DEGRADATION OF CHITOSAN BY γ-IRRADIATION OF CHITOSAN SWOLLEN IN HYDROGEN PEROXIDE SOLUTION

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ABSTRACT

Degradation of chitosan in swollen state with hydrogen peroxide solution (1–5 % w/v) by γ-irradiation was investigated. Molecular weight (Mw) of irradiated chitosan samples was determined by gel permeation chromatography (GPC). Fourier transform infrared (FT–IR) spectra and X–ray diffraction (XRD) patterns were utilized to study the structure of degraded chitosan. Results showed that low Mw chitosan of ~30–45 kDa was efficiently prepared by γ–irradiation of chitosan (Mw_0 ~ 91.7 kDa) swollen in hydrogen peroxide solutions at low dose less than 20 kGy. The main structure as well as the degree of deacetylation of degraded chitosan was almost unchanged. Furthermore, the radiation degradation yield (G_s) was remarkably enhanced with the presence of H_2O_2 as a sensitizer. Degradation of chitosan in swollen state with hydrogen peroxide solution by γ-irradiation is efficient due to synergistic effect of γ-ray/H_2O_2 at low dose.

Keywords: chitosan, degradation, hydrogen peroxide, γ-irradiation.

1. INTRODUCTION

Chitosan is commonly prepared by sodium hydroxide deacetylation of chitin from crab, shrimp shells and squid pens, and it consists of glucosamine and N-acetylglucosamine units linked by β(1–4) glycosidic bonds. Chitosan generally has high molecular weight (Mw) which in many cases limits its applications. The low Mw chitosan and its oligomer possess some special biological properties that differ from those of the ordinary high Mw chitosan such as antimicrobial activity [1–6], antioxidant activity [7–10], antitumor activity [11], immunity-enhancing effect [12, 13], plant elicitation and growth promotion effect [14–16], carriers for drug delivery [17, 18], and so on. Comprehensive information of biological activities of chitosan

and oligochitosan can be referred to the paper reviewed recently by Xia et al. (2011) [19]. In addition, Zeng et al. [20] reported that the absorption of chitosan in mice after oral administration increased with the decrease of the Mw and the increase of the water-solubility. They suggested that chitosan with low Mw and/or oligomers may be employed for different functional food. Thus, the development of a suitable process for reducing the Mw of chitosan, without altering chemical structure, is of great necessity. A variety of degradation methods of chitosan, including chemical method using HCl [21,22], HCl-H_3PO_4 [23], HNO_2 [24], H_2O_2 [25–27], enzymatic hydrolysis [8, 11, 22, 28–30], ultrasonic treatment [31–34], microwave irradiation [35–38], gamma irradiation [10, 15, 32, 39–42], etc has been studied. However, radiation (γ-ray, electron beam) has been considered as a useful tool for degradation of polymer from the viewpoint of environmentally friendly processing method [43] and large scale production [41]. Most of the previous research works on radiation degradation of chitosan was carried out by irradiation of chitosan in powder/flake form and/or in solution. Moreover, Kang et al. [40] and El-Sawy et al. [15] studied to irradiate chitosan in gelling state with acetic acid as solvent and hydrogen peroxide as sensitizer. However, they did not calculate radiation degradation yield (G_s) to compare with that of chitosan in powder form and in solution state. And furthermore, due to chitosan gelated with acid, it was rather difficult to obtain degraded chitosan product in powder form by drying.

Therefore, in the present study degradation of chitosan in swollen state with hydrogen peroxide solution by γ-ray Co-60 irradiation was carried out for the first time. G_s values of chitosan swollen in different concentration of hydrogen peroxide solution (1–5 %) and absorbed doses (5–20 kGy) were calculated. It was observed that the concentration of hydrogen peroxide was an important factor for increasing the G_s of chitosan. In addition, the degraded chitosan powder products were easily collected by drying of irradiated chitosan in forced air oven or even in open air at ambient temperature.

