International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 4, Issue 3, pp: 106-112, 2019
https://doi.org/10.5281/zenodo.2996503
http://www.ijzab.com

Research Article

EXPERIMENT ON THE CHEMICAL EXTRACTION OF CHITIN AND CHITOSAN FROM THE EXOSKELETONS OF INDIGENOUS PRAWN SPECIES AND THEIR BIOMEDICAL APPLICATION

Zoyeb Mohamed Zia* and Dr. M. Asrar Sheriff

Department of Zoology, The New College, Chennai - 600014, Tamilnadu, India

Article History: Received 1st May 2019; Accepted 9th May 2019; Published 16th May 2019

ABSTRACT

Prawn fisheries industry is a very commercially/economically important industry in India accounting for a large portion of the overall fisheries industry. The prawn processing industry produces a large amount of bio-waste which is a potential environmental pollutant. This waste consisting of carapace and shells can be converted into chitin and chitosan which are commercially viable substances with various bio-functionalities. In this experiment; Chitin and chitosan are extracted from the shells of *Fenneropenaeus indicus* and *Fenneropenaeus penicillatus* using the chemical method. Yield of chitin and chitosan is measured. Blood clotting activity is tested.

Keywords: Bio-waste, Blood clotting, Fisheries industry, Prawn shell.

INTRODUCTION

The fisheries industry especially the prawn industry is one of the most important industries economically in India contributing substantially to foreign exchange. The sea food industries process and package the harvested products, during processing s lot of potentially waste by-products are produced like shells. In India, the prawn processing industry produces around 100,000 tons of waste annually (Gopakumar, 2002). The prawn processing industry has seen rapid and significant growth over the years especially in south East Asia. A huge amount of bio-waste such as carapace and abdominal shells is produced by the prawn processing industry because prawns are usually sold as head-less or peeled or both (Pal *et al*., 2014).

These wastes are a potential bio-hazard and pollutant of the environment, the need of the hour is to reduce this risk and the potential threat to the environment by turning these wastes into useful products. A quick and effective method is to recycle these shells and extract commercially viable substances from them (Divya *et al*., 2014). Chitosan additionally happens normally in a few microorganisms, for example, parasites and yeast and is viewed as the biggest biomaterial after cellulose as far as usage and dissemination(Sakthidasan *et al*., 2018). The shell waste can be utilized to extract chitin and chitosan which are the primary constituents of the cuticle of crustaceans and also of fungi (Hirano *et al*., 1997). Chitin and chitosan are one of the most abundant bio- renewable resources. They are naturally occurring bio-polymers found to be the most abundant in the world after cellulose. Research and development in the field of chitin/chitosan and its application increased tremendously over the past few years. This spurred the development of new value added products and various applications in the field of food and feed, agriculture, pharmaceuticals, Medicine and recently in the field of nanotechnology (Pichyangkura & Chadchawan, 2015).

In seafood industries management of shellfish wastes is a huge problem. It is a laborious and expensive process. This has significantly affected the shell fish processing sector. Among the sea food industries, the crustacean industry lacks a proper and cost-effective outlet for its waste, marine organisms are an important source of dietary nutrients, medicines and now a day's many other economically viable products. The use of recovered products to produce valuable and biologically sustainable materials and to minimize waste is a challenge for current

*Corresponding Author: Mr. Zoyeb Mohamed Zia, Department of Zoology, The New College, Chennai - 600 014, Tamilnadu, India. Email: zoyebmdzia@gmail.com

