ca supplementation in fish diet [4], while $CaCO_3$ extracted from oyster shells is still used as food supplement for Ca replacement in fish diet and human. The extracted $CaCO_3$ can easily be absorbed in the intestine, and thus enhance the density of bone minerals, for instance in the lumber region, to fill in calcium deficiency especially among aged population [61].

5.2 As Protein Supplement

Useful feed ingredients could be obtained from shell wastes of crustaceans. This includes shell, heads and jointed legs of shrimps, which consist of essential amino acids such as lysine [62]. The product of shell fermentation can be used as feed ingredients in fish production [63]. Protein obtained during chitin extraction from shell wastes can be used to supplement fish or animal feed, while the wastes is being transformed into chitin, and then chitosan through deacetylatyion process. This is two-way advantage. While protein is being obtain, though a by-product, chitin is also being extracted as a primary product. Likewise, calcium is also obtained (Decalcification) and serve as a useful mineral source. However, the best approach to easily recover protein by-product is through enzymatic and microbial extraction so that the obtained by product (protein) will be safe (non-toxic) for other beneficial use [64].

5.3 For Fish Growth Improvement

Positive effect of Chitosan and chitosan nanoparticles as inclusions in fish diet at optimal dose depends largely on fish species. According to Abdel-Ghany and Salem [65], it was discovered that Japanese eel, *Paramigurunus dabryanus,* did not reflect any change in their growth performance, even after 3 months of experiment at varying inclusions, nonetheless, when used to feed *Paralichthys olivaceus*, *Cyprinus carpio* and *Dicentrachus labrax*, there was a tremendous growth increase after supplemented with Chitosan [65, 66]. According to Zaki et al., [66], chitosan has potential to bring about a healthy intestinal epithelium and induce growth through increase in microvilli absorbance surface. Conversely, high concentration of Chitosan in fish diet can lead to over growth of microvilli which can hamper fish growth performance resulting in intestinal blockage occasioned by over-growing microvilli [65]. In another study, *O. niloticus* demonstrated a unique response, with increase growth, even when 0.5 g/kg was used [62]. Moreover, it was recorded that, after fortified fish diet with chitosan at various concentrations (1800 mg/kg, 4000 mg/kg, 7500 mg/kg, 10000 mg/kg and 20000 mg/kg) of fish diets, and was used to feed *Carasius auratus* juvenile for 75 days; 4000 mg Chitosan per kg diet gives the optimum level of Chitosan supplementation [67]. So far, it was deduced that using chitosan nanoparticles at different dietary inclusion for *O. niloticus*, growth performance can be enhanced owing to increase feed intake and feed utilization. Likewise Chitosan nanoparticles can also increase fish growth through its positive impact on microvilli and goblet cells of intestinal epithelia [55, 68]. Thus the use of Chitosan and its nanoparticle form can induce fish resistance to pathogens and ultimately ensure fish health [69].

5.4 As Immunostimulants

Mortality rate reduction and immunity can be achieved with chitosan when properly administered [70, 65]. In a situation where stress condition is inevitable and has been causing increase mortality rate; for instance chitosan supplementation in *Oncorhyncus mykiss diet*, at 2.5 g/kg depicted a marked survival rate despite the stress condition [65]. In an experiment carried out by Ranja et al [71] in which dietary concentration of chitosan (0, 5, 10 and 20 g/kg diet) was fed to *Lates calcarifer* for 60 days, it was noted that all the treatment depicted a greater immune response than control group. Moreover, 10 g/kg group diet exhibit an exceptional innate immune response and hematological parameter on the 45th day of the experiment. This result is in tandem with the finding by Salem [65] and Zaki et al [66]. Also, another graded diet fortified with various concentrations of chitosan (0, 5, 10 and 15 g per kg diet) was fed to *Mugil cephalus*, the results shows that highest immune response was noticed on 10g and 15g chitosan concentration per kg [70]. Likewise, addition of chitosan to *Cyprinus carpio* diet improve its immune response [72]. Finally, Cha et al. [73] stated that, the ability of Chitosan to act as immunostimulant was credited to its ability to prompt non-specific or general immunity in a living cell. Again, Chitosan's potency against bacteria pathogens in *labeo rohita* enhances immunity and resistance against environmental change [74,75]. The same report was found about *Misgurnus angillicaudate* according to Yen et al [76]. Inherent immunity in *O. niloticus* was also boosted when applied Chitosan nanoparticles by increasing the total WBCs [55]. It was also discovered that when combine selenium with chitosan (CS-Se), the lysosome activity increases due to their synergistic effect and thus enhanced the immunity of *Paramisgurnus dabryanus* [77]. Abdel-Tawwab et al. [68] recorded the same result when fed *O. niliticus* with diet fortified with chitosan nanoparticles at varying concentrations (0.25, 0.5, 1.0 and 2.0 g/kg diet). In summary, the immunostimulatory action of chitosan nanoparticles on fish plays a notable beneficial role towards the growth of aquaculture [78].

