Journal of Advanced Zoology
ISSN: 0253-7214
Volume 44 Issue S-4 Year 2023 Page 1168-1175

Various Extraction Methods of Chitosan From An Aquatic Resource Shrimp Shell Waste

K. Ram Prasad[1], Dr. Suseela Meesala*[2]

[1]*Research Scholar, Department of Zoology, VSU PGC, Kavali*
[2*]*Assistant Professor, Department of Zoology, VSU PGC, Kavali*

***Corresponding Author:** Dr. Suseela Meesala*
Assistant Professor, Department of Zoology, VSU PGC, Kavali, Email: drsuseelameesala@gmail.com

Abstract

Natural biopolymer chitosan has free toxicity, biodegradability, and biocompatibility. Based on previously published works, the goal of this review is to investigate various chitosan production techniques. Drawing from published works, this review attempts to investigate several chitosan production techniques. This article is to provide a concise overview of the many approaches, including chemical and biological approaches, used in the manufacturing of chitosan. Chitosan can be made by processes that are chemical or biological in nature. Enzymatic and fermentation are biological techniques, and the mechanisms of the various chemical agents and reaction parameters employed, are chemical techniques. The three main procedures of demineralization, deproteinization, and deacetylation used to extract chitin and chitosan from raw shrimp wastes. The most important factor, according to study, is the concentration of alkali used for deacetylation, which is followed by the concentrations of acid and alkali used for demineralization and deproteinization.

Keywords: Chitosan, Chemical Method, Biological Method, Fermentation Method

INTRODUCTION:

Chitin, the precursor to chitosan, is the most abundant biopolymer in nature after cellulose and is present in a variety of eukaryotic species, including fungus, insects, and crustaceans (Kean, M. and Thanou, 2011; Ahmed and Ikram (2017)). According to Younes and Rinaudo (2015), chitin is unique due to the presence of the acetyl group, whereas chitosan is a chitin-partially deacetylated derivative made of ß-(14)-linked N-acetyl-D-glucosamine homopolymers.

A naturally occurring biopolymer having free toxicity, biocompatibility, and biodegradability is chitosan. It has a free amino group, which is the most effective chitin derivative with a range of biomedical uses, and may be chemically changed to make derivatives despite its biodegradability (Ahmad et al., 2020; Azuma et al., 2014). These derivatives are readily available on the market and are easily manufactured. According to Rinaudo (2006) and Islam et al., (2018), chitosan is a feasible environmentally friendly option for shell remediation and can be incorporated into various sustainable economic activities. There are several uses of chitin and chitosan in the pharmaceutical sector, tissue engineering, agriculture, water treatment, cosmetics, anti-tumor, and anti-microbial agents (Islam et al., 2018). It can also be used in the biotechnology, environmental chemistry, and wastewater treatment industries.

According to Elieh-Ali-Komi et al., (2016); Raghvendrakumar et al., (2017); Kim, (2011); Yao (2012), the most often mentioned sources in the literature for the raw materials needed to prepare chitosan are shrimp and crabs. Other species like crayfish, lobster, and oysters have also been used.

METHODS OF CHITOSAN EXTRACTION

Extraction of chitosan has documented by the three main procedures such as demineralization, deproteinization, and deacetylation from raw shrimp wastes under various conditions of temperature, time, and acid and alkali concentrations. Very few studies addressed about decolorization.
Before beginning the process of extracting the chitin and chitosan from shrimp wastes the shells must be free of unwanted materials, cleaned, dried, and ground. They must also be sieved through a 250-micron sieve (Sagheer et al., 2009).
Chitosan extraction takes place by two processes that are chemical or biological. Enzymatic and fermentation are biological techniques, and the mechanisms of the various chemical agents and reaction parameters employed, are chemical techniques. Chitin and chitosan yields varied depending on the demineralization and deproteinization conditions applied.

I.EXTRACTION OF CHITOSAN BY CHEMICAL METHOD

According to Lertsutthiwong et al., (2002); Lamarque et al., (2005); Trung, et al., (2006); Huang et al., (2004) the process of chitosan extraction starts with deprotienization following demineralization and deacetylation. The crushed shrimp shells were cooked for one hour in 2% (w/v) sodium hydroxide to dissolve the proteins and sugars. After that, they were allowed to cool for thirty minutes at room temperature. In order to remove the calcium carbonate, the demineralization process was performed using 1% HCl, 1:4 (w/v), for 24 hours. The resulting chitin was then rinsed with deionized water after the shells had been treated for one hour with 2% NaOH. In order to deacetylate the chitin, it was first cooked in 50% NaOH for two hours and then allowed to cool at ambient temperature for half an hour. To produce chitosan the sample was cleaned with 50% NaOH, filtered, and oven dried for six hours at 110 0C.

