The History of Chito/Chitin Oligosaccharides and Its Monomer

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Abstract

Chitooligosaccharides and chitin oligosaccharides are collectively referred to as aminooligosaccharides. The monomer of chito/chitin oligosaccharides are N-acetyl-D-glucosamine and glucosamine, two of the few nitrogenous sugars in nature. Chito/chitin oligosaccharides are mainly produced by hydrolysis from natural aminopolysaccharides (chitosan and chitin), which are extracted from crab and shrimp shells resource. Chitin and chitosan are well known as natural polysaccharides with abundant biological activities. Similarly, chito/chitin oligosaccharides also have a number of commercial uses as their biological activities. This chapter summarizes the history and chemical-based topics like definition, origin, structure, molecular weight, classification of chitin, chitosan, chito/chitin oligosaccharides and their monomers (N-acetyl-D-glucosamine and glucosamine). Due to the broad research interests and market demands, the discovery and development of biological activities of chito/chitin oligosaccharides are summarized as argument.

1.1 Introduction

1.1.1 Chitin

Chitin $(C_8H_{13}O_5N)_n$ is a natural polysaccharide composed of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc, NAG), chemical construction is shown in Fig. 1.1. Chitin is an abundant biopolymer on earth next to cellulose. Professor Henri Braconnot

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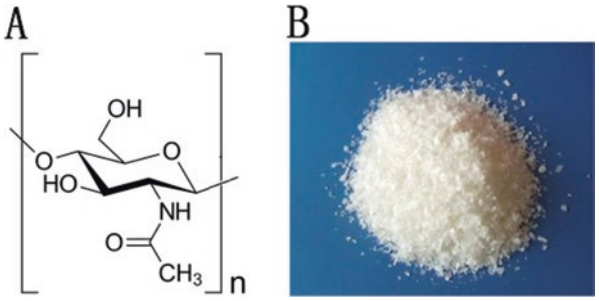


Fig. 1.1 Chitin. (**a**) Chemical construction of chitin; (**b**) Picture of chitin powder

firstly discovered and isolated chitin from mushrooms in 1811. After that, Odier found the same polysaccharide in the insects' exoskeleton and named it chitin in 1823. Chitin can be obtained from a wild range of sources. Exoskeletons of arthropods (crustaceans, insects and arachnids) and mollusks (beaks and endoskeleton of cephalopods) are main sources of chitin. And various microorganisms are also the sources of chitin, such as the cell walls polysaccharides of yeasts and fungi, and the spines of diatoms. However, commercially chitin product is mainly obtained from marine sources, e.g., crustacean shells from crabs and shrimps at present.

Like another common polysaccharide – cellulose, the structure of chitin often forms crystalline nanofibrils or whiskers. In terms of physiological function, chitin may be compared to the animal protein – keratin. Chitin has been proved to be used in a variety of industrial, pharmaceutical and biotechnology fields. Chitin and cellulose are polysaccharides similar on the structure; chitin is a linear polymer consists of β-(1,4)-N-acetyl-D-glucosamine. As the different crystalline microfibrils order, natural chitin occurs in three polymorphic forms: α-chitin, β-chitin and γ-chitin. α-Chitin is arranged in anti-parallel strands, and it is the most abundant and stable form found in nature. It acts as shell for insects, crabs, lobsters and shrimps, as well as structural skeletons in the cell walls of fungi and yeast. The β form is arranged in parallel chains. Consequently, β-Chitin is less stable than the α-Chitin. The source of different chitin is also not the same. The β-chitin has been found in the pens of squids, the extracellular fibers of diatoms and the spines and chaetae of certain annelids. γ-chitin is a mixture composed of α and β structures, which is the least common form. It has been found in the squid's stomach and in the cocoons of two beetles.

Chitin is a nitrogenous polysaccharide which has an acetamide group ($NH\text{-}CO\text{-}CH_3$) at C-2 in place of the hydroxyl group in cellulose. The pure chitin is white or yellowish, odorless and tasteless. Chitin exhibits excellent biodegradability and biocompatibility that is potential to be used in food, medicine or material industries. However, chitin is highly hydrophobic; thus, it is insoluble in water and even in most organic solvents. The water insolubility of chitin is one of the main limitations in the large-scale application. Therefore, the research about water-soluble derivatives of chitin has become one of the research hotpots.