5.5 As Antibacterial

Synthesized chitosan has good antibacterial properties. It can prevent bacterial growth in fish or meat and has a bacterial suppressing rate of 99.9%. Therefore, it has the potential to raise their keeping quality to 10 days especially during summer, without negative effect on those who consume them [46,79]. Chitosan can prevent *Bacillus cereus* spore germination and growth. The experiment was conducted on bacterial spore with concentrations corresponding to 10^2–10^3 CFU mL^{-1}, which is relevant to spore concentrations in food, the result shows that less chitosan was required to prevent bacteria growth, compared to higher spore numbers (equivalent to 10^8 CFU mL^{-1}) [80].

5.6 For Cholesterol Reduction

Chitosan can be used to reduce low density lipoprotein (LDL)-cholesterol level and its fat-binding properties [81]. Two weeks ingestion of chitosan oligosaccharide can lead to reduction in LDL-cholesterol by 6% and jack up high-density lipoprotein (HDL)-cholesterol by 10% [82]. Chitosan has ability to scavenge fats in the alimentary tract and pick up cholesterol debris through ionic bonding by its attraction to bile salts and acids, which helps to reduce weight. Chitosan is non-absorbable and effective only in the alimentary tract, hence increased fecal release of common fats is as a result of weight loss due to chitosan. This is applicable in man and animals including aquatic lives [82].

5.7 As Antioxidants

Chitosan with its several derivatives have antioxidant activity [42]. The ability of chitosan to reduce oxidation damages in cells as well as activating the inherent ability of fish such as *cyprino carpio* to scavenge dangerous free radicals when applied at a proper dose such as 0.5g/kg, 1g/kg, 2g/kg or 5g/kg diet, make it an excellent antioxidant of animal source. [65,76,83]. However, the study shows that it is necessary to include Vitamin C in order to improve Chitosan's antioxidant activity in *C. carpio* [83]. Chitosan nanoparticle on the other hand has greater potency, not because of its antioxidant activity alone but at the nanoscale, its physical and chemical properties have been enhanced and thus could inflict a deadly blow on toxic free radicals, especially in *O. niloticus,* and also enhance its inherent antioxidant activity [76]. Also, when fortified *Salmo salar* with chitosan nanoparticles, it reduces lipid oxidation, with greater scavenging activity against free radicals [68]. It was also reported that, as the dietary supplementation of chitosan nanoparticles increases, so also its antioxidant potency increases [68,84].

5.8 As Drug Carriers

The use of N-N-N trimethyl CS chloride (TMC) to enhance the drug delivery capacity of chitosan which works well at high or wide pH ranges than when only chitosan is used, has been adjudged as an excellent chitosan derivative for effective drug delivery [85]. For instance, microscopic examination of vaccinated *Paralichthyus olivaceus* fish using CS-DNA oral vaccination showed an excellent result, depicting that chitosan has the potential for effective drug delivery. Conversely, the literature revealed that certain part of the fish body could not be affected by the CS-DNA vaccine such as hind gut. It does not also induce immune–gene expression or serum circulation. Also, CS-DNA enhances the solubility of Chitosan in nucleic acid and optimizes cell reception of the embedded drug carried by Chitosan [57]. It was also gathered that the CS-DNA vaccine has the capacity to prevent disease pathogens such as bacteria and viruses from infecting fish with a high survival rate. This result was attributed to the ability of chitosan nanoparticles to induce immune-related genes. Chitosan nanoparticles is a potential drug carrier and antioxidant which help to enhance the immune system, it has an excellent capacity to infiltrate tissues and reach the targeted region. In addition, research has shown that chitosan nanoparticles is a useful oral DNA vaccine for fish [78, 86, 87]. It was also discovered that it is possible to egest or excrete chitosan and its nanoparticle form through the urinary system which makes them well applicable and useful in the medical and pharmaceutical industry [88]. Both substances (Cs and their nanoparticle form) can deliver drugs directly to the fish alimentary canal, such as vitamins, minerals, and vaccines, and also increase the mucoadhesive ability of the intestine, thus enhancing the drug absorption potential of the intestine [89]. Study also revealed a successful immunization of *Labeo rohita* against *Edwardisella tarda* using CSNPs-DNA vaccine which allows good expression of *E. tarda*'s immune genes [90].

