

Research Article

Study on Preparation of Water-Soluble Chitosan with Varying Molecular Weights and Its Antioxidant Activity

Dang Xuan Du  and Bui Xuan Vuong

Faculty of Pedagogy in Natural Sciences, Sai Gon University, 273 An Duong Vuong Street, Ho Chi Minh City 700000, Vietnam

Correspondence should be addressed to Dang Xuan Du; dangxuandu@sgu.edu.vn

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The preparation of water-soluble chitosan (WSC) with various molecular weights by gamma Co-60 irradiation of chitosan solution (5%) in the presence of hydrogen peroxide (1%) combined with acetylated reaction was carried out. The average molecular weight (M_w) of chitosan was measured by gel permeation chromatography (GPC). The chemical structure and the crystallinity of chitosan and WSC were characterized by Fourier-transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD), respectively. The antioxidant activity of WSC and chitosan was investigated using the free radical 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS^{•+}). Obtained results showed that chitosan with M_w of 18–90 kDa could be efficiently prepared by this technique in the dose range from 10 to 24.5 kGy. After the acetylated process, the resulting WSC possesses good solubility in a wide pH level of 2–12, and WSC with low molecular weight exhibited significantly higher antioxidant activity than the one with high molecular weight. In detail, the antioxidant activity was 14.7%, 70.5%, 84.2%, 89.4%, and 97.5% for WSC samples prepared from chitosan with M_w of 140.2 kDa, 91.4 kDa, 51.2 kDa, 35.3 kDa, and 18.1 kDa, respectively, at the same reaction time of 90 min. Moreover, the antioxidant activity of WSC was higher than that of chitosan. Thus, WSC prepared by gamma Co-60 irradiation and acetylated process can be potentially applied as a natural antioxidant agent.

1. Introduction

Antioxidant is construed as any substance that inhibits oxidation or reactions promoted by oxygen, peroxides, or free radicals. Nowadays, synthetic antioxidants such as *t*-butylhydroquinone, propyl gallate, butylated hydroxytoluene and butylated hydroxyanisole have been diversely utilized to counteract the deterioration of stored food and preserve cosmetics [1]. However, the application of synthetic antioxidants requires a strict adherence in utilized dose due to its possibly harmful side effects to the human health. Hence, study on extraction and application of antioxidants from natural origin to alternative to synthetic antioxidants was very necessary [2–4].

Chitosan is a natural polymer which has structure of polysaccharide consisting of *N*-glucosamine and *N*-acetylglucosamine units linked by β -1,4 glycoside. This polymer has been widely applied in many fields owing to its unique

properties such as biocompatibility, biodegradability, non-toxicity, antibacterial, antifungal, and antitumor. Moreover, chitosan also has the ability to stimulate disease resistance and heal the wound quickly [5]. However, a significant drawback of chitosan is it is soluble only in diluted-acid media. This cause restricted applications of chitosan, and therefore, modification of chitosan with the aim of enhancing its solubility has attracted a great deal of research attention [3, 6]. Recently, studies on chitosan modified with resultant products as natural antioxidants were carried out [2, 7–9]. However, these studies have only focused on preparation of low molecular weight chitosan or oligomers due to their easy solubility in water and high antioxidant activity [10]. In addition, the antioxidant activity of high molecular weight chitosan [8] and chitosan derivatives [11] has also been investigated. Generally, the lower the molecular weight of the chitosan, the higher the solubility obtained [9]. This means that, chitosan with low molecular

weight had a merit in applied ability due to soluble potency. However, if molecular weight was too low, like chitosan oligomer, it was also able to be a demerit in case of utilizing as a stabilizer to prepare metal nanoparticles; then, a high molecular weight of polymers had a better stability. Consequently, the preparation of water-soluble chitosan with molecular weight that was higher than chitosan oligomers for adding information and extending application was a necessary study.

The chitosan with varying molecular weight could be prepared from initial chitosan by a variety of techniques. Among them, gamma irradiation and hydrogen peroxide have some merits such as environment-friendly and being suitable for production of high-purity products and industrial manufacturing [12]. Furthermore, the simultaneous combination of gamma irradiation and hydrogen peroxide could efficiently degrade chitosan and reduce required dose owing to synergistic effect [13].

In this study, chitosan with varying molecular weight was prepared by hydrogen peroxide and gamma irradiation of chitosan solution containing hydrogen peroxide. Then, degraded chitosan has been acetylated by acetic anhydride to prepare water-soluble chitosan. The structure and solubility of resultant water-soluble chitosan have been investigated. Moreover, the antioxidant activity of water-soluble chitosan with varying molecular weight has also been evaluated.

