

Decoloration of chitosan by UV irradiation

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Received 28 September 2007; received in revised form 3 December 2007; accepted 4 December 2007

Available online 14 December 2007

Abstract

Decoloration of chitosan by UV irradiation, which was used to replace a bleaching step during chitosan preparation, was evaluated under four separate treatments (effect of irradiation time, chitosan/water ratio, stirring speed, and UV light source). The optimal decoloration condition was defined as that producing white chitosan with higher viscosity. Decoloration of chitosan could be achieved effectively using a UV-C light by stirring unbleached chitosan in water (1:8, w/v) for 5 min at 120 rpm. UV irradiation applied under the optimal conditions could be used to produce chitosan with desirable white color ($L^* = 76.95$, $a^* = -0.37$, and $b^* = 14.04$) and high viscosity (1301.7 mPa s at 0.5% w/v in 1.0% v/v acetic acid).

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Keywords: Chitosan; Color; Decoloration; UV irradiation; Viscosity

1. Introduction

Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crawfish. The typical production of chitosan from crustacean shell generally consists of three basic steps: demineralization (DM), deproteinization (DP), and deacetylation (DA) (No & Meyers, 1995); In chitin production, the DM and DP steps produce a colored chitin product. When a bleached chitinous product is desired, pigments can be removed with organic solvents or bleaching agents (No & Meyers, 1995). In general, the organic solvents are not as effective as the bleaching agents such as sodium hypochlorite, hydrogen peroxide or ozone in removing pigments from crab or crawfish shell (Brine & Austin, 1981; Moorjani, Achutha, & Khasim, 1975; No & Lee, 1995; No, Meyers, & Lee, 1989; Seo, King, & Prinyawiwatkul, 2007). As bleaching

agents considerably reduce the viscosity of the chitosan product (Moorjani et al., 1975; Seo et al., 2007), an alternative, yet effective and economical decoloration method that will yield decolorized chitosan with high viscosity should be developed.

Earlier investigations have revealed that higher molecular-weight (or viscosity) chitosans were more effective as food preservatives than lower molecular-weight chitosans in extending the shelf life of foods such as bread (Lee et al., 2002), pork (Lee, Park, & Ahn, 2003), sausage (Youn, Park, & Ahn, 2000), and fish (Jeon, Kamil, & Shahidi, 2002). Furthermore, some studies reported that chitosan was more effective in inhibiting growth of bacteria than were chitosan oligomers (No, Park, Lee, & Meyers, 2002; Uchida, Izume, & Ohtakara, 1989). Therefore, production of chitosan with high viscosity is a primary concern. Once high-viscosity chitosan is prepared, low-viscosity chitosan, if necessary, can be subsequently obtained by chemical or enzymatic hydrolysis (No, Nah, & Meyers, 2003).

Investigations from our laboratory (Youn, No, & Prinyawiwatkul, 2007) demonstrated that decolorized chitosan

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with high viscosity could be simply prepared by sun drying after the DA step without using a bleaching agent. Removal of pigments of chitosan with the aid of sun drying is mainly attributed to the UV radiation in the sun light (Sionkowska, 2006). However, applicability of sun drying for decoloration of chitosan is entirely dependent on the weather condition. It is realized that UV light irradiation may serve as an alternative and effective decoloration method that will yield decolorized chitosan without having to depend on weather conditions.

The objective of the present research was to prepare decolorized chitosan with high viscosity by UV irradiation without using a bleaching agent.

2. Materials and methods

2.1. Materials

Dried crab (*Chionoecetes opilio*) leg shell was obtained from Keumho Chemical (Seoul, Korea). The shell was ground through a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 2-mm mesh screen and subsequently sifted with 20 (0.841 mm) and 40 mesh (0.425 mm) sieves using a portable sieve shaker (JISICO, Seoul, Korea). The ground shell with 0.841–0.425 mm particle size was used throughout this research to obtain reproducible and consistent results.