1.1.2 Chitosan

Chitosan is the product of the deacetylation reaction of chitin, which is a linear polysaccharide composed of β-1,4-linked D-glucosamine (GlcN) and a small amount of N-acetyl-D-glucosamine (Fig. 1.2). Chitosan is generally produced by a deacetylation reaction of treating the chitin shells of shrimp and other crustaceans with a basic substance such as sodium hydroxide (Fig. 1.3). Chitosan was discovered as a transformation of chitin in water soluble form after chemical manipulation by Roughet in 1859. Later, in 1870, this transformation of chitin was named chitosan. The nature sources of chitin include shells of crabs and shrimps. Unlike chitin, natural chitosan not exist in animal species, and even is rarely found in nature, except fungi. Both chitosan and chitin present in the cell walls synthesis of fungi, e.g., Zygomycetes. Moreover, chitosan has two distinct forms in the fungal cell wall: free form of chitosan and chitosan bound to β-glucan.

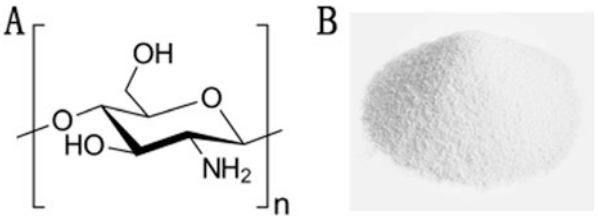


Fig. 1.2 Chitosan. (**a**) Chemical construction of Chitosan; (**b**) Picture of chitosan powder

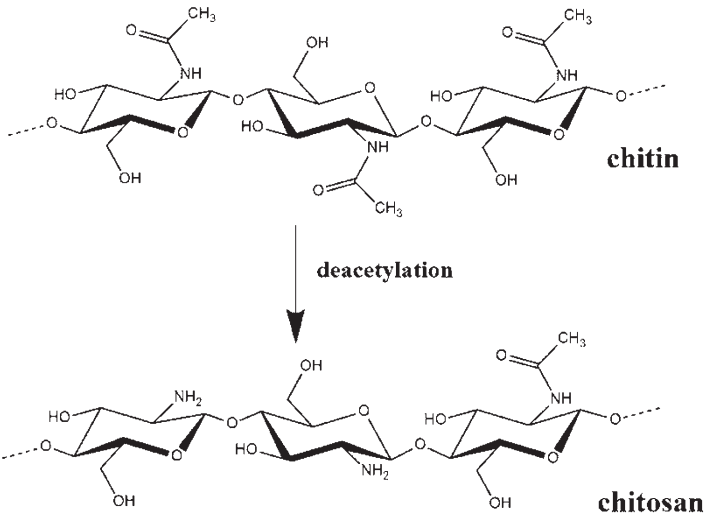


Fig. 1.3 Forming chitosan by partial deacetylation of chitin

Commercial chitosan product is mostly obtained from the deacetylation of natural chitin, which is completely or partially deacetylated by treatment with acid or alkali solution. Structurally, chitosan is a linear polysaccharide containing copolymer of β-1,4-linked D-glucosamine and N-acetyl-D-glucosamine. The degree of deacetylation (DD) is generally defined as the glucosamine/N-acetyl-D-glucosamine ratio, which goes up as chitin is converted to chitosan. Therefore, chitin and chitosan can be distinguished by the ratio above. When the content of N-acetyl-D-glucosamine is higher than that of glucosamine, the biopolymer is often called chitin; otherwise, the compound is called chitosan.

The physical and chemical properties of chitosan are similar to those of chitin as mentioned above. Due to the positive charges of the amino groups in glucosamine, chitosan is the only water-soluble cationic polysaccharide commercially available. Because of its distinctive physicochemical characteristics such as biocompatibility, biodegradability and low toxicity, chitosan is considered having great potential for the applications in various industries. However, the higher molecular weight and high viscosity of chitosan restrict its specific applications. Fortunately, chitosan can be further hydrolyzed into its low molecular weight (MW) derivatives, which exhibit more biologically active and solubility than chitosan.