2. Materials and Methods

2.1. Materials. Chitosan derived from shrimp shell with a weight average molecular weight (M_w) of 140.1 kDa and degree of deacetylation (DD%) of 91.3% was supplied by a company in Vung Tau province, Vietnam. Hydrogen peroxide (30%) was purchased from Merck, Germany. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was supplied by Biobasic, Canada. The other chemicals such as NaOH, NH_4OH (25%), lactic acid, and ethyl alcohol were of reagent grade.

2.2. Preparation of Chitosan with Varying Molecular Weights. In this study, the degradation of chitosan was carried out in two steps. In the first step, initial chitosan (CTS_0) was degraded in the swollen state by using 1% hydrogen peroxide with the ratio of chitosan/ H_2O_2 of 1/10 (w/v) for 24 h. The obtained product (CTS_1) was washed by distilled water and then dried naturally. In the second step, CTS_1 was dissolved in 3% lactic acid and stirred for 2 h. An amount of hydrogen peroxide was added in the mixture to create solution with concentration of chitosan, lactic acid, and H_2O_2 to be 5%, 2.5%, and 1% (w/v), respectively. Finally, this resulting mixture was irradiated by gamma SVST Co-60/B source with a dose rate of 1.33 kGy/h in the range of dose up to 24.5 kGy. The obtained chitosan at the different doses of this irradiated process, CTS_2 (3.5 kGy), CTS_3 (10.5 kGy), and CTS_4 (24.5 kGy), has been filtered by a stainless steel mesh and neutralized by 5% NH_4OH (v/v). Ethyl alcohol was added in solution during stirring to precipitate chitosan. This precipitant was filtered and washed several times with

alcohol and then dried in a force air oven at 60°C. The samples of dried chitosan were ground into powder for GPC measurement and preparation of water-soluble chitosan.

2.3. Preparation of Water-Soluble Chitosan. The chitosan samples from CTS_0 to CTS_4 were dissolved in lactic acid to create 150 mL solution with chitosan concentration of 5%. 40.5 mL ethyl alcohol and 4.5 mL anhydride acetic were added in solution. Then, the solution was stirred for 2 h and kept at ambient temperature overnight for acetylated process. The solutions after reaction were filtered, precipitated, washed, and dried. The dried chitosan samples were also ground into powder for FT-IR, XRD measurements, and antioxidant activity investigations.

2.4. Characterization. The M_w of chitosan samples was determined by using a GPC (LC-20AB Shimadzu, Japan) using an ultra-hydrogel column model 250 of Waters (USA) and detector RI-10A. The calibration of column was carried out by using pullulan with M_w of 780–3,80,000 Da. The mobile phase was buffer solution 0.25 M CH_3COOH /0.25 M CH_3COONa with a flow rate of 1 mL/min [14] and temperature at 30°C. The concentration of chitosan sample was ca. 0.1% (w/v).

IR spectra were taken on an FT-IR 8400S spectrometer (Shimadzu, Japan) using KBr pellets. The DD% was calculated based on FT-IR spectra according to the following equation [15]:

$$\text{DD}\% = 100 - \left[\frac{((A_{1320}/A_{1420}) - 0.3822)}{0.03133} \right], \quad (1)$$

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

X-ray diffraction (XRD) measurements of WSC were carried out on an X'Pert Pro X-ray diffractometer (PANalytical, Netherlands) and used a CuK_α target at 45 kV-40 mA with a scattering range (2θ) of 5°–40°.

The water solubility of WSC was estimated based on the solution transmittance at 600 nm that was recorded on a UV-vis spectrophotometer (V630, Jasco, Japan) against 0.5% WSC solution (w/v) using a quartz cell with an optimal path length of 1 cm [16].

2.5. Antioxidant Assay. Antioxidant assay was carried out by dissolving 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ATBS) in water to create the solution with a concentration of 7.4 mM. Next, 2 mL ATBS solution was mixed with 2 mL $\text{K}_2\text{S}_2\text{O}_8$ with a concentration of 2.6 mM to obtain the free radical cation $\text{ATBS}^{\bullet+}$. This $\text{ATBS}^{\bullet+}$ solution was kept in the dark for 24 h. Then, the $\text{ATBS}^{\bullet+}$ solution was diluted by water to receive an optical density (OD) of 1 ± 0.1 at a wavelength of 734 nm, after which the solution was also diluted with water for the control. For investigating antioxidant activity, the WSC samples with concentration of 0.2% were mixed with 0.1% acetic acid [9]. About 0.6 mL of solution sample was added into the cuvette that contained 1 mL of $\text{ATBS}^{\bullet+}$ solution.