2.2. Production of chitosan

The production of chitosan involved the demineralization (DM), deproteinization (DP), deacetylation (DA), and decoloration (DC) steps (No, Lee, Park, & Meyers, 2003). The ground crab leg shell was demineralized with 1 N HCl for 30 min at ambient temperature with a solid/solvent ratio of 1:15 (w/v). Following the DM step, the demineralized shell was collected on a 100-mesh sieve, washed to neutrality in running tap water, rinsed with deionized water, and filtered to remove excess moisture. The DP step was accomplished by treating the demineralized shell with 3% NaOH for 15 min at 15 psi/121 °C and a solid/solvent ratio of 1:10 (w/v). The unbleached chitin was collected, washed as mentioned above, and dried at 60 °C for 4 h in a forced-air oven.

The DA step was achieved by treating chitin under conditions of 15 psi/121 °C with 45% NaOH for 30 min and a solid/solvent ratio of 1:10 (w/v). The resulting chitosan residue was washed and filtered as mentioned above, and subjected to decoloration (DC) by UV irradiation.

2.3. Decoloration of chitosan by UV irradiation

Four separate treatments were sequentially conducted at different time intervals at room temperature. In Treatment 1 (selection of the optimum reaction time), 5 g wet chitosan and 40 mL water were placed in an open-glass petri dish [9 cm inside diameter (ID) × 1.8 cm height (H)], stirred at

about 120 rpm using a vortex mixer, and irradiated by 20 W UV-C light (lamp length, 41 cm; wavelength, 254 nm; Germical GL-15, Philips, Holland) for 5, 10, 15, 30, and 60 min.

In Treatment 2 (selection of the optimum water volume), 5 g wet chitosan and 0, 5, 10, 20, 40, 80, or 140 mL water (a chitosan:water ratio of 1:0, 1:1, 1:2, 1:4, 1:8, 1:16 or 1:28) were placed in a petri dish (14 cm ID × 2.3 cm H), stirred at 120 rpm, and irradiated by 20 W UV-C light for 5 min (based on the optimum reaction time from Treatment 1).

In Treatment 3 (selection of the optimum stirring speed), 5 g wet chitosan and 40 mL water (a 1:8 ratio) were placed in a petri dish (9 cm ID × 1.8 cm H), stirred at different speeds (120, 180, 300, and 420 rpm), and irradiated by 20 W UV-C light for 5 min. The chitosan:water ratio and irradiation time were selected based on results of Treatments 1 and 2.

In Treatment 4 (selection of the optimum light source), 5 g wet chitosan and 40 mL water were placed in a petri dish (9 cm ID × 1.8 cm H), stirred at 120 rpm, and irradiated by three different UV lights (A, B, C) for 5 min. The chitosan:water ratio, stirring speed, and irradiation time were selected based on results of Treatments 1–3. The wavelength of UV-A lamp (Philips actinic BL 15 W, Holland) and UV-B lamp (Philips TL-D 15 W, Holland) was 365 and 312 nm, respectively. Immediately following UV irradiation, all chitosans were filtered and dried at 60 °C for 4 h in a forced-air oven for determination of moisture, viscosity and color. From the four separate treatments, the optimal decoloration condition was selected and defined as that producing white chitosan with higher viscosity.

A schematic diagram of a UV-treatment system for chitosan decoloration is shown in Fig. 1. In Treatments

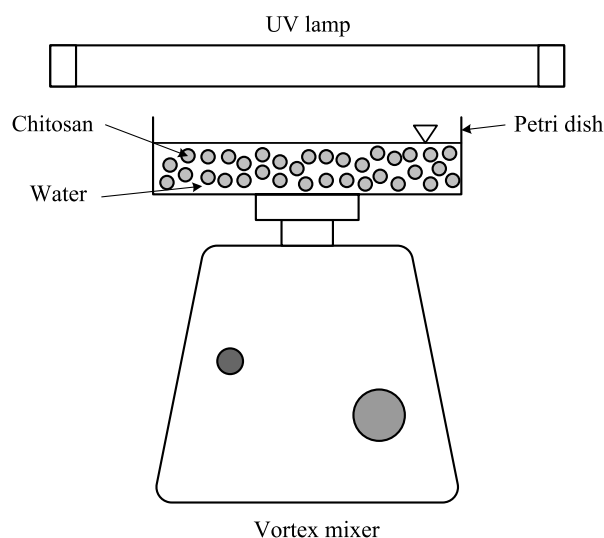


Fig. 1. A schematic diagram of a UV-treatment system for chitosan decoloration.