Marei et al. (2016) employed an extraction approach that begins with the demineralization procedure followed by deprotenization and deacetylation. The demineralization procedure, which entails an acid treatment with 1M HCl solution, 1:15 (w/v), at 25^{o}C, is the first step in the extraction technique. After that, distilled water was used to wash the sample until its pH was neutral. The deproteinization process was repeated multiple times using 1M NaOH at 100^{o}C for 8 hours. The final sample was cleaned by boiling it in acetone to eliminate all contaminants, after which it was rinsed with hot ethanol and distilled water until it reached a pH of neutral. In order to achieve constant weight, the resulting chitin was dried in an oven at 50^{o}C. After eight hours of deacetylation at 100^{o}C using 50% NaOH at a ratio of 1:15 (w/v), the mixture was filtered and cleaned with hot distilled water to reach a pH of neutral. The resultant chitosan sample was oven dried for 24 hours at 50^{o}C (Rodde et al., 2008; Abdou et al., 2008).

A. Demineralization by Chemical method

The removal of minerals, particularly calcium carbonate, is known as demineralization. Organic or inorganic acids could be used to conduct it (No et al., 1998; Percot et al., 2003). In order to produce chitin and chitosan, demineralization is thus one of the most crucial steps. Most research studies reported that the demineralization process is preferred under the effect of diluted hydrochloric acid. The carbonate salts in the shells can be demineralized with diluted hydrochloric acid by turning them into chloride salts and carbon dioxide. In certain research, organic acids like citric and acetic acids were used in single or double demineralization processes to demineralize shrimp shells (Younes and Rinaudo, 2015; Kumar et al., 2017).
The primary factors influencing the demineralization process's effectiveness are the degree of mineralization of shrimp shells, the concentration of acid, the temperature during extraction, and the duration of the process. The most important element in regulating the removal of minerals is the concentration of acid. Stopping the demineralization reaction requires neutralizing the pH of demineralized shells. The demineralization process' effectiveness determines the grade of chitin produced; the lower the mineral concentration, the greater the chitosan quality (Younes and Rinaudo, 2015). According to recent studies (Chang et al., 2017; Pujari and Pandharipande (2016); Varlamov et al., 2020), shrimp shell demineralization has been documented in acidic treatment conditions ranging from 1% to 50% acid concentration for 1–24 hours at 22–90 °C.

B. Deproteinization of Demineralized Shells by Chemical Method

The process of chemical deproteinization is a crucial step in the extraction of proteins from shrimp shells. One can perform chemical deproteinization either prior to or following the demineralization phase. The most recommended alkaline solution to utilize to break the connections between the chitin and proteins for protein removal or biopolymer hydrolysis is sodium hydroxide, while deproteinization can be carried out under the influence of several alkaline solutions or chemical reagents.

Reaction temperature, alkali concentration, and recovery time are the primary factors that influence the deproteinization process. According to Younes and Rinaudo (2015) and Rasweefali et al., (2022) poor protein removal reduces the quality of chitin and chitosan and limits their use in pharmaceutical and biological applications. Recent research has reported on the conditions for deproteinization of shrimp waste at 22–100 ◦C for 1–24 hours, with alkali concentrations ranging from 1% to 10% (Chang et al., (2017); Pujari and Pandharipande (2016); Varlamov et al., (2020).

C. Deacetylation by Chemical Method

Chitosan can be yielded by the chemical deacetylation of chitin, whereas the -NH_2 group replaces the acetyl group of C2 glucosamine (Hajji et al., 2014). The key distinction between chitosan and chitin is the level of acetylation. Either acidic or alkaline solutions can be used to deacetylate chitin. The degree of acetylation is the differentiation factor between chitin and chitosan. The Deacetylation of chitin can be achieved using either acidic or alkaline solutions.

However, deacetylation by the acidic medium is not the preferred option because it damages the glycosidic bonds and breaks the polymer chain. On the other hand, the concentrated alkaline deacetylation of chitin is a more efficient process for removing acetyl groups. Most of the literature reported conducting the deacetylation of chitin with a concentrated sodium hydroxide solution.

