Chronicle of Bioresource Management 2021, 5(4):170-174

Chronicle of Bioresource Management
An International E-magazine

December 2021 | Popular Article

Open Access

Corresponding Author
S. Lokesh
e-mail: kasturifish@gmail.com

Citation: Lokesh et al., 2021. Chito-Oligosaccharides: Derivations and Bio-Perspectives. Chronicle of Bioresource Management 5(4), 170-174.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

Keywords:
Biopreservative, hydrolysis, immunostimulant, prebiotic

Article History
Article ID: CBM92
Received on 09th November 2021
Received in revised form on 03rd December 2021
Accepted in final form on 20th December 2021

Chito-Oligosaccharides: Derivations and Bio-Perspectives

S. Lokesh*, Kasturi Chattopadhyay and K. A. Martin Xavier

Abstract

Natural biopolymers are a potent reserve for ecologically safe food material. Being available from replenishable resources and higher biocompatibility and biodegradability, they fetch a huge demand to be exploited as a health constructive agent in several industries. Chito-oligosachharides are very short chained water soluble compounds, often called as chitosan oligomers or chitooligomers, prepared from partial hydrolysis of unstable glycosidic bonds of chitosan. Chemical and enzymatic hydrolysis are the two hydrolytic methods employed to prepare the oligomers from chitin and chitosan. Smaller molecular size, lower viscosity and greater solubility in aqueous solutions, have attracted the interest of many researchers to utilize them further for various biological applications including food and medicine. Inherent anti-microbial properties and film-forming ability of chitin oligomers make it an ideal natural polymer that can be an alternative to chemically synthesized antimicrobial agents used in food industries.

1. Introduction

Chitin is a bio-polymer derived from crustacean shell wastes which is water insoluble and its partial deacetylation yields chitosan which is a useful cationic polysaccharide. Further, hydrolysis (depolymerization) of chitosan yields very short chained water soluble compounds called chitooligosaccharide (COS) or chitosan oligomers or chitooligomers. Chemically it is a homo- or heterooligomers of N-acetyl-D- glucosamine (GlcNAc) and D-glucosamine (GlcN) linked by β-1,4-O-glycoside bond and usually prepared from partial hydrolysis of unstable glycosidic bonds of chitosan (Jung and Park, 2014). Smaller molecular size, lower viscosity and greater solubility in aqueous solutions, along with other useful properties of these oligo-saccharides have been listed as figure 1 (A and B) that have attracted the interest of many researchers to utilize them further for various biological applications including food and medicine. In terms of biomedical applications, chitin derivatives in several forms including crystallites are applied as biomaterials for artificial bones, cartilage, skin regeneration and drug delivery agents (Dash et al., 2011; Parvez et al., 2012). They are also used as biosorbent for wastewater treatment (Nechita, 2017),

Author's Address
ICAR- Central Institute of Fisheries Education (CIFE), Mumbai, Maharashtra (400 061), India

plant elicitors in agricultural production (Katiyar et al., 2014) and as food preservatives in the form of edible films (Azuma et al., 2015, Muzzarelli and Muzzarelli, 2005) Cost of these oligosaccharides is around Rs. 200-250 kg^{-1} commercially which is quite reasonable. However they may vary in prices depending on the quality.

2. Methods of Preparations

Chemical hydrolysis by using acids and enzymatic hydrolysis are the two hydrolytic methods employed to prepare the oligomers from chitin and chitosan. To assist the chitin hydrolysis and to enhance the yield of the chitin oligomer different treatments in the presence of acids or enzymes under controlled conditions is done.

2.1. Physical treatment

Chitin shells are refined by conventional chemical treatments by removing protein and minerals by aqueous sodium hydroxide and hydrochloride treatments, respectively. The resultant mixture undergoes mechanical disintegration carried out by a pair of blender or grinder to form oligomers. Chitin slurry turned into gel indicating disorganization due to the large surface-to-volume ratio formed after one phase of mechanical grinder treatment. This method exhibits efficient hydrolysis with minimum residual waste however some limitations such as occurrence of deacetylation, the lower degree of polymerization (DP), and formation of dimer as an adverse effect on bioactivity.

2.1.1. Ultrasonication

Ultrasonication is a process used to intensify chemical or enzymatic hydrolysis. During acid hydrolysis, chitin is mixed with HCl and sonicated at 50 or 60 Hz (275W) which completely dissolved within 30 minutes in HCl solution. It is found to be advantageous, as it preserves the chemical nature of the polysaccharide by breaking the sensitive chemical bonds and reducing its molecular weight.