1–4, petri dishes containing chitosan and water were positioned 4 cm below the UV light source. The intensity of UV lights was measured by the light intensity meter (VLX-3W, Cole-Parmer Instrument, Vernon Hills, IL, USA).

2.4. Measurement of moisture, viscosity and color

The moisture content was determined using a halogen moisture analyzer (HG53, Mettler Toledo, Greifensee, Switzerland). Viscosity was determined with a Brookfield viscometer, model LVDV-II+ (Brookfield Engineering Labs., Stoughton, MA, USA). Chitosan solution was prepared in 1% (v/v) acetic acid at a 0.5% (w/v) concentration on a moisture-free basis. Viscosity measurements were made using a small sample adapter at a shear rate of 1.32 or 2.64 s⁻¹, depending on the viscosity range, in the solution (8 mL) at 25 ± 0.3 °C, and reported in mPa s. Color measurements were measured with a portable Minolta Chroma Meter CR-200 (Minolta Camera Co. Ltd, Osaka, Japan) using standard illuminant C or D₆₅, and reported as *L*^{*} (lightness), *a*^{*} (+ for redness and – for greenness) and *b*^{*} (yellowness). Three measurements were made at different locations on each sample.

2.5. Spectroscopic study

The IR spectra of the chitosans before and after UV irradiation were obtained with a JASCO FTIR spectrometer (300E, Tokyo, Japan). Chitosan samples (particle size less than 100 mesh) were mixed with KBr to form a homogeneous mixture for the FTIR measurements.

2.6. Statistical analysis

All experiments were carried out in triplicate, and means ± standard deviations were reported. Means of the main effects were separated by Duncan's multiple-range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) software package.

3. Results and discussion

3.1. Effect of the irradiation time (Treatment 1)

The moisture content of all chitosans, after decoloration by UV irradiation and subsequent drying at 60 °C for 4 h, was less than 4%. The viscosity and color values for the chitosans decolorized by UV-C irradiation for 5–60 min are shown in Table 1. The viscosity of chitosan solutions decreased significantly with increasing irradiation times, particularly during the first 15 min. Compared with that of non-irradiated chitosan (0 min), the viscosity of chitosans irradiated for 5, 10, 15, 30, and 60 min decreased by 42%, 71%, 84%, 90%, and 94%, respectively. Regardless of irradiation time, irradiation treatment yielded chitosans with significantly whiter, less red, and less yellow compared to the non-irradiated chitosan. An increase in irradiation times from 5 to 60 min resulted in an increase in the *L*^{*} value (78.54 to 82.20) and a decrease in the *a*^{*} value (–1.18 to –1.99), without affecting the *b*^{*} value. Although some significant differences in color *L*^{*} and *a*^{*} values were observed among irradiated chitosans by the instrumental measurement, these differences were not easily discerned by visual observation. Therefore, an irradiation time of 5 min, which resulted in a white chitosan product with the highest viscosity, was considered appropriate for decoloration of chitosan. Based on these results, all subsequent studies (Treatments 2–4) were conducted with 5 min irradiation time.

Wang, Huang, and Wang (2005) observed that the intrinsic viscosity of chitosan solution (2% in 1% acetic acid) exposed to UV (mercury lamp emitting at 254 nm) irradiation for 30 and 180 min decreased by 17.2% and 26.4%, respectively. In our present study, the viscosity of chitosans irradiated for 5, 10, 15, 30, and 60 min decreased by 42%, 71%, 84%, 90%, and 94%, respectively (Table 1). Even with the same irradiation time (30 min), the rates of viscosity decrease between these two studies differ, and are probably due to differences in UV treatment conditions, particularly the form of chitosans treated under the UV treatment (solu-

Table 1
Effect of the irradiation time on viscosity and color of chitosans decolorized^a by UV-C light (Treatment 1)

Time (min)	Viscosity (mPa s) ^{b,c}	Color value ^b		
		<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]
0	2229.0 ± 13.7f	67.05 ± 0.73a	7.14 ± 0.13d	13.55 ± 0.24b
5	1288.7 ± 1.2e	78.54 ± 1.48b	–1.18 ± 0.41c	10.93 ± 0.93a
10	648.4 ± 3.1d	79.12 ± 0.48b	–1.34 ± 0.26bc	10.96 ± 0.50a
15	351.9 ± 0.5c	79.56 ± 0.64b	–1.65 ± 0.03ab	11.36 ± 0.91a
30	229.8 ± 0.7b	81.59 ± 1.01c	–1.92 ± 0.06a	11.09 ± 0.81a
60	142.4 ± 0.1a	82.20 ± 1.05c	–1.99 ± 0.12a	11.34 ± 0.46a

^a Decoloration was achieved using a UV-C light source by stirring chitosan in water (1:8 w/v) at 120 rpm.

