Paper ID-301

Application of Gamma Radiation and Physicochemical Treatment to Improve the Bioactive Properties of Chitosan Extracted from Shrimp Shell

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Summary

The aim of this study is to exploit suitable chitosan extraction method from chitin implying different physicochemical treatments and to improve different bioactive properties of this extracted chitosan (CS) by applying gamma radiation. Chitosan was extracted from chitin by eight different methods varying different physicochemical parameters and assessed with respect to degree of de-acetylation, time and reagents. The method where chitin was repeatedly treated with 121˚C for 30 min with 20M NaOH, produced highest DD value (92%) with the least consumption of time and chemicals and thus selected as the best suitable extraction method. Antimicrobial activity as determined by well-diffusion method, was found insignificant in all the samples.

For further quality improvement, chitosan with highest DD value was irradiated with different doses (i.e., 5.0, 10.0, 15.0 and 20.0 kGy) of gamma radiation. No significant changes in DD value of chitosan was observed upon irradiation. Molecular weight of wet chitosan decreased from $1.16x10^5$ to 1.786×10^3, 1.518×10^3, 1.134×10^3 and 1.046×10^3 Dalton, respectively, as the radiation dose increased. Radiation treatment of chitosan samples increased the antimicrobial activity significantly in concentration dependent manner on both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria. One and half percent (1.5%) chitosan solution treated with a radiation dose of 5.0-10.0 kGy showed highest antimicrobial activity. Solubility, water binding capacity (WBC) and fat binding capacity (FBC) also improved due to irradiation of chitosan

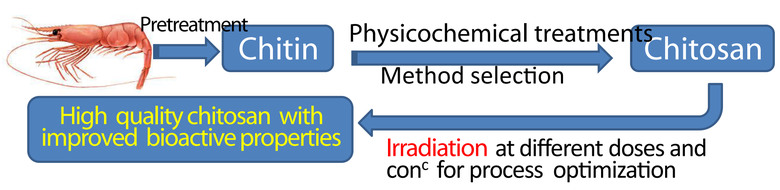
Introduction

Chitin is a renewable natural nitrogenous polysaccharide found in bio-shield of crustaceans and insects as well in the cell wall of fungus. Among them, shrimp-shell is the most potential source of chitin. It is long chain biopolymer of a N-acetyl glucosamine linked with β-(1 4) glycosidic linkage.

Chitosan, a cationic polymer can be derived upon N-deacetylation of chitin by alkalization method. Radiation processing of different polymers including chitosan can make many value added products with improved quality. Therefore, research on irradiation on these polymers recently drawing special attention in different fields including food preservation, agriculture and medical applications.

Objectives

- Utilization of chitin-rich shrimp waste as renewable resource for production of valuable products.
- Conversion of Shrimp-chitin into chitosan by various physicochemical treatments and selection of the most suitable method (cheap, time saving and high DD value) for chitosan extraction.
- Radiation modification of this chitosan to improve its bioactive properties (e.g., antimicrobial activity)

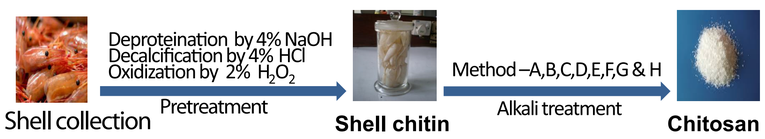


Methods and Materials

Extraction of Chitosan from Shell chitin

After Shell collection, chitin sample was prepared by pretreatment. From this chitin, chitosan was extracted by 8 alkalization methods as follows-

A. NaOH(20M): 121˚C, 30min→Wash→Chitosan
B. NaOH(20M): 121˚C, 30min→Wash→121˚C, 30min→Wash→Chitosan
C. NaOH(26M): 121˚C, 30min→Wash→Chitosan
D. NaOH(26M): 121˚C, 30min→Wash→121˚C, 30min→Wash→Chitosan
E. NaOH(20M+0.2%H_2O_2): RT, 4d→Wash→Chitosan
F. NaOH(20M+0.2%H_2O_2): RT,4d→Wash→121˚C, 30min→Wash→Chitosan
G. NaOH(20M+0.2%H_2O_2): RT, 4d→Wash→ RT, 4d→Wash→Chitosan
H. NaOH(20M+0.2%H_2O_2): RT, 4d→Wash→ 121˚C,30min→Wash→Chitosan



Irradiation of Chitosan

Chitosan sample was irradiated with a series of doses (5, 10, 15, 20, 30, 40 and 50 kGy) at different concentration from a Cobalt-60 gamma source (~65kCi). Ceric-Cerus dosimetry was performed to ensure the dose level.