1.1.3 Chitooligosaccharides

Chitooligosaccharides (COS) are the degradation products of chitosan, which exhibit various biological activity, such as antitumor, antioxidant, immunostimulatory, antimicrobial and elicitors of plant immunity. Since the discovery of chitosan in the late 1870s, many scientists have put a lot of efforts on research of its physicochemical properties. Under different reaction conditions, chitooligosaccharides can be prepared with different physicochemical properties. According to the academic definition of the oligosaccharides, the degrees of polymerization (DP) of an oligosaccharide should be between 2 and 10. However, in industrial production and commercial application, chitosan with DP degrees of polymerization (DP) less than 20 and an average molecular weight less than 3900 Da are also classified named chitooligosaccharides (or chitosan oligosaccharides, chitosan oligomer). The DD of chitooligosaccharides is generally higher than 75%. Chitooligosaccharides has three types of reactive functional groups: an amino/acetamido group, a primary hydroxyl group, and a secondary hydroxyl group at the C-2, C-3, and C-6 positions, respectively. Chitooligosaccharides and modified chitooligosaccharides are both found to be much easier to use in large-scale applications (Fig. 1.4).

The most important factors that affect the physicochemical properties of chitooligosaccharides are DP and DD, which are also correlated with their biological actives. The DP, especially the corresponding molecular weight, contributes to the change in the physicochemical properties of chitooligosaccharides. Low DP chitooligosaccharides is more soluble than chitosan (often with relatively high DP). Compared with chitosan, chitooligosaccharides is more easily absorbed in the intestine and rapidly enters to blood circulation. Thus, molecular weight is considered to

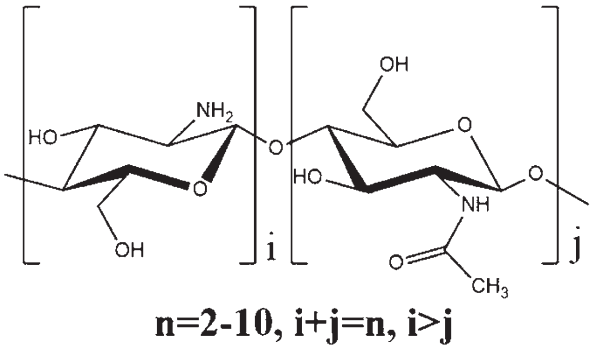
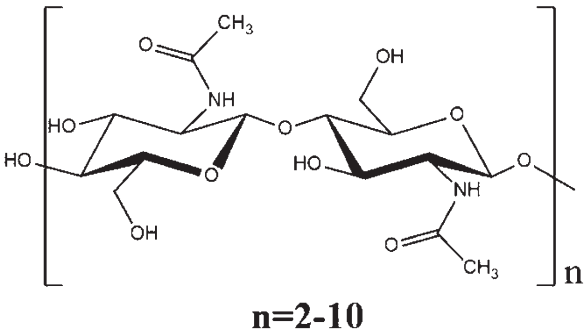


Fig. 1.4 Chemical construction of chitooligosaccharide

Fig. 1.5 Chemical construction of chitin oligosaccharides



be a major feature of chitooligosaccharides and is highly correlated with their biological activity. DD is also one of the important parameters of chitooligosaccharides. The higher DD chitooligosaccharides have more free amino groups and more positive charges in the solution. DD is the most important parameter determining the solubility of chitooligosaccharides. The rate and extent of biodegradation of chitooligosaccharides in organisms are related to DD, and the decrease in degradation rate leads to an increase in DD (Fig. 1.5).

1.1.4 Chitin Oligosaccharides

Chitin oligosaccharides (N-acetyl COS, ChOS) are the partially degraded products of chitin by chemical, physical or enzymatic methods. In general, he degree of polymerization (DP) of chitinoligosaccharides is 2–10. The indicator of chitinoligosaccharides and chitooligosaccharides is the degree of deacetylation. The degree of acetylation of chitinoligosaccharides is generally high (>75%). Chitin oligosaccharides has attracted considerable attention in recent years due to their water-solubility and temperature and pH stability, as well as excellent biological activities, such as antimicrobial, anti-tumor and hypoglycemic activities. In addition to these