^b mPa s, milliPascal seconds; means ± standard deviation of three replicates. In each column, mean values with different letters are significantly different at *P* < 0.05.

^c 0.5% w/v in 1.0% v/v acetic acid, on a moisture-free basis. Viscosity was measured at a shear rate of 1.32 s⁻¹.

tion vs. solid). During UV irradiation of polymers, the excited molecules are formed in the first step and then the secondary processes, such as chain scission, cross-linking and oxidation, take place (Tse, Ng, & Yu, 2006). It is obvious in our study that the UV irradiation caused chain scission of the chitosan molecule, which is reflected by the decrease in chitosan viscosity (Table 1).

The main component of carotenoid pigments present in chitosan is astaxanthin (No et al., 1989). The oxidative degradation of carbon–carbon double bonds caused decoloration of carotenoids (Henry et al., 2000). Therefore, decoloration of chitosan was likely due to the oxidative destruction of double bonds present in astaxanthin by UV light (Youn et al., 2007). Decoloration of chitosan with a bleaching agent significantly reduces the viscosity of chitosan product (Nadarajah, Prinyawiwatkul, No, Sathivel, & Xu, 2006). The viscosity (1288.7 mPa s) of chitosan decolorized by UV irradiation for 5 min in the present study was 37.9 times higher than that (34 mPa s) of the chitosan bleached with 10% sodium hypochlorite for 5 min (Youn et al., 2007). Therefore, Moorjani et al. (1975) recommended that it is not desirable to bleach chitosans at any stage.

Youn et al. (2007) reported that decoloration of chitosan could be achieved effectively by sun drying after deacetylation. The color values of chitosan prepared under 4 h sun drying were $L^* = 86.53$, $a^* = -0.98$, and $b^* = 10.40$. These color a^* and b^* values are comparable to those

($a^* = -1.18$ and $b^* = 10.93$) of chitosan irradiated for 5 min in our present study (Table 1). However, the viscosity (424 mPa s) of chitosan reported by Youn et al. (2007) was much lower than that (1288.7 mPa s) of chitosan decolorized by UV irradiation for 5 min in this present study.

3.2. Effect of chitosan/water ratios (Treatment 2)

The effect of chitosan/water ratios on viscosity and color of chitosans decolorized by UV-C irradiation for 5 min is shown in Table 2. Chitosan is insoluble in water because of its large molecular weight, stable crystalline structure, and strong intermolecular hydrogen bonding (Muzzarelli, 1973), but it is soluble in weak organic acids. In this study, water was, however, used as a dispersing medium of chitosan with constant stirring to obtain uniform decoloration of chitosan.

As shown in Table 2, the highest viscosity was obtained from the chitosan/water ratio of 1:8. Further decrease or increase of the chitosan/water ratio rather yielded less viscous chitosan products. The chitosan/water ratio did not affect the L^* (76.68–79.24) and b^* (9.10–11.00) values. However, a ratio of 1:2, 1:4, and 1:8 yielded chitosans with less redness (decreased a^* value) compared with other ratios. Further reduction of the chitosan/water ratio to 1:1 was found to be insufficient to wet the chitosan particles and resulted in chitosan with more redness (increased a^* value). In view of both viscosity and color values, a chito-

Table 2

Effect of the chitosan/water ratio on viscosity and color of chitosans decolorized^a by UV-C irradiation for 5 min (Treatment 2)