Determination of Degree of De-acetylation

Performed by Potentiometric Titration Method

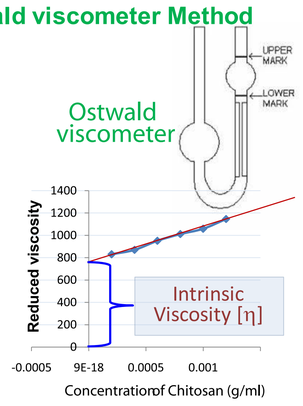
Preparation of chitosan soln (0.1 M HCl + 0.05% Chitosan)
↓
Step-wise titration @100µl NaOH (0.1M), (pH: 1.5 → 11.0)
↓
Measurement pH in every step upon addition of NaOH
↓
Plot with pH vs NaOH vol and slope of pH vs NaOH vol
↓
Two Peaks detection from plot of slope vs NaOH (V1, V2)
↓
DD value (%) = 16.1x(V2-V1)xMol of NaOH/Wt of Chitosan

Determination of Molecular Weight (MW)

➢ Performed by Ostwald viscometer Method

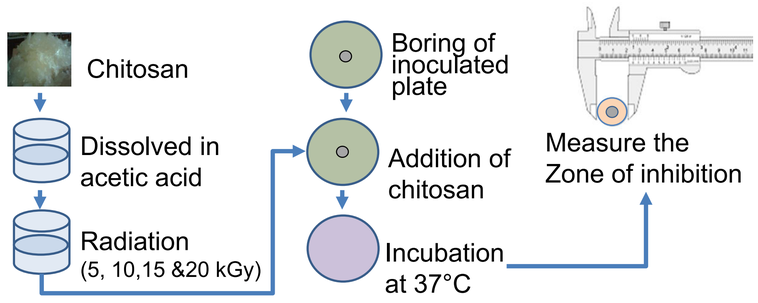
Preparation Chitosan soln (dif. Conc)
↓
Load & Run viscometer
↓
Flow time (at 30°C)
↓
Intrinsic viscosity, [η]
↓
Calculate MW by $[\eta]=KM^{\alpha}$

(Mark Houwink Formula where K&α are constant)



Determination of Antimicrobial Activity

Performed by Well Diffusion Method



Measurement of WBC/FBC Binding Capacity

➢ Performed by Well Diffusion Method

Weigh 0.5 g of chitosan in a cen. tube
↓
Add 10ml of water & vortex for 1min
↓
Incubate at 37C for 30min with int. shake
↓
Centrifuge at 3000 rpm for 25 min
↓
Decantate sup. and weigh tube again

$$WBC(\%) = \frac{WaterBound(g)\times 100}{InitialSampleWeight(g)}$$

$$FBC(\%) = \frac{WaterBound(g)\times 100}{InitialSampleWeight(g)}$$

Result and Discussion

Parameters Optimization for Suitable Extraction of Chitosan from chitin:

Chitosan was extracted from chitin applying 8 different methods varying different physicochemical parameters. Method-B was found to be most suitable method that produced highest DD value (92%) within short time (4h).

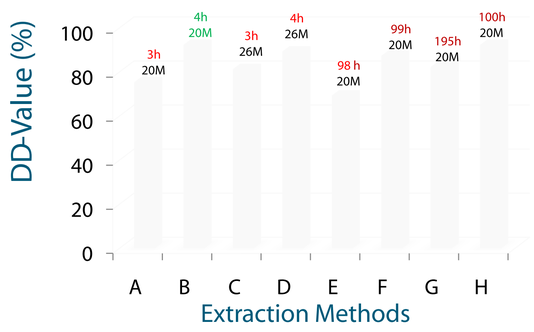


Figure 1: DD-Value obtained by different extraction Methods

Irradiation of Chitosan decreased its MW

To improve the quality, chitosan of best quality was irradiated at different doses. MW decreased as the radiation dose increased much in wet condition than in dry condition (Fig 2a & 2b).

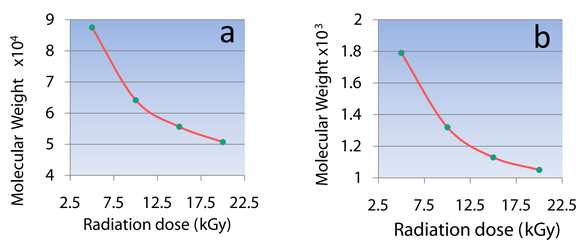


Figure 2: Effect of gamma radiation on the MW of chitosan Irradiated in (a) dry and (b) wet condition

Radiation increased the antimicrobial activity of chitosan in dry condition

Radiation treatment improved the antimicrobial activity of chitosan significantly in dry condition (Fig 3). When dry chitosan was irradiated, activity on both Gram negative (E.coli) and Gram Positive bacteria (S. aureus) gradually increased as the dose increased up to the last dose (50kGy) applied .