5.9 For the Treatment of Fish Farms Wastewaters

Heavy metals have been one of the major water quality threats in fish farming. They have high atomic mass and denser than water at about ratio of 5:1, and are very harmful to fish [91]. Aluminium, although has been used over times but due to its negative side effect of being responsible for Azheimer disease in man, when consumed fish raised in water that has been treated with aluminium. However, chitosan, due to its chelating property is able to remove heavy metals including aluminium from fish pond water. Therefore, when synthesizing Chitosan with iron III oxide, it forms a powerful composite with superior ability to absorb lead, cadmium and aluminium. This synergistic combination was made possible due to the hydroxyl and amino group present in chitosan [58,92].

Chitosan nanoparticle is also useful in the removal of Copper from water. However, the concentration of cupper in the solution also determine the concentration of chitosan nanoparticles to use [93]. In addition, while both chitosan and chitosan nanoparticles can be used for the removal of heavy metal in water, the research shows that chitosan nanoparticle is far more efficient due to its high chelating power. This was confirmed by Seyed-mohammadi et al. [94] in his research which depicted the optimum condition at which chitosan and chitosan nanoparticle can effectively absorbed Zn (90.80% and 99.19% respectively) at Ph. 7 and 25^0C. The nano-scale size, crystalline nature of the nanoparticles, numbers and types of the functional group present also contributes to this [59]. This is where Nanotechnology plays a significant role in the valorization of crustacean, gastropods and other animal shells waste yields, to ensure effective and efficient utilization, towards improved aquaculture.

5.10 As Biopackaging Agent

Polymeric materials of animal shells origin are suitable to produce safe and environment-friendly plastic, which can be used in human or veterinary medicine, household, and industry, especially as biopackaging materials [95]. Packaging films made from fossil products should be substituted for biodegradable materials to improve environmental wellness. In view of this, biopolymers can be used as natural alternative to outdated ones which are plagued with several limitations. Chitosan-based food packaging includes systems that has ability to prevent microbial growth. This is necessary especially in fish processing, packaging and transportation in order to maintain high quality fish and fisheries products. The antimicrobial biopackaging materials obtained from chitosan has been one of the best packaging materials so far [96].

6. CONCLUSIONS

Shell produced from mollusks and crustaceans these days are mostly seen as wastes, despite the fact that they were once items of historical value, and some have been used as legal tender in exchange for goods and services. Nevertheless, shell waste repurposing and valorization are highly important to determine strategies in order to make them a standard valuable resources as highlighted in this paper, most especially in fisheries and aquaculture. Ecofriendly and non-toxic chitin extraction and deacetylation methods have been discussed in detail and was recommended. Collection method is never a problem, since these wastes can be found in large quantities at the fishing jetties and processing companies; as well as restaurants, artisanal fishermen, and domestic sources. Effort should therefore be made to collect them in a well-organized manner, exploring every collection avenues earlier stated to ensure a stable supply of shell, in order to give room for a large scale chitin and chitosan production. As earlier discussed, chitosan can be synthesized into nanoparticles, and nanocomposites when synergized with other nanoparticles. These can find good applications in aquaculture as earlier discussed. Finally, chitin and chitosan are potential sources of several opportunities which has great possibility to take aquaculture to a higher pedestal. To gain full advantage and control, sustainable shell waste bioeconomy and redirection of use will requires an integrated support from both state and federal government through necessary legislation just as it applies in fishery legislation.