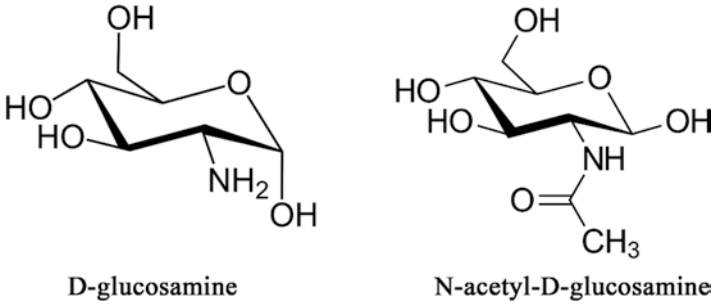


Fig. 1.6 Chemical construction of glucosamine and N-acetyl-D-glucosamine

biological activities, ChOS are also considered as effective plant elicitors and potential angiogenic inhibitor, involving in the promotion of bifidus proliferation, the characteristics of which could be widely used in agricultural, food and pharmaceutical industrials. Thus, production of ChOS is a promising way for the disposal and recycling of enormous waste chitin materials (Fig. 1.6).

1.1.5 Glucosamine and N-acetyl-D-Glucosamine

Glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) are the monomers of chito/chitin oligosaccharides. Glucosamine and N-acetyl-D-glucosamine are natural amino monosaccharides and derivatives from glucose. Glucosamine was first prepared by the hydrolysis of chitin with concentrated hydrochloric acid by Georg Ledderhose in 1876. And the chemical construction of glucosamine was determined by Walter Haworth in 1939. Glucosamine/N-acetyl-D-glucosamine is not only part of the constitutional unit of the structural polysaccharides (chitosan and chitin), but also one of the most abundant bioactive monosaccharides with significant biological activity. Since glucosamine is the precursor for glycosaminoglycan, which is a major component of cartilage. Research has focused on the potential for supplemental glucosamine to beneficially influence cartilage structure and alleviate arthritis. In addition, N-acetyl-D-glucosamine is a major component of the bacterial and fungal cell walls, which has a significant effect on the treatment of inflammatory diseases. Thus, glucosamine/N-acetyl-D-glucosamine have been widely used as dietary supplement and pharmaceutical drug for decades. In the United States, glucosamine/N-acetyl-D-glucosamine is one of the most common dietary supplements used by adults.

For a long time, glucosamine/N-acetyl-D-glucosamine is produced commercially by the enzymatic/chemical hydrolysis of crustacean exoskeletons (Fig. 1.7). The crab shell is first deproteinized and decalcified by acid-base treatment to obtain chitin. Chitin is then processed by acid hydrolysis or enzymatic hydrolysis to obtain N-acetyl-D-glucosamine. N-acetyl-D-glucosamine are finally deacetylated by concentrated alkali or enzyme to obtain glucosamine. Hydrolysis process has many potential problems such as environmental pollution, product allergy and shortage of raw material supply. Although the use of enzymes alleviated the above problems in

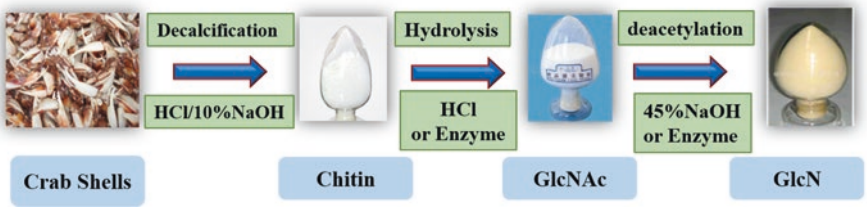


Fig. 1.7 Preparation of glucosamine/N-acetyl-D-glucosamine by hydrolysis

Fig. 1.8 Preparation of glucosamine/N-acetyl-D-glucosamine by fermentation

hydrolysis process, cost and production efficiency still need to be resolved. Synthetic biology is a newly research field with potential to product a certain substance in cell by optimizing intracellular inheritance and regulating metabolic processes. Thus glucosamine/N-acetyl-D-glucosamine also can be produced by microbial fermentation using recombinant microorganism such as *Escherichia coli* and *Bacillus subtilis*. Fermentation method as an environmentally friendly process is attracting increasing attention. In addition, N-acetyl-D-glucosamine deacetylase has been discovered in recent years and has potential for industrial preparation of glucosamine by enzymatic deacetylation. The enzymatic deacetylation combined fermentation will completely solve the pollution and the source of raw materials problem of glucosamine preparation (Fig. 1.8).

