Degradation of chitosan for rice crops application (Postprint)

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Abstract

A variety of techniques including chemical and enzymatic hydrolysis, and radiation degradation processes can be used to prepare low molecular weight chitosan. Degradation of chitosan by radiation can be carried out in solid state and liquid state. Radiation degraded polysaccharides has been reported to exhibit growth-stimulating activity like phytohormones thatinduce the promotion in germination, shoot and root elongation in variety of plants. In this study, the chitosan was irradiated in solid state (powder form) by gamma rays within the dose range of 25 75 kGy. And the irradiated chitosan was then irradiated in solution form in the presence of hydrogen peroxide. The effects of irradiation on the molecular weight and viscosity of the chitosan were investigated using Ubbelohde Capillary Viscometer. The molecular weight and viscosity of the chitosan decreased with increment of absorbed doses. In the presence of hydrogen peroxide, the molecular weight of chitosan could be further decreased. The effect of radiation degraded chitosan on the growth promotion of rice was investigated and it was shown during seedling period of 15 days for transplanting whereby the growth is 15% 20% faster than using chemicals growth promoters.

Full Text

Preamble

Degradation of chitosan for rice crops application

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Abstract

A variety of techniques including chemical and enzymatic hydrolysis, and radiation degradation processes can be used to prepare low molecular weight chitosan. Degradation of chitosan by radiation can be carried out in both solid state and liquid state. Radiation-degraded polysaccharides have been reported to exhibit growth-stimulating activity similar to phytohormones that promote germination, shoot and root elongation in various plants. In this study, chitosan was irradiated in solid state (powder form) by gamma rays within the dose range of 25–75 kGy. The irradiated chitosan was subsequently irradiated in solution form in the presence of hydrogen peroxide. The effects of irradiation on the molecular weight and viscosity of chitosan were investigated using an Ubbelohde Capillary Viscometer. The molecular weight and viscosity of chitosan decreased with increasing absorbed doses. In the presence of hydrogen peroxide, the molecular weight of chitosan could be further reduced. The effect of radiation-degraded chitosan on the growth promotion of rice was investigated, and it was shown that during the 15-day seedling period for transplanting, growth was 15%–20% faster compared to using chemical growth promoters.

Key words: Radiation, Degradation, Rice crops, Growth promoter

Introduction

Chitosan is a cationic polymer that is the second most abundant polymer in nature after cellulose. It is a linear copolymer polysaccharide consisting of β(1-4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units. Chitosan has been identified for use in many primary industries such as agriculture, paper, textiles, pharmaceuticals, and wastewater treatment [1–3]. It is an interesting biomaterial due to its good biocompatibility, biodegradability, low toxicity, hemostatic potential, good film-forming characteristics, and anti-infectional activity. The unique properties of chitosan arise from its amino groups that carry positive charges at pH values below 6.5, enabling binding to negatively charged materials such as enzymes, cells, polysaccharides, nucleic acids, hair, and skin. However, in some fields (especially industrial, medical, and food applications), chitosan is limited by its high molecular weight and low solubility in aqueous media [4,5]. Low-molecular-weight chitosan can be prepared by chemical [6], enzymatic [7], or radiation [8] degradation of high-molecular-weight chitosan.

Radiation has been found to be one of the most effective tools for modification of polysaccharides, offering a safer, more environmentally friendly, and easier method to modify polymers without using chemicals or high temperature treatment. Natural polymers that have potential for modification include cellulose, starch, guar gum, gum acacia, chitin-chitosan, alginates, carrageenans, and their derivatives, which occur abundantly. These polymers are now being explored for potential applications in food, medicine, and cosmetic industries due to their unique structures, biodegradability, and biocompatibility. Irradiation of natural polymers leads to scission of the main chain that can produce oligomers with lower molecular weight and viscosity. In some applications, such as plant growth promoters in agriculture and drug delivery systems, low molecular weight of chitosan or alginate is needed for the polymer to function successfully.

Oligosaccharides derived from depolymerization of polysaccharides through enzymatic hydrolysis have been reported to exhibit growth-stimulating activity like phytohormones that induce promotion of germination, shoot and root elongation in various plants [9–11]. Radiation-degraded polysaccharides show similar effects as plant growth promoters. For example, radiation-degraded alginate solution (4%) at 100 kGy has been shown to significantly enhance growth of rice seedlings in hydroponic systems [12]. The suitable concentration range of degraded alginate between 20–100 ppm has impacted 15% and 60% weight gain in rice and peanuts, respectively. A similar study was conducted by Relleve et al. [13] using irradiated carrageenan for rice seedlings under non-circulating hydroponic conditions.