According to Younes and Rinaudo (2015) and Rasweefali et al. (2022) the degree of deacetylation directly relates to the quality of chitosan and is dependent on the alkali concentration, chitin supply, temperature, and duration of chemical deacetylation. Recent studies have used concentrated sodium hydroxide at concentrations ranging from 30% to 65% for 40 min to 72 h at 22–100 ◦C to report the deacetylation conditions of chitin (Chang et al., 2017; Pujari and Pandharipande 2016; Varlamov et al., 2020).

Table 1 shows the degree of deacetylation percentages (DD%) and the chemical processes used to extract chitosan from shrimp waste.

Shell Origin	Extraction Condition Minutes	DD (%)	Reference
Shrimp shell waste	2% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, at DT 65 °C	39.10	Hossain and Uddin (2020)
Shrimp shell waste	2% HCl for DM, 4% NaOH for DP, and 60% NaOH for DA, at DT 65 °C	40.00	-do-
Shrimp shell waste	3% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, at DT 65 °C	41.00	-do-
Shrimp shell waste	3% HCl for DM, 4% NaOH for DP, and 60% NaOH for DA, at DT 65 °C	42.00	-do-
Shrimp shell waste	4% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, at DT 65 °C	61.00	-do-
Shrimp shell waste	4% HCl for DM, 4% NaOH for DP, and 60% NaOH for DA, at DT 65 °C	70.00	-do-
Shrimp shell waste	5% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, at DT 65 °C	58.00	-do-
Shrimp shell waste	5% HCl for DM, 4% NaOH for DP, and 60% NaOH for DA, at DT 65 °C	67.00	-do-
Penaeus monodon	(2%, 3%, 4%) HCl for DM, 4% NaOH for DP, and 30% NaOH for DA, at DT 65 °C	45.50	Hossain and Iqbal (2014)
Penaeus monodon	(2%, 3%, 4%) HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, at DT 65 °C	61.24	-do-
Penaeus	(2%, 3%, 4%) HCl for DM, 4% NaOH for DP, and	79.57	-do-

monodon	50% NaOH for DA, at DT 65 °C		
Penaeus monodon	(2%, 3%, 4%) HCl for DM, 4% NaOH for DP, and 60% NaOH for DA, at DT 65 °C	81.24	-do-
Shrimp shell waste	6% HCl for DM, 3.5% NaOH for DP, and 50% NaOH for DA, at DT 70 °C	76.26	Patria (2013)
Shrimp shell waste	6% HCl for DM, 3.5% NaOH for DP, and 50% NaOH for DA, at DT 80 °C	81.37	-do-
Shrimp shell waste	6% HCl for DM, 3.5% NaOH for DP, and 50% NaOH for DA, at DT 90 °C	81.25	-do-
Shrimp shell waste	6% HCl for DM, 3.5% NaOH for DP, and 50% NaOH for DA, at DT 100 °C	84.87	-do-
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 16 mesh size, then DP under 4% NaOH, and 45% NaOH for DA, at 600 watts in the microwave for 15 min	81.00	Santos (2019)
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 32 mesh size, then DP under 4% NaOH, and 45% NaOH for DA, at 600 watts in the microwave for 15 min	72.00	-do-
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 60 mesh size, then DP under 4% NaOH, and 45% NaOH for DA, at 600 watts in the microwave for 15 min	78.00	-do-
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 16 mesh size, then DP with 4% NaOH, and 45% NaOH for DA at 600 watts in the microwave for six pulses of 5 min	81.00	-do-
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 32 mesh size, then DP with 4% NaOH, and 45% NaOH for DA at 600 watts in the microwave for six pulses of 5 min	92.00	-do-
Litopenaeus vannamei	DM with 2% HCl for shrimp shells with 60 mesh size, then DP with 4% NaOH, and 45% NaOH for DA at 600 watts in the microwave for six pulses of 5 min	89.00	-do-
Pink shrimp shells	3% HCl for DM, 4% NaOH for DP, heating at 2-atmospheric pressure in the autoclave for 1 h and then steeping in 40% NaOH for DA for 4 days	97.00	Abdou (2008)
Brown shrimp shells	3% HCl for DM, 4% NaOH for DP, heating at 2-atmospheric pressure in the autoclave for 1 h and then steeping in 40% NaOH for DA for 4 days	92.00	-do-
Pink shrimp shells	3% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, and then autoclaved at 2-atmospheric pressure for 3 h	94.00	-do-
Brown shrimp shells	3% HCl for DM, 4% NaOH for DP, and 40% NaOH for DA, and then autoclaved at 2-atmospheric pressure for 3 h	90.00	-do-

Where DM, DP, DA, and DT are demineralization, deproteinization, deacetylation, and deacetylation temperature, respectively.

