Factors Affecting the Antimicrobial Activity of Chitosan and Its Mechanism of Action: Postprint

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Abstract

Chitosan is the deacetylated form of chitin, a biopolymer obtainable from crustaceans, insects, and fungi, which exhibits favorable physicochemical properties and numerous biological activities, and demonstrates antimicrobial activity against various fungi, Gram-positive bacteria, and Gram-negative bacteria. The electrostatic interaction between chitosan and anions on the microbial surface constitutes an important factor determining the antifungal and antibacterial activity of chitosan, yet is influenced by microbial type, chitosan molecular weight, degree of deacetylation, and other parameters. Leveraging its antifungal and antibacterial properties, chitosan is employed in animal production via feed additives or gel injection, demonstrating considerable application potential for enhancing animal production performance and immune function, as well as for disease treatment. This review primarily summarizes the latest mechanisms of antimicrobial activity and influencing factors of chitosan, providing a theoretical basis for its application in animal production.

Full Text

Factors Influencing the Antimicrobial Activity of Chitosan and Its Mechanism of Action

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Abstract

Chitosan is the deacetylated form of chitin, a biopolymer derived from crustaceans, insects, and fungi. It possesses favorable physicochemical properties

and numerous biological activities, including antimicrobial effects against various fungi, Gram-positive bacteria, and Gram-negative bacteria. Electrostatic interactions between chitosan and anionic components on microbial surfaces constitute a critical determinant of its antifungal and antibacterial activity, though these interactions are influenced by microbial type, chitosan molecular weight, and degree of deacetylation. Leveraging its antimicrobial properties, chitosan can be applied in animal production through feed additives or gel injections, offering significant potential for improving animal performance, immune function, and disease treatment. This review synthesizes recent findings on the mechanisms and influencing factors of chitosan' s antimicrobial activity, providing a theoretical foundation for its application in animal production.

Keywords: chitosan; antimicrobial activity; fungi; animal production; mechanism of action

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Introduction

As the use of green biopolymers continues to expand, chitosan has attracted considerable attention. Derived from chitin—the most abundant natural polysaccharide—chitosan is a biopolymer extracted from crustaceans, fungi, and insects. It is obtained through deacetylation of chitin using 30–60% sodium hydroxide. Chitosan exhibits excellent adsorption, hygroscopicity, film-forming capacity, and permeability, along with favorable biocompatibility, biodegradability, and low allergenicity. Additionally, it demonstrates antimicrobial, antitumor, and hypocholesterolemic functions. Its potent antimicrobial properties make it particularly promising for livestock applications, especially in modulating rumen fermentation, enhancing immunity, and preventing diseases in ruminants. This paper focuses on recent research regarding chitosan' s biological activities, mechanisms of action, and influencing factors to establish a theoretical basis for its use in ruminant nutrition and health.

1.1 Structural Properties

Chitosan is the deacetylated derivative of chitin, obtained by removing the acetyl group at the C-2 position, with the chemical name (1,4)-2-amino-2-deoxy- -D-glucan. Typically, chitin with a deacetylation degree exceeding 55% is classified as chitosan. Therefore, chitosan is an N-deacetylated form of chitin and a linear polysaccharide with varying degrees of N-acetylation, composed of less than 20% -(1,4)-2-acetamido-D-glucopyranose and over 80% -(1,4)-2-amino-D-glucopyranose. It is a random copolymer of D-glucosamine and N-acetyl-D-glucosamine units. The ratio between these two monomer units (degree of deacetylation) and the molecular weight play crucial roles in chitosan' s antimicrobial activity and solubility in aqueous media.

Chitosan contains three types of reactive functional groups: amino/acetamido groups at the C-2 position, and primary and secondary hydroxyl groups at the C-3 and C-6 positions. The amino group content is the primary factor underlying structural and physicochemical differences and is associated with chelating, flocculating, and biological functions. High molecular weight chitosan is poorly water-soluble and highly viscous in dilute acidic solutions, limiting its biological applications. Modifying chitosan to produce derivatives represents an important strategy for improving its solubility and antimicrobial activity. Due to the differential reactivity of the primary amino and hydroxyl groups of D-glucosamine, numerous chitosan derivatives can be synthesized directly, thereby enhancing solubility and antimicrobial function.

1.2 Biological Activities

Chitosan has garnered widespread attention for its diverse biological activities, with applications spanning food, pharmaceutical, agricultural, and environmental industries. These include use as agricultural chemicals, plant defense response activators, flocculants in wastewater treatment, chitosan-based water purification membranes, food packaging and preservation materials, drug emulsifiers, and wound dressings.

