
Oligosaccharide Chitosan: Viscosity, Molecular Weight, Antibacterial Activity, and Impact of γ Radiation

Vu Ngoc Boi^{1,*}, Nguyen Thi My Trang¹, Dang Xuan Cuong², Vu Thi Hoan³, Le Hai⁴

¹Faculty of Food Technology, Nha Trang University, Nha Trang, Vietnam

²Organic Matter from Marine Resource, Nha Trang Institute of Technology Application and Research, Vietnam Academy of Science and Technology (VAST), Ha Noi, Vietnam

³Institute of Biotechnology and Food, Industrial University of Ho Chi Minh City, Ho Chi Minh, Vietnam

⁴Da Lat Nuclear Research Institute, Vietnam Atomic Energy Institute, Da Lat, Vietnam

Email address:

minhboint@yahoo.com (Vu N. Boi), trangntm@ntu.edu.vn (N. T. My Trang), cuong_mails@yahoo.com.vn (D. X. Cuong),

yenhoan08@yahoo.com (Vu T. Hoan), lehaicnxb@yahoo.com (Le Hai)

*Corresponding author

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Abstract: Chitosan is a bioactive polymer produced from shrimp and crab shells, etc. According to VASEP (Vietnam Association of Seafood Exporters and Producers), the production of raw shrimp cultured in Vietnam was about 800,000 tons in 2018. Therefore, the shrimp processing industry has generated about 320,000 tons of wastes, including heads and shells. If wastes are not utilized and managed in proper ways, it can lead to serious environmental problems. In our study, shrimp shells were used to produce chitosan and further obtained oligochitosan for application in food preservation. The cobalt-60 radiation technology has been used to segment chitosan into oligochitosan. The radiation dose applied to chitosan solution was in the range of 25 ÷ 50 kGy and in the range of 66 ÷ 166 kGy for chitosan flakes. The results showed that the chitosan solution had higher segmental efficiency compared to that of chitosan flakes. The antibacterial activities of oligosaccharide chitosan segmented from chitosan flakes were higher than those of oligosaccharide chitosan segmented from chitosan solution. The highest antibacterial activities were observed in the oligochitosan segmented from chitosan flakes at the radiation dose of 66 kGy for all tested bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*. In addition, oligochitosan segmented from chitosan flakes at the radiation dose of 66 kGy had higher antibacterial activities on bacteria gram (-) than bacteria gram (+). The strongest antibacterial activities on *L. monocytogenes* and *B. subtilis* at the concentration of 0.3125%.

Keywords: Radiation, Chitosan, Oligochitosan, Antibacterial, Bacteria Gram (-), Bacteria Gram (+)

1. Introduction

Chitosan is a biological polymer produced from shrimp and crab shells, etc [1-3]. Oligochitosan can be segmented from chitosan by using different technologies such as enzyme technology or cobalt-60 radiation technology [3-6]. Oligochitosan is believed to have antibacterial activities. Therefore, some researches have been carried out on using oligosaccharide chitosan in vegetable preservation and antifungal, etc [7-10]. The advantage of using cobalt-60

radiation technology to segment chitosan is that after segmentation oligochitosan products can be used immediately in the preservation process without purification or removal of impurities.

The aim of this study was to the impact of cobalt-60 radiation on the viscosity, the molecular weight, and antibacterial activity of oligosaccharide chitosan.

2. Material and Methods

2.1. Material

+ Chitosan: Chitosan was whiteness, fine flakes, 0.51% of protein, 0.5% of ash, the water content of 13.2%, deacetyl degree of 93.5%, soluble degree of 99.5%, the viscosity of 1050 cPs, and turbidity of (NTU) 12.1.

+ Bacteria strains: The bacteria strains were used for the antibacterial evaluation consisting of *E. coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis* that supplied by the Institute of Veterinary Research and Development of Central Vietnam.

The chemicals in the analysis were purchased from Sigma – Aldrich, except for distilled water and 96% ethanol of Vietnam.