II. Biological methods:

The preparation of chitosan from crustacean wastes can be done biologically in addition to chemically. Two approaches are available in the biological approach. The enzymatic method is one approach, and the fermentation process is another.

A. Enzymatic methods

The enzymatic procedure is identical to the previously mentioned chemical approach. The sole distinction is that in the deproteinization and demineralization processes, enzymes such as papain, trypsin, alcalase, and pepsin are employed in place of other chemicals (Yadav, 2009). It is necessary to do further processes, such as centrifugation after enzyme inactivation. The pellet's chitin and the supernatant's protein will be separated by centrifugation. To extract the pure chitin, the pellet is successively rinsed with water, ethanol, and acetone.

Chemical and enzymatic approaches both use acid to remove CaCO3 from the shell, as previously mentioned (Younes et al., 2014). This is the same demineralization mechanism. Yet, this approach uses enzymes for the deproteinization and deacetylation process at a moderate temperature, typically between 25 and 59 o C, in place of an alkaline and high reaction temperature (Younes et al., 2014; Beaney, 2007).

For enzymatic deproteinization, a variety of proteinases have been developed (Ahmed and Ikram, 2017; Kim, 2011). These enzymes are typically extracts from microorganisms or fish entrails, such as the intestines of grey triggerfish (Balistes capriscus) and sardinella (Sardinella aurita) (Younes et al., 2014). Deacetylases, on the other hand, can also be isolated from microorganisms or fish intestines (Ahmed and Ikram, 2017; Kim, 2011; Tsigos et al., 2000; Zhao et al., 2010). One example of this is Alcalase, which was isolated from Bacillus licheniformis (Younes et al., 2015). According to reports, another source of enzymes for deproteinization and deacetylation reactions is genetically modified microbes (Inzali et al., 2017).

B. Fermentation methods

Fermentation uses a variety of microbes, including Lactobacillus plantarum, Pseudomonas aeruginosa, Bacillus subtilis, and others, to produce chitosan (Rao, 2009; Yang, 2009). Prior to using the fermenting procedure, the shrimp waste must be gathered and crushed. Subsequently, distilled water, a suitable carbon source, and the pulverized material are combined, and a suitable microbe for fermentation is cultivated at 370C. The filtrate is separated via filtration following a two to three day incubation period. Proteins are present in the filtrate. The residue, on the other hand, is gathered and largely consists of chitin. After being cleaned, chitin is next converted to chitosan by deacetylation.

The demineralization of the shrimp waste occurs during fermentation and is caused by substances that the fermenting bacteria secrete. For instance, in the fermentation of lactic acid, the calcium carbonate from the waste shrimp combines with the lactic acid generated by the lactic acid-fermenting bacteria to form CaCO3. Washing will then readily remove the precipitated calcium lactate. As is often the case with fish and shrimp waste, the fermentation method's deproteinization of the chitin waste largely happens by autolysis (Hayes et al., 2008; Kandra et al., 2012; Sini et al., 2007).

Most publications employing biological methods for chitin/chitosan preparation have therefore turned to fermentation methods instead of enzymatic methods due to the limitations of the aforementioned methods (Jo et al., 2010; Doan et al., 2019). The classification of fermentation techniques can be made into two subcategories: lactic acid fermentation techniques and non-lactic acid fermentation techniques. This is based on whether the microbial strains employed in the research secrete lactic acid or other organic acids as the acid(s) for the demineralization reaction (Arbia et al., 2013). The fermentation procedures have typically been observed to take seven days or longer to complete (Arbia et al., 2013).

ADVANTAGES AND DISADVANTAGES:

Chemical methods have the advantage of using shorter reaction times, more straightforward production processes (especially when it comes to pre- and post-reaction steps like product purification), and the production of chitosan with medium to lower MW and higher DD (which exhibits stronger biological properties).