2. EXPERIMENTAL

2.1. Materials

Chitosan from shrimp shell chitin with Mw_0 of 91.7 kDa (polydispersity index, $PI_0 \sim 2.26$) and degree of deacetylation (DD) of 91.3 % was purchased from Chitosan Co., Vung Tau province, Vietnam. Before use, chitosan was dried in a forced air oven at 60 ^oC until reaching constant weight in order to remove moisture content. Hydrogen peroxide solution (30 %, w/v) was of reagent grade supplied by Merck, Germany. All the other chemicals were of reagent grade and used as received. Distilled water was used in all experiment.

2.2. Degradation of chitosan

Chitosan samples (1 g) in powder form were swollen in 5 ml aqueous H_2O_2 solution with concentration of 0 (water), 1, 3 and 5 % (w/v) for 30 min. The mixture ratio of chitosan and H_2O_2 solution of 1/5 (w/v) was selected and this ratio almost reached to the saturated capacity of water binding of chitosan after 30 min mixing with water [44]. Then, the swelled chitosan samples were irradiated by ^{60}Co gamma rays on the Gamma Chamber 5000, BRIT, India at the Nuclear Research Institute, Dalat with the absorbed dose from 5 to 20 kGy and dose rate of 3.6 kGy/h at ambient temperature. Then, the irradiated chitosan samples were dried at 60 ^oC in a forced air oven and ground into fine powder for characterization.

2.3. Characterizations

The weight-average molecular weight (Mw) of chitosan samples was measured by gel permeation chromatography (GPC) on an Agilent 1100 instrument with detector RI G1362A and two columns of ultrahydrogel model 250 and 500 from Waters (USA). The standards used to calibrate the column were pullulan (Mw 780–380.000). The eluent was aqueous solution containing 0.25 M CH_3COOH/0.25 M CH_3COONa with flow rate of 1 $ml.min^{-1}$ and temperature at 30 ^oC [45]. The chitosan sample concentration was ca. 0.1 % (w/v), and the injection volume was of 50 µl.

IR spectra were taken on a Shimadzu FT–IR 8400S spectrophotometer in the range between 4000 cm^{-1} and 400 cm^{-1} using KBr pellets. The DD (%) of degraded chitosan was calculated based on FT–IR spectra according to the following equation [46]:

$$A_{1320}/A_{1420} = 0.3822 + 0.0313\times(100–DD) \quad (1)$$

where A_{1320} and A_{1420} were absorbance of chitosan at 1320 cm^{-1} and 1420 cm^{-1}, respectively.

X–ray diffraction (XRD) patterns of degraded chitosan were measured by an X'Pert Pro X–ray diffractrometer (PANanatical, Nertherlands) over a range of diffraction angle 2θ from 5^o to 40^o and used a CuK_α target at 45 kV–40 mA at 25 ^oC.

3. RESULTS AND DISCUSSION

3.1. Reduction in Mw of chitosan

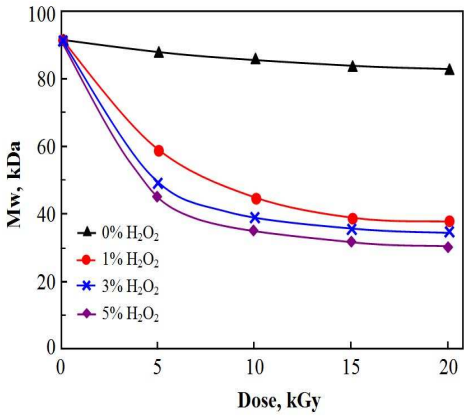

Figure 1. The molecular weight of chitosan versus treatment dose (the dose rate 3.6 kGy/h).