Recent studies have demonstrated that chitosan possesses antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, immune-enhancing, antitumor, drug delivery, and mineral absorption-promoting properties. However, no single type of chitosan exhibits all these activities simultaneously, driving continued research interest. Furthermore, different chitosan derivatives and enzymatic products possess distinct structural and physicochemical properties that may reveal novel bioactivities or discoveries beyond those of known compounds.

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2. Mode of Action

The specific antimicrobial mechanism of chitosan remains difficult to elucidate due to the influence of various factors. Research indicates that the most critical factors affecting its mode of action are microbial type, molecular weight, and degree of deacetylation. Microorganisms can be broadly categorized into four groups: Gram-positive bacteria, Gram-negative bacteria, chitosan-sensitive fungi, and chitosan-resistant fungi. Numerous studies demonstrate that chitosan acts on fungal or bacterial cell surfaces, ultimately altering membrane permeability. This interaction is primarily electrostatic, occurring between the positively charged amino groups of protonated chitosan and negatively charged molecules on the cell surface. Typically, altered cell surface permeability leads to leakage of intracellular components and subsequent cell death. Studies also show that chitosan can affect DNA expression by binding to nucleic acids. However, before triggering these intracellular responses, chitosan must penetrate the plasma

membrane, which largely depends on microbial species and molecular weight.

2.1 Mode of Action on Fungi

Unlike Gram-positive and Gram-negative bacteria, the difference in mode of action between chitosan-sensitive and chitosan-resistant fungi is less distinct. Initially, chitosan affects the cell membrane through electrostatic interactions with negatively charged phospholipids. Once the membrane is compromised, chitosan can enter the cell, leading to inhibition of DNA or RNA synthesis and disruption of protein synthesis. Palma-Guerrero et al. observed membrane disruption, intracellular material leakage, and chitosan penetration in chitosan-sensitive fungi. In contrast, chitosan-resistant fungi appear to prevent chitosan from crossing the cell membrane, causing it to remain on the outer surface. This inability to disrupt membranes in resistant fungi stems from differences in membrane fluidity. Studies on the phospholipid fatty acid composition of fungal membranes revealed that increased chitosan activity correlates with higher unsaturated fatty acid content. When testing chitosan' s antimicrobial activity against *Neurospora crassa* mutants with reduced unsaturated fatty acid content, the antimicrobial effect decreased compared to wild-type strains, demonstrating that membrane fluidity influences chitosan activity and that the mode of action depends largely on the fungal species.

Notably, chitosan-sensitive and chitosan-resistant fungi belong to different taxonomic families, a classification method based on fatty acid composition that has proven valuable for fungal taxonomy. This suggests that such classification could predict or explain chitosan' s antifungal activity. The same research group also demonstrated that chitosan uptake in sensitive fungi is ATP-dependent, as evidenced by monitoring uptake at 4°C or in the presence of azide, both of which inhibit ATP production and prevent chitosan translocation across the plasma membrane. At room temperature without azide, chitosan uptake occurs within 30 minutes, indicating that absorption involves not only diffusion but also ATP-dependent processes. Once inside the cell, chitosan affects DNA/RNA synthesis and protein synthesis.

2.2 Mode of Action on Gram-Positive Bacteria

In Gram-positive bacteria, chitosan non-covalently binds to teichoic acids in the peptidoglycan layer. These surface teichoic acids play important roles in cell division and other physiological aspects of Gram-positive bacteria, though chitosan' s direct effect on the cell membrane remains uncertain. Some researchers argue that chitosan is unlikely to affect the cell membrane because its hydrodynamic diameter exceeds the expected pore size in the peptidoglycan structure. However, Park et al. found that low molecular weight chitosan samples (<5 kDa)

induced apoptosis in *Bacillus megaterium* by blocking DNA synthesis, indicating that molecular weight is a critical factor influencing membrane permeability and mode of action. Studies using *Staphylococcus aureus* mutants deficient in teichoic acid biosynthesis genes demonstrated the importance of teichoic acids for chitosan activity, as these mutants showed greater resistance compared to wild-type strains. This suggests that polyanionic teichoic acids enhance chitosan' s antimicrobial activity against Gram-positive bacteria. Teichoic acid functions include protection against environmental stress, controlling enzyme activity and cation concentration in the cell membrane, and mediating receptor binding. Electrostatic interactions between chitosan and teichoic acids may disrupt these functions, leading to cellular dysfunction. Similar cationic biocides also exhibit antimicrobial activity against Gram-positive bacteria due to these anionic teichoic acids. These findings indicate that chitosan' s primary mode of action involves electrostatic interactions with teichoic acids, disrupting multiple cellular functions and causing cell death.