Nevertheless, the comparison also demonstrates the drawbacks of chemical methods, as the reaction process typically involves the use of some toxic or corrosive chemicals, such as HCl and NaOH. As a result, the chemical process's byproducts, such as reaction liquors, which contain high concentrations of Na+ due to the use of NaOH, may pose serious environmental risks if they are not disposed of carefully or if they are not easily reused or recycled (Yao, 2012; Arbia et al., 2013).

RESULTS AND DISCUSSION:

The three phases of reaction involved in chemical techniques of chitosan manufacture are demineralization, deproteinization, and deacetylation. Demineralization by using HCl at concentrations as high as 10% w/v to react for two to three hours while stirring in order to remove the CaCO3 from the shell. Deproteinization is

the process of reacting with a hot alkali solution, such as 1%–10% (w/w) aqueous NaOH solution, at temperatures between 65 and 100ºC for 0.5–12 hours, in order to remove the protein and other organic components in the shell aside from chitin. Deacetylation is a process using a 40%–50% (w/w) heated alkali solution, such as NaOH solution, to convert chitin to chitosan. Besides chemical methods, biological methods (i.e. enzymatic methods and fermentation methods) are also available to prepare chitosan from crustacean byproducts

The sources of chitin and the strains/enzymes utilized to prepare chitin and chitosan were thoroughly outlined in earlier research discovered that the biologically produced chitin/chitosan often only achieves 70% - 90% CaCO3 removal and 40% - 94% protein removal, requiring 3-7 days to complete the entire process. An extra step of product purification by a cycle of chemical methods must be added after the biological reaction process is finished in order to further remove the leftover CaCO3 and proteins from the chitin/chitosan products because biological methods do not always remove CaCO3, protein impurities, and acetyl functional groups in shells as thoroughly as chemical methods do.

CONCLUSION:

Based on the prediction analysis, the concentration of alkali used for deacetylation is the most important parameter. The concentrations of acid and alkali used for demineralization and deproteinization are the most important parameters, respectively.

REFERENCES:

1. Abdou, E.S., Nagy, K.S.A., Elsabee, M.Z. (2008). Extraction and characterization of chitin and chitosan from local sources. *Bioresour. Technol.,* 99(5), 1359– 1367.
2. Ahmad, S.I.; Ahmad, R.; Khan, M.S.; Kant, R.; Shahid, S.; Gautam, L.; Hasan, G.M.; Hassan, I. (2020). Chitin and its derivatives: Structural properties and biomedical applications. *Int. J. Biol. Macromol.* 2020, 164, 526–539. [CrossRef]
3. Ahmed S. and Ikram S. (2017). Chitosan: Derivatives, Composites and Applications, 1st ed., John Wiley & Sons, Incorporated, *Hoboken*, NJ, 2017.
4. Al Sagheer, F.; Al-Sughayer, M.; Muslim, S.; Elsabee, M. (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydr. Polym*. 2009, 77, 410–419. [CrossRef]
5. Arbia W., Arbia L., Adour L., and Amrane A. (2013). Chitin extraction from crustacean shells using biological methods - A review. *Food Technology and Biotechnology* 51(1) (2013) 12-25.
6. Azuma, K.; Ifuku, S.; Osaki, T.; Okamoto, Y.; Minami, S. (2014). Preparation and Biomedical Applications of Chitin and Chitosan Nanofibers. *J. Biomed. Nanotechnol.* 2014, 10, 2891–2920. [CrossRef]
7. Beaney P.D., Gan Q., Magee T.R.A., Healy M., and Lizardi-Mendoza J. (2007). Modification of chitin properties for enzymatic deacetylation. *Journal of Chemical Technology & Biotechnology* 82(2) (2007) 165-173.
8. Chang, C.C.; Chang, K.C.; Lin, W.C.; Wu, M.H.(2017). Carbon footprint analysis in the aquaculture industry: Assessment of an ecological shrimp farm. *J. Clean. Prod.* 2017, 168, 1101–1107. [CrossRef]
9. Doan C.T., Tran T.N., Vo T.P.K., Nguyen A.D., and Wang S.L., (2019). Chitin extraction from shrimp waste by liquid fermentation using an alkaline protease-producing 39 strain, Brevibacillus parabrevis. *International Journal of Biological Macromolecules* 131 (2019) 706-715.
10. Elieh-Ali-Komi D and Hamblin M.R. (2016). Chitin and chitosan: Production and application of versatile biomedical nanomaterials. *International Journal of Advanced Research* 4(3) (2016) 411.
11. Hajji, S., Ghorbel-Bellaaj O., Younes I., Jellouli K., Nasri M., (2015). Chitin extraction from crab shells by Bacillus bacteria. Biological activities of fermented crab supernatants. *Int. J. Biol. Macromolec.,* 79, 167–173.
12. Hayes M, Carney B, Slater J, Brück W. (2008). Mining marine shellfish wastes for bioactive molecules: Chitin and chitosan Part A: extraction methods. *Biotechnology Journal: Healthcare Nutrition Technology*, 2008; 3(7):871-7. doi: 10.1002/biot.200700197.
13. Hossain M and Iqbal A (2014). Production and characterization of chitosan from shrimp waste. *J. Bangladesh Agric. Univ.* 2014, 12, 153–160. [CrossRef]
14. Hossain S and Uddin K (2020). Isolation and Extraction of Chitosan from Shrimp Shells. *Int. J. Adv. Res.* 2020, 8, 657–664. [CrossRef]

