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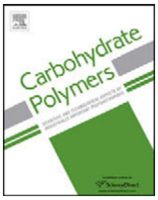

Short communication

Degradation of chitosan in solution by gamma irradiation in the presence of hydrogen peroxide

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ABSTRACT

Chitosan from squid pens with deacetylation degree (DD) of 70% and average molecular weight (M_{w0}) 90.5×10^3 in dilute lactic acid solution containing H_2O_2 (1%) was effectively degraded by irradiation with gamma ^{60}Co radiation (1.33 kGy/h) at doses in the range 4–16 kGy. Based on the results of M_w measured by gel permeation chromatography (GPC), it was concluded that there was particularly strong synergy between H_2O_2 and radiation for degradation at the lower radiation doses studied. Radiation scission yields (Gs) were found out to be 2.2 μmol/J and 0.2 μmol/J for 5% chitosan with and without 1% H_2O_2, respectively. The DD of degraded chitosan measured from IR spectra was almost unchanged by the treatment.

1. Introduction

Chitosan, poly-β-(1-4)-D-glucosamine can be obtained by full or partial deacetylation of chitin, the natural polymer widely existing in crustacean shells, cell walls of fungi, insects and yeast (Liu et al., 2006; Wang, Huang, & Wang, 2005). Due to many unique properties such as biocompatibility, biodegradability, bioactivity and non-toxicity, chitin/chitosan has been studied and utilized extensively in biotechnology, water treatment, agriculture, pharmacy, and the food industry (Kumar, 2000; Rinaudo, 2006; Shahidi, Arachchi, & Jeon, 1999). Due to its antimicrobial activity in particular, chitosan has been used to prepare edible film and coating by blending with other ecofriendly and biodegradable polymers such as poly(vinylalcohol) (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997), gelatin (Arvanitoyannis, Nakayama, & Aiba, 1998), and starch (Vasconez, Flores, Campos, Alvarado, & Gershenson, 2009). Properties of chitosan depend not only on its structure but also on molecular weight. Generally, chitosan with high molecular weight is not soluble in water, which limits its applications especially in medicine and food industry (Yue, Yao, Wei, & Mo, 2008). Degraded chitosan with low molecular weight is soluble in water and has significant differences in antimicrobial (Zheng & Zhu, 2003), antitumor (Qin, Du, & Xiao, 2002; Qin, Du, Xiao, Li, & Gao, 2002) and plant growth promoting activity (Chmielewski et al., 2007; El-Sawy, Abd El-Rehim, Elbarbary, & Hegazy, 2010; Hien, 2004) compared to high molecular weight chitosan. There are some methods for degradation of chitosan, such as acidic hydrolysis (Tommeraas, Varum, Christensen, & Smidrod, 2001), enzymatic hydrolysis (Kim & Rajapakse, 2005; Qin, Du, & Xiao, 2002; Qin, Du, Xiao, et al., 2002), oxidation by hydrogen peroxide (Tian, Liu, Hu, & Zhao, 2004; Qin, Du, & Xiao, 2002; Qin, Du, Xiao, et al., 2002), radiation (Choi, Ahn, Lee, Byun, & Park, 2002; El-Sawy et al., 2010; Feng, Du, Li, Hu, & Kennedy, 2008; Hien, 2004; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005). According to Kim and Rajapakse (2005), chemical processes have some drawbacks due to low production yields and a higher risk of environmental pollution. The cost of production of oligochitosan by enzymatic methods is generally higher than that of oxidative processes (Makuuchi, 2010). Degradation of chitosan using hydrogen peroxide (H_2O_2) has been extensively studied because this method is easy and non-hazardous (Qin, Du, & Xiao, 2002; Qin, Du, Xiao, et al., 2002). However, this will change the structure of chitosan especially the glucosidic ring when H_2O_2 is used excessive at high temperature. On the other hand, irradiation induced degradation of chitosan has gained considerable attention due to its advantages: the process is reliable, carried out at room temperature and can apply in large scale. Nevertheless, in order to prepare oligochitosan with M_w generally less than 10,000 the required irradiation dose has to be relatively high from 50 to 100 kGy (Choi et al., 2002; Hien, 2004). In this article, the synergistic effect for the degradation of chitosan in solution with γ-ray and hydrogen peroxide has been studied. The objective of the work is the preparation of low molecular weight chitosan and/or oligochitosan using low irradiation doses.

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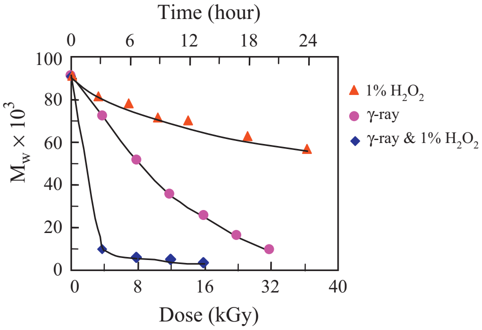

Fig. 1. The molecular weight of chitosan versus treatment time and dose.