1.2 Biological Activities of Chito/Chitin Oligosaccharides

1.2.1 Antimicrobial Activity

COS has antimicrobial activity and is a natural antimicrobial agent that inhibits the growth of various pathogenic microorganisms. COS and its derivatives were reported to have antimicrobial activity in the 1980s (Allan and Hadwiger 1979). Since then COS has received much attention due to its antimicrobial activity. The antimicrobial activity of COS is mainly related to its Mw, DP, DD and chemical modification. The antimicrobial activity of COS is superior to that of chitosan and

chitin, and the COS with higher molecular weight shows higher antimicrobial activity. There are two main mechanisms for the inhibition of COS. One is that the COS itself has positive charges and can adhere to the surface of the cell, thereby affecting the normal metabolism of the microorganism (Chung et al. 2004; Je and Kim 2006). The second is that COS with a molecular weight of less than 5000 Da block the transcription process of DNA, thereby inhibiting the growth of microorganisms (Liu et al. 2001). The antimicrobial activity of COS remains to be further studied, and its research is of great significance. The development of new antimicrobial drugs using COS has great potential.

1.2.2 Antioxidant Activity

COS and its derivatives have strong reducing ability and can effectively scavenge hydroxyl radicals and superoxide anions. Because COS has positive charges, it provides positrons to free radical, which makes the free radical in a stable state, thus blocking the radical chain reaction and effectively reducing the oxidative damage. COS and its derivatives have stronger antioxidant activity than chitosan. The antioxidant activity of COS is related to its DD, DP and MW. The smaller the molecular weight of COS is, the stronger its antioxidant activity will be, and the COS with low MW (5 kDa) has highest antioxidant capacity (Mengibar et al. 2013). The low DP of COS shows good antioxidant activity, and the COS of DP between 10 and 12 has the strongest scavenging ability (Li et al. 2012). Because of its strong antioxidant activity, COS can effectively remove free radicals from the body, which has very important research and application value.

1.2.3 Anti-inflammatory Activity

The anti-inflammatory activity of COS depends on their physicochemical properties. The MW and DP of COS have great effects on their anti-inflammatory ability. COS with MW lower than 10 kDa and low DP have better anti-inflammatory activity, and their anti-inflammatory activity is directly related to the dose used. Studies have shown that Similar proportions of monoacetylated and deacetylated oligomers is necessary for the mixtures of chitooligosaccharides to achieve anti-inflammatory effects, and it directly depends on the preparation method to which chitosan was submitted (Sanchez et al. 2018). The use of COS to develop new anti-inflammatory agents has great potential in biological and medical applications.

1.2.4 Anti-tumor/Anticancer Activity

Studies in the twentieth century have shown that COS or can effectively inhibit the growth of cancer cells. Highly charged COS effectively inhibits the growth of cancer cells (Huang et al. 2006). Mw of COS plays a key role in its anti-tumor activity,

and its tumor suppressive effect is negatively correlated with MW (Salah et al. 2013). The COS with low molecular weight has higher antitumor activity, and as the MW decreases, the tumor suppressing ability of COS is obviously enhanced. Since COS has significant antitumor activity and has the potential to treat cancer, the development of new anticancer agents using COS is of great significance. At present, the anticancer activity and its mechanism of COS are not fully understood. Therefore, whether COS can be used as an anticancer agent requires further research.

1.2.5 Immunostimulatory Activities

Studies have shown that COS has immunomodulatory activities and its immunological activity is related to its molecular weight. COS promotes the secretion of NO and pro-inflammatory cytokines through the PI3K-Akt and the MAPK pathway as well as NF-κB activation (Yang et al. 2017). NO is involved in the clearance of invading pathogens by macrophages, and proinflammatory cytokines are also involved in the destruction of pathogens. Low molecular weight chitosan can induce the expression and secretion of NO and proinflammatory cytokines in cells. After treatment with COS, the amount of NO and pro-inflammatory cytokines in the cells increased significantly, and the amount increased was positively correlated with the dose of COS used. The immunomodulatory activity of COS makes it be of great application value in medicine.

