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Biochemical activities of 6-carboxy β-chitin derived from squid pens

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ABSTRACT

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical)-mediated 6-carboxy β-chitin derivatives (T-chitin) with different carboxylate content were successfully synthesized by controlling the addition level of NaClO as the primary oxidant. The structural and biochemical properties of the derivatives were investigated. The carboxylate contents of the derivatives calculated by electrical conductivity titration were 1.33, 1.68, 1.80, and 2.08 mmol/g, respectively. The yield of T-chitin with carboxylate content of 2.08 mmol/g reached 74.55%. T-chitin exhibited stronger bile acid binding capacities than that of β-chitin. The scavenging ability of T-chitin against hydroxyl radicals improved with increasing concentration, and EC_{50} values were below 1.2 mg/mL. All T-chitin exhibited a strong ferrous ion chelating effect. At 8 mg/mL, the chelating effects of T-chitin with carboxylate content of 0.81 mmol/g reached 80.15%. These results showed that T-chitin had good bile acid binding capacity and antioxidant activities and it may be a potential antioxidant *in vitro*.

1. Introduction

Chitin, the partly deacetylated (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (Muzzarelli et al., 2012), is the second most abundant natural biomass resources derived from exoskeletons of arthropods and from cell walls of fungi (Mathur & Narang, 1990; Muzzarelli, 1988; Roberts, 1992; Tharanathana & Kittur, 2003). Most natural chitins have the α-type crystal structure, while the β-type chitin is present in squid pens and tubeworms (Muzzarelli, 1977). Chitin has great potential to be used in the repair of implant devices, wound dressing materials, immunoadjuvants, non-allergenic drug carriers, and regenerative medical components for bones and wounded skin (Muzzarelli et al., 2007; Muzzarelli, 2009, 2010; Yang et al., 2012). Chitin has been dissolved in various solvents, and various chemical modifications of chitin are known such as sulfation (Jayakumar, Nwe, Tokura, & Tamura, 2007), TEMPO-mediated oxidation (Bragd, van Bekkum, & Besemer, 2004; Kato, Kaminaga, Matsuoa, & Isogai, 2004; Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999), trimethylsilylation (Kurita, Sugita, Kodaira, Hirakawa, & Yang, 2005), selective *N*-acetylation (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994), dibutyrylation (Castagnino et al., 2008) and tosylation (Zou & Khor, 2005), and the derivatives have been intensively studied for industrial as well as scientific interest. Chemical modification of chitin to generate new functional biopolymers is of interest because the modification would not change the fundamental skeleton of chitin, would increase water-solubility, keep the original physicochemical and biochemical properties and finally would bring new or improved properties. Owing to their versatile biological activity, excellent biocompatibility, and complete biodegradability in combination with low toxicity, both chitin and its modified derivatives have extensive applications in medicine, agriculture, and food industries as well (Muzzarelli et al., 2007; Muzzarelli, 2009, 2010; Tanodekaew et al., 2004).

Polysaccharides have been demonstrated to play an important role in directly reacting with and quenching free radicals to prevent oxidative damages and oxidative stress related human diseases (Shin, Lee, Lee, & Lee, 2005; Tsiapali et al., 2001). Some recent studies showed that water-insoluble α-chitin become water-soluble by the TEMPO/NaBr/NaClO oxidation system through partial or complete conversion of the C6 primary hydroxyls to carboxylate groups (Bragd et al., 2004; Kato et al., 2004; Muzzarelli et al., 1999). However, few studies on the antioxidant properties and bile acid binding capacity of the TEMPO-oxidized chitin prepared from squid cartilage are available. Compared with α-chitin, β-chitin is attractive

Abbreviations: TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl radical; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TEMPO-mediated 6-carboxy β-chitin, T-chitin; EDTA, ethylenediaminetetraacetic acid; PMS, phenazine methosulfate.

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as another form of chitin by weak intermolecular forces (Rudall, 1963) and has been confirmed to exhibit higher reactivity and processability under various modification conditions, as well as higher affinity for solvents (Kurita et al., 1994). β-Chitin is much more reactive and advantageous than α-chitin as a starting material to prepare fully substituted chitin. To date, TEMPO-mediated oxidation has not been evaluated for its potential in improving the antioxidant properties and bile acid binding capacities of β-chitin.