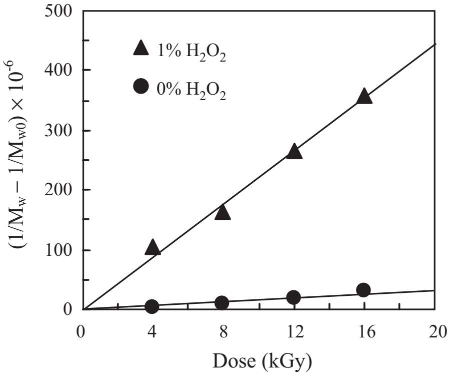

Fig. 2. The relationship between $(1/M_w - 1/M_{w0})$ and dose.

2. Materials and methods

2.1. Materials

β-Chitosan was prepared from squid pens with DD of 70.4% and $M_w \sim 90{,}500$. Lactic acid, H_2O_2 and other chemicals were of reagent grade.

2.2. Preparation of chitosan solution and irradiation

Chitosan (5 g) was dissolved in 80 ml lactic acid solution 3% (w/v), then a certain amount of H_2O_2 was added to obtain a solution containing chitosan 5% (w/v) and H_2O_2 1% (w/v). Then, the resulting solution was irradiated with a gamma ^{60}Co source at the absorbed doses up to 16 kGy with a dose rate of 1.33 kGy/h at ambient temperature.

2.3. Characterization

The molecular weight (M_w) of degraded chitosan was characterized by an Agilent1100 gel permeation chromatography (GPC) (Agilent Technologies, USA) with detector RI G1362A and the columns Ultrahydrogel model 250 and 500 from Waters (USA). The standards for calibration of the columns were pullulan (M_w 780–380 × 10^3). The eluent was aqueous solution 0.25 M CH_3COOH/0.25 M CH_3COONa with the flow rate of 1.0 ml min^{-1} and temperature at 30 °C (Knaul, Kasaai, Bui, & Creber, 1998). The chitosan sample concentration was 0.1% (w/v).

IR spectra was taken on Equinox55 (Bruker, Germany) FT-IR spectrometer using KBr pellets.

The degree of deacetylation was calculated based on IR spectra according to the following equation (Brugnerotto et al., 2001):

$$\frac{A_{1320}}{A_{1420}} = 0.3822 + 0.0313 \times (100 - \mathrm{DD}\%)$$

where A_{1320} and A_{1420} are absorbances of chitosan at 1340 cm^{-1} and 1420 cm^{-1}, respectively.

3. Results and discussion

3.1. Synergistic degradation of chitosan by γ-ray and hydrogen peroxide

Fig. 1 shows the degradation of chitosan molecular weight versus reaction time with H_2O_2 and absorbed dose with γ-ray. It was obvious that the percentage of chitosan molecular weight decreased by the combination of γ-ray and H_2O_2 was much higher than that by γ-irradiation or H_2O_2 1% alone. This result showed that the synergistic effect for degradation of chitosan with γ-ray and H_2O_2 gained the most effective outcome, especially at the lowest absorbed dose studied of 4 kGy. Based on the data in Fig. 1, percentage of chitosan molecular weight decrease was calculated and shown in Table 1. The synergistic effect defined as the additional % molecular weight reduction over and above that obtained from the sum of H_2O_2 treatment applied separately was 58.4% at 4 kGy and gradually decreased to 0.2% at 16 kGy. That means that synergistic degradation is more effective at lower doses. The mechanism for the synergistic effect was presented by Kang, Dai, Zhang, and Chen (2007). Particularly, the degradation of chitosan by the γ-ray irradiation alone can be ascribed to the direct action of radiation on the chitosan chains. Besides, when chitosan was degraded by gamma irradiation in the presence of H_2O_2, the radiolysis of H_2O_2 and water resulted in the formation of hydroxyl radicals, which were the primary reason for the degradation of chitosan. As the powerful oxidizing species, hydroxyl radicals react with chitosan by abstraction of carbon-bound hydrogens and then the resulting carbohydrate radicals cause breakage of the glucosidic bonds by rearrangement (Ulanski & von Sonntag, 2000), which reduced molecular weight of chitosan very effectively. The same tendency was obtained by Kang et al. (2007) when they studied the degradation of chitosan under γ-ray irradiation at dose from 10 kGy to 100 kGy in the presence of 2%, 10% and 30% H_2O_2. However, they prepared chitosan in suspension form. Therefore, the dose and the H_2O_2 concentration were rather high compared to our results. In addition, El-Sawy et al. (2010) studied the degradation of chitosan by mixing chitosan powder in paste form with H_2O_2. Then chitosan samples were irradiated at dose from 20 to 200 kGy, which was also higher as compared to the dose in our study. These results may be explained that the hydroxyl radicals were more mobile in solution than in suspension or paste form. Thus, the synergistic effect for the degradation of chitosan in solution was more efficient than in suspension or paste form.

The radiation scission yield G_s (mol/J) was calculated as following equation (Janik, Kasprzak, Al-Zier, & Rosiak, 2003):

$$\left(\frac{1}{M_w} - \frac{1}{M_{w0}}\right) = \frac{G_s \times D \times d \times 1000}{2C}$$

where M_{w0} and M_w are the molecular weights of original and degraded chitosan, respectively; D is the absorbed dose (kGy); d is the solution density (g/cm^3); C is the solution concentration (g/l).