2.1.2. Microwave irradiation

The irradiation technique is the most established technique to produce chitin oligomers. Here the chitin is mixed with Hcl and irradiated in a microwave device at 700 to 2100 W for around 24 hours. This method is modified by implying a pre-warming technique where 38% HCl was slightly warmed at 850 W in an oven for a particular period followed by adding chitin powder and further irradiated at 57.5 °C for 38 mins. This irradiation technique was applied prior to enzymatic hydrolysis for efficient cleavage of chitin.

2.1.3. Gamma irradiation

Polymeric chitin irradiated at different doses varying from 15 to 210 kGy, to accelerate chitin hydrolysis, prior to depolymerization with chitinases. Gamma irradiation was found to be superior to other methods due to the high hydrolysis rate, improved chitin properties, and no additives used for the irradiation process.

2.1.4. Supercritical water

Polymeric chitin reacted with supercritical water at a temperature above 374°C followed by mechano-chemical grinding by ball mill which resultant in easy degradation. The resultant mixture acts as an effective substrate for hydrolysis due to its lower particle size.

2.1.5. Steam explosion

Steam explosion applied to the treated mixture of chitin powder and distilled water at 180 °C and 1 MPa for different durations. The resultant chitin suspension was then mixed with the buffer that acted as a suitable substrate for chitin hydrolysis with chitinase. It can significantly lower the crystallinity without undergoing sufficient depolymerisation.

2.2. Chemical treatment

Aqueous solutions like phosphoric acid, hydrochloric acids, alkaline solution, and methanol decrystallise chitin increases solubility and accelerate hydrolysis. Chitin oligomers produced by acid hydrolysis of chitin employing strong inorganic acids such as concentrated hydrochloric acid, aqueous sulfuric acid, phosphoric acid, or methane sulfonic acid to cleave polymeric chitin chain. The acid strength, incubation time, and temperature are the key parameters in the process. Acid strength ranging from 3 to 12 N assists to hydrolyze chitin at temperatures ranging between 20 and 90 °C for time durations varying between 5 min and 7 h. Acids bring out the dissolution of firm fibril structure resulting in cleavage of glycosidic bonds of native chitin. The optimal concentration for the preparation of chitin oligomer is 2.5 to 3 N of Hydrochloric acid. The method involves adding the chitin powder to an acid solution and stirring the mixture under reflux in a water bath kept at the desired temperature. The hydrolysis is stopped by cooling the reaction mixture in an ice bath or on dry ice, following which, the chitin oligomers are isolated by: 1) Lyophilization under vacuum, 2) re-dissolving the dried product in deionized water, and 3) neutralizing the solution with NaOH followed by filtration to remove

impurities from the oligomers. These steps are repeated to remove the residual hydrochloric acids.

The chitin before depolymerization consists of alternating crystalline and amorphous regions. Initially, the amorphous regions are rapidly hydrolyzed within 5 min to produce regular-sized segments with a central crystalline region attached to amorphous tails at both ends. The amorphous tails are then gradually degraded, leading to the accumulation of chitin oligomers, as well as a crystalline chitin core consisting of multiple chitin chains. Single chitin chains may then be slowly separated from the chitin core, and once separated, be rapidly hydrolyzed to yield chitin oligomers within 30 to 60 min. Although the hydrolysis process found to be effective, some of the disadvantages such as the occurrence of deacetylation (that produces chitosan oligomers instead of chitin oligomers), production of acidic waste streams, the lower yield of the high degree of polymerization (DP) oligomers, and requiring skilled labour force for purification.

2.3. Enzymatic treatment

Higher DP chitin oligomers can be produced under milder conditions using chitinolytic enzymes. Many Bacterium such as *Serratia proteamaculans* 568, *Serratia marcescens* 2170, *Rhizobium* sp. GRH2, *Bacillus cereus* TKU027 used to produce chitinases. A plant enzyme, hevamine possesses both chitinase and lysozyme activities. Chitinase can also be extracted from fungi *Lecanicillium lecanii* and *Lecanicillium fungicola*. While *Trichoderma reesei* fungi are reported to have hydrolases (cellulases and β-glucanases). In addition, non-chitinase enzymes like cellulase, hemicellulase, pepsin, papain, lysozyme, and pectinase hydrolyze chitin to yield chitin oligomers. Prior to hydrolysis, the substrate is prepared by adding chitin powder to a phosphate or acetate buffer solution, so that its concentration is between 0.5 and 2.0% and pH 5.0 to 5.5. The enzyme is subsequently mixed with the substrate at an appropriate amount so that, the hydrolysis by chitinases, lysozyme, pectinase, and pepsin requires incubation at temperatures between 37 and 44 °C. To cease the reaction, the hydrolysis mixture is heated to 90 °C or boiled for 10 min, and subsequently centrifuged and filtered to separate the supernatant containing the oligomers and unhydrolysed chitin.