HIGHLIGHTS

- Massive wastes of Crustacean and Mollusc shells in Nigeria coastal and inland water environment were reviewed and discussed.
- Dearth of adequate knowledge on effective valorization of chitinous waste shells, apart from historical use was reviewed and the way forward was provided.
- Re-utilization possibilities of chitinous waste shells of Crustaceans and Molluscs through eco-friendly and sustainable technology to improve fish production were established.
- A comprehensive review of Extraction methods of chitin and chitosan from chitinous shell wastes were explored.
- Application of chitosan and chitosan nanoparticles for a robust aquaculture practice, economic development and job creations were outlines and discussed.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Food and Agriculture Organization of the United Nations World Food and Agriculture Statistical Yearbook 2021; Food and Agriculture Organization: Rome, Italy; 2021. ISBN 978-92-5-134332-6.
2. Yan N, Chen X. Sustainability: Don't Waste Seafood Waste. Nature. 2015; 524:155–157. Available:https://doi.org/10.1038/524155a.
3. Summa D, Lanzoni M, Castaldelli G, Fano EA, Tamburini E. Trends and opportunities of bivalve shells' waste valorization in a prospect of circular blue bioeconomy. Resources. 2022; 11:48. Available:https://doi.org/10.3390/resources 11050048
4. Morris JP, Backeljau T, Chapelle G. Shells from aquaculture. A valuable biomaterial, not a nuisance waste product. Rev Aquacult. 2019; 11:42–57. Available:https://doi.org/10.1111/raq.12225.
5. Vélez-Henao JA, Weinland F, Reintjes N. Life cycle assessment of aquaculture bivalve shellfish production—a critical review of methodological trends. Int. J. Life Cycle Assess. 2021; 26:1943–1958. Available:https://doi.org/10.1007/s11367-021-01978-y.
6. Zhan J, Lu J, Wang D. Review of shell waste reutilization to promote sustainable shellfish aquaculture. Rev. Aquacult. 2022; 14:477–488. Available:https://doi.org/10.1111/raq.12610
7. Jimoh AA, Lemomu IP. Shellfish resources in Nigeria. Proceeding of the 25th Annual Conference and exhibition of the Fisheries Society of Nigeria, at Administrative Staff College of Nigeria (ASCON), Topo-Badagry, Lagos, Nigeria, 25th – 29th October. Pages 683-693; 2010.
8. Bonnard M, Boury B, Parrot I. Key Insights, tools, and future prospects on oyster shell end-of-life: A critical analysis of sustainable solutions. Environ. Sci. Technol. 2020;54: 26–38. Available:https://doi.org/10.1021/acs.est.9b 03736.
9. Hardy BL, Moncel MH, Kerfant C, Lebon M, Bellot-Gurlet L, Mélard N. Direct evidence of Neanderthal fibre technology and its cognitive and behavioral implications. Sci. Rep. 2020; 10:4889. Available:https://doi.org/10.1038/s41598-020-61839-w
10. Arrighi S, Moroni A, Tassoni L, Boschin F, Badino F, Bortolini E, Boscato P, Crezzini J, Figus C, Forte M. Bone tools, ornaments and other unusual objects during the middle to upper palaeolithic transition in Italy. Quat. Int. 2020; 551:169–187. Available:https://doi.org/10.1016/j.quaint.2 019.11.016.
11. Gamble LH. The origin and use of shell bead money in California. J. Anthropol. Archaeol. 2020; 60:101237. Available:https://doi.org/10.1016/j.jaa.2020 .101237
12. Maurer B. Primitive and nonmetallic money. In handbook of the history of money and Currency; Battilossi S, Cassis Y, Yago K. Eds.; Springer: Singapore. 2020;87–104, ISBN 9789811305955
13. Guo P. Marriage-Related Exchanges and the Agency of Women among the