Ratio (w/v)	Viscosity (mPa s) ^{b,c}	Color value ^b		
		L^*	a^*	b^*
1:0	896.8 ± 1.0e	76.85 ± 1.17a	0.86 ± 0.21d	11.00 ± 1.72a
1:1	835.8 ± 3.6c	76.68 ± 1.67a	0.24 ± 0.09c	10.09 ± 0.67a
1:2	777.3 ± 0.5b	77.33 ± 3.94a	-0.73 ± 0.08a	9.46 ± 0.91a
1:4	841.5 ± 0.6d	77.30 ± 0.71a	-0.74 ± 0.43a	9.66 ± 3.02a
1:8	1115.7 ± 1.5g	79.24 ± 1.47a	-0.83 ± 0.10a	9.10 ± 1.76a
1:16	1063.7 ± 1.2f	76.75 ± 1.27a	-0.34 ± 0.14b	10.55 ± 1.36a
1:28	704.5 ± 0.6a	78.36 ± 1.40a	-0.24 ± 0.04b	10.32 ± 2.62a

^a Decoloration was achieved using a UV-C light source by stirring chitosan in water for 5 min at 120 rpm.

^b mPa s, milliPascal seconds; means ± standard deviation of three replicates. In each column, mean values with different letters are significantly different at $P < 0.05$.

^c 0.5% w/v in 1.0% v/v acetic acid, on a moisture-free basis. Viscosity was measured at a shear rate of 2.64 s^{-1} .

Table 3

Effect of the stirring speed (rpm) on viscosity and color of chitosans decolorized^a by UV-C irradiation for 5 min (Treatment 3)

Rpm	Viscosity (mPa s) ^{b,c}	Color value ^b		
		L^*	a^*	b^*
120	1115.7 ± 1.5d	79.24 ± 1.47a	-0.83 ± 0.10a	9.10 ± 1.76a
180	775.5 ± 5.7c	79.13 ± 2.14a	-0.79 ± 0.27a	10.96 ± 1.16a
300	587.4 ± 1.5b	80.02 ± 1.51a	-0.44 ± 0.35a	11.96 ± 1.81a
420	462.7 ± 1.8a	78.65 ± 1.71a	-0.65 ± 0.15a	11.65 ± 1.27a

^a Decoloration was achieved using a UV-C light source by stirring chitosan in water (1:8 w/v) for 5 min.

^b mPa s, milliPascal seconds; means ± standard deviation of three replicates. In each column, mean values with different letters are significantly different at $P < 0.05$.

^c 0.5% w/v in 1.0% v/v acetic acid, on a moisture-free basis. Viscosity was measured at a shear rate of 2.64 s^{-1} .

Table 4
Effect of the UV light source on viscosity and color of chitosans decolorized^a by UV irradiation for 5 min (Treatment 4)

Light source	Viscosity (mPa s) ^{b,c}	Color value ^b		
		L^*	a^*	b^*
A	1449.7 ± 18.0c	77.73 ± 0.32b	0.53 ± 0.04b	14.33 ± 0.23a
B	1350.7 ± 7.8b	76.13 ± 0.56a	1.45 ± 0.33c	14.07 ± 0.16a
C	1301.7 ± 12.6a	76.95 ± 0.51ab	−0.37 ± 0.30a	14.05 ± 0.62a

^a Decoloration was achieved using a UV light by stirring chitosan in water (1:8 w/v) for 5 min at 120 rpm.

^b mPa s, milliPascal seconds; means ± standard deviation of three replicates. In each column, mean values with different letters are significantly different at $P < 0.05$.

^c 0.5% w/v in 1.0% v/v acetic acid, on a moisture-free basis. Viscosity was measured at a shear rate of 2.64 s^{-1} .

san/water ratio of 1:8 was considered to be an optimum ratio in obtaining a white chitosan product with the highest viscosity under the conditions investigated in this study.

3.3. Effect of the stirring speed (Treatment 3)

The effect of the stirring speed on viscosity and color of chitosans decolorized by UV-C irradiation for 5 min is given in Table 3. Stirring was applied in order to ensure a uniform irradiation distribution (Ye, Koutchma, Parisi, Larkin, & Forney, 2007). Increased stirring speed significantly reduced the viscosity of chitosans without affecting their color as indicated by no changes in the L^* , a^* , and b^* values. The viscosity of chitosans at 180, 300, and 420 rpm decreased by 30%, 47%, and 58%, respectively, compared with that of chitosan at 120 rpm. These results suggest the use of a lower stirring speed (120 rpm) to obtain chitosan with higher viscosity. As shown in Table 3, nearly complete decoloration of chitosan may be accomplished by UV irradiation for 5 min, irrespective of the stirring speed. Decrease in chitosan viscosity with increasing stirring speed may have been due to increased surface contact between chitosan and UV light.