From Fig. 2, G_s values for 5% of chitosan solution with and without 1% H_2O_2 were 2.2 and 0.2 μmol/J, respectively. Thus, with the presence of 1% H_2O_2, the G_s value of chitosan was increased about 10 times. The lowest G_s value of about 0.1 μmol/J is for chitosan irradiated in dry powder or flake (Hien, 2004; Ulanski & Rosiak, 1992). Therefore, irradiation technique may be suitable for the preparation of low molecular weight chitosan (Chmielewski et al., 2007; Zainol, Akil, & Mastor, 2009). However, G_s value increases with the decreasing chitosan concentration in solution, mainly because of

Table 1
Results of degradation of chitosan using H_2O_2, γ-ray and synergistic effect (γ-ray and H_2O_2).

Samples	4 kGy (3 h)	8 kGy (6 h)	12 kGy (9 h)	16 kGy (12 h)
Molecular weight decrease (%) = 100 × ($M_{w0} - M_w$)/M_{w0}				
A (1% H_2O_2)[a]	10.4	14.5	21.3	23.5
B (γ-ray)[b]	21.7	44.8	61.9	73.4
C (A and B)	90.5	93.8	96.2	97.1
Synergistic effect D (%)				
D = C–(A + B)	58.4	34.5	13.0	0.2

[a] Time (h) for H_2O_2.
[b] Time (h) and/or dose (kGy) for γ-ray.

Table 2
The DD values and M_w of chitosan in solution containing 1% H_2O_2 irradiated with different doses.

Dose (kGy)	0	4	8	16
M_w	90,500	8600	5600	2700
DD (%)	70.4	70.5	73.2	74.6

the enhancement of $^{\bullet}OH$ mobility with reduced viscosity of dilute solution of polymer (Wasikiewicz et al., 2005). Therefore, irradiation of chitosan in solution is convenient for the production of oligochitosan.

3.2. IR spectral analysis

Table 2 shows that degradation of chitosan in solution containing 1% H_2O_2 by γ-ray irradiation with the dose up to 16 kGy did not significantly affect the DD of chitosan. However, degradation of chitosan to prepare oligochitosan only by H_2O_2 will accompany with structure changes (Qin, Du, & Xiao, 2002; Qin, Du, Xiao, et al., 2002). There was no significant change in the backbone of chitosan with M_w of 51×10^3, but the degraded chitosan with M_w of 3.5×10^3 lost about 15% amino groups. This was also different from our results where amine groups were almost unchanged with M_w of 2.7×10^3 (Fig. 3 and Table 2). Interestingly, it was found that oligochitosan with M_w of 2.7×10^3 as prepared is completely soluble in water.

Vander, Varum, Domard, Gueddari, and Moerschbacher (1998) reported that oligochitosan with intermediate DD had higher effect of anti-infection of disease for plants. The oligochitosan obtained in this study also exhibited strong effect against disease infection caused by *Pyricularia grisea* fungus and growth-promotion on rice (data not shown). Furthermore, according to results for the effect of radiation degraded chitosan on the growth of faba bean plants reported by El-Sawy et al. (2010), the obtained oligochitosan with M_w less than 10,000 with the formation of carboxyl groups and partial elimination of amino groups had a positive effect on plants growth and also on the productivity. Recently, Yin, Zhao, and Du (2010) considered oligochitosan as a potential plant disease vaccine in their review.

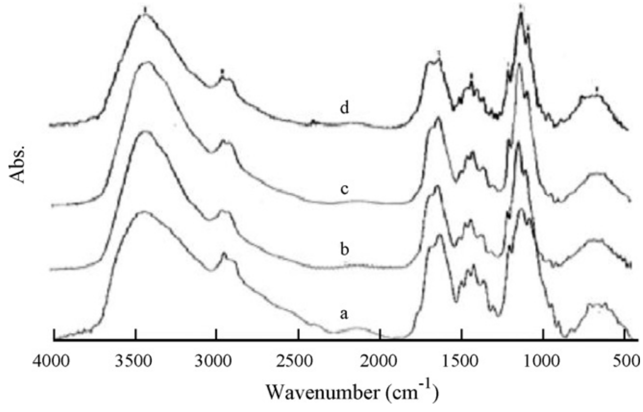

Fig. 3. FT-IR spectra of chitosan: initial chitosan (a); after irradiation with different doses in 1% H_2O_2: 4 kGy (b), 8 kGy (c) and 16 kGy (d).

4. Conclusions

Chitosan was effectively degraded by γ-ray irradiation in the presence of H_2O_2. The G_s value for chitosan was increased from 0.2 (γ-ray only) to 2.2 μmol/J (γ-ray and H_2O_2). The synergistic effect was 58.4% at 4 kGy and decreased to 0.2% at 16 kGy. The DD of degraded chitosan was not significantly changed for the doses up to 16 kGy. Degradation of chitosan in solution containing hydrogen peroxide by γ-irradiation is an efficient method for the preparation of oligochitosan.

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