2.2. Methodologies

The segment chitosan flakes and chitosan solution in 1% acetic acid were by using gamma 60Co radiation equipment (GC-5000) (Made in India) at the Da Lat Nuclear Research Institute. Radioactivity used was at 4.000 Ci, dose rate of $\sim 3,6$ kGy/hour, operating in semi-automatic mechanism. Radiation chamber volume of 4.4 liters. The radiation temperature was controlled by water at temperature of 25°C [10].

The irradiation of chitosan flakes and chitosan solutions was by various doses for collecting oligosaccharide chitosan, for example, 10, 30, 70, 166 kGy for chitosan flakes and 10, 15, 20, 25 and 50 kGy for chitosan solutions. After the irradiation, all samples were filtered, segmented by different solvents (methanol and acetone), and evaluated on its viscosity and molecular weight.

The irradiation of chitosan solution and chitosan flakes was at 50 kGy and by various doses (66 kGy, 76 kGy, 100 kGy, 158 kGy, and 166 kGy), respectively, for collecting the solution of antibacterial oligosaccharide chitosan that evaluated by using the method of minimum inhibitory concentration (MIC).

The molecular weight determination of chitosan and oligosaccharide chitosan was on the viscometer Ubbelohde [11]. The determination of solution viscosity was on the machine.

Segment separation was by using specific solvents [10].

Antibacterial activities determination of oligosaccharide chitosan was by using the method of minimum inhibitory concentration (MIC), as in [12, 13]. Results assessment: The evaluation of antibacterial activity was according to the following levels: +++++: 100% inhibition (non-presented bacteria colonies); ++++: inhibition from 75% to <100%; ++: inhibition from 50 to <75%; +: inhibition from 25 to <50%; (-): negative, and positive evaluation in levels +++ and +++++.

2.3. Data Analysis

The data sets presented were mean of three different replicates. Means were calculated and graphed by using MS. Excel 2007 software.

3. Results and Discussion

3.1. The Viscosity

When the radiation dose increased, the viscosity was decreased for chitosan solution preparing from chitosan of flakes and solution. The solution viscosity of flakes chitosan was higher than one of solution chitosan. The concentration of chitosan also decided the viscosity of the radiated chitosan concentration. The concentration of chitosan flakes affected the viscosity of solution more than chitosan solution, as in Figures 1 and 2.

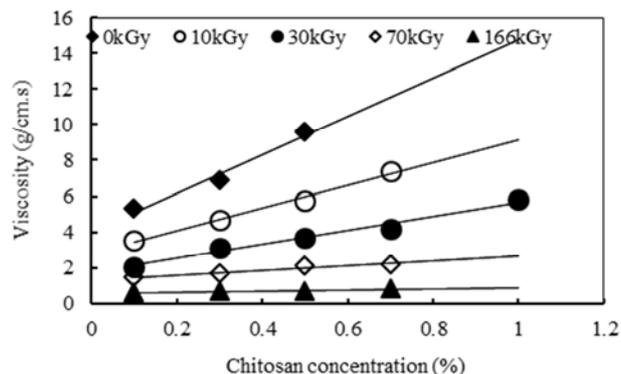


Figure 1. The viscosity of solution preparing from chitosan flakes according to chitosan concentration and radiation dose.

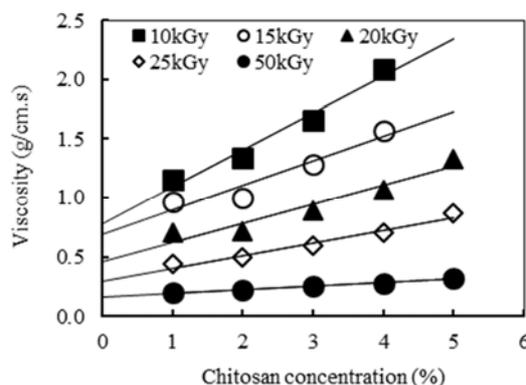


Figure 2. The viscosity of solution preparing from chitosan solution according to chitosan concentration and radiation dose.

