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Review Article

A REVIEW: NATURALLY AVAILABLE SOURCES OF CHITOSAN AND ANALYSIS OF CHITOSAN DERIVATIVES FOR ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Chitosan is a natural polymer which can be derived from chitin by deacetylation process. Chitin is naturally found in the shells of fish, prawn, shrimp etc. which are being generated regularly as waste from fish industries as well as from household garbage that not only effects the environment but also human health. It can also be extracted from bacteria and fungi by an enzyme called chitosanases present within the species. Chitosan has many applications in pharmaceutical, agricultural, food and water industry. Production of chitosan by these sources are not only cheap but can also reduce the environmental problems to some extent. Also chitosan and its derivatives are proposed to have antimicrobial activity This study provides an overview about a variety of sources that has been used for chitosan production and its applications with antibacterial activity.

INTRODUCTION

In recent years pollution has come out to be one of a major environmental problem. Pollutants are basically the waste materials and can either be a foreign substances or naturally occurring contaminants. These pollutants not only come from the industries and the chemical compounds but also from daily household wastes. Fish or prawn shells are one of those wastes which are not only generated from household garbage on a daily basis but also from fish processing industries. These industries generate a huge amount of shell waste per processing which usually cause environmental hazard. It is estimated that the shell-fish industry produces about 60,000-80,000 tons of waste. Though these wastes are biodegradable but disposal of this huge amount of waste can be a serious environmental problem. Over the period of time these wastes can not only produce obnoxious smell but also attract pathogenic insects, flies and rodents, thus creating an unhygienic atmosphere (Tarafdar *et al.* 2013). As these fish shell wastes contains chitin, protein and minerals so by a quick recycling process chitin can be extracted from the shells. Further this chitin, which is the second most abundant natural biopolymer after cellulose can produce chitosan. Chitosan is a natural linear polysaccharidepolymer, consist of chitin-monomer and chitosan- monomer. It is composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (figure1). Chitosan is produced commercially by deacetylation of chitin and the percentage of Degree of Deacetylation in commercial chitosans ranges from 60 to 100%. This reaction occurs in two stages under first-order kinetic control. Activation energy for the first step is higher than the second; and its estimated value is 48.76 kJ/mol at 25-120°C. This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product. The molecular weight of commercially produced chitosan is between 3800-20,000 Daltons. The amino group in chitosan has a pKa value of ~6.5, which leads to addition of proton in acidic group to change in neutral solution with a charge density dependent on pH and the percentage of Degree of Deacetylation value. This makes chitosan water-soluble. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable.

Further research also revealed that chitosan is a fiber like substance which differs with cellulose only by the amine (-NH2) group in the position C-2 of chitosan instead of the

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hydroxyl (-OH) group found in cellulose. As chitosan is positively charged it also possesses the ability to bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules (Li *et al.* 1992). Not only fish shells but chitosan can also be isolated from microorganisms, which can be a fungus or a bacterium. It involves isolation of particular bacteria or fungi from specific locations and these strains need further development and extraction for chitosan isolation.

