

Extraction of Chitin and Chitosan from Marine Waste and its Applications on Food and Agriculture Products

¹Arya Aji, ²Senthilkumar Rajagopal, ³Thabitha Aavula, ³Vignesh Sakthivel and ¹Rajadurai Murugan

¹Department of Food Technology, Faculty of Life and Allied Health Sciences, M.S. Ramaiah University of Applied Science, Bangalore, Karnataka, India

²Department of Biotechnology, School of Applied Sciences, REVA University, Bangalore, Karnataka, India

³Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Kelambakkam 603103, Chennai, Tamil Nadu, India

ABSTRACT

Chitin and chitosan are linear polysaccharides, structurally similar to cellulose, and have a set of unique characteristics such as biocompatibility, biodegradability to harmless products, non-toxicity, physiological inertness, antibacterial properties, heavy metal ion chelation, gel-forming properties, and, hydrophilicity and remarkable affinity to proteins. The motive of this review was to provide a literature review about chitin and chitosan. And also gives scientific research information about the context. This review reveals the chemistry of chitin and chitosan, various marine sources, different extraction methods and the different applications of chitin and chitosan in the food and agriculture industry.

KEYWORDS

Chemical method, enzymatic method, food and agriculture, marine sources, ion chelation

Copyright © 2022 Arya Aji et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Chitin is a derivative of cellulose, which is a natural polysaccharide and is structurally the same as cellulose¹. Chitin is a linear polysaccharide, divided into chitin and chitosan monomers, the sources not only from marine sources but also in some bacteria and fungus. Chitin and chitosan are said to be wastes produced by processing units for seafood, such as crabs, shrimp, crustaceans, etc. In the beginning, they were only treated as waste on the seashore because they were biodegradable materials. It still takes time to penetrate the soil, all of which will lead to unacceptable odour, green problems and air pollution. After so much research and investigation, waste is transformed into economically valuable products and applications².

Chitin and chitosan are plentiful and sustainable polymers with excellent biodegradability, biocompatibility, non-toxicity and adsorption characteristics. Chitin and chitosan also have good properties, such as fat-blocking properties, antiviral, antimicrobial, antifungal and free-radical scavenging activities³. Antioxidant activity in biomedical applications is used in wound healing, tissue engineering,



stem cell technology, drug, gene delivery, etc⁴. Chitin is a biopolymer that can be transformed into valuable products through different chemical processes, which can be used in various applications. Products include hydrogels⁵. Hydrogel is a cross-linked hydrophilic polymer that is insoluble in water. They have strong water absorption but maintain a clear structure.

Chitin and chitosan have been chemically and structurally modified to increase their solubility in common organic solvents⁶. Chitin and chitosan are classified primarily into two types: These types are distinguished by infrared and solid-state NMR spectroscopy, as well as X-ray diffraction⁷. There is an edible film known for its pork preservation, which is mainly to prevent the growth of microorganisms and improve the quality of canned food. Wax coatings are used for fruits and vegetables to prevent perishable foods from spoiling and microbial attack. Wax coatings can cause serious health problems, such as cancer and digestive problems in some people. As a solution to minimize the use of wax coatings on fruits and vegetables in 2009, Tripathi developed an edible antibacterial coating based on chitosan and Polyvinyl Alcohol (PVA) and a proven and effective edible coating, suitable for minimally processed tomatoes and coating can be considered a promising food packaging material. Hence, the need of the hour is to find the alternate and nontoxic coating and packaging material for food and agricultural products.

Extraction of chitin and chitosan by using different methods: The deacetylation process is carried out in 40% sodium hydroxide at 120°C for 13 hrs. The result of this treatment is 70% acetylated chitin. About 60,00080,000 tons of chitin are produced from solid shellfish waste every year⁸. Many countries such as India, Poland, Japan and Australia produce chitin commercially⁹. Waste from shellfish processing units is generally considered useless material and piled upon these ashore. Which causes environmental pollution? One of the key steps in chitin production is to extract chitin from natural resources (Fig. 1). Chemical and physical traction methods have their advantages and disadvantages. Today, only a few laboratories perform and track biological extraction¹⁰.