15. Huang M. (2004). Uptake and cytotoxicity of chitosan molecules and nanoparticles: Effects of molecular weight and degree of deacetylation. *Pharmaceutical Research,* 21(2), 344-353.
16. Inzali H., Aung S.P.S., Win K.C., Le Nu W., Ngwe H., and Nwe N. (2017). Enzymes from Genetically Modified Microorganisms for Production of Chitin, Chitosan, and Chitooligosaccharide, Chitosan: Derivatives, Composites and Applications, *John Wiley & Sons, Newark, NJ*, 2017, pp. 167-181.
17. Islam, A.; Islam, M.; Zakaria, M.; Paul, S.; Mamun, A.(2019). Extraction and Worth Evaluation of Chitosan from Shrimp and Prawn Co-products. *Am. J. Food Technol.* 2019, 15, 43–48. [CrossRef]
18. Jo G.H., Park R.D., and Jung W.J. (2010). Enzymatic production of chitin from crustacean shell waste, in: S.-K. Kim (Ed.), Chitin, Chitosan, Oligosaccharides and Their Derivatives, *Taylor & Francis, Boca Raton, FL*, 2010, pp. 37-45.
19. Kandra P, Challa MM, Jyothi HKP.(2012). Efficient use of shrimp waste: present and future trends. *Applied Microbiology and Biotechnology*, 2012;93(1):17-29. doi: 10.1007/s00253-011-3651-2.
20. Kean T and Thanou M., (2011). Chitin and chitosan: Sources, production and medical applications, in: P.A. Williams (Ed.), Renewable resources for functional polymers and biomaterials: Polysaccharides, proteins and polyesters, *Royal Society of Chemistry, Cambridge, England*, 2011, pp. 292-318.
21. Kim S.K. (2011). Chitin, chitosan, oligosaccharides and their derivatives: Biological activities and applications. *CRC Press, Boca Raton, FL*, 2011.
22. Kumar, M.Y. and Ravi, A. (2017). Extraction and Characterization of Chitosan from Shrimp Waste for Application in the Feed Industry. *Int. J. Sci. Environ. Technol.* 2017, 6, 2548–2557. Available online: www.ijset.net (accessed on 13 July 2022).
23. Lertsutthiwong, P., How, N.C., Chandrkrachan, S. and Stevens, W.F. (2002). Effect of chemical treatment on the characteristics of shrimp chitosan. *Journal of metals, materials and minerals,* 12(1), 11-18
24. Marei, N.H., Abd El-Samie, E., Salah, T., Saad, G.R., and Elwahy, A.H.M., (2016). Isolation and characterization of chitosan from different local insectsin Egypt. *International Journal of Biological Macromolecules,* 82, 871–877.
25. No H.K. and Meyers S.P. (1995). Preparation and characterization of chitin and chitosan - A review. *Journal of Aquatic Food Product Technology* 4(2) (1995) 27-52.
26. No, H.K. and Hur, E.Y.(1998). Control of Foam Formation by Antifoam during Demineralization of Crustacean Shell in Preparation of Chitin. *J. Agric. Food Chem.* 1998, 46, 3844–3846. [CrossRef]
27. Patria A. (2013). Production and Characterization of Chitosan from Shrimp Shells Waste. *AACL Bioflux* 2013, 6, 339–344. Available online: http://www.bioflux.com.ro/aacl (accessed on 15 July 2022).
28. Percot A.; Viton C. and Domard A.(2003). Characterization of Shrimp Shell Deproteinization. *Biomacromolecules.* 2003, 4, 1380–1385. [CrossRef]
29. Pujari N. and Pandharipande S.L. (2016). Review on Synthesis, Characterisation and Bioactivity of Chitosan. IJESRT *Int. J. Eng. Sci. Res. Technol*. 2016, 5, 334. [CrossRef]
30. Puvvada Y. S., Vankayalapati S., and Sukhavasi S. (2012). Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. *International Current Pharmaceutical Journal,* 1(9), 258-263.
31. Raghvendrakumar M., Yadu Nath V., Aswathy V., Parvathy P., Sunija S., Neelakandan M., Nitheesha S. and Vishnu K. (2017). Chitosan as promising materials for biomedical application: Review, Research & Development in Material Science 2(4)(2017) 16.
32. Rao MS, Munoz J, and Stevens WF (2000). Critical factors in chitin production by fermentation of shrimp biowaste. *Applied Microbiology and Biotechnology*, 2000; 54(6):808-13. doi: 10.1007/s002530000449.
33. Rasweefali M.; Sabu, S.; Azad, K.M.; Rahman, M.R.; Sunooj, K.; Sasidharan, A. and Anoop, K. (2022). Influence of deproteinization and demineralization process sequences on the physicochemical and structural characteristics of chitin isolated from Deep-sea mud shrimp (Solenocera hextii). *Adv. Biomark. Sci. Technol.* 2022, 4, 12–27. [CrossRef]
34. Rinaudo, M.(2006). Chitin and chitosan: Properties and applications. *Prog. Polym.* Sci. 2006, 31, 603–632. [CrossRef]
35. Rodde, R.H., Einbu, A., and Vårum, K.M. (2008). A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (Pandalus borealis). *Carbohydr. Polym.,* 71(3), 388–393.
36. Santos V.P.; Maia P.; Alencar N.D.S.; Farias L.; Andrade R.F.S.; Souza D.; Ribaux D.R.; Franco L.D.O. and Campos-Takaki G.M. (2019). Recovery of chitin and chitosan from shrimp waste with microwave technique and versatile application. *Arq. Inst. Biol.* 2019, 86, 1–5. [CrossRef]