2.3 Mode of Action on Gram-Negative Bacteria

Two mechanisms are proposed for chitosan' s activity on the outer membrane of Gram-negative bacteria. First, at pH values above its pKa, chitosan chelates various cations, potentially disrupting cell wall integrity and interfering with nutrient uptake (e.g., calcium and magnesium ions). Second, chitosan undergoes electrostatic interactions with the anionic lipopolysaccharide components of the outer membrane. The dominant mechanism remains uncertain. Recent studies show that chitosan disrupts inner membrane function, causing leakage of intracellular contents. Additionally, chitosan has been observed to cross the cell membranes of Gram-negative bacteria, suggesting it may interfere with DNA/RNA synthesis and trigger intracellular responses.

In summary, electrostatic interactions between anionic cell surfaces and chitosan constitute a critical determinant of its antimicrobial activity against fungi and bacteria. This is reflected in the enhanced antimicrobial activity observed with increasing deacetylation degree and the relatively low activity of chitin. Due to its protonated amino groups, chitosan (pKa 6.3–6.5) exhibits optimal antimicrobial properties at low pH, which also explains why quaternized chitosan derivatives are generally more effective than chitosan itself. Despite the importance of electrostatic interactions, chitosan can also non-covalently bind cholesterol, suggesting that other non-covalent interactions may contribute to antimicrobial activity.

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3. Factors Influencing Chitosan' s Antimicrobial Activity

Chitosan exhibits antimicrobial activity against various fungi and bacteria, though its efficacy and mode of action are influenced by numerous factors.

Generally, chitosan is active primarily at the cell surface, but depending on microbial type and molecular weight, it may trigger additional antimicrobial effects such as inhibiting DNA/RNA synthesis or disrupting protein synthesis.

3.1 Antifungal Activity

Recent studies testing chitosan samples with similar deacetylation degrees but different molecular weights against three fungal species revealed that low molecular weight chitosan (41.2 kDa) was most effective, though the same molecular weight showed varying effects against different fungi. This indicates that the mode of action depends heavily on fungal species. Another study reported that low molecular weight chitosan (17.4 kDa) was more effective against mycelial growth of *Rhizopus stolonifer*, whereas high molecular weight chitosan (307 kDa) was more effective against spore development in *Agrostis stolonifera*.

Beyond molecular weight, deacetylation degree is a critical factor for antifungal activity. Multiple studies demonstrate that increasing deacetylation enhances antifungal efficacy. Generally, high deacetylation degree combined with low molecular weight increases antifungal activity, as confirmed in studies testing chitosan samples against *Candida albicans*. Electrostatic interactions between chitosan and negatively charged phospholipids are central to its mode of action, and increased deacetylation provides more free amino groups, generating additional positive charges and strengthening these interactions. However, like molecular weight, the optimal deacetylation degree depends on fungal type, as demonstrated by Younes et al. in their examination of molecular weight and deacetylation effects on three fungal species.

3.2 Activity Against Gram-Positive Bacteria

Similar to antifungal activity, chitosan' s antibacterial efficacy against Gram-positive bacteria depends largely on molecular weight, acetylation degree, environmental conditions, and bacterial species. Generally, chitosan oligosaccharides exhibit lower antibacterial activity than chitosan, and chitosan' s effectiveness varies significantly by bacterial type. Longer chains (chitosan) prove more effective than oligosaccharides, though comparisons across different molecular weights suggest that medium to high molecular weight chitosan shows superior activity, indicating that optimal chain length depends on the bacterial species.

Studies on the effects of molecular weight and acetylation degree on three *Bacillus cereus* strains demonstrated that low molecular weight chitosan (36–93.3 kDa) with high deacetylation was most effective. Deacetylation degree influences electrostatic interactions between chitosan and teichoic acids in Gram-positive bacteria; thus, increased deacetylation enhances antimicrobial activity. This has been confirmed in *Bacillus cereus*, *Listeria monocytogenes*, *Staphylo-*

coccus aureus, and *Streptococcus agalactiae*. The underlying mechanism mirrors that of antifungal activity: higher deacetylation provides more protonatable free amino groups, creating a positively charged polymer. This effect also correlates with increased antimicrobial activity at lower pH values.