Added otherwise by 0.6 mL distilled water into a cuvette that contained 1 mL ABTS^{•+} solution, we could receive a control sample. The OD of the samples was measured over time by using a UV-vis spectrophotometer at a max absorbance wavelength of 734 nm [17]. The efficiency of free radical scavenging was calculated by the following equation:

$$\text{Efficiency (\%)} = 100 \times \left(\frac{\text{OD}_{\text{AC}} - \text{OD}_{\text{AS}}}{\text{OD}_{\text{AC}}} \right), \quad (2)$$

where OD_{AC} is the OD of the control sample (ABTS^{•+} and water without WSC) and OD_{AS} is the OD of the solution containing ABTS^{•+} and WSC [17, 18].

2.6. Determination of Reducing Power. In order to clarify the rule of antioxidant activity in another way, the reducing power of WSC was also determined based on the ferricyanide method [19]. The WSC sample was diluted to create solution with 0.3% concentration (w/v). 1 mL of this solution was added to 2.5 mL of phosphate buffer (200 mM, pH = 6.6) and then 2.5 mL of potassium ferricyanide. This mixture was incubated for 20 min at 50°C in a water bath. Next, 2.5 mL of 10% trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 min. 5 mL of upper layer solution was mixed with 1.0 mL of 0.1% ferric chloride and water. The obtained solution was measured for absorbance at 700 nm. The higher the absorbance, the stronger the reducing power obtained.

3. Results and Discussion

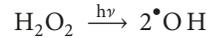
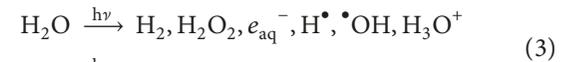
3.1. Preparation of Chitosan of Different Molecular Weights. According to the previous studies, the degradation of chitosan in the heterogeneous state in the presence of hydrogen peroxide solution is quite efficient [9, 20]. However, if the concentration of hydrogen peroxide was at the high level, the main chain structure of chitosan may be changed [20]. The way around this situation is the choice of 1% H₂O₂ [9, 13]. In addition, the degradation of chitosan in the heterogeneous state was almost supposed that it occurred in the amorphous region, and as a result, the reduction of molar mass of chitosan is only reached to a certain extent. Reasoning further along this line, the degradation of chitosan at crystal regions regularly occurs in the homogeneous state, i.e., in solution. For this study, the degradation of chitosan in the heterogeneous state and homogeneous state was performed in two steps in turn.

As shown in Table 1, the degradation of chitosan by 1% hydrogen peroxide is fairly efficient. Moreover, the DD of chitosan was almost unchanged. According to Qin et al. [20], the structure of the degraded chitosan with a M_w less than 50 kDa that was obtained by the high concentration of hydrogen peroxide as well as the long degradation time had been remarkably changed. Hence, the H₂O₂ concentration of 1% was chosen to prevent the change of the structure of chitosan. Moreover, the obtained chitosan with a molecular weight of 91 kDa was suitable for preparation of WSC with varying molecular weight. Since M_w was lower than 10 kDa, chitosan was in the form of an oligomer that may be soluble in water [9]; then, the acetylation of chitosan for its improvement of solubility was unnecessary.

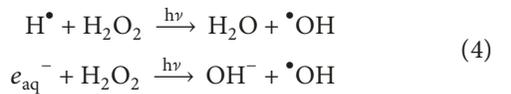
TABLE 1: Degradation of chitosan in the swollen state (first step) and in solution (second step).

	First step (1% H ₂ O ₂)		Second step (1% H ₂ O ₂ and γ -ray)		
	CTS ₀	CTS ₁	3.5 kGy CTS ₂	10.5 kGy CTS ₃	24.5 kGy CTS ₄
M_w (kDa)	140.1	91.7	51.7	35.1	18.6
DD (%)	91.1	91.3	90.3	91.0	89.5