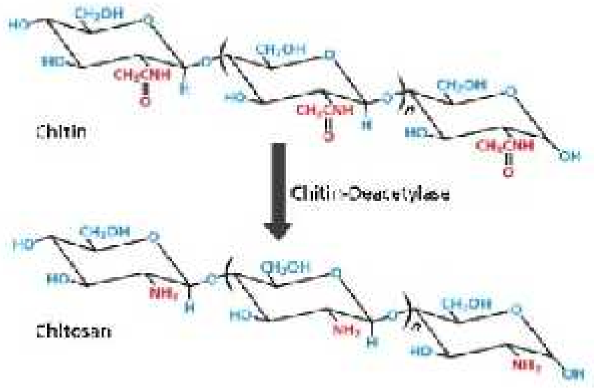

Fig 1 Chemical structure of chitosan

An Overview on Available Sources of Chitosan

Among the various sources available for chitosan production, prawn is one of the most promising and highly discussed. In study different sources of prawn, like *Penaeus monodon* commonly known as Giant Tiger Prawn and *Penaeus indica* or Indian prawn (Tarafdar *et al.* 2013) and *Fenneropenaeus indicus* (Paul *et al.* 2014) were observed for chitosan extraction. Shrimps are also one of promising source of chitosan obtained from *Penaeuscarinatus* and *Penaeusmonodon* specimens (Puvvada *et al.* 2012). Not only shrimps and prawns but some crab also releases chitosan and *Sesarma plicatum* is one of those species. This chitosan shows the antimicrobial activity against the fish pathogenic bacteria (*Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *V. cholerae*, *Micrococcus sp.*, and *Streptococcus sp.*,) and fungi (*Aspergillus flavus* & *Rhizopus sp.*,) (Sakthivel *et al.* 2015). As previously mentioned microorganisms like fungi and bacteria can also release chitosan. Various strains of *Aspergillus Niger* MTCC which includes *Aspergillus niger* MTCC 872, *Aspergillus niger* MTCC 1785 and *Aspergillus niger* MTCC 2208 and *Salmonella typhi*, *Salmonella paratyphi*-A, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were obtained for chitosan production (Kumaresapillai *et al.* 2010). Some researchers also isolated bacterial strains like *Bacillus sp.* and *Serratia sp* (Kaur *et al.* 2012) which also showed chitosan degrading ability with a yield of 16% and 10% respectively. These fungal or bacterial strains release chitosan because they show chitin deacetylase activity and contain chitosanase enzyme. It can be said that the strains used in this work are promising sources of chitin and these strains can be adapted by industries for producing a good quality and economically cheap chitosan.

The above discussed sources has different physical properties which varies from species to species. These parameter are discussed in the following table (table 1).

Fig 2 Schematic diagram of naturally available sources

Applications of Commercially and Biologically Available Chitosan

Aaccording to researchers chitosan has unique biocompatible, non-antigenic, non-toxic, bio-functional and bio-degradable characteristics and thus also has the ability to form gels and can be transformed into films with controlled permeability and good mechanical properties. Due to this chitosan has been considered as a material with potential applications in several fields such as in pharmaceutical industry such as wound healing agents, drug carriers and in drug delivery systems, drug targeting polymer, as a potential biomaterial for nerve repair. It can also be used as a chelating agent for heavy metal adsorption, membrane filter for water treatment and bio-degradable coating or film for food packaging and food preservation, for water purification etc (Paul *et al.* 2014). Commercially it also has many application in agricultural, water filtration, horticulture, food technology (Gavhane *et al.* 2013). It can also be employed in production of biofertilizers and biopesticides of economical benefits. It also has fat blocking property, anti viral, antimicrobial, antifungal, radical scavenging activity and antioxidant activity. The above discussed biological soures of chitosan also has many useful industrial applications. Some of them are discussed below.

Chitosan extracted from prawn shells shows properties like antibacterial, antifungal and anti-oxidant activity of this chitosan can be used in food industries and also as natural additives. It has been used for development of antimicrobial films for use in packaging materials for foods, medical supplies, or as laminated coating. The anti-coagulant activity is useful in biomedical applications like wound dressing, surgical sutures and for other treatments like reducing oxidative stress in live cells, antitumor activity, anti-inflammatory effect, HIV-1 inhibitors, antihypertensive, hypoglycemic and hypolipidemic effect etc. (Tarafdar *et al.* 2013, Paul *et al.* 2014). Due to its physicochemical properties and excellent antimicrobial property it can be used in various fields of pharmaceutical industry, food packaging, water treatment, drug delivery and also against human pathogens. From the above table it can be said the chitosan from shrimp shell is of a good quality. As the value of Degree of Deacetylation (89.79%) is high it shows high amount of protein and high quality yield of chitosan which is suitable for the pharmaceutical application. The viscosity of the prepared chitosan falls under the category which is suitable for drug delivery (table 1).

Table 1 Different physical parameters of chitosan produced from different sources

PARAMETER	PRAWN *Fenneropenaeus indicus*	SHRIMP *Panaeusmonodon*	FISH *Labeo rohita*	CRAB *Sesarma plicatum*	FUNGI *Aspergillus Niger* MTCC Strains	BACTERIA *Bacillus sp/ Serratia sp*
Yield	57.69%	34%	7.72%	41.37%		16% / 10%
Molecular weight	159653g/mol.	1,599,558.029	$1.01*10^5$ g/mol.	-	-	-
Moisture content	4%		-	-	10.9%	-
Ash value	1.86	0.25%	-	-	0.89%	-
Loss on drying	2%	9.34	-	-	-	-
Ph	6.7	8.5	-	-	-	-
Solubility	Acetic acid		-	-	Acetic acid	-
Degree of deacetylation	87%	89.79%	78.2%	-	85%	-

Also in pharmaceutical industry this chitosan has applications in the wound healing, vaccine for oral, parenteral, nasal, buccal, transdermal, vaginal, topical etc., drug delivery system, adsorption, covalent linking, encapsulation. It can also be formulated as nanoparticles, microspheres, membrane sponge etc. (Puvvada *et al.* 2012).