Chemical extraction: The first step in the chemical extraction process is to wash and clean the shells from various sources and grind them to powder¹¹. The chemical extraction process consists of three main steps i.e., deproteinization, demineralization and discolouration.

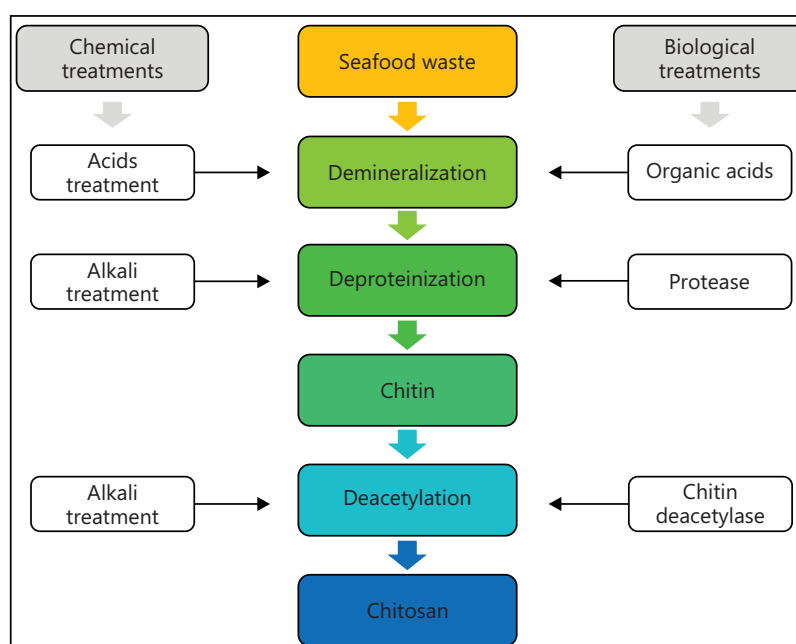


Fig. 1: Formation of chitosan in flow formation

Chemical deproteinization: The main reagent range for deproteinization is 0.125-5.0 M of NaOH. The concentration can vary depending on the temperature and the time required to complete the process. Sodium hydroxide also aids in the hydrolysis of biopolymers, molecular weight reduction and partial deacetylation of chitin. It is the process of destroying protons from chemical bonds. Chemicals were used in this chemical deproteinization process. Protein removal occurs during this process. Protein is removed because some proteins can cause allergies in humans. Therefore, this process is considered an important step. Different types of chemicals were used and applied to test the efficacy of deproteinization¹².

Chemical demineralization: The process of using sulphuric acid, hydrochloric acid, nitric acid, acetic acid, formic acid and other acids to remove minerals such as calcium carbonate is called demineralization. This is a full acid treatment. During the method of demineralization process, calcium carbonate breaks down and turns into calcium chloride, which emits carbon dioxide¹³.

Decolouration: It is not common to do this only when there are indeed colours or pigments in the final product. With the help of an organic acetone solvent, the pigment can be removed. The pigment can be a carotenoid¹⁰.

Biological extraction: The chemical extraction process generally requires high energy and high cost to purify chitin and can affect the physical and chemical properties of the chitin or the extracted product¹⁰. To overcome all these problems, scientists have developed safe, environmentally friendly and cheaper biological processes that can produce high-quality extraction products without negative effects, such as without losing the physical and chemical properties of chitin and chitosan¹⁴.

To show that the biological extraction method is better, many comparative studies have been conducted between chemical extraction and biological extraction in shrimp shells. There are two extraction methods in the biological extraction process, enzymatic deproteinization and the Use of microbial fermentation¹⁵.

Enzymatic deproteinization: The use of enzymes, such as the protease during periods of extraction of Titina, is mainly carried out when the chitin is extracted from the marine debris. The main source of proteolytic enzymes is plants, animals and some microorganisms¹⁶. Several proteolytic enzymes were used, such as trypsin, pepsin, papain, etc.