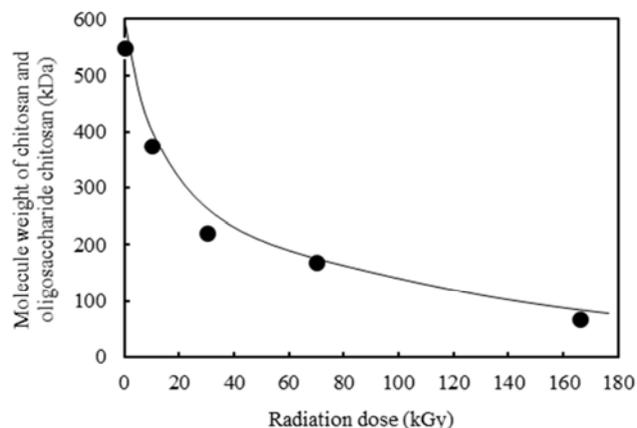


Figure 3. The molecular weight of chitosan oligosaccharide chitosan preparing from chitosan flakes according to the radiation dose.

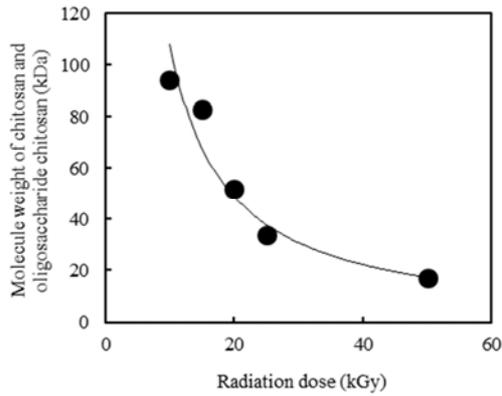


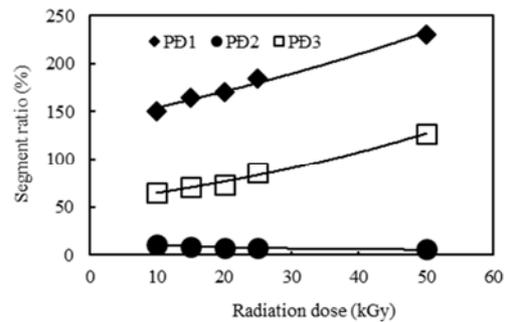
Figure 4. The molecular weight of chitosan and oligosaccharide chitosan preparing from chitosan solution according to the radiation dose.

3.2. The Molecule Weight

The radiation dose affected the weight molecular of chitosan ($p < 0.05$). The molecular weight of chitosan decreased with increasing the radiation dose for both chitosan flakes and chitosan solution (Figures 3 and 4, respectively). The molecular weight of chitosan flakes decreased from about 549 kDa to 67 kDa (decreasing 8.2 times) at the radiation dose of 166 kGy (Figure 3). It means that chitosan had segmented into smaller molecular weight chitosan (called oligosaccharide chitosan) and similar to those notices of Ulanski et al. (1992) [14] and Le Hai et al. (2003) [10]. After radiation, the molecular weight was 94 kDa at the radiation dose of 10 kGy, which decreased 5.8 times compared to the control sample. At radiation dose of 15 kGy, 20 kGy, 25 kGy, and 50 kGy, the molecular weight of chitosan corresponded about 83 kDa, 52 kDa, 34 kDa, and 17 kDa, respectively, for solution chitosan (Figure 4). It means the $\gamma 60$ radiation segmented chitosan in forms of either chitosan flakes or chitosan solution. The $\gamma 60$ radiation impacted on solution chitosan more than chitosan flakes.

Oligosaccharide chitosan in solution was continuously precipitated in different solvents, for example, methanol and acetone. The results showed acetone precipitated the lowest oligosaccharide chitosan of the molecular weight and the content, compared to methanol solvent. The molecular weight of oligosaccharide chitosan was arranged in the increasing order for a molecular weight as follows: acetone, the methanol

to chitosan solution ratio (9/1 (v/v)), and the methanol to chitosan solution ratio (2/1 (v/v)). The average molecular weight was in the range of 15 - 70 kDa for the 1st segment and 11 - 50 kDa for the 2nd segment. The average molecular weight of 3rd segment was under 11 kDa (Figures 5 and 6). The content of oligosaccharide chitosan in the precipitation segment by using the methanol to chitosan solution ratio (2/1 (v/v)) was 40 and 1.8 times, compared to the precipitation segment by using acetone and the methanol to chitosan solution ratio (9/1 (v/v)), respectively. The oligosaccharide chitosan in 1st, 2nd, and 3rd segment was black color, gelatinous form, and ivory-white color powder, respectively. The oligosaccharide chitosan in the 3rd segment solubilized well in water with a molecular weight under 11 kDa. The solution viscosity was a positive correlation to the chitosan concentration and a negative correlation to radiation dose for both chitosan flakes and chitosan solution.