The further degradation of chitosan was carried out in solution by combination of H₂O₂ and gamma irradiation. The results showed that M_w of obtained chitosan at the different doses of 3.5 kGy, 10.5 kGy, and 24.5 kGy were 51.7 kDa, 35.1 kDa, and 18.6 kDa, respectively. Meanwhile, the DD of resulting chitosan samples was hardly different. This outcome indicated that at only 1% concentration of H₂O₂ combined with gamma ray, the reduction of molar mass is very efficient even when the applied dose is quite low, less than 25 kGy. Nguyen et al. [9] and Nguyen et al. [13] degraded chitosan by simultaneously combining H₂O₂ and gamma ray; as a result, they obtained chitosan oligomers at the low dose, about 12 kGy with 1% concentration of H₂O₂ used. Although these reductions were further than those in our study, the degree of M_w degradation (%) is almost the same owing to the fact that initial chitosan in those studies has different molecular weights as well as origin (i.e., crab shell in their studies and shrimp shell in this study). However, the degree of M_w reduction (%) in their studies and in this study is almost the same owing to the fact that initial chitosan in those studies was different in molecular weight as well as origin (from crab shell in their studies and shrimp shell in this study). The phenomenon of the increase in degraded efficiency with the presence of H₂O₂ was suggested through synergistic effect [13]. Accordingly, the hydroxyl radicals formed through the radiolysis of water and H₂O₂ are mainly responsible for degradation of chitosan:



Additionally formation of hydroxyl radicals, e_{aq}^- and H^\bullet can react with H₂O₂ during irradiation:



Hydroxyl radicals plays a significant role as a powerful oxidative agent, reacting with chitosan chain by abstraction of hydrogen atoms linked with carbon to form carbohydrate radicals (Figure 1) that can then cause the direct breakage of the glycosidic linkage by rearrangement [21]. The obtained chitosan samples with varying molecular weight and DD of ~90% then were utilized for preparation of WSC through *N*-acetylation process.

3.2. Preparation of Water-Soluble Chitosan. The chitosan samples with different molar masses CTS₀, CTS₁, CTS₂, CTS₃, and CTS₄ were *N*-acetylated by acetic anhydride to

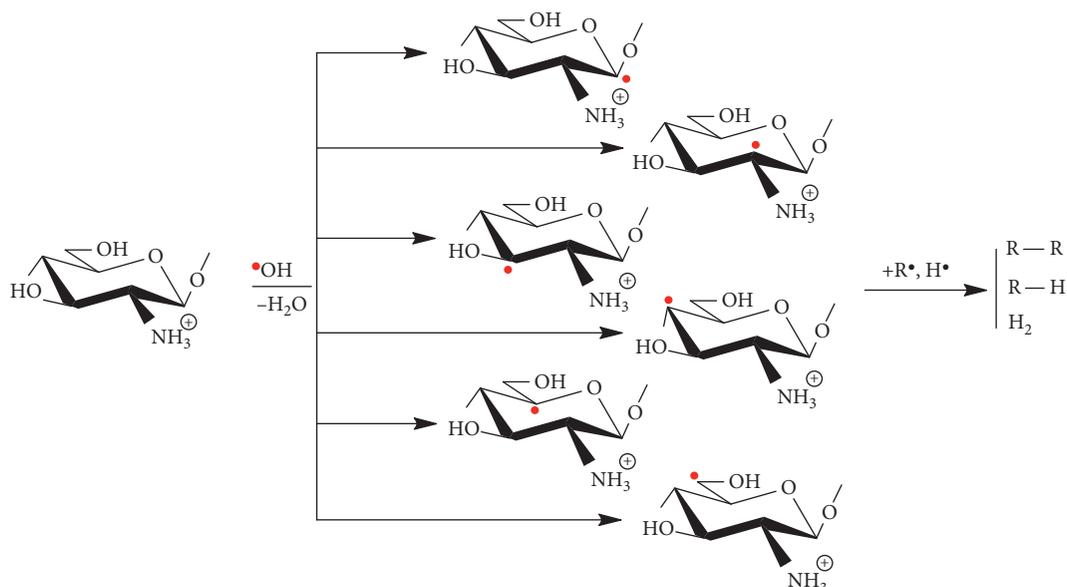


FIGURE 1: The mechanism of abstraction of hydrogen by hydroxyl radical [21] in the degradation of chitosan.