During chitin hydrolysis, the enzymes will degrade the polysaccharide chain by enzymes like endo-chitinases or exo-chitinases. Endo-chitinases involves random cleavage of glycosidic linkages of chitin, generating free ends and Chitin oligomers, while exo-chitinases release dimers (two units of GlcNAc) from the reducing (C1) or non-reducing (C4) ends. End products of endo and exoenzymes are oligomers and N-acetylglucosamine.

Enzymes exhibit some disadvantages: specific enzymes such as chitinase and chitosanases are not readily available commercially and tend to be very expensive. Furthermore, the presence of protein residues after hydrolysis potentially limits biomedical application due to possible allergen and pyrogenicity, which will warrant significant further purification that will make the whole process economically unviable. However, this method has a key advantage of minimal chemical waste productions during hydrolysis.

3. Bio-Applications: Food and Pharmaceutics

COS can be used in the food industry as packaging material (packaging films) and food preservative due to its inherit antioxidant activity as well as antimicrobial activity against different microbes (*E coli, B cereus, S aureus, Serratia liquefaciens, Lactobacillus plantarum* etc*)* which allows overall protection of foods from microbial deterioration and help them extending their shelf life. So it can be very scientifically termed as excellent biopreservatives for food grade applications. The antimicrobial property gets increased when the degree of acetylation is higher and the molecular weight is lower. Higher deacetylated chitooligomers have many free amines, which can bind to the negatively charged residues at the microbial cell wall which leads to the formation of large clusters of microorganisms that precipitates down, resulting in death of the microorganism by blocking their nutrition transport flow. These water-soluble bioactives are also easily incorporated into dairy products and beverages due to all its positive traits.

Recently, COS have also been a centre of attraction in the subject of pharmaceutical and medicinal applications, due to their high solubility and nontoxic properties which make them impart several positive health benefit and physiological effects like lowering high blood cholesterol, high pressures, improving calcium uptakes, improving bone strengths, controlling arthritis and providing protective effects against life threatening infections, tumors, diabetes etc. Antioxidant properties of Chito-oligosaccharides are closely related to the presence of amino and hydroxyl groups, that can react with unstable

free radicals to form stable macromolecule radicals which is the sole cause of increased hypercolesterol concentration in blood due to generation of reactive oxygen species. Conjugation with antioxidant elements like phenolic acids further enhances the antioxidant activities by triggering the enhanced synthesis of catalase and superoxide dismutase and decreasing lipid peroxidation which are associated with structure of phenolic acids and the substitutions on the aromatic ring of the side chain. Chito-oligosacharides also has a huge potential to be used as an effective prebiotic source as it is non-digestible by intestinal enzymes, which supports growth of beneficial probiotic bacteria in the human intestinal tract such as *Bifidobacterium* and *Lactobacillus*. Chito-oligosaccharides (COSs) have also been clinically evaluated for their immune stimulating effects after oral intake. In addition, COS has also been reported to be applied as a supplement in dietary therapy against obesity by inhibiting adipocytes differentiation or altering adipose tissue gene expression, reducing serum levels of triglyceride and cholesterol and alleviating lipid accumulation in the liver. COS derivatives have strong affinity for biological systems with distinctive properties without any negative side effects like allergies. They are highly specific in actions as bioactive molecules when prepared through hydrolysis and biodegradable methods.