3.4. Effect of the UV light source (Treatment 4)

The effect of the UV light source on viscosity and color of chitosans decolorized by UV irradiation for 5 min is given in Table 4. The highest viscosity was obtained with UV-A light followed by UV-B and UV-C light sources. However, decoloration of chitosan with UV-A and UV-B light sources was less effective compared to that with UV-C light source due to higher a^* values (more redness). In general, a bleached chitosan product is required for commercial applications. The UV-C light produced white chitosan which is more desirable for commercial applications. The light intensities of UV-A, UV-B and UV-C lights used in this experiment were 1.2, 2.24 and 4.4 mW/cm^2 , respectively. Therefore, the UV-C light had the shortest wavelength and the highest light intensity which could impart more negative effect on the viscosity and more positive effect on decoloration of chitosan.

Data from Tables 1–4 collectively indicate that a white chitosan product with high viscosity can be simply pro-

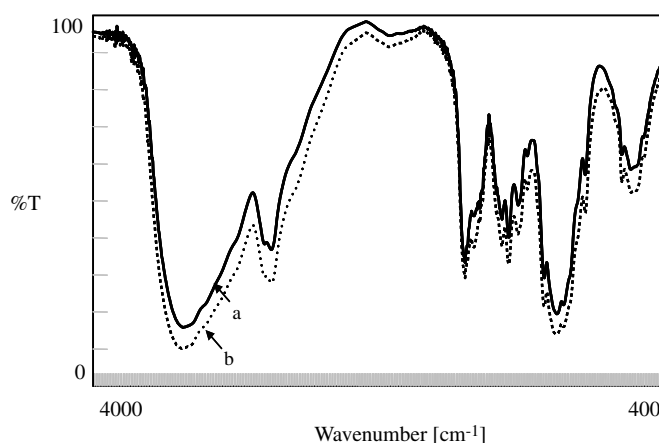


Fig. 2. The FTIR spectra of chitosans (a) before and (b) after 5-min irradiation under UV-C light (chitosan:water at 1:8 w/v and stirring speed of 120 rpm).

duced by UV irradiation after deacetylation. In summary, decoloration of chitosan was effectively achieved by stirring deacetylated chitosan in water (1:8 w/v) for 5 min at 120 rpm under UV-C light. The IR absorption spectra (Fig. 2) of the chitosans before and after UV irradiation showed that the position and absorption intensity of the bands did not change upon UV irradiation, as also observed with chitosan film by Sionkowska et al. (2006). Production of decolorized chitosan by UV irradiation without using a bleaching agent would considerably reduce production cost due to reduction in chemical usage, process time, and voluminous wastewater discharge.

4. Conclusions

This study demonstrated that decolorized chitosan with high viscosity could be prepared with the aid of UV light without using a bleaching agent. UV irradiation applied in this study produced a chitosan with desirable white color and high viscosity. Use of a bleaching agent in general causes significantly reduced viscosity of chitosan in addition to the observed undesirable light brown color. Youn et al. (2007) reported that a white chitosan product with high viscosity can be simply produced by sun drying after the deacetylation step without using a bleaching agent. Although production of white-colored chitosan with the

aid of sun drying and without using a bleaching agent would considerably reduce production cost due to reduction in chemical usage, process time, and voluminous wastewater discharge, applicability of sun drying is entirely dependent on the weather condition. On the other hand, the proposed UV-C irradiation method can be applicable without having to depend on weather conditions. Nevertheless, the proposed UV-irradiation method reported here needs to be further examined in actual chitosan plants, especially as conditions used in a large-scale commercial chitosan production will, in all likelihood, vary from those based on laboratory-scale trials. Energy saver due to effective decoloration of chitosan in a short time (5 min) by UV-C irradiation without using a bleaching agent may help offset the cost of installation of UV irradiation facility in the long run.

Acknowledgment

We thank Dr. Young Seek Park at Daegu University for his valuable comments in carrying out this experiment.

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