To remove the protein during the extraction of chitin. These enzymes help minimize the polymerization of solution and deacetylation during the isolation of the chitin¹⁷. Usually, they are applied for a discouraging step or after a desalination step to improve the performance of these enzymes. Both raw enzymes are extracted from visceral fish and the brute enzyme is relatively cheap than the purified enzymes. Some bacteria are also sources of raw enzymes¹⁷. Therefore, the use of protease enzymes in the extraction of chitin is economical and low risk for the environment, since all products are naturally obtained. Several studies are showing the advantage of the use of bacterial protease for the extraction of chitin¹⁸.

A comparative study of the two processes shows that the efficiency of the enzymatic process is reduced compared to chemical methods. Through enzymatic extraction, 510% of the protein will be retained after deproteinization. To overcome this situation, NaOH was added to improve the purity of the final product. Many studies have also shown that processes such as demineralization and deproteinization don't have a significant impact on the performance of the final product¹⁹.

Fermentation: Fermentation is used to remove proteins from the shells and enzymes are mainly used in this process. The enzymes used in this process are very expensive, to overcome the situation of microbial strains, microbial strains are the added. The microbial strains used can determine fermentation, such as

one-stage, two-stage, continuous or co-fermentation¹⁵. The fermentation process can be divided into two steps, such as lactic acid fermentation and non-lactic acid fermentation

Lactic acid fermentation: Bacterial species such as *Lactobacillus* are used for the fermentation of crustacean shells. These bacteria produce proteases and lactic acid during fermentation. The change in glucose produces lactic acid, which generally lowers the pH, thus reducing the growth of spoilage microorganisms. The fertility of the fermentation process depends on several features, such as the number of microorganisms, the microbial composition of the inoculant, the pH, the development and concentration of the carbon source during the fermentation process, the temperature and the time required to complete the process²⁰.

Non-lactic acid fermentation: Except for the specious lactic acid-like fungi, bacteria like *Pseudomonas*, *Bacillus*²¹ and *Aspergillus*²² are used for the fermentation of crustacean shells in non-lactic acid fermentation.

Applications on agriculture and agriculture products: Chitin and chitosan are known for their properties to protect plants and crops from harmful pathogens and insects. Both chitin and chitosan play an inevitable role in the agricultural field. The main function of chitin and chitosan is the characteristics of the seeds, allowing the growth of microbial insecticides, such as *Trichoderma harzianum* and P1, which are reported to be active against leaf diseases.

Chitin is often mixed with other products to promote the growth of microorganisms such as *T. harzianum* and partially inhibit the growth of certain pathogens, such as *R. Solani*. It has a dual role, such as improving and allowing the production of suitable microorganisms and reducing the growth of unsuitable microorganisms through true inhibition and greater population control²³.

Chitosan is primarily considered a natural fertilizer, so it is also considered a natural defence of plants against harmful pathogens, etc²⁴. Chitosan is used primarily in the field of plant and crop protection to protect against a variety of insects and pathogens. In 1990, research was conducted on chitosan products used for plant protection, which targeted pathogens that cause root and harmful effects on crops and plants during the growing and harvesting stages²⁵.

They concluded that killing bacteria would hinder the growth of bacteria, but the mechanism is not yet fully understood and research is still ongoing. Chitosan is an excellent antifungal agent due to its chelating properties. Plants containing chitosan have a defensive effect on pathogens and infected fungi²⁶. All of these can be concluded that chitosan can be used to control diseases in plants and is an excellent inducer. Chitosan can be used as a biological fertilizer, used alone or in combination with other products and can be used to control pathogens and pests that cause crop loss and damage plants during the growth stage and post-harvest season²⁷.

Chitosan also has the effect of inhibiting viruses and other plant and crop infections. Chitosan can be used in large quantities as biological pesticides and may not have any advisory effect on humans and other animals. Chitosan uses the differences in the agricultural field as a root application agent, seed soaking agent and sprays to control and inhibit the growth and invasion of pathogens and insects. It can also prevent fungi that cause root and crop infections²⁷.

Chitin is also used to prevent and control the release of pesticides and pesticides after use, so chitosan can be mixed or mixed with other products such as alginate, chewing gum and starch²⁸. It is the most common method to prevent or control the release of pesticides. Chitosan and its products are used in many plant diseases (more than 60 diseases of various plants)²⁹. The chitosan has a high inhibitory activity,

also observed in several stages such as the mycelium, the formation of spores, the survival of the spore and the germination and is also used to paint soy, cotton, cucumber, wheat, rice and other seeds can. It can also improve the natural behaviour of the soils used for crops, such as plants, potatoes, soy, lettuce and spinach. The plants can be used directly as liquid fertilizers³⁰.