Langalanga, Solomon Islands. Oceania. 2020; 90:273–291.
Available:https://doi.org/10.1002/ocea.5281 (2020)
14. Silva Mota EL, Nóbrega Alves RR, Pereira Dias TL. Fishing, trade, and local ecological knowledge of the marine gastropod, cassis Tuberosa—a target species of the international shell trade. Ethnobiol. Conserv. 2020; 9:23.
Available:https://doi.org/10.15451/ec2020-06- 9.23-1-11.
15. Nijman V, Spaan D, Nekaris KAI. Large-scale trade in legally protected marine mollusc shells from Java and Bali, Indonesia. Plos One. 2015; 10:e0140593.
Available:https://doi.org/10.1371/journal.pone.0140593.
16. Watson GJ, Bonner A, Murray JM, Hebblethwaite Z. Offsetting the impact of the marine ornamental trade: A case study of two temperate top shells (Osilinus Lineatus and Gibbula; 2012.
17. Moruf RO, Aminu MU, Garba AA, Olubob TF. Coastal shellfish as harvestable resources in Nigeria: A review. 47nd Annual conference Proceedings of the Nigeria Society for Animal Production in Collaboration with the University of Jos and Federal college of Forestry, Jos. Theme: Securing Animal Agriculture Amidst Global Challenges. 2022;1708-1712.
18. Jimoh AA, Lemomu IP. Shellfish resources in Nigeria. Proceeding of the 25th Annual Conference and exhibition of the Fisheries Society of Nigeria, at Administrative Staff College of Nigeria (ASCON), Topo-Badagry, Lagos, Nigeria. 2010;683-693.
19. Elegbede I, MaxemilieNgo-Massou V, Kies F, Jolaosho TL. Marine and Coastal Resources C. Springer nature Encyclopedia of Sustainable Management. 2023;1-5.
Available:https://doi.org/10.1007/978-3-030-02006-4-304-1.
20. Ateme ME. Developing marine and coastal resources in Nigeria: Prospects and challenges.Maritime Technology and Research. 2021;3(4):335-347.
Available:https://doi.org/10.33175/mtr.2021.244473.
21. Olaoye OJ, Ojebiyi WG. Marine fisheries resources in Nigeria: A Review.in: Marine Ecology – Biotic and Abiotic interactions. (Edited by Turkoglu and Ismen). Intechopen. 2018;155-172.
Available:http://dx.doi.org/10.5772/intechopen.7503.
22. Isebor CE. Marine biodiversity in Nigeria – The known and the unknown. National report. Nigerian Institute for Oceanography and Marine Research; 2004.
Available:http://hdl.handle.net/1834/359.
23. Crustacean Classification. 2021. www.easyscienceforkids.com//all about crustaceans. Accessed on July, 2024
24. Merriam-Webster Inc. (2006).Encyclopedia Britannica Online. http://www.merriam-webster.com. Accessed on July, 2024.
25. Pakizeh M, Moradi A, Ghassemi T. Chemical extraction and modification of chitin and chitosan from shrimp shells. Europ. Polym. J. 2021;159(5):110709.
26. El-Naggar MM, Abou-Elmagd WSI, Suloma A, El-Shabaka HA, Khalil MT, Abd El-Rahman FA. Optimization and physico-chemical characterization of chitosan and chitosan nanoparticles extracted from the crayfish Procambarus clarkii Wastes. J. Shellfish Res. 2019;38(2):385-395
27. Casadidio C, Peregrina DV, Gigliobianco MR, Deng S, Censi R, Martino PD. Chitin and Chitosans: Characteristics, eco-friendly processes, and applications in cosmetic science. Marine Drug, MDPI. 2019;17(369):1-30.
DOI: 10.3390/md17060369.
28. Gortari MC, Hours RA. Biotechnological processes for chitin recovery out of crustacean waste: A mini-review. Electron. J. Biotechnol. 2013; 16:14.
29. Cheong JY, Azwady AN, Rusea G, Noormasshela U, Shaziera AN, Azleen A, Muskhazli M. The availability of astaxanthin from shrimp shell wastes through microbial fermentations, aeromonas hydrophila and cell disruptions. Int. J. Agric. Biol. 2014; 16:277–284.
30. Manni L, Ghorbel-Bellaaj O, Jellouli K, Younes I, Nasri M. Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste b treatment with crude protease from bacillus cereus sv1. Appl. Biochem. Biotechnol. 2010;162:345–357
31. Nwe N, Furuike T, Tamura H. Isolation and characterization of chitin and chitosan from marine origin. In Advances in Food and Nutrition Research; Elsevier: Amsterdam, the Netherlands. 2014;72:1–15
32. Arbia W, Arbia L, Adour L, Amrane A. Chitin extraction from crustacean shells using biological methods—A review. Food Technol. Biotechnol. 2013; 51:12–25.