create WSC samples denoted by WSC₀, WSC₁, WSC₂, WSC₃, and WSC₄, respectively. Unlike chitosan insoluble in water before reaction, all WSC samples shown in Figure 2 had good solubility in water despite different soluble time periods (Table 2). The transmittance (T) of all WSC samples was more than 98%. This means that water solubility of CTS was remarkably improved by virtue of *N*-acetylated process. Furthermore, these WSC samples also were soluble in the slightly alkaline media (pH of 7–12) regardless of its different molecular weights (data not showed). According to the results previously published by Lu et al. [6], the impact of molecular weight of WSC on solubility in water and in alkali media was ineffective and effective, respectively. In terms of the solubility in water, we have the similar result. For the alkaline media, the higher molecular weight of WSC has the lower solubility obtained [6]. However, all WSC samples in our study were soluble in weakly alkaline media. It may be due to the fact that the range of molecular weight in our study is far lower compared to that of their study. The WSC samples with $M_w > 180$ kDa in their study were slightly soluble in pH > 9 media. In our study, all WSC samples were prepared from chitosan with M_w less than 140 kDa, and therefore, they accounted for good solubility in dilute alkaline media. Feng et al. [3] also studied solubility of *N*-acetylated chitosan with DD~50%, prepared by acetylation of chitosan degraded by cellulase enzyme. They reported that the only *N*-acetylated chitosan with low M_w (<18 kDa) has a high solubility and retained over a wide pH range. The solubility of the others ($M_w > 18$ kDa) in the alkaline decreased with increasing M_w . Unlike the results of Feng et al., WSC prepared in our study has a high solubility in over a wide pH range despite the M_w from 18 to 140 kDa. Apart from the molecular weight, the solubility of chitosan depends on the other factors such as intermolecular interactions (van der Waals forces), intermolecular interaction forces, the degree of acetylation, and initial soluble media [2, 3].

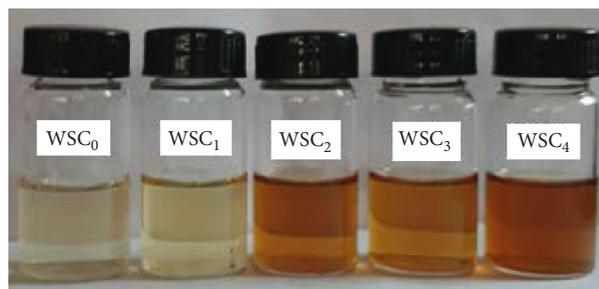


FIGURE 2: WSC samples dissolved in water at 25°C with the concentration 0.5% (w/v).

TABLE 2: DD and soluble time of WSC samples and CTS₄.

Samples	DD (%)	Soluble time at 25°C (h)	T (%)
WSC ₀	56.3	2.5	99.93
WSC ₁	57.7	2.0	99.95
WSC ₂	52.3	2.0	99.97
WSC ₃	49.2	1.5	99.98
WSC ₄	48.7	1.5	99.98

The FT-IR spectra of the WSC samples as well as CTS₀ (initial chitosan) and CTS₁ (degraded by only H₂O₂) are shown in Figure 3. The broad band around 3427 cm⁻¹ is assigned to O–H and N–H bond stretching. The band at 1159–895 cm⁻¹ is assigned to the special absorption peaks of β (1–4) glycoside bond, which is characteristic of polysaccharide structure of chitosan [22]. The peaks at 1072 and 1028 cm⁻¹ are assigned to stretching vibration of C–O and stretching of the C–O–C in the glucopyranose ring, respectively. Peaks at 1320 and 1420 cm⁻¹ are assigned to the absorbance of C–N of CH₃CONH (amid III) and symmetrical deformation of CH₂ as well as CH₃. The peaks at 1650 cm⁻¹ and 1597 cm⁻¹ are, respectively, concerned to stretching vibration of amide I and amide II groups [3]. The