However, there are many disadvantages to the application and use of chitosan in agriculture, one of which is the solubility and standardization of chitosan commercially available. The bioactivity of the chitosan is based on different frames, such as the pH of the solution, the viscosity, the objective of the microorganism, the concentration of chitosan, the degree of molecular weight and the degree of acetylation²⁷. Different studies have been carried out on the antimicrobial activity of chitosan against several microorganisms, such as bacteria, yeast, fungi and viruses³¹. In general, most studies have increased antimicrobial activity as the molecular weight decreases³². One other important problems that limit the application of chitosan in agriculture are its water-soluble characteristics and some studies remain on some of these problems and some of the applications and the application of chitosan in agriculture³³.

Applications on the food products: Chitosan or poly (β -(14) N-acetyl D-glucosamine is a very important natural polysaccharide, which was first discovered in 1884. This biopolymer is synthesized by various living organisms and is the most abundant natural fibre chitin polysaccharides. Chitin does not melt and almost does not dissolve into different conformations during the transformation process. Its solubility is an important issue in the development and characterization of chitin processing and use. Chitin as a whole has more application, it becomes chitosan through deacetylation part under alkaline conditions⁷.

Changing the application of chitin and chitosan has resulted in several examples of drug delivery, wound dressings or biofilms. The direct application of chitin in the food industry is impossible, which is why chitin is converted to chitosan through different commercial chemical procedures. Not only in the food industry, chitin and chitosan are also used in agriculture, medicine and the environment.

This is because chitin and chitosan have some chemical properties, such as insoluble in water, cationic biopolymer behaviour and biological pH. Positive overall charge and easy to form gelatin. The reuse of wastewater will increase the economic performance of the country, reduce poverty and reduce economic sustainability. It also helps improve social well-being³⁴.

Wastewater comes from various industries and processing units such as food, textile and vegetable oil processing, as well as from domestic wastewater. It is an important part of environmental pollution. The use of chitosan can reuse wastewater through different chemical procedures. In most companies that do not have adequate treatment, this water is discharged into the water body as sewage³⁵. Adsorption is the adhesion or immobilization of molecules or the electrostatic combination of fluids and solid surfaces so that inactive adsorbents of biological origin or natural products can be used to remove compounds, metal ions or other materials and the removed biological materials and adsorbents can be used³⁶.

The increase in the degree of deacetylation of chitosan is related to a large number of amino groups, which are the main absorption centre and the degree of acetylation and pH are the main factors affecting the absorption capacity of chitosan³⁷. Chitosan biopolymer is a biocompatible, non-allergenic and bio-functional molecule, which has attracted widespread attention as a possible preservation method for foods of natural origin³⁸. Based on scientific procedures for the impact of multiple technologies on different food categories, chitosan extracted from shrimp is recommended as GRAS (Genetically Recognized as Safe, GRAS notification number: GRN000443)³⁹. Chitosan is mainly used to increase the shelf life of bread by observing the retrogradation of starch and preventing the growth of microorganisms. Use 0.5, 1.0 or 1.5% chitosan diluted in 1.0 ethanol to coat 493 kDa chitosan molecules in shelf-life baguettes.

Their results show that chitosan is effective in coating 1% chitosan baguettes. Compared with the control during storage, the barrier properties are less weight loss, hardening and regeneration at 25°C for 36 hrs. Chitosan is derived from many biological waste products used in many energy-saving methods. Compared to other biopolymers, chitosan is much cheaper.