Note: PD_i: Segment i, and i was from 1 to 3.

Figure 5. Changes in segment ratio of oligochitosan as a function of radiation dose (chitosan solution).

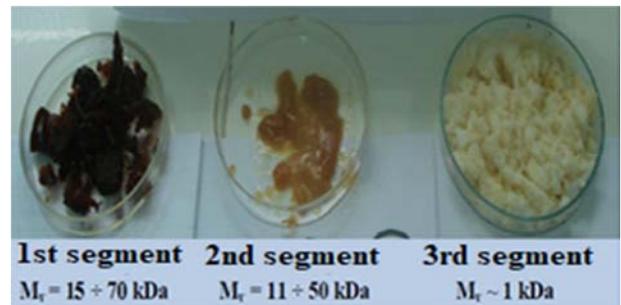
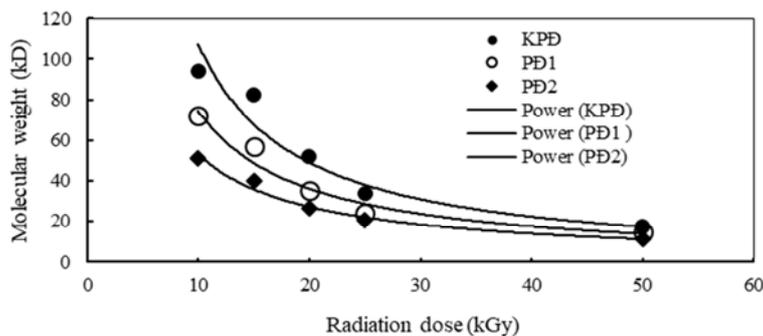


Figure 6. Pictures of different segments of oligochitosan at the radiation dose of 50 kGy.



Note: KPĐ, PDD1, and PDD2 was the none-segment, 1st segment, and 2nd segment, respectively.

Figure 7. Changes in molecular weight of oligochitosan of segment 1 and 2 as affected by the radiation dose.

3.3. Antibacterial Activities

The results showed that oligosaccharide chitosan from chitosan solution and chitosan flakes exhibited antibacterial activity on *E. coli* O157: H7, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, and *B. subtilis*. Antibacterial activity of M1.1 (66 kGy) and M1.6 (166 kGy) was higher than in other samples. M1.3 (100 kGy) and M1.5 (158 kGy)

were no resistance *E. coli* O157:H7 (Table 1). *L. monocytogenes*, *S. aureus*, and *B. subtilis* were impacted by oligosaccharide chitosan preparing at the different conditions of radiation. Oligosaccharide chitosan exhibited the highest antibacterial on *Bacillus subtilis* in comparison to other bacterial. 1st, 2nd, and 3rd segment of oligosaccharide chitosan were oxidation fast and decreased the activity to near zero.

Table 1. Impact of oligosaccharide chitosan concentration on the growth of bacteria.

Sample	Minimum inhibitory concentration				
	<i>E. coli</i> O157:H7	<i>S. typhimurium</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Oligosaccharide chitosan obtaining from the radiated chitosan solution					
Oligosaccharide chitosan 10%	1/8	1/4	1/4	1/4	1/8
Oligosaccharide chitosan 5%, pH 6.5	1/2	1/2	1/2	1/2	1/4
Oligosaccharide chitosan obtaining from the radiated chitosan flakes (5% solution)					
M1.1 (66kGy)	1/8	1/2	1/8	1/8	1/16
M1.2 (76kGy)	1/4	1/2	1/4	1/4	1/8
M1.3 (100kGy)	-	1/2	1/2	1/8	1/8
M1.5 (158kGy)	-	1/2	1/2	1/8	1/8
M1.6 (166kGy)	1/8	1/2	1/8	1/8	1/16
The control (non-radiation)	1/4	-	1/4	1/4	1/4

Table 2. The minimum inhibition concentration of oligosaccharide chitosan from radiated chitosan flakes on the bacteria growth.