research and development. The shell fish wastes are dumped abundantly into the sea, which is major cause of pollution in coastal areas (Gimeno *et al*., 2007). Crustacean shell waste mainly consists of 30-40% proteins,30-50% calcium carbonate and 20-30% chitin depending on species and season (Cho *et al*., 1998). Crustacean shells are the most important source of chitin due to their high content and ready availability (Muzzarelli, 1997). Chitin is an abundant polysaccharide made of N-acetyl-D-glucoseamine units linked by (1.4) linkage and it is the most abundant biopolymer on earth next to cellulose. There has been a strong demand for chitin and chitosan all over the world, especially in Japan. Recently the major source of chitin has been crustacean shells like crab shells and shrimp shells. About 10^{11} tons of chitin is produced in the biosphere annually (Wang *et al*., 2010). Chitin is a versatile environmentally friendly modern material. It is a naturally occurring high molecular weight linear homo-polysaccharide composed of N-acetyl-D-glucoseamine units linked by alpha (1, 4) linkages. Chitin and chitosan derivatives are natural biodegradable and biocompatible biopolymers that have been used in virtually every field of economy (eg. Water treatment, pulp and paper industry, biotechnology, agriculture, food sciences and membrane technology).

Chitin is a major constituent of the exoskeleton, or external skeleton, of many arthropods such as insects, spiders, and crustaceans. Exoskeletons made of this durable and firm compound support and protect the delicate soft tissues of these animals, which lack an internal skeleton. Chitin is a polysaccharide, a type of carbohydrate that has a basic structure of a repeating chain of sugar molecules. Chitin is analogous in structure to cellulose, the compound that provides structural support to plant tissues. In addition to being found in arthropod exoskeletons, chitin is also found in the cell walls of some species of fungi. Chitin can be found in a number of organisms both in the animal and plant kingdom. The traditional source of chitin is shells of shellfish, krill etc. it is presents in amounts varying from trace amounts up to about 40% of the body weight of an organism. Crustacean shell waste is the most important source of chitin due to its high content and ready availability (Subasingle, 1995). However chitin presents in crustacean shells is associated with proteins, minerals (mainly calcium carbonate) and lipids like pigments.

Chitosan is a natural, non-toxic co-polymer of glucosamine and N-acetylglucosamine obtained after partial de N-acetylating of chitin. Which is a major constituent in the shells of shellfish and found in the waste of the marine food processing industry (Tharanathan & Kittur, 2003), in spite of its abundance and many biological applications, the application of chitosan is restricted due to its high molecular mass and viscosity resulting in its low absorption in *in vitro* studies. Recent studies on chitosan depolymerisation gained a lot of attraction, since the obtained products are water soluble and possess various bio functionalities (Sukwattanasinitt *et al*., 2002). Chitin and chitosan are natural resources waiting for a market. They were waste products of the crabbing and shrimp canning industry. Chitin produced as processing waste from shellfish, krill, clams, oysters, squid, and fungi. Commercially chitin and chitosan are of great importance owing to their relatively high percentage of nitrogen compared to synthetically substituted cellulose.

Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, but lower toxicity toward mammalian cells. Ever since the broad-spectrum antibacterial activity of chitosan was first proposed by Allen, along with great commercial potential, the antimicrobial property of chitosan and its derivatives have been attracting great attention from researchers. Investigation of the antimicrobial properties of chitosan has been a long journey of scientific exploration and technological development. The journey began two decades ago, with studies on the biological phenomena arising from food borne and soilborne pathogenic fungi in the food and agriculture industries. Chitosans showed were good in antioxidant properties, especially antioxidant activity, scavenging ability on hydroxyl radicals and chelating ability on ferrous ions. In addition, the prolonged Ndeacetylation resulted in chitosan with more effective antioxidant properties. Chitosan with presumed antioxidant properties may be used as a source of antioxidants, as a possible food supplement or ingredient or in the pharmaceutical industry. Antioxidant properties of chitosan derivatives have been studied. Furthermore, antioxidant properties of fungal chitosan from shiitake stipes have also been studied.

Nowadays, bio sorption is a strongly explored technique; it is defined as passive, not involving metabolically mediated processes, with the property to bind metals by living or dead biomass. Considerable attention has been paid to the recovery and removal of valuable heavy metal ions from industrial and municipal wastewater by using various bio substances or natural products, particularly because of the low cost and high availability of these materials, without needing arduous regeneration process for reuse, being capable of binding heavy metals by sorption, chelation and ion exchange processes. These low-cost abundant natural materials such as chitin, chitosan, alginate, cellulose, peat and biomass require little processing and are abundant in nature, mainly when obtained as by-products and waste from industry.