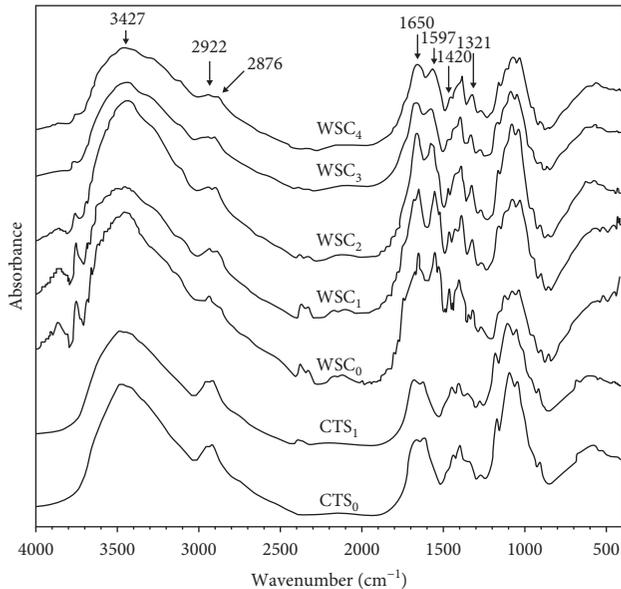


FIGURE 3: FT-IR spectra of the initial chitosan (CTS₀), only H₂O₂-degraded chitosan (CTS₁), and WSC samples prepared from CTS₀ (WSC₀), CTS₁ (WSC₁), and degraded chitosan at doses of 3.5 kGy (WSC₂), 10.5 kGy (WSC₃), and 24.5 kGy (WSC₄).

C–H stretching of methyl and methane were assigned at 2922 and 2876, respectively. In terms of mainly chemical structure, CTS₁ is hardly different from CTS₀. This accounts for unchanged DD (Table 1). Compared to initial chitosan (CTS₀) and CTS₁, the principle functional groups of WSC were still present after *N*-acetylation. This implied that the main polysaccharide chain structure remained after degradation and *N*-acetylation. However, there is change of absorbance at some bands. Especially, the peaks at 1650 and 1597 cm⁻¹ owing to –CONH₂ stretching vibration were significantly enhanced. This revealed that the acetylated reaction mainly occurred at the amino group of chitosan [15]. On the contrary, the absorbance at 2922 and 2876 cm⁻¹ due to C–H stretching of methyl and methane were quite weakened. It indicated that *N*-acetylation reaction impacts on inter-macromolecular hydrogen bonds and interchain hydrogen bonds. This is due to of water solubility by virtue of reduced crystallinity of chitosan [6].

The X-ray diffraction (XRD) patterns of WSC and initial chitosan (CTS₀) are shown in Figure 4. In the XRD pattern of CTS₀, a broad peak of 2θ around 20° and another peak around 9° were assigned to the diffraction of the plane of the crystal region in the chitosan structure. This pattern refers to as the L-2 polymorph of chitosan [9, 23]. As can be seen in Figure 4, the diffraction intensity of peaks in WSC patterns is weaker than that of in CTS₀—even disappearance at 2θ around 9°. These results showed that *N*-acetylation and degradation caused the destruction of the crystallinity of chitosan. Consequently, the lower degree of crystallinity of WSC obtained from these processes induced the higher solubility attained in aqueous media. This is similar to the previous reports [6, 13]. For the WSC₁, the higher intensity can be observed at 2θ around 20° compared to WSC₀ and CTS₀. This result may be due to the fact that the first step of

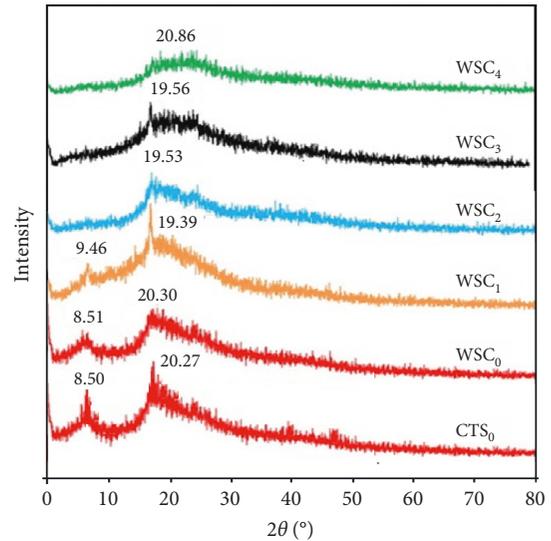


FIGURE 4: XRD patterns of CTS₀ and the WSC samples.

degradation by only H₂O₂ mainly occurs at the amorphous region. Accordingly, the obtained chitosan has relatively higher crystallinity similar to the outcome previously reported by Qin et al. [20].