Sample	Dilution factor				Inhibition
	1/2	1/4	1/8	1/16	
<i>E. coli</i> O157:H7					
M1.1 (66 kGy)	++++	++++	+	-	1/4
M1.6 (166 kGy)	++++	++	-	-	1/2
<i>Salmonella typhimurium</i>					
M1.1 (66 kGy)	+++	++	+	+	1/2
M1.6 (166 kGy)	+++	+	+	+	1/2
<i>Listeria monocytogenes</i>					
M1.1 (66 kGy)	++++	++++	++++	++++	1/16
M1.6 (166 kGy)	++++	++++	++++	++++	1/16
<i>Staphylococcus aureus</i>					
M1.1 (66 kGy)	++++	++++	+	-	1/4
M1.6 (166 kGy)	++++	+++	+	-	1/4
<i>Bacillus subtilis</i>					
M1.1 (66 kGy)	++++	++++	++++	+++	1/16
M1.6 (166 kGy)	++++	++++	++++	++	1/8

At the lowest dilution ratio of 1/16 (v/v), M1.1 (66 kGy) (++++) was the resistance of *Listeria monocytogenes* and *Bacillus subtilis* and M1.6 (166 kGy) was the resistance of *Listeria monocytogenes* (Table 2). At the dilution ratio of 1/8 (v/v), M1.6 (166 kGy) resisted *Bacillus subtilis*. For *Staphylococcus aureus*, M1.1 (66 kGy) and M1.6 (166 kGy) exhibited resistance activity at the dilution ratio of 1/4 (v/v). M1.1 (66 kGy) and M1.6 (166 kGy) was the resistance of *Salmonella typhimurium* at the dilution ratio of 1/2 (v/v). For *E. coli*, M1.1 (66 kGy) and M1.6 (166 kGy) exhibited activity at the dilution ratio of 1/4 and 1/2 (v/v), respectively. Therefore, the impact of oligosaccharide chitosan on *Salmonella typhimurium* was weak than other bacterial, and *E. coli* was affected by M1.6 (166 kGy) less than M1.1 (66 kGy).

The oligosaccharide chitosan from chitosan flakes possessed higher antibacterial activity than that from chitosan solution at the same concentration. The antibacterial activity of oligosaccharide chitosan at the radiation dose of 66 kGy

was higher than that at the radiation dose. The oligosaccharide chitosan affected gram-negative bacteria more than gram-positive bacteria, and the highest impact on *L. monocytogenes* and *B. subtilis*, compared to other bacteria. The minimum inhibition concentration of oligosaccharide chitosan on *L. monocytogenes* and *B. subtilis* was 0.3125%. For most other bacteria strains, the minimum inhibition concentration of oligosaccharide chitosan was in the range of 0.625 to 1.25%. Numerous previous studies noticed on antibacterial activity of oligosaccharide chitosan, as in [15, 16]. Antibacterial activity of oligosaccharide chitosan depended on the molecular weight of oligosaccharide chitosan, the chitosan destroying method, and the material extracting of chitosan [17, 18]. The difference caused the diverse on antibacterial activity of oligosaccharide chitosan. The antibacterial mechanism of oligosaccharide chitosan was to base on the membrane disruption [15], the inhibition of RNA and protein synthesis, the breakage of the intracellular

component [16], the ionic interaction [19], and free amino groups of oligosaccharide chitosan [20]. According to [21], cationic formation based on the amino protonation leading the bacterial killing. All mechanisms had a common to be interaction with the cell membrane of bacterial and mechanism inhibition in microorganisms. Antimicrobial activity depended not only on the physical chemistry properties of chitosan and their derivatives, but also on the concentration of metal ions (K^+ , Na^+ , Mg^{2+} , and Ca^{2+}) [22].