Chitosan's extraordinary properties have created an inevitable strong position in food packaging applications. Deacetylation is the most commonly used and most economical method of extracting chitin from chitosan. Chitosan is widely used to extend the life of food. The addition of Ca^{2+} ions changes the osmotic rate of CO_2 through the chitosan film, increasing the effective lifetime of the raw material of the fruit, preparing a series of pictures of chitosan and preserving the preparation of glycerol. Corning with a chitosan film by immersing it in a 1% polysaccharide solution containing $0.1^\circ\text{C} +$ prevents changes in the functional properties of the vegetable. In addition, the pellets are also useful for the preparation of food packaging paper that acts as a suppressant of microbial growth⁴⁰. Chitosan is widely used in food in the food industry because it is non-toxic to warm-blooded animals. Microcrystalline chitin is a special kind of chitin, which has the characteristics of emulsification, excellent thickening and gelling agent and can stabilize food. In most foods, chitosan is used as dietary fibre. The use of microcrystalline chitosan reduces the problems associated with flavour, colour and shelf. Life from other fibre sources. Chitosan plays an important role in the production of protein-enriched bread. And this does not add emulsifiers and butter agents and other ingredients³.

Chitosan is incorporated in the production of poultry feed at a level of 0.5% decreasing the food consumption ratio and increasing body weight by 12% in comparison with birds fed with a chitin-free diet. Similarly, nutritional studies in the US have shown that chicks fed on a diet containing dried whey and chitin, utilized whey more efficiently and gained more weight than those fed a similar but chitin-free diet. Trials also showed that small amounts of chitin added to the diets of chicks and calves enabled the animals to digest milk lactose through increased growth of specific intestinal bacteria⁹. These bacteria prevent the growth of other types of organisms and produce the enzymes necessary to digest lactose. Since some people and many animals are intolerant to lactose, this property may be very important. At present, there is no comprehensive study on the metabolism of chitin and chitosan in the human body, so the application of these polymers in the food processing industry needs to be further explored^{10,40}. In the last 30 years, the application of chitin and chitosan has come a long way. And based on the excellent physical and chemical properties of chitin and chitosan, various investigations have been conducted⁴¹.

CONCLUSION

The research efforts have increased the applications of marine debris and oligosaccharides such as chitin and chitosan in various fields such as industry and biomedicine and these have been applied and used successfully in chitin- and chitosan-based nanomaterial used in tissue engineering, drug delivery, cancerdiagnostics and wound dressings. They are also used as edible packaging films and food coating materials and have also proven to be a successful preservative, which can be used alone or in combination with other preservatives. This review is expected to provide information on the use of marine debris (such as chitin and chitosan) to discover new materials with new properties so that they can be used invaluable applications.

SIGNIFICANCE STATEMENT

Chitin and chitosan are the chiefs, the most abundant natural polymers after cellulose, while they may have diverse foundations, like exoskeletons of crustaceans, molluscs, insects and fungi, the major resource for obtaining this polymer is the shell of crustaceans. Chitosans have shown effective for the production of bioactive materials due to unmatched traits such as non-toxicity, biodegradability, chelating, anticoagulant, antioxidant and antibacterial capabilities, as well as biocompatibility. This mini-review is

focused on the main analytical techniques used for the development of chitosan nano-film, moreover, an overview of the major applications of chitosan-based active food packaging systems is also discussed.