33. Mhamdi S, Ktari N, Hajji S, Nasri M, Kamoun AS. Alkaline proteases from a newly isolated micromonospora chaiyaphumensis: Characterization and application as a detergent additive and for chitin extraction from shrimp shell waste. Int. J. Biol. Macromol. 2017; 94:415–422.
34. Hamdi M, Hammami A, Hajji S, Jridi M, Nasri M, Nasri R. Chitin extraction from blue crab (Portunus segnis) and shrimp (*Penaeus kerathurus*) shells using digestive alkaline proteases from P. Segnis viscera. Int. J. Biol. Macromol. 2017;101: 455–463.
35. Popović NT, Lorencin V, Strunjak-Perović I, Čož-Rakovac R. Shell waste management and utilization: Mitigating organic pollution and enhancing sustainability. Applied Sciences, MDPI. 2023;13(623):1-20. Available:https://doi.org/ 10.3390/app13010623.
36. Castro R, Guerrero-Legarreta I, Bórquez R. Chitin extraction from allopetrolisthes punctatus crab using lactic fermentation. Biotechnol. Rep. 2018;20:e00287
37. Ghorbel-Bellaaj O, Jellouli K, Younes I, Manni L, Salem MO, and Nasri MA. Solvent-stable metallo-protease produced by pseudomonas aeruginosa grown on shrimp shell waste and its application in chitin extraction Appl. Biochem. Biotechnol. 2011a;164:410–425
38. Hajji S, Ghorbel-Bellaaj O, Younes I, Jellouli K, Nasri M. Chitin extraction from crab shells by bacillus bacteria. Biological activities of fermented crab supernatants. Int. J. Biol. Macromol. 2015; 79:167–173.
39. Teng WL, Khor E, Tan TK, Lim LY, Tan SC. Concurrent production of chitin from shrimp shells and fungi. Carbohydr. Res. 2001; 332:305–316.
40. Hembach L, Cord-Landwehr S, Moerschbacher BM. Enzymatic production of all fourteen partially acetylated chitosan tetramers using different chitin deacetylases acting in forward or reverse mode. Sci. Rep. 2017; 7:17692.
41. Li Q, Dunn ET, Grandmaison EW, Goosen MFA. Applications and properties of chitosan. J. Bioact. Compat. Polym. 1992; 7(4):370-397.
42. El-Naggar M, Fareda Medhat F, Taha A. Application of chitosan and chitosan nanoparticles in Fish aquaculture. Egyptian Journal of Aquatic Biology and Fisheries. ISSSN 1110-6131, 2022:26 (1): 23-43. Available: www.ejbf.journal.ekb.eg
43. Zargar V, Asghari M, Dashti A. A review on chitin and chitosan polymers: Structure, chemistry, solubility, derivatives, and applications. Chembioeng Rev. 2015; 2:204–226
44. Weinhold MX, Thöming J. On conformational analysis of chitosan. Carbohydr. Polym. 2011; 84:1237–1243.
45. Kang Y, Wu X, Ji X, Bo S, Liu Y. Strategy to improve the characterization of chitosan by size exclusion chromatography coupled with multi angle laser light scattering. Carbohydr. Polym. 2018; 202:99–105.
46. Rinaudo M. Chitin and chitosan: Properties and applications. Prog. Polym. Sci. 2006; 31:603–632. Available:https://doi.org/10.1016/j.prog polymsci.2006.06.001.
47. Kim S, Rajapakse N. Enzymatic Production and Biological Activities of Chitosan Oligosaccharides (COS): A Review. Carbo hydr. Polym. 2005; 62:357–368. Available:https://doi.org/10.1016/j.carbpol. 2005.08.012
48. Heidari F, Razavi M, Bahrololoom ME, Tahriri M, Rasoulianboroujeni M, Koturi H, Tayebi L. Preparation of natural chitosan from shrimp shell with different deacetylation degree. Mater. Res. Innov. 2018; 22:177–181.
49. Zaj A.C.A, Hanuza J, Wandas M, and Dymi´nska L. Determination of n-acetylation degree in chitosan using Raman spectroscopy. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2015;134: 114–120.
50. Dimzon IK, Knepper TP. Degree of deacetylation of chitosan by infrared spectroscopy and partial least squares. Int. J. Biol. Macromol. 2015; 72:939–945.
51. Rathore A, Mahesh G. Public perception of nanotechnology: A contrast between developed and developing countries. Technol. Soc. 2021;67:10751
52. Augustine R, Dan P, Schlachet I, Rouxel D, Menu P, Sosnik A. Chitosan ascorbate hydrogel improves water uptake capacity and cell adhesion of electro spun poly (epsilon-caprolactone) membranes, Int. J. Pharmaceut. 2019; 559:420 426.
53. Alishahi A, Mirvaghefi A, Tehrani MR, Farahmand H, Koshio S, Dorkoosh FA, Elsabee MZ. Chitosan nanoparticle to carry vitamin C through the gastrointestinal tract and induce the non-specific immunity system of rainbow trout (*Oncorhynchus mykiss*). Carb. Polym. 2011;86(1):142-146.