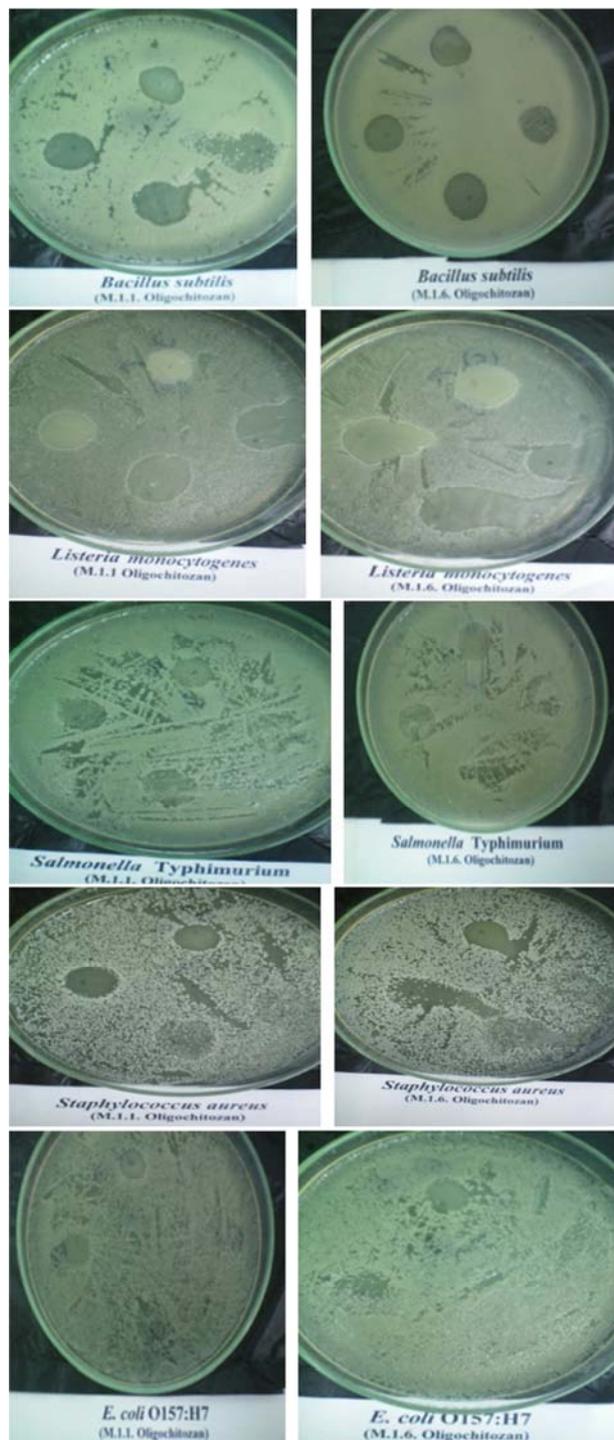


Figure 8. Some pictures of the antibacterial of oligosaccharide chitosan.

4. Conclusion

The γ 60 radiation destroyed chitosan to oligosaccharide chitosan that got the molecular weight under 100 kDa. The viscosity and the molecular weight of oligosaccharide chitosan were negative proportional to the radiation dose, positively proportional to the chitosan concentration. Acetone and the mixture of methanol and aqueous were useful in the segment of oligosaccharide chitosan. The average molecular weight of the 3rd segment was under 11 kDa. Oligosaccharide chitosan produced by radiation of chitosan flakes (dried chitosan) had higher antibacterial activity than that of oligosaccharide chitosan produced by radiation of chitosan solution. The radiated oligosaccharide chitosan at the radiation dose of 66 kGy had the highest antibacterial activity. Oligosaccharide chitosan at the radiation dose of 66 kGy had antibacterial activity on all tested bacteria strains: *E. coli* O157:H7, *Salmonella typhimurium*, *L. monocytogenes*, *S. aureus*, and *B. subtilis*. Antibacterial activity on bacteria gram (-) was higher than bacteria gram (+), in particular, the antibacterial activity was observed on *L. monocytogenes* and *B. subtilis*, which are most common spoilage bacteria on the food system. Oligosaccharide chitosan at the concentration of 0.3125% inhibited *L. monocytogenes* and *B. subtilis*.

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