REFERENCES

1. Dutt, S.C. and R. Roy, 2002. A critical review on idealization and modelling for interaction among soil-foundation-structure system. *Comput. Struct.*, 80: 1579-1594.
2. Ramachandran, S., V. Narasimman and P. Rajesh, 2022. Low molecular weight sulfated chitosan isolation, characterization and anti-tuberculosis activity derived from *Sepioteuthis lessoniana*. *Int. J. Biol. Macromol.*, 206: 29-39.
3. Dutta, S., K. Firdous and S. Chakraborty, 2021. Screening of synergistic and antimicrobial effect of Cr (VI) and Ni (II) tolerant bacteria *Bacillus cereus*. *J. Appl. Biol. Biotechnol.*, 9: 69-77.
4. Richeldi, L., R.M. du Bois, G. Raghu, A. Azuma and K.K. Brown, *et al.*, 2014. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N. Engl. J. Med.*, 370: 2071-2082.
5. Raghu, G., B. Rochwerg, Y. Zhang, C.A.C. Garcia and A. Azuma, *et al.*, 2015. An official ATS/ERS/JRS/ALAT clinical practice guideline: Treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. *Am. J. Respir. Crit. Care Med.*, 192: e3-e19.
6. Al-Baghdadi, M.A.R.S., 2005. Modelling of proton exchange membrane fuel cell performance based on semi-empirical equations. *Renewable Energy*, 30: 1587-1599.
7. Rinaudo, M., 2008. Main properties and current applications of some polysaccharides as biomaterials. *Polym. Int.*, 57: 397-430.
8. Yanes, L.L., D.G. Romero, J.W. Iles, R. Iliescu, C. Gomez-Sanchez and J.F. Reckelhoff, 2006. Sexual dimorphism in the renin-angiotensin system in aging spontaneously hypertensive rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 291: R383-R390.
9. Chen, J.F., and J.G. Teng, 2001. Anchorage strength models for FRP and steel plates bonded to concrete. *J. Struct. Eng.*, 127: 784-791.
10. Dhillon, G.S., S. Kaur, S.K. Brar and M. Verma, 2013. Green synthesis approach: Extraction of chitosan from fungus mycelia. *Crit. Rev. Biotechnol.*, 33: 379-403.
11. Islam, M.S. and M. Tanaka, 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. *Mar. Pollut. Bull.*, 48: 624-649.
12. Gomathy, V., V. Manigandan, N. Vignesh, A. Thabitha and R. Saravanan, 2021. Evaluation of antibacterial, teratogenicity and antibiofilm effect of sulfated chitosans extracted from marine waste against microorganism. *J. Bioact. Compatible Polym.*, 36: 249-258.
13. Percot, A., C. Viton and A. Domard, 2003. Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, 4: 12-18.
14. Khanafari, A., R. Marandi and S. Sanatei, 2008. Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. *Iran. J. Environ. Health Sci. Eng.*, 5: 19-24.
15. Arbia, W., L. Arbia, L. Adour and A. Amrane, 2013. Chitin extraction from crustacean shells using biological methods-A review. *Food Technol. Biotechnol.*, 51: 12-25.
16. Sedighi, M., H. Jalili, M. Darvish, S. Sadeghi and S.O. Ranaei-Siadat, 2019. Enzymatic hydrolysis of microalgae proteins using serine proteases: A study to characterize kinetic parameters. *Food Chem.*, 284: 334-339.
17. Marković, K., M. Hruškar and N. Vahčić, 2006. Lycopene content of tomato products and their contribution to the lycopene intake of Croatians. *Nutr. Res.*, 26: 556-560.
18. Kunz, A., A. Labes, J. Wiese, T. Bruhn, G. Bringmann and J. Imhoff, 2014. Nature's lab for derivatization: New and revised structures of a variety of streptophenazines produced by a sponge-derived *Streptomyces* strain. *Mar. Drugs*, 12: 1699-1714.
19. Kurita, K., K. Sugita, N. Kodaira, M. Hirakawa and J. Yang, 2005. Preparation and evaluation of trimethylsilylated chitin as a versatile precursor for facile chemical modifications. *Biomacromolecules*, 6: 1414-1418.