37. Sini TK, Santhosh S and Mathew PT. (2007). Study on the production of chitin and chitosan from shrimp shell by using Bacillus subtilis fermentation. *Carbohydrate Research,* 2007; 342(16):2423-9. doi: 10.1016/j.carres.2007.06.028.
38. Tsigos I., Martinou A., Kafetzopoulos D.and Bouriotis V. (2000). Chitin deacetylases: New, versatile tools in biotechnology, *Trends in Biotechnology* 18(7) (2000) 305- 312.
39. Varlamov V.P.; Ilina A.V.; Shagdarova B.T.; Lunkov A.P. and Mysyakina I.S. (2020). Chitin/Chitosan and Its Derivatives: Fundamental Problems and Practical Approaches. *Biochemistry* .2020, 85, 154–176. [CrossRef]
40. Yadav M, Goswami P, Paritosh K, Kumar M, Pareek N, and Vivekanand V. (2019). Seafood waste: A source for preparation of commercially employable chitin/chitosan materials. *Bioresources and Bioprocessing,* 2019;6(1):8. doi: 10.1186/s40643-019-0243-y.
41. Yang JK, Shih L, Tzeng YM and Wang SL. (2000). Production and purification of protease from a Bacillus subtilis that can deproteinize crustacean wastes. *Enzyme and Microbial Technology,* 2000; 26(5-6):406-13. doi: 10.1016/s0141-0229(99)00164-7.
42. Yao K (2012). Chitosan-based hydrogels: Functions and applications, *CRC Press, Boca Raton, FL,* 2012.
43. Younes I., and Rinaudo M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications, *Marine Drugs* 13(3) (2015) 1133-1174.
44. Younes I., Hajji S., Frachet V., Rinaudo M., Jellouli K. and Nasri M. (2014). Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. *International Journal of Biological Macromolecules* 69 (2014) 489-498.
45. Younes I. and Rinaudo M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs,* 13, 1133–1174.
46. Zhao Y., Park R.-D., and Muzzarelli R.A.A. (2010). Chitin Deacetylases: Properties and Applications. *Marine Drugs* 8(1) (2010) 24.