Figure 1 shows the reduction of chitosan Mw versus the treatment doses at different concentrations of H_2O_2. Accordingly, the Mw of chitosan decreased as the increase of the absorbed dose and concentration of H_2O_2 solution. It was also observed from figure 1 that Mw of chitosan dropped rapidly at the dose range from 0 to about 7 kGy for all three concentrations of H_2O_2 used and then it decreased slowly up to 20 kGy, whereas the Mw of the irradiated chitosan sample swelled in water decreased insignificantly. It was very surprised that chitosan swelled in water degraded by γ-irradiation with the extent less than that of dried chitosan powder, G_s of about 0.021 µmol/J (Table 1) was nearly five times smaller compared to that (0.1

μmol/J) of chitosan in powder form [39]. The reason may be due to the radiation degradation process occurred simultaneously with the radiation crosslinking process for chitosan swelled in water, but they happened in different level. Furthermore, the G_s values calculated based on two different equations namely one used for solid chitosan [39] and the another one (Eq. 2) used for chitosan in solution [38, 41] will receive different results that could not be appropriate to compare with each other. Therefore, further study of the effect of water on radiation degradation of chitosan should be carried out.

It was also obvious in Figure 1 that the higher the H_2O_2 concentration the higher the extent of the decrease of chitosan Mw attained. This can be explained due the synergistic effect of degradation occurred when chitosan was irradiated in the presence of H_2O_2 [15, 40, 41]. The mechanism of the synergistic effect of H_2O_2/γ–ray for degradation of chitosan was described in detail by Kang et al. 2007 [40] and Duy et al. (2011) [41]. Briefly, ˙OH radicals resulted from the radiolysis of water and H_2O_2 were one of the main agents for the degradation process of chitosan in solution as well as in swollen state. Assuming that chitosan swelled in hydrogen peroxide solution is supposedly as a solution, then the G_s (mol/J) of chitosan can be calculated based on the following equation [47]:

$$1/M_w - 1/M_{w0} = G_s \times D \times d/2C \quad (2)$$

where M_{w0} and M_w are the weight-average molecular weight of polymer before and after irradiation, d is the solution density (kg/dm^3), D is the absorbed dose [Gy (J/kg)], and C is the concentration of polymer in solution (g/dm^3). In this swollen mixture of chitosan and H_2O_2 solution, d was measured to be of 0.705 kg/dm^3 and C was of 117.65 g/dm^3. The calculated G_s values were presented in Table 1.

Table 1. G_s values of irradiated chitosan at the different doses and H_2O_2 concentration.

Dose (kGy)	G_s(μmol/J)			
	γ-ray/ water	γ-ray/1% H_2O_2	γ-ray/3% H_2O_2	γ-ray/5% H_2O_2
5	0.031	0.405	0.636	0.741
10	0.024	0.379	0.493	0.591
15	0.022	0.329	0.377	0.454
20	0.019	0.259	0.296	0.375

Results in Table 1 indicated that G_s values of chitosan in swollen state with water were from 0.02 to 0.03 μmol/J in the dose range from 5 to 20 kGy, whereas G_s values of chitosan in swollen state with H_2O_2 solution were drastically high from 0.26 to 0.74 μmol/J. Results in Table 1 also showed that the higher concentration of H_2O_2 solution the higher the G_s values obtained at the same dose. It was also recognized that at the same concentration of H_2O_2 the G_s values decreased as the increase of the dose. Duy et al. (2011) studied on synergistic degradation of chitosan by γ-irradiation of chitosan solution of 3% in the presence of H_2O_2 with concentration of 0, 0.25, 0.5 and 1 % [41]. They reported that the higher the concentration of H_2O_2 used the higher the G_s values obtained and particularly at dose of 12 kGy, G_s values were of 0.08, 0.55, 0.70 and 1.04 μmol/J for H_2O_2 of 0, 0.25, 0.5 and 1 %, respectively. Furthermore, Duy et al. (2011) also found that the G_s values decreased with the increase of dose. The reason

was due to that most of the existing H_2O_2 content decomposed at low dose less than 10 kGy and subsequently the resultant content of $^{\bullet}OH$ radicals for the degradation of chitosan was less at higher dose. Therefore, it may not be appropriate to calculate the average G_s value for the whole process of radiation degradation of chitosan containing H_2O_2 because the scission reaction of chitosan containing H_2O_2 by γ-irradiation occurs by mixed first-and second-order kinetics [48]. Thus, the presence of H_2O_2 during irradiation can cause significant enhancement of G_s, typically in swollen state. Therefore, degradation of chitosan in swollen state with H_2O_2 solution by γ-irradiation at low dose can be potentially applied on large scale.