The chemical modification of the amino and hydroxyl groups can generate products for pharmaceutical applications, for example: sulfated chitosans possess a wide range of biological activities. Thus, chitosan sulfates as the nearest structural analogues of the natural blood anticoagulant heparin, demonstrate anticoagulant, antisclerotic and antiviral activities.

Chitosan, a derivative of the biopolymer chitin, has been extensively applied in biomedical and pharmaceutical research because of its low toxicity and good biocompatibility. It is able to accelerate the re epithelialization and normal skin regeneration, and to

confer considerable antibacterial activity against a broad spectrum of bacteria. Chitosan (poly D-glucosamine, deacetylated derivative of chitin) and its oligomers are well known for their interesting biological properties, which have led to various applications such as drug delivery carriers, surgical thread, bone healing materials, and especially wound dressing.

Chitooligosaccharides (COSs), derivatives of chitosan, can be obtained by either enzymatic or acidic hydrolysis. COSs has been the choice of interest among many researchers due to their potential biological activities such as immunity enhancing and antitumor, antioxidant and radical scavenging activity and hepatoprotective activity. Chitosan, a biopolymer of glucosamine derived from chitin that is chemically similar to that of cellulose, is not digestible by mammalian digestive enzymes and acts as a dietary fiber in gastrointestinal tract. It is well known for its cholesterol-lowering effect. However, relative less information is available about the effect of chitosan on plasma lipids and glucose control in diabetic subjects. Previous study has reported that chitosan reduced the concentration of plasma cholesterol in animals and type II diabetes patients in combination with hypercholesterolemia. Increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation was proposed to be the mechanisms responsible for the hypocholesterolemic properties. One of the recently reports demonstrated that chitosan has a hypoglycemic effect in STZ-induced diabetic animals. Other studies also found that low molecular weight chitosan (average MW about 2.0 X104 Da) as well as chitosan oligosaccharides, can reduce plasma glucose level in diabetic animals.

Chitosan is used to prepare hydrogels, films, fibers or sponges, most of the materials are used in the biomedical domain, for which biocompatibility is essential. Many systems are described in the literature, but we can cite only a few of the most promising. Chitosan is much easier to process than chitin, but the stability of chitosan materials is generally lower, owing to their more hydrophilic character and, especially, pH sensitivity. To control both their mechanical and chemical properties, various techniques are used, as mentioned previously for chitin. Often, the methods are adapted from the cellulose world. To produce 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 Kg of NaOH are required in addition to nitrogen, process water (0.5 t) and cooling water (0.9 t). Important items for estimating the production cost include transportation, which varies depending on labor and location. In India, the Central Institute of fisheries Technology, Kerala, initiated research on chitin and chitosan. From their investigation, they found that dry prawn waste contained 23% and dry squilla contained 15% chitin. They have also reported that the chitinous solid waste fraction of the average Indian landing of shell fish ranges from 60 000 to 80 000 tones. Chitin and chitosan are now produced commercially in India, Japan, Poland, Norway and Australia. Crustacean shell waste is usually dried on the beaches. It encourages not only environmental pollution but also reduces the recoverable components from their bio waste. Solid Crustacean shell waste undergoes rapid putrefaction because of its alkaline nature (pH 7.5–8.0). Due to high perishability of Crustacean shell waste, implemented processing is needed. Improving the design and operation of biological treatment process for shrimp waste in real life application presents many challenges, including working within the following constraints: the need for robust operation, environmental parameters, and low cost operation. Therefore, extensive research should be carried out to explore bioactive compounds and their activities from Crustacean shell waste.

MATERIALS AND METHODS

Chemicals used

4% sodium hydroxide (4g in 100 ml distilled water. 4% hydrochloric acid (4ml made up to 100ml using distilled water). 50% sodium hydroxide (50g in 100ml distilled water).

Collection and processing

F.indicus and *F.penicillatus* were procured from the local kuppam fish market. They were washed thoroughly. They were shelled. The shells were washed thoroughly. The shells were sundried for 3 days. The shells were ground into a powder.