The effect of radiation-degraded polysaccharide on plant growth promotion is not well understood. However, radiation-degraded polysaccharide can be controlled to a certain molecular weight to produce oligosaccharides that are mobile and easily taken up by plants. In this paper, the effects of radiation-processed oligochitosan on rice seedling growth and its use as a fungicide are reported.

2. Materials and Methods

2.1 Materials

Chitosan powder was purchased from Nibong Tebal with the following properties: 90.6% degree of deacetylation (DDA), 10% water content, and containing 0.1 ppm As, 0.1 ppm Cd, 0.8 ppm Cu, 3.16 ppm Ni, and 0.1 ppm Pb. Hydrogen peroxide (H_2O_2) was purchased from Sigma, sodium acetate and acetic acid from Fisher Scientific, and lactic acid from OFT Chemicals Sdn. Bhd. All reagents were used as received without further purification.

2.2 Preparation of oligochitosan

Chitosan powder was irradiated in PE plastic bags with a gamma ^{60}Co source using the Sinagama Facility (Nuclear Malaysia Agency) at doses of 25, 50, and 75 kGy with a dose rate of 2.15 $\text{kGy} \cdot \text{h}^{-1}$. The irradiated chitosan powder at the optimum dose was then dissolved in lactic acid prior to irradiation using gamma rays from ^{60}Co at 12 kGy combined with a small amount of hydrogen peroxide. The irradiated chitosan powder was later dissolved in the lactic acid solution and stirred overnight for complete hydration of chitosan.

2.3 Molecular weight and intrinsic viscosity measurement

Intrinsic viscosity and viscosity-average molecular weight were examined using an automatic Ubbelohde capillary viscometer system that allows automatic reading of flow times without using a stopwatch. The measurement was conducted at (25$±$0.1)°C. The reduced viscosity and inherent viscosity were plotted against chitosan concentration. The intrinsic viscosity value was calculated by extrapolating the graphs of reduced viscosity and inherent viscosity to zero concentration, and the average of the two intercept values was calculated. The intrinsic viscosity [ ] as a function of viscosity-average molecular weight (Mv) is represented by the Mark-Houwink-Sakurada equation:

$$[\eta] = KM_v^{\alpha} \quad (1)$$

where K = 3$×10^{-2}$ mL/g and α = 0.83, determined in 0.2M CH_3COOH/0.1M CH_3COONa solution. The solution was prepared 24 h before measurement and could only be used within 24 h.

2.4 Study of rice seedling growth

MR219 rice seeds supplied by FECLRA Berhad were used throughout this study. Three different treatments of rice seeds were performed: (i) rice seeds were soaked in commercial grade growth promoter solution for 24 h as control (C); (ii) rice seeds were soaked in oligochitosan (O) for 24 h; (iii) rice seeds from treatment (i) were sprayed with commercial grade nutrient solution; and (iv) rice seeds from treatment (ii) were sprayed with oligochitosan on day 8 and day 13. Rice seeds from control (C) and oligochitosan (O) treatments were planted in trays (~300 seeds per tray) containing rice husk as the planting medium.

2.5 Field trial for blast disease study

For the blast disease study, nine treatments were applied across a total area of 24 hectares with 1.0 hectare per plot, prepared in triplicates as shown in Table 1 .

Ulanski and Rosiak [14] reported that chitosan irradiated in the solid state was only degraded and that crosslinking was negligible. The degradation rate of polysaccharide depends on many factors such as the type of ionizing source (gamma rays, electron beam, or X-rays), radiation dose, and sample condition. Reduction of molecular weight was found to be lower when the sample was irradiated in liquid state [15].

3. Results and Discussion

3.1 Effect of irradiation on molecular weight

Radiation technology has been used to produce high-performance polymeric materials with unique physical and chemical properties. Upon radiation, two main reactions influence the final properties of polymers: (a) scission of the main chain, also known as degradation, and (b) cross-linking, the opposite process to degradation. The former results in reduction of molecular weight while the latter causes an increment in molecular weight. The degradation process usually occurs when natural polymers are subjected to ionizing radiation.

Figure 1 [Figure 1: see original paper] illustrates the effect of radiation on chitosan in powder form after exposure to gamma radiation at various doses up to 75 kGy. The most pronounced decrease occurred at lower doses up to 50 kGy. At 50 kGy, the molecular weight dropped from 67,352 Da to 14,946 Da. This reduction was due to chain scission of the chitosan backbone where degradation took place. Further increment in irradiation dose did not compensate for the decrement in molecular weight of chitosan.

The drop in molecular weight was followed by a decrement in intrinsic viscosity of chitosan. Changes in intrinsic viscosity of chitosan with irradiation dose are depicted in Fig. 1. A sudden fall of intrinsic viscosity was observed at 50 kGy, where the intrinsic viscosity reduced from 369.5 mL/g to 98.5 mL/g. After 50 kGy, the intrinsic viscosity of chitosan remained stable. These data suggest that the optimum radiation dose for chitosan powder was 50 kGy.