20. Muffler, K. and R. Ulber., 2005. Downstream Processing in Marine Biotechnology. In: Marine Biotechnology II, Ulber, R. and Y. Gal (Eds.), Springer, Berlin, Heidelberg, Germany, ISBN: 978-3-540-31395-3, pp: 63-103.
21. Sini, T.K., S. Santhosh and P.T. Mathew, 2007. Study on the production of chitin and chitosan from shrimp shell by using *Bacillus subtilis* fermentation. Carbohydr. Res., 342: 2423-2429.
22. Mahmoud, M.A., P.A. Parker, W.H. Woodall and D.M. Hawkins, 2007. A change point method for linear profile data. Qual. Reliab. Eng. Int., 23: 247-268.
23. Venkataprasanna, K.S., J. Prakash, S. Vignesh, G. Bharath and M. Venkatesan *et al.*, 2020. Fabrication of chitosan/PVA/GO/CuO patch for potential wound healing application. Int. J. Biol. Macromol., 143: 744-762.
24. Xu, J., N. Zhang, K. Wang, Q. Xian, J. Dong, X. Qi and X. Chen, 2022. Chitinase Chi 2 positively regulates cucumber resistance against *Fusarium oxysporum* f. sp. cucumerinum. Genes, Vol. 13. 10.3390/genes13010062.
25. The Brassica Rapa Genome Sequencing Project Consortium, X. Wang, H. Wang, J. Wang, R. Sun and J. Wu *et al.*, 2011. The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet., 43: 1035-1039.
26. Rabea, E.I., M.E.T. Badawy, C.V. Stevens, G. Smagghe and W. Steurbaut, 2003. Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules, 4: 1457-1465.
27. Divya, K. and M.S. Jisha, 2017. Chitosan nanoparticles preparation and applications. Environ. Chem. Lett., 16: 101-112.
28. Global Burden of Disease Cancer Collaboration, 2018. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the global burden of disease study. JAMA Oncol., 4: 1553-1568.
29. Grande-Tovar, C.D., C. Chaves-Lopez, A. Serio, C. Rossi and A. Paparella, 2018. Chitosan coatings enriched with essential oils: Effects on fungi involved in fruit decay and mechanisms of action. Trends Food Sci. Technol., 78: 61-71.
30. Morin-Crini, N., E. Lichtfouse, G. Torri and G. Crini, 2019. Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry. Environ. Chem. Lett., 17: 1667-1692.
31. Sahariah, P. and M. Másson, 2017. Antimicrobial chitosan and chitosan derivatives: A review of the structure-activity relationship. Biomacromolecules, 18: 3846-3868.
32. Badawy, M.E.I., E.I. Rabea, M.A.M. El-Nouby, R.I.A. Ismail and N.E.M. Taktak, 2017. Strawberry shelf life, composition, and enzymes activity in response to edible chitosan coatings. Int. J. Fruit Sci., 17: 117-136.
33. Manigandan, V., J. Nataraj, R. Karthik, T. Manivasagam and R. Saravanan *et al.*, 2019. Low molecular weight sulfated chitosan: Neuroprotective effect on rotenone-induced *in vitro* Parkinson's disease. Neurotoxic. Res., 35: 505-515.
34. Karthik, R., V. Manigandan, R. Saravanan, R.P. Rajesh and B. Chandrika, 2016. Structural characterization and *in vitro* biomedical activities of sulfated chitosan from *Sepia pharaonis*. Int. J. Biol. Macromol., 84: 319-328.
35. Richards, S., J. Dawson and M. Stutter, 2019. The potential use of natural vs commercial biosorbent material to remediate stream waters by removing heavy metal contaminants. J. Environ. Manage., 231: 275-281.
36. Józwiak, T., A. Mielcarek, W. Janczukowicz, J. Rodziewicz, J. Majkowska-Gadomska and M. Chojnowska, 2018. Hydrogel chitosan sorbent application for nutrient removal from soilless plant cultivation wastewater. Environ. Sci. Pollut. Res., 25: 18484-18497.
37. Hirano, S., C. Itakura, H. Seino, Y. Akiyama, I. Nonata, N. Kanbara and T. Kawahami, 1990. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem., 38: 1214-1217.

38. Raafat, D. and H.G. Sahl, 2009. Chitosan and its antimicrobial potential-A critical literature survey. *Microb. Biotechnol.*, 2: 186-201.
39. Zakaria, S., H.C. Chin, W.H.W. Ahmad, H. Kaco, W.C. Soon and H.C. Chi, 2015. Mechanical and antibacterial properties of paper coated with chitosan. *Sains Malaysiana*, 44: 905-911.
40. Dutta, J., S. Tripathi and P.K. Dutta, 2012. Progress in antimicrobial activities of chitin, chitosan and its oligosaccharides: A systematic study needs for food applications. *Food Sci. Technol. Int.*, 18: 3-34.
41. Subhadrappa, N., R. Saravanan, P. Ramasamy, A. Srinivasan, V. Shanmugam and A. Shanmugam, 2014. Hepatoprotective effect of β -chitosan from gladius of *Sepioteuthis lessoniana* against carbon tetrachloride-induced oxidative stress in wistar rats. *Appl. Biochem. Biotechnol.*, 172: 9-20.