Deproteinization

10 gms of prawn shell powder is taken in a conical flask.45 ml of 4% sodium hydroxide is added to the conical flask. It is incubated for 21 hours at 25-36^{o} Celsius. The liquid is filtered out and the filtrate is collected separately in a conical flask.

Demineralization

To the remaining filtrate in the conical flask 4%hydrochloric acid was added (1:4 w/v ratio). It was incubated at 25-36^{o}c for 21 hours. The liquid was filtered out and the solid filtrate was collected separately. The filtrate was washed and dried. This filtrate is chitin. The weight of the chitin produced was measured and noted.

Deacetylation

To the chitin crystals in the conical flask 50%sodium hydroxide (1:20 w/v ratio) was added. it was incubated at 25-36^{o}c for 3 days. The liquid was filtered out and the solid filtrate was collected separately in a conical flask. The filtrate was washed and dried. The weight of chitosan was measured and noted.

Deacetylation

To the chitin crystals in the conical flask 50%sodium hydroxide (1:20 w/v ratio) was added. It was incubated at 25-36^{o}c for 3 days. The liquid was filtered out and the solid filtrate was collected separately in a conical flask.

Figure 1. *F. penicillatus.*

Figure 2. Shells.

Figure 3. Washed, dried and ground.

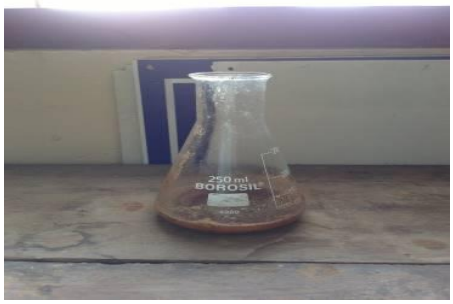

Figure 4. Deproteinization.

Figure 5. Demineralisation.

Figure 6. Chitin.

Figure 7. Deacetylation.

Figure 8. Chitosan.

Figure 9. *F.indicus.*

Figure 10. Shells.

Figure 11. Dried, wash and powdered.

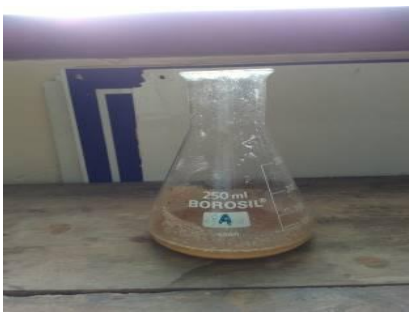

Figure 12. Deproteinisation.

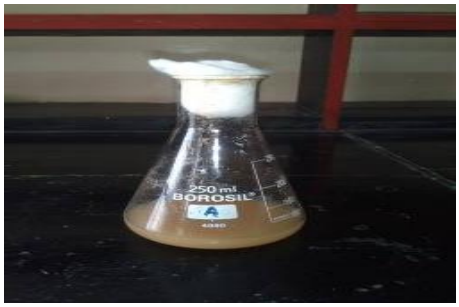

Figure 13. Demineralisation.

Figure 14. Chitin.

Figure 15. Deacetylation.

Figure 16. Chitosan.

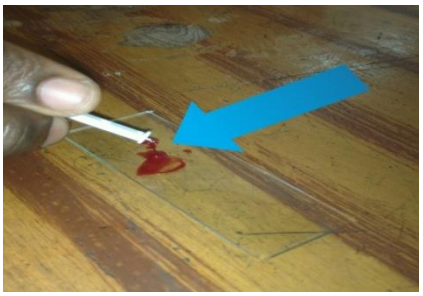

Figure 17. A blood clot (fibrin thread) is formed in 165 secs in the control.

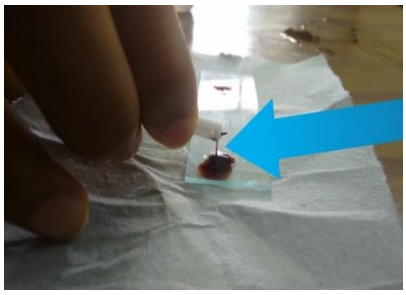

Figure 18. A blood clot (fibrin thread) is formed in 60 secs on adding *F.indicus* chitosan.