3.3. Antioxidative Activity Assay. Free radicals containing reactive oxygen species are unstable agents. They react readily with other groups or substances in body, causing cell damage. Thus, one of the most effective defense mechanisms of the body against diseases is removal of free radicals by using antioxidants. In this study, the antioxidant activity of WSC was investigated by free-radical scavenging of ABTS^{•+}. As can be seen in Figure 5, initial ABTS solution was transparent, whereas the color of ABTS^{•+} solution was dark green (Figure 5(a)). The color of ABTS^{•+} in aqueous solution was green. When ABTS^{•+} solution was added into WSC solution, a variation in color occurred. The color of ABTS^{•+} solution in the presence of WSC was decreased. Moreover, the ABTS^{•+} samples in WSC₃ and WSC₄ were almost colorless (Figure 5(b)). It proved that WSC scavenged ABTS^{•+}. As a result, the green color of ABTS^{•+} solution in the presence of WSC was weaker than that of ABTS^{•+} without WSC. The higher the molecular weight of WSC was, the lower the free-radical scavenging activity attained. The efficiency of antioxidant activity is one of the parameters using to evaluate free-radical scavenging activity of WSC. In this study, the efficiency of antioxidant activity was calculated by equation (2) [9]. It was obvious that the efficiency of antioxidant activity of WSC samples increased in a time-dependent manner, and this parameter also increased versus the decrease of molecular weight (Figure 6). In particular, the WSC₄ sample with the lowest molecular weight exhibited the highest efficiency of antioxidant activity, attained 92% after only 10 min and 98% after 90 min (Figure 6). Besides, the efficiency of antioxidant activity of the WSC₀ sample was entirely lower than that of CTS₄ sample and the others. The reason may be due to the

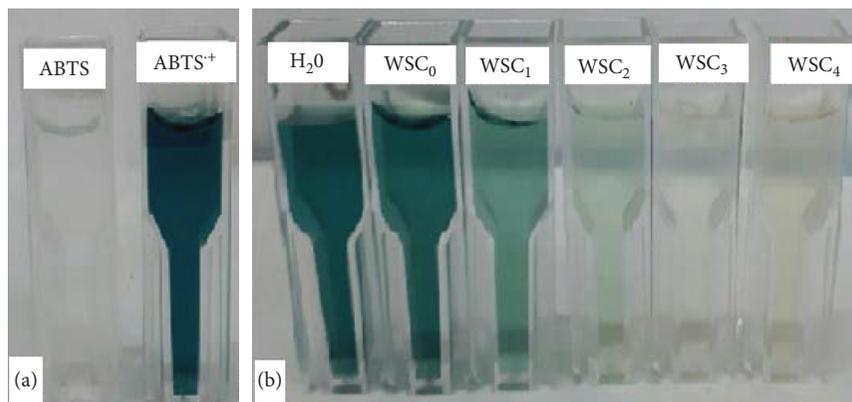


FIGURE 5: ABTS and $ABTS^{\bullet+}$ solution (a) and $ABTS^{\bullet+}$ with the presence of WSC and H_2O (b).

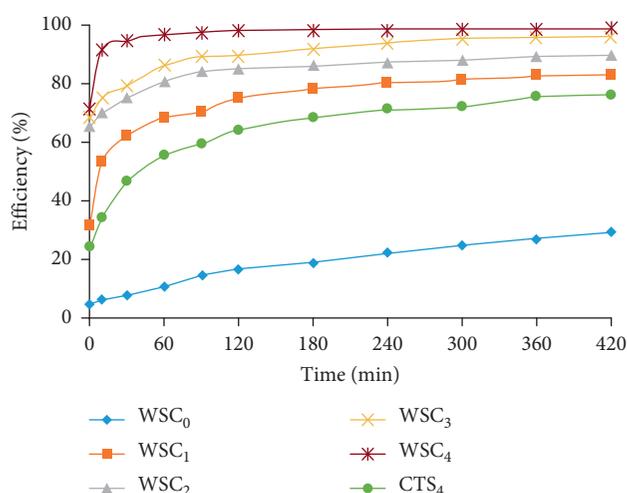


FIGURE 6: Efficiency of antioxidant activity of WSC and CTS_4 with reaction time.

significant difference of M_w . Feng et al. [3] also reported that the antioxidant activity of WSC with M_w of 281.0 and 1.8 kDa was significantly different. The WSC with M_w of 281 kDa has a relatively low antioxidant activity. Whereas, the other with M_w of 1.8 kDa had a quite high antioxidant activity approximated with α -tocopherol [3]. The antioxidant activity of a compound was also suggested in various mechanisms such as prevention of chain initiation, decomposition of peroxides, biding of transition metal ion catalysts, radical scavenging, and reductive capacity [3].