3.3 Activity Against Gram-Negative Bacteria

Chitosan' s antimicrobial activity against Gram-negative bacteria, similar to that against Gram-positive bacteria, shows molecular weight effects that depend heavily on bacterial species. Chitosan oligosaccharides exhibit lower activity compared to chitosan. One study investigating oligochitosan (2, 3, 6, 10, and 16 kDa) and low molecular weight chitosan (60 kDa) against *Bacteroides thetaiotaomicron* and *Bacteroides vulgatus* found that antimicrobial activity increased with oligochitosan molecular weight, though low molecular weight chitosan remained more effective than oligochitosans. Tayel et al. reported similar results and further observed that differences between various chitosan molecular weights were minimal and largely bacterial species-dependent, with cell membrane disruption being the primary bacteriostatic mechanism.

Regarding deacetylation degree, increased deacetylation consistently enhances antimicrobial activity against Gram-negative bacteria, including *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus*.

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4. Conclusion

Chitosan is a highly studied multifunctional biopolymer exhibiting numerous advantageous properties across various fields. Among its most promising applications are the antimicrobial activities of chitosan and its derivatives. This antimicrobial action is influenced not only by fungal and bacterial species but also primarily by deacetylation degree, molecular weight, and the derivatization and preparation methods employed. By integrating these properties, chitosan can be rationally applied in animal production to improve performance, such as by altering rumen fermentation patterns and increasing feed utilization efficiency. Furthermore, chitosan shows substantial potential for disease prevention and treatment in animals.

References

[1] STREIT F, KOCH F, LARANJEIRA M C M, et al. Production of fungal chitosan in liquid cultivation using apple pomace as substrate[J]. Brazilian Journal of Microbiology, 2009, 40(1): 20–25.

[2] SONG C, YU H, ZHANG M, et al. Physicochemical properties and antioxidant activity of chitosan from the blowfly *Chrysomya megacephala* larvae[J]. International Journal of Biological Macromolecules, 2013, 60: 347–354.

[3] DE MOURA C M, DE MOURA J M, SOARES N M, et al. Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: used to produce biofilm[J]. Chemical Engineering and Processing: Process Intensification, 2011, 50(4): 351–355.

[4] ANITHA A, SOWMYA S, KUMAR P T S, et al. Chitin and chitosan in selected biomedical applications[J]. Progress in Polymer Science, 2014, 39(9): 1644–1667.

[5] VERLEE A, MINCKE S, STEVENS C V. Recent developments in antibacterial and antifungal chitosan and its derivatives[J]. Carbohydrate Polymers, 2017, 164: 268–283.

[6] CHIEN R C, YEN M T, MAU J L. Antimicrobial and antitumor activities of chitosan from shiitake stipes, compared to commercial chitosan from crab shells[J]. Carbohydrate Polymers, 2016, 138: 259–264.

[7] HAMDINE M, HEUZEY M C, BÉGIN A. Effect of organic and inorganic acids on concentrated chitosan solutions gels[J]. International Journal of Biological Macromolecules, 2005, 37(3): 134–142.

[8] XIA W S, LIU P, ZHANG J L, et al. Biological activities chitosan chitooligosaccharides[J]. Food Hydrocolloids, 2011, 25(2): 170–179.

[9] HADWIGER L A. Multiple effects of chitosan on plant systems: solid science or hype[J]. Plant Science, 2013, 208: 42–49.

[10] FITZA K N E, PAYN K G, STEENKAMP E T, et al. Chitosan application improves resistance to *Fusarium circinatum* in *Pinus patula*[J]. South African Journal of Botany, 2013, 85: 70–78.

[11] GUAN W, TIAN S C. The Modified chitosan for dyeing wastewater treatment via adsorption and flocculation[J]. Science of Advanced Materials, 2017, 9(9): 1603–1609.

[12] THAKUR V K, VOICU S I. Recent advances in cellulose and chitosan based membranes for water purification: a concise review[J]. Carbohydrate Polymers, 2016, 146: 148–165.

[13] MITELUT A C, TĂNASE E E, POPA V I, et al. Sustainable alternative for packaging: chitosan biopolymer-a review[J]. Agrolife Scientific Journal, 2015, 4(2): 52–61.

[14] CHEUNG R C F, NG T B, WONG J H, et al. Chitosan: an update on potential biomedical and pharmaceutical applications[J]. Marine Drugs, 2015, 13(8): 5156–5186.