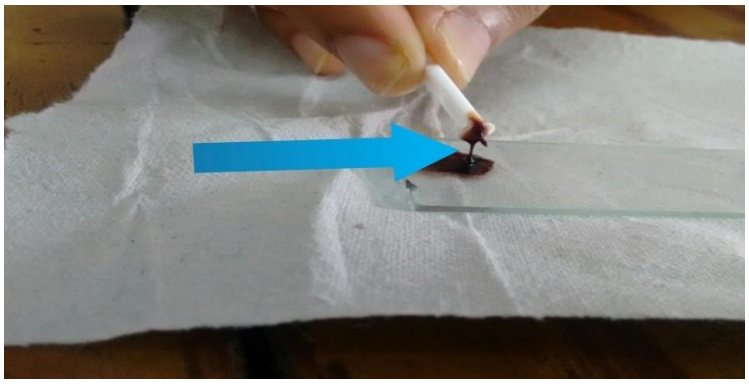

Figure 19. A blood clot (fibrin thread is formed in 45 secs on adding *F. penicillatus* chitosan.

RESULTS AND DISCUSSION

Chitin yield was 60% (6 gms from 10 gms) and chitosan yield was 50% (5 gms from 10 gms) for *F.indicus.* Chitin yield was 50% and chitosan yield was 40% for *F.penicillatus.* The clotting time of blood was found to be around 165 secs by the slide test. Chitosan was found to accelerate the clotting of blood. Blood was seen to clot in around 60 secs on adding chitosan from *F.indicus.* Blood was seen to clot in around 45 secs on adding chitosan from *F.penicillatus.*

Chitin is a natural polysaccharides synthesized by a number of living organisms and functions as a structural polysaccharides while chitosan is a natural, non-toxic copolymer prepared from chitin by deacetylation (Figure 1-16), the antimicrobial activity of chitin, chitosan and their derivatives against various groups like bacteria, yeast and fungi has received considerable attention in recent years.

Various chemical modifications have been investigated to try and improve the extraction/preparation of chitin and chitosan (Kamala *et al*., 2013; Paul *et al*., 2014). In the present study the yield of chitin and chitosan extracted was lower than that reported by Kamala *et al*., (2013). For other species which may be due to the difference in chemical extraction procedures as well as in the presence of these two biopolymers in the shells of different prawn species. The current chemical procedures used are harsh, non-environmentally friendly and involve relatively toxic chemicals like HCl, acetic acid and sodium hydroxide. Hence eco-friendly biological and microbial techniques for

the extraction of chitin and chitosan from prawn shells are the need of the hour in order to prevent further environmental problems (Arbia *et al*., 2013; Xie *et al*., 2001).

The results of the present study prove that prawn chitosan is a blood coagulating agent (Figure 18-19) and hence may have medicinal use, as an external application for accelerated wound healing (Sanandam *et al*., 2013).

CONCLUSION

The prawn shell waste contains chitinous material which can be processed into chitin/chitosan which is high value added products. Chitin/Chitosan can be applied and used in various field like medicine, cosmetics, composites, food preservation, nano-particles and neutraceutical products. To further promote the development of the chitin-chitosan industry, extensive research in their application and marketing of products are crucial. This technology for utilization of prawn waste can go a long way in solving environmental problems caused by the fisheries industry. Bio hazardous prawn waste can be converted in such useful products that are not only commercially valuable but also reduce pollution, risk of potential pathogen infections etc. Prawn industry specially is economically very important contributing substantially to foreign exchange. The Indian prawns *F.indicus* and red tailed shrimp *F.penicillatus* are the most commonly available species in the market. Prawn processing industries produce a large amount of bio waste which causes pollution. Recycling prawn shell waste and extracting commercially viable substances like chitin and chitosan with multiple bio-medical applications is the need of the hour. Prawn shell waste of *F.indicus* and *F.penicillatus* obtained from the local fish market in Chennai India was processed and standard chemical methods were used to extract/prepare chitin and chitosan. Chitin and chitosan were extracted by chemical methods involving deproteinization, demineralization and acetylation. Effect of chitosan on clotting time of blood is studied. Glucosamine hydrochloride preparation was done from chitin.