3.2. FT–IR spectra

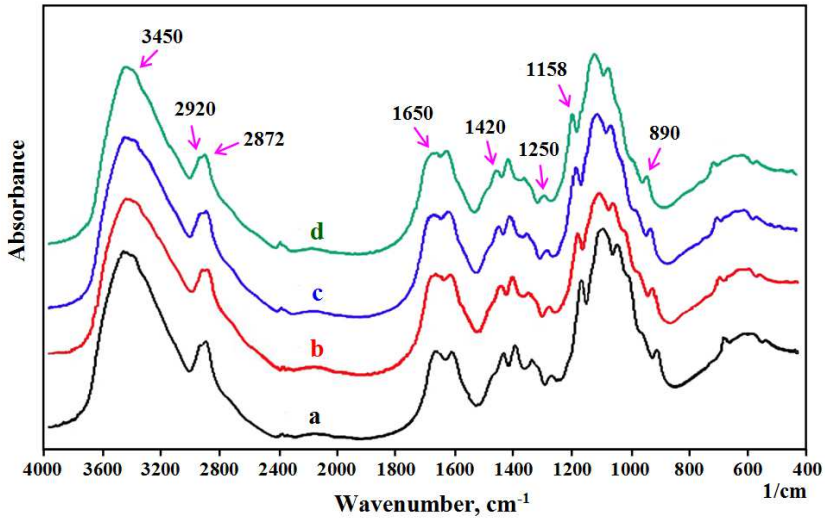

Figure 2. FT–IR spectra of the initial (a) and degraded chitosan in swollen state with 1 % (b), 3 % (c) and 5 % (d) concentration of H_2O_2 irradiated at dose of 10 kGy.

Infrared spectroscopy has been extensively applied to characterize the structure and calculate DD of chitosan. Figure 2 describes the FT–IR spectra of initial chitosan and degraded chitosan irradiated at 10 kGy in swelled state with the different concentration of H_2O_2. It was observed in Figure 2 that the spectra of degraded chitosan exhibited most of the characteristic bands as the initial chitosan. The bands in the range of 1158–890 cm^{-1} are corresponded to the characteristics of its polysaccharide structure. Peaks at 3450, 1650 and 1250 cm^{-1} are assigned to the hydroxyl, carbonyl or carboxyl, methyl and C–O–C groups, respectively [49]. The FT–IR spectra indicated that there is no change in main structure of degraded chitosan compared to that of initial one. Peaks at 2920, 2872, 1423 and 1265 cm^{-1} indicated the D–glucopyranose ring CH_2 group's symmetric and asymmetric vibration [38] was not altered. It indicated that there is no ring opening reaction. Furthermore, the carboxyl groups assigned as a oxidative product of glucopyranose rings cleavage-peak at 1730 cm^{-1} were absent [26]. It confirmed again that the oxidation of glucopyranose rings to acidic functional groups did not occur. In addition, the results of DD in Table 2 also indicated that the DD of degraded chitosan was not significantly different compared with that of the initial chitosan.

Table 2. Degree of deacetylation (DD%) of the initial chitosan and degraded chitosan in swollen state versus concentration of H_2O_2 at 10 kGy.