Nguyen et al. [9] reported the preparation of water-soluble oligochitosan and its antioxidant activity. Results showed that water-soluble oligochitosan mainly prepared by degradation of chitosan using hydrogen peroxide and gamma irradiation at low dose <25 kGy. The obtained oligochitosan with M_w of 35.29 kDa, 7.03 kDa, and 4.07 kDa had the scavenge percentage against $ATBS^{\bullet+}$ radical in 90 min of 69.6%, 84.5%, and 99.2%, respectively. In terms of the rule of efficiency of antioxidant activity versus molecular weight of chitosan, their study was consistent with our study. However, the difference is that the M_w of oligochitosan in

their study was lower compared to that in our study despite the same efficiency of antioxidant activity (more than 90%) and good solubility. Chitosan with low molecular weight such as oligochitosan (M_w less than 10 kDa) in studies reported by Je et al. [2], Feng et al. [3], and Nguyen et al. [9] generally had a good antioxidant activity. However, its antimicrobial property was less than that of chitosan with medium molecular weight ($M_w \sim 10$ –100 kDa). In addition, in the case of chitosan using as a stabilizer for synthesis of metal nanoparticles, a high M_w has a better stability. In our study, WSC with a molecular weight of 18–90 kDa has good solubility in water so that it has suitably potential to be used as a stabilizer or an antioxidant substance. The other point is the difference of the method for preparation of water-soluble chitosan. In our method, apart from degradation by hydrogen peroxide and gamma Co-60 irradiation like the method in Nguyen et al. [9], chitosan also was acetylated to increase solubility and M_w was kept constant at medium molecular weight. Therefore, in terms of this approach, the obtained chitosan has good solubility and high antioxidant activity at the relatively higher molecular weight.

In terms of reason for antioxidant activity, many authors mentioned in their studies [3, 9, 24] that the hydroxyl and amino groups in chitosan molecules play important roles in free-radical scavenging activity. According to these authors, the more activated the hydroxyl and amino group are, the more the antioxidant activity. The hydroxyl and amino group could be activated in case intramolecular hydrogen bond and van der Waals forces reduced sharply. In our study, acetylated process destroyed crystallinity of chitosan as mentioned above. This may significantly decline intramolecular hydrogen bond. As a result, hydroxyl and amino groups could be more mobile. They facilitate effective reactions with $ABTS^{\bullet+}$ radical [11]. In addition, the residual hydroxyl groups in polysaccharides can react with radicals via a H abstraction. In a similar way, the residual free amino groups can absorb a hydrogen ion to form ammonium groups, which in turn react with radicals through an addition reaction [25].

The reducing capacity of a substance may be construed as a significant indicator of its potential antioxidant activity [26]. The substances with good reducing capacity are

TABLE 3: OD of solution determined by reducing power of WSC samples and CTS₄.

Samples	WSC ₀	WSC ₁	WSC ₂	WSC ₃	WSC ₄	CTS ₄
OD	0.03	0.20	0.36	0.44	0.47	0.12

electron donors. They can easily react with free radicals to convert them to more stable states and terminate radical chain reactions [2]. As shown in Table 3, the reducing power of WSC samples increased with the decrease of M_w . It is obvious that reducing power of CST₄ and WSC samples was in good agreement with the efficiency of antioxidant activity. The stronger the reducing power of WSC, the higher the efficiency of antioxidant activity attained. Besides, reducing power of CTS₄ was also more than WSC₀ but less than the others. It implicated that WSC has generally given a higher reducing power than chitosan. However, in this case, CTS₄ having a higher reducing power than WSC₀ may be due to the limit of M_w [8, 10, 27]. In WSC₀ with the higher M_w , the structure chain was twisted making a mobile degree of amino and hydroxyl groups with decreasing vigour. It could be accounted for a negligible reducing power of WSC₀. Rao et al. [26] studied irradiation of chitosan-glucose mixture and antioxidant activity of the resultant Maillard products. They reported that the mixture irradiated at high dose had a high reducing power. They also suggested that the intermediate reduction compounds of Maillard reaction products broke the radical chain by donation of a hydrogen atom [26].

4. Conclusions

Water-soluble chitosan (WSC) with M_w in the range of 18–140 kDa was successfully prepared by combination of degradation and acetylation in which the gamma Co-60 irradiation and hydrogen peroxide were used to adjust the molecular weight. The solubility and antioxidant activity of the obtained WSC were significantly enhanced. All WSC samples showed good solubility in water with the transmittance more than 98%. The WSC₄ sample exhibited the highest efficiency of antioxidant activity, attained 92% after only 10 min and 98% after 90 min. Owing to the water solubility and high efficiency of antioxidant activity, WSC can be potentially used for various purposes of applications, especially as an effective antioxidant or a stabilizer in synthesizing metal nanoparticles.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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