54. Wang Y, Li J. Effects of chitosan nanoparticles on survival, growth and meat quality of tilapia, Oreochromis nilotica. Nanotoxicol. 2011;5(3):425-431.
55. Abd El-Naby FS, Naiel MAE, Al-Sagheer AA, Negm SS. Dietary chitosan nanoparticles enhance the growth, production performance, and immunity in Oreochromis niloticus. Aquacult. 2019;501: 82-89.
56. El-Naggar M. Effects of chitosan nanoparticles on the growth rate and reproductive performance of the Nile Tilapia, Oreochromis niloticus. Doctoral Dissertation. Faculty of Science, Ain Shams University, Cairo, Egypt; 2020.
57. Wu Y, Rashidpour A, Almajano MP, Metón I. Chitosan-based drug delivery system: Applications in fish biotechnology, Polymers. 2020;12(5):1177.
58. Fan HL, Zhou SF, Jiao WZ, Qi GS, Liu YZ. Removal of heavy metal ions by magnetic chitosan nanoparticles prepared continuously via high-gravity reactive precipitation method. Carb. Polym. 2017; 174:1192-1200.
59. Zareie C, Eshkalak SK, Darzi GN, Baei MS, Younesi H, Ramakrishna S. Uptake of Pb(II) Ions from simulated aqueous solution via nanochitosan. Coatings. 2019; 9(12):862.
60. Udo I, Anwana U. Effects of chitosan and chitosan nanoparticles on water Quality, growth performance, survival Rate and meat quality of the African Catfish, Clarias gariepinus, Nanosci. 2018; 17:12-25.
61. Silva T, Mesquita-Guimarães J, Henriques B, Silva FS, Fredel MC. The potential use of oyster shell waste in new value-added by-product. Resources. 2019; 8:13. Available:https://doi.org/10.3390/resources 8010013.
62. Malaweera BO, Wijesundara WM. Use of seafood processing by-products in the animal feed industry. In Seafood Processing By-Products; Kim, S.-K., Ed.; Springer: New York, NY, USA. 2014;315–339. ISBN 978-1-4614-9589-5.
63. Amar B, Philip RB, Singh IS. Efficacy of fermented prawn shell waste as a feed ingredient for indian White Prawn, Fenneropenaeus Indicus. Aquac. Nutr. 2006; 12:433–442. Available:https://doi.org/10.1111/j.1365-2095.2006.00445.x..
64. Paul MK, Mini KD, Antony AC, Mathew J. Deproteinization of shrimp shell waste by kurthia gibsonii mb126 immobilized Chitinase. J. Pure Appl. Microbiol. 2022;16: 909–923. Available:https://doi.org/10.22207/JPAM.16 .2.11
65. Abdel-Ghany HM, Salem MES. Effects of dietary chitosan supplementation of farmed fish; A review. Rev. Aquacult. 2020;12(1): 438-452.
66. Zaki MA, Salem MES, Gaber MM, Nour AM. Effect of chitosan supplemented diet on survival, growth, feed utilization, body composition and histology of Sea bass (*Dicentrarchus labrax*). World J. Eng. Technol. 2015;3(4):38-47.
67. Chen Y, Zhu X, Yang Y, Han D, Jin J, and Xie S. Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance in gibel carp (Carassius auratus gibelio). Aquacult. Nutr. 2014;20(5):532-546.
68. Abdel-Tawwab M, Razek NA, Abdel-Rahman AM. Immunostimulatory effect of dietary chitosan nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus (L*.). Fish Shellfish Immunol. 2019; 88:254-258.
69. El-Naggar M, Salaah S, El-Shabaka H, El-Rahman FA, Khalil M, Suloma A. Efficacy of dietary chitosan and chitosan nanoparticles supplementation on health status of Nile tilapia, *Oreochromis niloticus (L.)*. Aquacult. Rep. 2021; 19:100828.
70. Meshkini S, Tafy AA, Tukmechi A, Farhang-Pajuh F. Effects of chitosan on hematological parameters and stress resistance in rainbow trout (Oncorhynchus mykiss). Vet Res. Forum. 2012;3(1):49-54.
71. Ranjan R, Prasad KP, Vani T, Kumar R. Effect of dietary chitosan on haematology, innate immunity and disease resistance of Asian seabass Lates calcarifer (Bloch). Aquacult. Res. 2014;45(6):983-993.
72. Alishahi M, Rad E, Zarei M, and Ghorbanpour M. Effect of dietary chitosan on immune response and disease resistance in Cyprinus carpio. Iran. J. Vet. Med. 2014;8:125-133
73. Cha SH, Lee JS, Song CB, Lee KJ, Jeon YJ. Effects of chitosan coated diet on improving water quality and innate immunity in the olive flounder, Paralichthys olivaceus. Aquacult. 2008;278(1–4):110-118.
74. Mastan SA. Use of Immunostimulants in aquaculture disease management. 2015; 2(4):277- 280