3.2 Effect of H_2O_2 on chitosan

The reduction of molecular weight can be further enhanced by adding hydrogen peroxide (H_2O_2), as shown in Table 1. The irradiated chitosan powders were dissolved in lactic acid and then irradiated at 12 kGy in the presence of a small amount of H_2O_2. The results clearly show that irradiation in liquid state decreased the molecular weight rapidly to 8,034 Da compared to irradiated powder chitosan. A marked drop in molecular weight was observed for irradiated chitosan solution at 12 kGy in the presence of 0.1% H_2O_2, where the molecular weight reduced to 2,436 Da. With further increment in the amount of H_2O_2, the molecular weight of chitosan decreased further. It was evident that the combination of gamma irradiation and hydrogen peroxide in liquid state resulted in higher decrement in molecular weight compared to chitosan irradiated using gamma irradiation alone.

Under gamma irradiation in liquid state in the presence of H_2O_2, the primary reactions might occur as follows:

$$\mathrm{H_2O} \rightarrow e^-_{aq}, \mathrm{OH}\cdot, \mathrm{H}\cdot, \mathrm{H_2O_2}, \mathrm{H_2}, \mathrm{H}^+, \mathrm{OH}^-, \mathrm{H_3O}^+$$

$$\mathrm{H} \cdot + \mathrm{H_2O_2} \rightarrow \mathrm{H_2O} + \cdot\mathrm{OH}$$

$$e^-_{aq} + \mathrm{H_2O_2} \rightarrow \cdot\mathrm{OH} + \mathrm{OH}^-$$

$$\mathrm{H_2O_2} \rightarrow 2 \cdot \mathrm{OH}$$

$$\mathrm{R} + \cdot\mathrm{OH} \rightarrow \cdot\mathrm{R} + \mathrm{H_2O}$$

$$R + \cdot H \rightarrow \cdot R + H_2$$

$$\cdot R \rightarrow R_1 + R_2$$

The OH radical is a much more powerful oxidant. This radical, derived from the radiolysis of water, and H_2O_2 will break the chitosan backbone and lead to shorter chains. Thus, the addition of hydrogen peroxide can further decrease the molecular weight of chitosan. The same phenomenon was observed in the intrinsic viscosity measurements when chitosan was irradiated in H_2O_2 solution.

Table 1 Effect of hydrogen peroxide on chitosan solution treated with gamma rays and gamma rays/H_2O_2. Chitosan powder was pre-irradiated in powder form at 50 kGy prior to irradiation in liquid format at 12 kGy and 24 kGy.

Sample	Mv (Da$\times10^{3}$)	Intrinsic viscosity (mL/g)
12 kGy, without H_2O_2	8.034	-
24 kGy, without H_2O_2	-	-
12 kGy, 0.1% H_2O_2	2.436	-
12 kGy, 0.3% H_2O_2	-	-

3.3 Rate of rice seedling growth

Figure 2 [Figure 2: see original paper] shows that the growth rate of rice seedlings treated with oligochitosan is faster than that of rice seeds treated with commercial grade growth promoter. The usual height of rice seedlings for transplanting is around 14 cm at day 15, which can now be achieved at day 11 using oligochitosan. This shortens the rice seedling period by 4 days prior to transplanting. During this period, no additional nutrients were added to the rice seeds treated with oligochitosan. In contrast, rice seeds treated with commercial grade growth promoter required two nutrient sprayings.

3.4 Blast disease infection

From the blast lesion index against rice treatment as shown in Fig. 3, the control (T1) had the highest rank of blast infection. Treatment with oligochitosan and fungicide showed lower average rank of blast infection compared to the control. T6, T7, and T8 with oligochitosan treatment on seed and leaf gave lower numbers of plants infected with blast. The lowest rank of blast infection was observed in treatment 7 (soak in oligochitosan (200 ppm) + spray oligochitosan on leaves (40 ppm) on days 20, 30, and 40). This study demonstrated a significant effect of oligochitosan in protecting rice plants from severe blast infection in the field.

4. Conclusion

Radiation technology has proven to be an effective method for reducing the molecular weight of chitosan and other natural polymers. The molecular weight of chitosan decreased with increasing radiation dose, accompanied by a reduction in intrinsic viscosity. Radiation treatment of chitosan in the presence of hydrogen peroxide could effectively enhance the reduction of its molecular weight. Oligochitosan was shown to act as a growth promoter during the rice seedling period from day 1 to day 15. Based on the blast lesion index, oligochitosan treatment demonstrated effectiveness as an elicitor that can protect rice plants from blast disease.

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