Fig 3 Applications of chitosan extracted from various sourcs

Antimicrobial Activity of Chitosan and Its Derivatives

Though chitosan shows all the above mentioned characteristics and it also involves different mode of actions to achieve this but all the mechanism are not fully understood Considering the fact that chitosan shows biocidal activity it can be said that the interaction between positively charged chitosan and negatively charged microbial cell membranes is the most acceptable antimicrobial mechanism and the most widely studied (Tsai and Su, 1999; Goy *et al.*, 2009). This activity mainly depends on factors, such as concentration, degree of deacetylation, molecular weight, the solvent used and pH of chitosan solution. When the pH of chitosan is lower than it's pKa value it shows a positive charged density. Here the protonated amino groups ($NH3+$) at the C2 position in the glucose monomer of chitosan chains forms a polycationic structure, which interacts with anionic compounds and macromolecular structures of bacteria. Due to this interaction the bacterial surface gets altered, membrane permeability increases or even decreases, and promotes leakage of intracellular substances (e.g., proteins including lactate dehydrogenase, nucleic acids and glucose). It was suggested that positively charged chitosan which contains protonated $NH3+$ sites interacts with cellular DNA thereby allowing chitosan to transport into the cells and finally inhibits the transcription. According to researchers the chitosan-derivatives which are free of quaternization have good solubility in aqueous solution at neutral pH and shows excellent antimicrobial activity (Martins *et al.* 2014).

A derivative of chitosan, N,N,N-trimethylchitosan (also known as salt form), is particularly found to be interesting for uses in antimicrobial applications because it has a higher density of permanent positive charges in the chain, hence, a stronger electrostatic interaction with the microorganism's walls (Sadeghi *et al.*, 2008; Silva *et al.*, 2010). TMC is soluble in a wide range of pH and forms films with good mechanical properties (Britto and Assis, 2007b). This TMC can be obtained by the covalent addition of a quaternary ammonium group (Curti *et al.*, 2003), or by methylation of the primary amine groups in the parent polymer (Britto and Assis, 2007a). Researchers concluded that the antimicrobial activity of commercial chitosan is lower than that of TMC in all concentrations which were tested (Sadeghi *et al.*, 2008; Goy *et al.*, 2009). The main feature which made the difference between the two is the density of charges. Also the commercial chitosan has an intrinsic pKa value around 6.5 and approximately 24% of its primary amine groups are positively charged in acid medium, whereas all primary and quaternized amine groups of TMC are protonated, even in neutral solution (Mao *et al.*, 2006). Other than the quaternary salts, aqua-soluble derivatives like hydroxypropyl and carboxymethyl chitosan also exist. Hydroxypropyl chitosan derivativeshas high degree of substitution (DS) and are water insoluble, but after influencing with maleic acid they become soluble in neutral pH with antibacterial activity higher than that of commercial chitosan.

DISCUSSION

This naturally occurring product has gained a great attention in recent years due to its huge number of applications in various fields. From the above study it can be concluded that various products can be generated using waste shells as starting materials. Not only fish shells but the above discussed bacterial or fungal strains can also be genetically manipulated for getting better results to be used by the industries in economical chitosan production as an alternative to fish shells. The Chitosan produced from these sources were observed to have many important properties with commercial applications, clinical and pharmaceutical uses. Still many research is going on about this subject as chitosan has shown to be a very promising biomaterial with a great scope of industrial applications and in many other fields.

References

Britto D. and Assis O.B.G. 2007a A novel method for obtaining a quaternary salt of chitosan. *Carbohydrate Polymers*, 69(2) 305-310.

Britto D. and Assis O.B.G. 2007b Synthesis and mechanical properties of quaternary salts of chitosan - based films for food application. *International Journal of Biological Macromolecules*, 41(2) 198-203.

Fng De-Zeng, Jun Liu, Huan Yan 2013 Mechanism Study on Flocculating Organnic Pollutants By Chitosan with Different Molecular In Wastewater. *American Journal of Engineering Research*. 02 91-95.

Gavhane Y N, Gurav A S and Adhikrao Y V 2013 Chitosan and Its Applications: A Review of Literature.