1.2.6 Wound Healing and Tissue Regeneration Properties

Both chitin and chitosan have functions to promote wound healing and tissue regeneration, which is related to their immunostimulatory activity (Okamoto et al. 2003). Compared with chitin, Chitinoligosaccharides and N-acetyl-D-glucosamine, chitosan with higher deacetylation degree, chitooligosaccharides and glucosamine have stronger effects on fibroblast activation and can significantly stimulate the production of fibroblasts (Minagawa et al. 2007), thereby accelerating wound healing. Studies have shown that COS-modified membranes can accelerate wound healing while improving antibacterial ability (Luo et al. 2016). The osteogenesis effect of the COS membrane is comparable to that of the gelatin membrane and is considered to be a superior material for osteogenic differentiation of stem cells (Ratanavaraporn et al. 2009). COS has great application prospects in medicine, due to its ability to promote wound healing and tissue regeneration.

1.2.7 Hypocholesterolemic Activity

Several studies have attributed some metabolic syndrome to a high level of cholesterol, researchers are also extensively examining the hypocholesterolemic activity of COS and its derivatives. COS has shown angiotensin converting enzyme (ACE)

inhibiting effects because of its positive charge and its affinity towards the ACE functional site. Pyo-Jam et al. (Park et al. 2003) studied the ACE inhibitory activity of three different degrees of deacetylation COS mixtures, indicating that the relatively lowest degree of deacetylation COS, which was prepared from 50% deacetylated chitosan, exhibited the highest ACE inhibitory activity. Zhang et al. (Zhang et al. 2012) investigated the hypolipidemic activities of different MW chitosan in rats fed high-fat diets. The studies showed that hypolipidemic activity of lower MW of chitosan was better than higher MW of chitosan which might be partially attributed to the increase of serum and liver LPL activities.

1.2.8 Elicitors of Plant Immunity

COS is an essential and natural plant immunity elicitor. Many studies reported that N-acetyl COS was the potent elicitor for rice cells, it induced a set of defense reactions including depolarization of ion fluxes, membrane potential, phytoalexin synthesis at extremely low concentration et al. The effects of COS depend on their concentration or dosage, DP, application ways, application time, and growth period of plants (Liaqat and Eltem 2017). Previous research has found that the defense reactions of N-acetyl COS are dependent on their physicochemical properties. N-acetyl COS which has higher DP (DP>3) show stronger defense activities than the lower DP or the deacetylated COS (Yamada et al. 1993). Lan et al. used COS as an elicitor to enhance barley germination for improving the quality of malt (Lan et al. 2016). The study showed that malt quality was significantly improved by COS in seed priming at 1 mg/L. In addition, much previous studies have indicated that COS has great potential to provide biopesticides and biofertilizers (Zong et al. 2017; Zou et al. 2016).

1.3 Future Perspectives and Limitations

Aminooligosaccharides (chito/chitin oligosaccharides) have broad application prospects as bioactive compounds in many fields. Therefore, exploring the clear structure-activity relationship of aminooligosaccharides, preparing aminooligosaccharides products with specific DP or DD are the future development trends. Aminooligosaccharides have many possibilities for application in foods, agriculture, cosmetics and medicine. It is essential to develop novel methods for large scale production of aminooligosaccharides products with desired characteristics. In addition, the pure standards of aminooligosaccharides are quite expensive, in fact, aminooligosaccharides standards with DP higher than 6 are difficult to obtain in large-scale at present. For this reason, it is still a hard work to correctly analyses the chito/chitin oligosaccharides products mixtures with broad DP. Although aminooligosaccharides related research has made great progress, it is still a difficult task to produce purified products with good characteristics. Therefore, the large-scale production of purified aminooligosaccharides, proper characterization of the

aminooligosaccharides products, the reduction in production costs and knowing the detail mechanism of action of aminooligosaccharides is valuable for further study.

The biopolymers, chitosan and chitin, still have a lot of usefulness and many application properties in the application of biological materials. However, due to their properties of solubility, adsorption and viscosity, the wide application of these glycans is limited. At the same time, chitinoligosaccharides, chitooligosaccharides and their derivatives exhibit their versatility, complete solubility and low viscosity properties, which are superior to their polymers, chitosan and chitin, in these respects; so COS have received widespread attention. Especially in biomedicine and pharmaceutical applications. This is closely related to its degree of deacetylation, molecular weight, degree of polymerization and cationic nature. Although COS have great application prospects, it is not currently possible to mass-produce chitooligosaccharides and single COS, and the study on the physiological activities of chitooligosaccharides is also limited. A novel and efficient method for the preparation of COS, the separation of chitooligosaccharides and the mechanism of action of COS should be the focus of further research.

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