ACKNOWLEDGMENT

The authors express sincere thanks to the management, Principal and HOD of Zoology, The New College for the facilities provided to carry out this research work.

REFERENCES

Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods–a review. *Food Technology and Biotechnology, 51*(1), 12-25.

Cho, Y.I., No, H.K., & Meyers, S.P. (1998). Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *Journal of Agricultural and Food Chemistry, 46*(9), 3839-3843.

Divya, K., Sharrel, R., & Jisha, S. (2014). A simple and effective method for extraction of high purity chitosan from shrimp shell waste. Paper presented at the Proceedings of the international conference on advances in applied science and environmental engineering-ASEE.

Gimeno, M., Ramírez-Hernández, J.Y., Mártinez-Ibarra, C., Pacheco, N., García-Arrazola, R., Bárzana, E., & Shirai, K. (2007). One-solvent extraction of astaxanthin from lactic acid fermented shrimp wastes. *Journal of Agricultural and Food Chemistry, 55*(25), 10345-10350.

Gopakumar, K. (2002). Textbook of fish processing technology. 1- 491.

Hirano, S., Kim, J., & Srinivasan, S. (1997). High performance proton exchange membrane fuel cells with sputter-deposited Pt layer electrodes. *Electrochimica Acta, 42*(10), 1587-1593.

Kamala, K., Sivaperumal, P., & Rajaram, R. (2013). Extraction and characterization of water soluble chitosan from parapeneopsis stylifera shrimp shell waste and its antibacterial activity. *International Journal Science and Research Publication, 3*, 1-8.

Muzzarelli, R. (1997). Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cellular and Molecular Life Sciences CMLS, 53*(2), 131-140.

Pal, J., Verma, H.O., Munka, V.K., Maurya, S.K., Roy, D., & Kumar, J. (2014). Biological method of chitin extraction from shrimp waste an eco-friendly low cost technology and its advanced application. *International Journal of Fisheries and Aquatic Studies, 1*(6), 104-107.

Paul, S., Jayan, A., Sasikumar, C.S., & Cherian, S.M. (2014). Extraction and purification of chitosan from chitin isolated from sea prawn *Fenneropenaeus indicus*. *Extraction, 7*(4), 201-2014.

Pichyangkura, R., & Chadchawan, S. (2015). Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae, 196*, 49-65.

Sakthidasan, V., Pugazhendy, K., Tamizhazhagan, V., & Jayanthi. (2018). Antioxidant properties of Portunus sanguinolentus chitosan fabricates crab shell. *International Journal of Zoology and Applied Biosciences, 3*(2), 345-351.

Sanandam, M., Salunkhe, A., Shejale, K., & Patil, D. (2013). Chitosan bandage for faster blood clotting and wound healing. *International Journal of Advanced Biotechnology and Research. 4*(1), 47-50.

Subasingle, S. (1995). The development of crustacean and mollusc industries for chitin and chitosan resources. Chitin and chitosan. *Penerbit Universiti Kebangsaan, Malaysia*, 27-34.

Sukwattanasinitt, M., Zhu, H., Sashiwa, H., & Aiba, S.I. (2002). Utilization of commercial non-chitinase enzymes from fungi for preparation of 2-acetamido-2-deoxy-D-glucose from β-chitin. *Carbohydrate Research, 337*(2), 133-137.

Tharanathan, R.N., & Kittur, F.S. (2003). Chitin the undisputed biomolecule of great potential. *Critical Reviwe Food Science and Nutrition*, 43(1):61-87.

Wang, S.L., Chang, T.J., & Liang, T.W. (2010). Conversion and degradation of shellfish wastes by *Serratia* sp. TKU016 fermentation for the production of enzymes and bioactive materials. *Biodegradation, 21*(3), 321-333.

Xie, W., Xu, P., & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. *Bioorganic & Medicinal Chemistry Letters, 11*(13), 1699-1701.