75. Najafabad MK, Imanpoor MR, Taghizadeh V, Alishahi A. Effect of dietary chitosan on growth performance, hematological parameters, intestinal histology and stress resistance of Caspian kutum (Rutilus frisii kutum Kamenskii, 1901) fingerlings. Fish Physiol. Biochem. 2016;42(4):1063-1071.
76. Yan J, Guo C, Dawood MAO, Gao J. Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in Misgurnus anguillicaudatus. Benef. Microbe. 2017;8(3):439-449.
77. Victor H, Zhao B, Mu Y, Dai X, Wen Z, Gao Y, Chu Z. Effects of Se chitosan on the growth performance and intestinal health of the loach Paramisgurnus dabryanus (Sauvage). Aquacult. 2019;498:263-270
78. Ahmed F, Soliman FM, Adly MA, Soliman HAM, El-Matbouli M, Saleh M. Recent progress in biomedical applications of chitosan and its nanocomposites in aquaculture: A review. Res. Vet. Sci. 2019; 126:68-82.
79. Coma V. Recent developments in chitin and chitosan bio-based materials used fob food preservation. In Polysaccharide Building Blocks; Habibi, Y., Lucia, L.A., Eds.; Wiley:Hoboken, NJ, USA. 2012;143–175. ISBN 978-0-470-87419-6
80. Mellegård H, From C. Christensen BE, Granum PE. Inhibition of bacillus cereus spore outgrowth and multiplication by chitosan. Int. J. Food Microbiol. 2011;149: 218–225. Available:https://doi.org/10.1016/j.ijfoodmicro.06.013.
81. Preuss H, Kaats G. Chitosan as a dietary supplement for weight loss: A review. Curr. Nutr. Food Sci. 2006; 2:297–311. Available:https://doi.org/10.2174/157340106778017869.
82. Kim S, Rajapakse N. Enzymatic production and biological activities of chitosan oligosaccharides (COS): A Review. Carbo hydr. Polym. 2005; 62:357–368. Available:https://doi.org/10.1016/j.carbpol.2005.08.012.
83. Mehrpak M, Banaee M, Haghi BN, Noori. A. Protective effects of vitamin C and chitosan against cadmium-induced oxidative stress in the liver of Common Carp (Cyprinus carpio). Iran. J. Toxicol. 2015; 9:1360-1367.
84. Niaz T, Hafeez Z, Imran M. Prospectives of antihypertensive nano-ceuticals as alternative therapeutics. Curr. Drug Targets. 2017; 18:1269–1280. Available:https://doi.org/10.2174/1389450117666160711163119.
85. Kulkarni AD, Patel HM, Surana SJ, Vanjari YH, Belgamwar VS, Pardeshi CV. N, N,N-Trimethyl chitosan: An advanced polymer with myriad of opportunities in nanomedicine. Carb. Polym. 2017;157: 875-902.
86. Vimal S, Abdul Majeed S, Nambi KSN, Madan N, Farook MA, Venkatesan C Taju G, Venu S, Subburaj R, Thirunavukkarasu AR, Hameed ASS. Delivery of DNA vaccine using chitosan-tripolyphosphate (CS/TPP) nanoparticles in Asian sea bass, Lates calcarifer (Bloch, 1790) for protection against nodavirus infection. Aquacult. 2014;420-421:240-246.
87. Zhao L, Su C, Zhu B, Jia Y. Development and optimization of insulin chitosan nanoparticles. Trop.J. Pharm. Res. 2014; 13(1):3-8.
88. Ji J, Torrealba D, Ruyra À, Roher N. Nanodelivery systems as new tools for immunostimulant or vaccine administration: Targeting the fish immune system. Biol. 2015;4(4):664-696.
89. Ferosekhan S, Gupta S, Singh A, Rather M, Kumari R, Kothari D, Pal A, Jadhao S. RNA-loaded chitosan nanoparticles for enhanced growth, immunostimulation and disease resistance in fish. Curr. Nanosci. 2014;10(3):453-464.
90. Kole S, Kumari R, Anand D, Kumar S, Sharma R, Tripathi G, Makesh M, Rajendran K.V, Bedekar MK. Nanoconjugation of bicistronic DNA vaccine against Edwardsiella tarda using chitosan nanoparticles: Evaluation of its protective efficacy and immune modulatory effects in Labeo rohita vaccinated by different delivery routes. Vaccine. 2018; 36(16):2155-2165.
91. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ, Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. Exp. Suppl. 2012; 101:133-164.
92. Iber BT, Okomoda VT, Rozaimah SA, Kasan NA. Eco-friendly approaches to aquaculture wastewater treatment: Assessment of natural coagulants vis-a-vis chitosan. Bioresour. Technol. Rep. 2021; 15:100702.
93. Abd-Elhakeem MA, Ramadan MM, Basaad FS. Removing of heavy metals from wates by chitosan nanoparticles. J. Adv Chem. 2016;11(7):3765 3771.

94. Seyedmohammadi J, Motavassel M, Maddahi MH, Nikmanesh S. Application of nanochitosan and chitosan particles for adsorption of Zn (II) ions pollutant from aqueous solution to protect environment. Model. Earth Syst. Environ. 2016;2(3):165.
95. Audrézet F, Pochon X, Floerl O, Le Guen MJ, Trochel B, Gambarini V, Lear G, Zaiko. A.Eco-Plastics in the Sea: Succession of Micro and Macro Fouling on a Biodegradable Polymer Augmented With Oyster Shell. Front. Mar. Sci. 2022;9: 891183. Available:https://doi.org/10.3389/fmars.2022.891183.
96. Martinez LMT. Handbook of Ecomaterials; Springer: New York, NY, USA; ISBN 978-3-319-68254-9; 2019.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/120695