Samples	Initial chitosan	Degraded chitosan		
		1% H_2O_2	3% H_2O_2	5% H_2O_2
DD%	91.3 ± 0.3	90.0 ± 0.4	91.0 ± 0.3	91.2 ± 0.5

3.3. XRD analysis

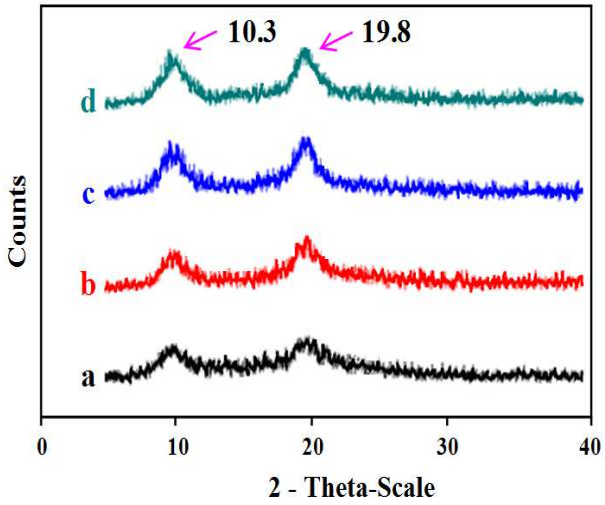

Figure 3. XRD patterns of the initial (a) and degraded chitosan in swollen state with 1 % (b), 3 % (c) and 5 % (d) concentration of H_2O_2 irradiated at 10 kGy.

The XRD patterns of the initial chitosan and degraded chitosan are illustrated in Figure 3. It revealed that XRD patterns of degraded chitosan also have two peaks, $2\theta = 10.3^o$ and 19.8^o, that are similar to characteristic peaks of the initial chitosan [15, 40, 41]. Accordingly, the crystalline structure of degraded chitosan was also unchanged.

The obtained low Mw chitosan has been tested as feed additive for chicken (data not shown). Preliminary obtained results showed that low Mw chitosan improved the weight gain and reduced the death rate of chicken. It is expected that the resultant low Mw chitosan will act as an effective immunity-enhancing agent for domestic animal and also for aquaculture of shrimp and fish [12, 13].

4. CONCLUSION

Chitosan with low Mw of 30–45 kDa can be efficiently prepared by γ-irradiation of chitosan swollen in 1–5 % H_2O_2 solution with ratio 1/5 (w/v) at low dose of about 10 kGy. The structure and degree of deacetylation of degraded chitosan were almost unchanged. The

radiation degradation yield G_s was remarkably enhanced with the presence of H_2O_2 as a sensitizer. Degradation of chitosan in swollen state with H_2O_2 solution by γ-irradiation is practically promising to apply for production of low Mw chitosan on large scale.

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TÓM TẮT

NGHIÊN CỨU CẮT MẠCH CHITOSAN Ở DẠNG TRƯƠNG TRONG DUNG DỊCH H_2O_2 BẰNG BỨC XẠ GAMMA Co-60

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Nghiên cứu cắt mạch chitosan ở dạng trương trong dung dịch H_2O_2 (1-5%) bằng bức xạ gamma Co-60 được thực hiện. Khối lượng phân tử (Mw) của các mẫu chitosan chiếu xạ được đo bằng phương pháp sắc kí gel thấm qua (GPC). Phổ hồng ngoại và giản đồ nhiễu xạ tia X được sử dụng để khảo sát đặc trưng cấu của chitosan chiếu xạ. Kết quả cho thấy chitosan có Mw thấp (~30 – 40 kDa) nhận được bằng phương pháp chiếu xạ chitosan (Mw_0 ~91,7 kDa) trương trong dung dịch H_2O_2 ở liều xạ thấp hơn 20 kGy. Cấu trúc phân tử và độ deacetyl của chitosan chiếu xạ thay đổi không đáng kể. Hơn nữa, hiệu suất cắt mạch bức xạ được tăng lên rõ rệt khi có mặt H_2O_2. Cắt mạch chitosan ở dạng trương trong dung dịch H_2O_2 bằng bức xạ gamma Co-60 là rất hiệu quả để chế tạo chitosan Mw thấp.

Từ khóa: chitosan, cắt mạch, H_2O_2, bức xạ gamma Co-60.