International Journal of Research in Pharmaceutical and Biomedical Sciences. 4(1) 312-331.

Goy, R. C., Britto, D. and Assis, O. B. G. 2009 A review of the antimicrobial activity of chitosan. *Polímeros: Ciência e Tecnologia.* 19(3) 241-247.

Hadi A G 2014 Metal Anion Removal Cr(Vi) from Wastewater using chitosan extracted from fish shells. *Journal of Babylon University/Pure and Applied Sciences*.22 1799-1804.

Hossain MS, Iqbal A 2014 Production and characterization of chitosan from shrimp waste. *Journal of the Bangladesh Agricultural University* 12(1) 153–160.

Jia, Z.; Shen, D.; Xu, W. 2001 Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydrate. *Research journal* 333 1–6.

Kaur K, Dattajirao V, Shrivastava V, Bhardwaj U 2012 Isolation and Characterization of Chitosan-Producing Bacteria from Beaches of Chennai, India. Hindawi Publishing Corporation. *Enzyme Research*.1-6.

Kumaresapillai N, Bashab RA, Sathish R 2010 Production and Evaluation of Chitosan from *Aspergillus niger* MTCC Strains. *Iranian Journal of Pharmaceutical Research*. 10(3) 553-558

Li, Q., Dunn, ET., Grandmaison, EW., Goosen, MFA 1992 Applications and properties of chitosan. *Journal of Bioactive and Compatible Polymer*. 7:370-397.

Martins F Alessandro, Facchi P Suelen, Follmann DM Heveline, Pereira GB Antonio, Rubira F Adley and Muniz C Edvani 2014 Antimicrobial Activity of Chitosan Derivatives Containing N-Quaternized Moieties in Its Backbone: A Review. *International Journal of Molecular Sciences*. 15 20800-20832.

Muslim T, Rahman MH, Begum HA, Rahman MA 2012 Chitosan and Carboxymethyl Chitosan from Fish Scales of *Labeo rohita. Dhaka University Journal Science* 61(1) 145-148.

Paul S, Jayan A, Sasikumar CS, Cherian SM 2014 Extraction and purification of chitosan from chitin isolated from sea prawn (*Fenneropenaeus indicus*). *Asian journal of pharmaceutical and clinical research*.7 201-204.

Prashanth, KVH.; Tharanathan, RN 2007 Chitin/chitosan: Modifications and their unlimited application potential—An overview. *Trends Food Science Technology*. 18 117–131.

Puvvada YS, Vankayalapati S, 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. Rajalakshmi A, Krithiga N, Jayachitra A 2013 Antioxidant

Activity of the Chitosan Extracted from Shrimp Exoskeleton. *Middle-East Journal of Scientific Research*. 16(10) 1446-1451.

Sadeghi, A. M. M., Amini, M., Avadi, M. R., Siedi, F., Rafiee-Tehrani. M. and Junginger, H. E., 2008 Synthesis, characterization, and antibacterial effects of trimethylated and triethylated 6-NH2-6 deoxy chitosan.

Journal of Bioactive and Compatible Polymers, 23(3) 262-275.

Sakthivel D, Vijayakumar N, Anandan V 2015 Extraction of Chitin and Chitosan from Mangrove Crab *Sesarma plicatum* from Thengaithittu Estuary Pondicherry Southeast Coast of India. *Human Journals Research Article*.4 12-24.

Silva, L. P., Britto, D., Seleghim, M. H. R. and Assis, O. B. G. 2010 In vitro activity of water-soluble quaternary chitosan chloride salt against E.coli. *World Journal of Microbiology and Biotechnology*, 26(11) 2089-2092.

Sun Y, Zhang J, Wang S 2009 Purification and characterization of the chitosanase from *Aeromonas* sp. HG08. *African Journal of Biotechnology*. 8(12) 2830-2834.

Tarafdar A, Biswas G 2013 Extraction of Chitosan from Prawn Shell Wastes and Examination of its Viable Commercial Applications. *International Journal on Theoretical and Applied Research in Mechanical Engineering*. 2 17-24.

Tsai, G. J. and Su W. H. 1999 Antibacterial activity of shrimp chitosan against Escherichia coli. *Journal of Food Protection,* 62(3), 239-243.

Zakaria MB, Zakaria SN, Musa M, Hamilin H, Zulkifly NSH 2012 Identification of Chitosan-Degrading Microbes for the Production of Chitooligomer. 3rd International Conference on Chemistry and Chemical Engineering. 38 127-131.

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