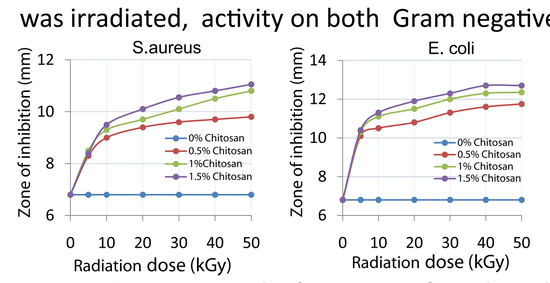


Figure 3: Antimicrobial activity of irradiated dry chitosan

Radiation attributed the antimicrobial activity of chitosan in wet condition critically

Radiation treatment improved the antimicrobial activity of chitosan in wet condition significantly. When chitosan was irradiated at 0.5% concentration with different doses, the antimicrobial activity increases initially at 5 and 10 kGy, but later on activity decreased rapidly as the dose increased (Fig 4). When chitosan was irradiated at 1.0% concentration with different doses, after gaining antimicrobial activity at 5 and 10 kGy it started losing its potency slowly as the dose increased gradually and finally become 0 at 40-50 kGy. Interestingly when the sample was irradiated at 1.5% concentration the antimicrobial activity substantially gained at 5-10kGy and sustained up to high dose (50 kGy) In the case of Gram negative bacteria. On the other hand potency seems to be decreased against Gram positive bacteria

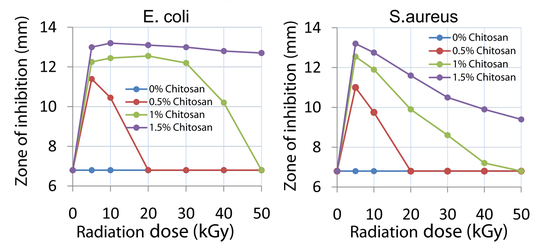


Figure 4: Antimicrobial activity of chitosan irradiated at 0.5%, 1.0% and 1.5% concentration

When chitosan was irradiated at 4% concentration, antimicrobial activity increased significantly at 5 and 10kGy and activity persisted up to 40 kGy with slowly increment which still against both on Gram Positive and negative bacteria up to 40 kGy with slowly increment which still against both on Gram positive and negative bacteria (Fig 5).

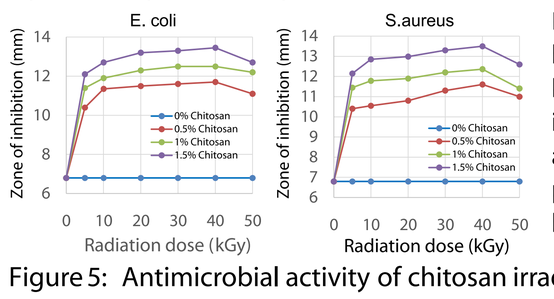


Figure 5: Antimicrobial activity of chitosan irradiated at 4.0% con^c

When chitosan was irradiated at 10% concentration, antimicrobial activity increased at 10 kGy and gradually increased up to the last dose 50kGy applied But the level of antimicrobial activity is yet less than that of treated at 4% concentration (Fig 6).

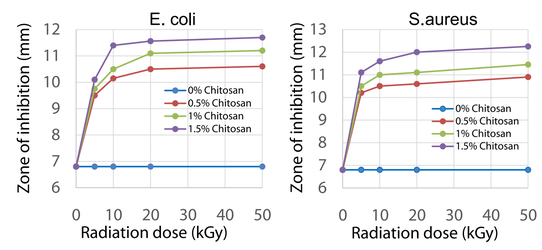


Figure 6: Antimicrobial activity of chitosan irradiated at10.0% con^c

Irradiation improve WBC and FBC of chitosan

Gamma radiation also influenced the water and fat binding capacity (WBC and FBC) of chitosan. Both of WBC and FBC increased gradually as the radiation dose increased. WBC of chitosan is more influenced by irradiation than FBC as depicted in Fig 7.

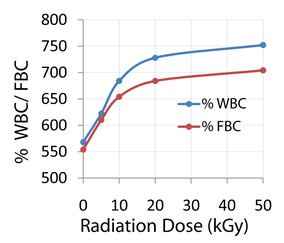


Figure 7: Effect of gamma radiation on WBC and FBC of chitosan

Conclusion

- Repeated alkali treatment (20M NaOH) at 121°C for 30 min was found to be the best extraction method.
- MW decreased as the radiation dose increased gradually.
- Gamma radiation critically influences the antimicrobial activity of chitosan on organism type, radiation dose and chitosan concentration dependant manner.
- It is more feasible and economic to irradiate the product at 5-10 kGy at a concentration of 4-5% of chitosan as this product is active against both Gram positive and Gram negative bacteria with minimum energy consumption.
- WBC, FBC & solubility of chitosan can be increased by applying gamma radiation.