[15] ZHONG L, GAO J T, HE Q F, et al. Enhanced antibacterial and wound healing activities of microporous chitosan-Ag/ZnO composite dressing[J]. Carbohydrate Polymers, 2017, 156: 460-469.

[16] MA Z, GARRIDO-MAESTU A, JEONG K C. Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles antimicrobial agents: a review[J]. Carbohydrate Polymers, 2017, 176: 257-265.

[17] SOUZA V G L, FERNANDO A L, PIRES J R A, et al. Physical properties of chitosan films incorporated with natural antioxidants[J]. Industrial Crops and Products, 2017, 107: 565-572.

[18] TEIXEIRA Q, PEREIRA L, CASTRO al. Anti-inflammatory Chitosan/Poly- -glutamic acid nanoparticles control inflammation while remodeling extracellular matrix degenerated intervertebral disc[J]. Acta Biomaterialia, 2016, 42: 168-179.

[19] MUZZARELLI R A A, ORLANDINI F, PACETTI D, et al. Chitosan taurocholate capacity to bind lipids and to undergo enzymatic hydrolysis: an in vitro model[J]. Carbohydrate Polymers, 2006, 66(3): 363-371.

[20] PARK J H, SARAVANAKUMAR G, KIM K, et al. Targeted delivery of low molecular drugs using chitosan and its derivatives[J]. Advanced Drug Delivery Reviews, 2010, 62(1): 28-41.

[21] BRAVO-OSUNA I, TEUTONICO D, ARPICCO S, et al. Characterization of chitosan thiolation and application to thiol quantification onto nanoparticle surface[J]. International Journal of Pharmaceutics, 2007, 340(1/2): 173-181.

[22] KONG M, CHEN X G, XING K, et al. Antimicrobial properties of chitosan and mode of action: a state review[J]. International Journal Microbiology, 2010, 144(1): 51-63.

[23] PALMA-GUERRERO J, LOPEZ-JIMENEZ J A, PÉREZ-BERNÁ A J, et al. Membrane fluidity determines sensitivity filamentous fungi chitosan[J]. Molecular Microbiology, 2010, 75(4): 1021-1032.

[24] PARK S C, NAH J W, PARK Y. pH-dependent mode of antibacterial actions of low molecular weight water-soluble chitosan (LMWSC) against various pathogens[J]. Macromolecular Research, 2011, 19(8): 853-860.

[25] WANG Q, ZUO J H, WANG Q, et al. Inhibitory effect of chitosan on growth of the fungal phytopathogen, *Sclerotinia sclerotiorum*, and sclerotinia rot of carrot[J]. Journal of Integrative Agriculture, 2015, 14(4): 691-697.

[26] KRAJEWSKA B, WYDRO P, JAŃCZYK A. Probing the modes of antibacterial activity of chitosan. Effects of pH and molecular weight on chitosan interactions with membrane lipids in langmuir films[J]. Biomacromolecules, 2011, 12(11): 4144-4152.

[27] COSTA E M, SILVA S, PINA C, et al. Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens[J]. Anaerobe,

2012, 18(3): 305-309.

[28] MÁRQUEZ I G, AKUAKU J, CRUZ I, et al. Disruption of protein synthesis as antifungal action chitosan[J]. International Journal Microbiology, 2013, 164(1): 108-112.

[29] RUESS L, HÄGGBLOM M M, ZAPATA E J G, et al. Fatty acids of fungi and nematodes-possible biomarkers chain?[J]. Soil Biology Biochemistry, 2002, 34(6): 745-756.

[30] PALMA-GUERRERO J, HUANG I C, JANSSON H B, et al. Chitosan permeabilizes the plasma membrane and kills cells of *Neurospora crassa* in an energy dependent manner[J]. Fungal Genetics and Biology, 2009, 46(8): 585-594.

[31] RAAFAT D, VON BARGEN K, HAAS A, et al. Insights into the mode of action of chitosan antibacterial compound[J]. Applied Environmental Microbiology, 2008, 74(12): 3764-3773.

[32] BROWN S, MARIA J P S, Jr., WALKER S. Wall teichoic acids of gram-positive bacteria[J]. Annual Review of Microbiology, 2013, 67(1): 313-336.

[33] XIA G Q, KOHLER T, PESCHEL A. The wall teichoic acid and lipoteichoic acid polymers *Staphylococcus aureus*[J]. International Journal Medical Microbiology, 2010, 300(2/3): 148-154.