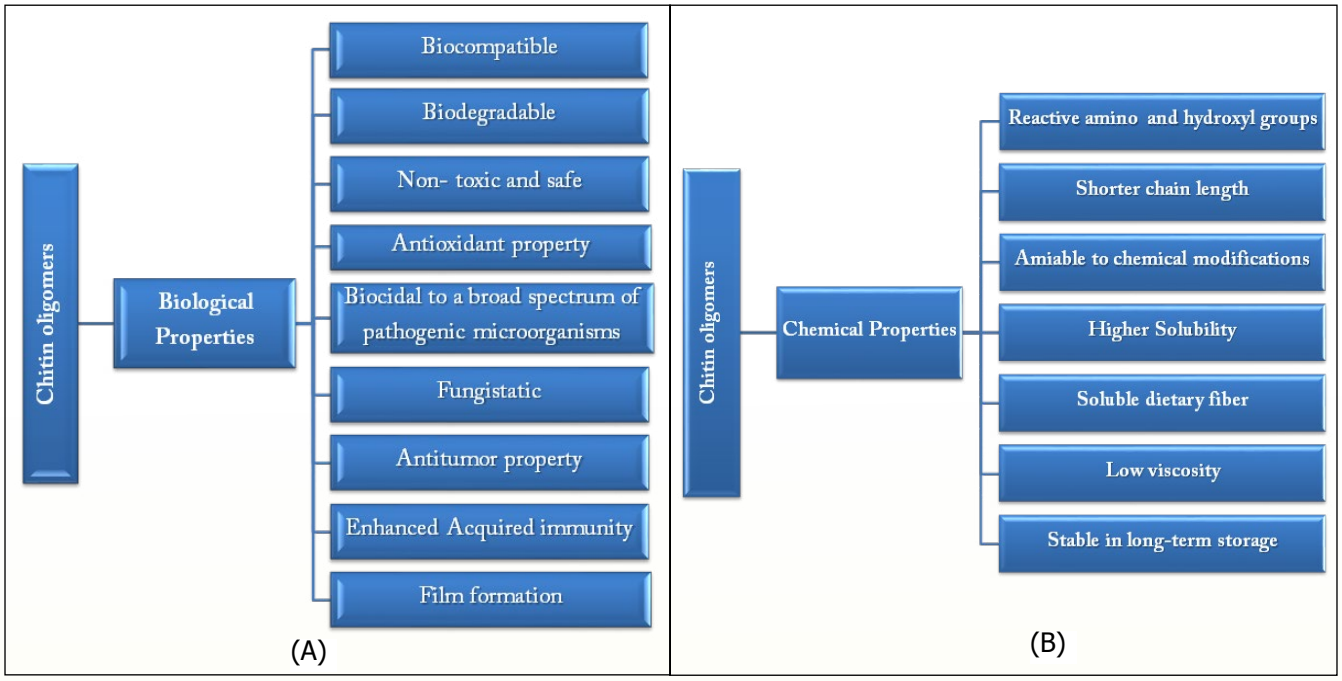

Figure 1: Biological (A) and chemical (B) properties of chito-oligosaccharides

4. Conclusion

Research on chitin oligomers has increased in the last few years due to its promising and multiple biological applications all around the world, gaining a broad potentiality in various fields. Native antimicrobial properties in chitin oligomers can be enhanced by physical and chemical modifications. On a commercial basis, N-acetyl chitin oligomers (NAc-COS) would be preferred since it is prepared directly from chitin without the need for a deacetylation step, while shorter chained chitosan oligosaccharides (COS) must be prepared from chitosan.

5. References

Azuma, K., Osaki, T., Kurozumi, S., Kiyose, M., Tsuka, T., Murahata, Y., Imagawa, T., Itoh, N., Minami, S., Sato, K., Okamoto, Y., 2015. Anti-inflammatory effects of orally administered glucosamine oligomer in an experimental model of inflammatory bowel disease. Carbohydrate polymers 115(1), 448–456.

Dash, M., Chiellini, F., Ottenbrite, R., Chiellini, E., 2011. Chitosan-A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science 36(8), 981–1014.

Jung, W.J., Park, R.D., 2014. Bioproduction of

chitooligosaccharides: present and perspectives. Marine drugs 12(11), 5328–5356.

Katiyar, D., Hemantaranjan, A., Singh, B., Bhanu, A.N., 2014. A future perspective in crop protection: Chitosan and its oligosaccharides. Advances in Plants and Agriculture Research 1(1), 1–8.

Muzzarelli, R.A.A., Muzzarelli, C., 2005. Chitin and chitosan: Research opportunities and challenges. Edited by Dutta PK. New Delhi, India: New age international.

Nechita, P., 2017. Applications of chitosan in wastewater treatment. Biological activities and application of marine polysaccharides 1, 209–228.

Parvez, S., Rahman, M., Khan, M., Khan, M., Islam, J., Ahmed, M., Rahman, M., Ahmed, B., 2012. Preparation and characterization of artificial skin using chitosan and gelatin composites for potential biomedical application. Polymer Bulletin 69(6), 715–731.