[34] NEUHAUS F C, BADDILEY J. A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria[J]. Microbiology and Molecular Biology Reviews, 2003, 67(4): 686-723.

[35] WANG X H, DU Y M, FAN L H, et al. Chitosan- metal complexes as antimicrobial agent: synthesis, characterization structure-activity study[J]. Polymer Bulletin, 2005, 55(1/2): 105-113.

[36] LIU H, DU Y M, WANG X H, et al. Chitosan kills bacteria through cell membrane damage[J]. International Journal of Food Microbiology, 2004, 95(2): 147-155.

[37] MANSILLA A Y, ALBERTENGO L, RODRÍGUEZ M S, et al. Evidence on antimicrobial properties and mode of action of a chitosan obtained from crustacean exoskeletons on *Pseudomonas syringae* tomato DC3000[J]. Applied Microbiology Biotechnology, 2013, 97(15): 6957-6966.

[38] RAAFAT D, SAHL H G. Chitosan and its antimicrobial potential-a critical literature survey[J]. Microbial Biotechnology, 2010, 2(2): 186-201.

[39] DE SOUZA R H F V, TAKAKI M, DE OLIVEIRA PEDRO R, et al. Hydrophobic effect of amphiphilic derivatives of chitosan on the antifungal activity against *Aspergillus flavus* and *Aspergillus parasiticus*[J]. Molecules, 2013, 18(4): 4437-4450.

[40] LI, K., XING, R., LIU, S., QIN, Y., MENG, X., & LI, P. Microwave-assisted degradation of chitosan for a possible use in inhibiting crop pathogenic fungi[J]. International Journal of Biological Macromolecules, 2012, 51, 767–773.

[41] HERNÁNDEZ-LAUZARDO A N, BAUTISTA-BAÑOS S, VELÁZQUEZ-DEL VALLE M G, et al. Antifungal effects of chitosan with different molecular weights on in vitro development *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill[J]. Carbohydrate Polymers, 2008, 73(4): 541–547.

[42] HONGPATTARAKERE T, RIYAPHAN O. Effect of deacetylation conditions on antimicrobial activity of chitosans prepared from carapace of black tiger shrimp (*Penaeus monodon*)[J]. Songklanakarin Journal of Science & Technology, 2008, 30(Suppl.1): 1–9.

[43] YOUNES, ISLEM, RINAUDO, et al. Chitin and chitosan preparation from marine sources. Structure, properties and applications[J]. Marine Drugs, 2015, 13(3), 1133–1174.

[44] GOY R C, MORAIS S T B, ASSIS O B G. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth[J]. Revista Brasileira de Farmacognosia, 2016, 26(1): 122–127.

[45] LIN S B, CHEN S H, PENG K C. Preparation of antibacterial chitooligosaccharide by altering the degree of deacetylation of -chitosan in a *Trichoderma harzianum* chitinase-hydrolysing process[J]. Journal Science Agriculture, 2010, 89(2): 238–244.

[46] NO H K, PARK N Y, LEE S H, et al. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights[J]. International Journal Microbiology, 2002, 74(1/2): 65–72.

[47] NO H K, PARK N Y, LEE S H, et al. Antibacterial activities of chitosans and chitosan oligomers with different molecular weights on spoilage bacteria isolated from tofu[J]. Journal of Food Science, 2010, 67(4): 1511–1514.

[48] MELLEGÅRD H, FROM C, CHRISTENSEN B E, et al. Inhibition of *Bacillus cereus* spore outgrowth and multiplication chitosan[J]. International Journal Microbiology, 2011, 149(3): 218–225.

[49] TSAI G J, SU W H, CHEN H C, et al. Antimicrobial activity of shrimp chitin and chitosan from different treatments applications preservation[J]. Fisheries Science, 2010, 68(1): 170–177.

[50] LI J H, WU Y G, ZHAO L Q. Antibacterial activity and mechanism of chitosan with ultra high molecular weight[J]. Carbohydrate Polymers, 2016, 148: 200–205.

[51] ŠIMŮNEK J, BRANDYSOVÁ V, KOPPOVÁ I, et al. The antimicrobial action of chitosan, low molar mass chitosan, and chitooligosaccharides human colonic bacteria[J]. Folia Microbiologica, 2012, 57(4): 341–345.

[52] TAYEL A A, MOUSSA S, OPWIS K, et al. Inhibition of microbial pathogens by fungal chitosan[J]. International Journal of Biological Macromolecules, 2010, 47(1): 10-14.

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