In this paper, therefore, we studied the preparation of 6-carboxy β-chitin (T-chitin) by TEMPO/NaBr/NaClO oxidation, and investigated effects of different carboxylate content on antioxidant activities and bile acid binding capacities of T-chitin.

2. Materials and methods

2.1. Chemicals

Squid pens were provided by Hangzhou Baokai Biochemical Co., Ltd. Bile acid (derived from taurocholate, ≥98%), furfural, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2,6,6-tetamethyl-1-piperdinyloxy (TEMPO), hydrogen peroxide (H_2O_2), potassium ferricyanide ($K_3Fe(CN)_6$), sodium bromide (NaBr), sodium hypochlorite solution (activated chlorine 5.5%), trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), ferrozine, ethylenediaminetetraacetic acid (EDTA), ascorbic acid, phenazine methosulfate (PMS), and ferrous sulfate ($FeSO_4$) were purchased from Aladdin-reagent Co., Ltd. (Shanghai, China). All other chemical were analytical grade and used without further purification. All water used in extraction and analysis had been distilled and deionized.

2.2. Preparation of β-chitin from squid pens

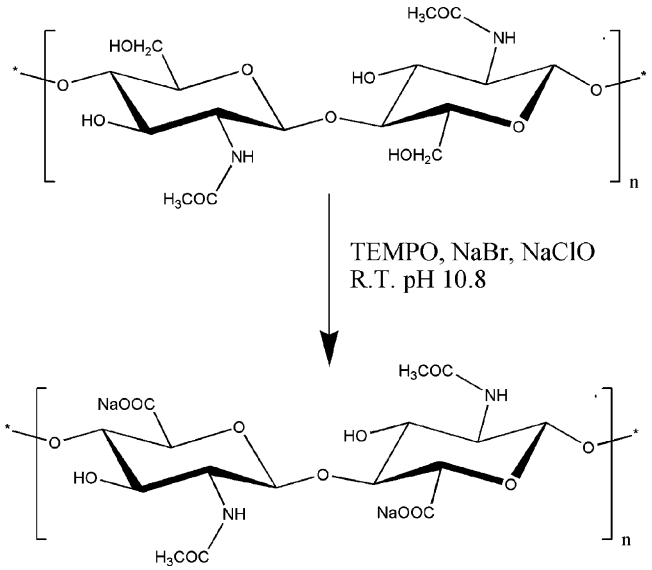
The β-chitins was extracted from squid pens according to the previous procedure (Huang, Zhao, Hu, Mao, & Mei, 2012). The β-chitin product obtained was filtered under reduced pressure, washed with deionized water, and dried under vacuum at 60 °C for 12 h.

2.3. TEMPO-mediated oxidation

TEMPO-mediated oxidation was carried out using a modification of the method as previously described (Fan, Saito, & Isogai, 2009; Fan, Fukuzumi, Saito, & Isogai, 2012; Kato et al., 2004; Tamura, Wada, & Isogai, 2009; Wang et al., 2011). β-Chitin (2 g) was suspended in water (100 mL) containing TEMPO (40 mg, 0.25 mmol) and sodium bromide (0.8 g, 8 mmol), 0.5 M NaOH was added to adjust the pH value to 10.8 and the suspension was stirred at room temperature. TEMPO-mediated oxidation was started by adding a designed amount of the NaClO solution (8–20 mmol NaClO per gram of β-chitin). The pH of the mixture was maintained to be 10.8 by adding 0.5 M NaOH until no NaOH consumption was observed. After the pH was adjusted to 7 with 0.5 M HCl, the reaction mixture was dialyzed (regenerated cellulose tubing; Mw cut-off 5000) against distilled water for 48 h, filtered, and insoluble material (solid) were decanted. The filtrate was concentrated and precipitated by adding 100% ethanol of five times as much as the volume of the solution, then followed by drying in vacuum at 45 °C. The precipitate was washed with 100% ethanol to obtain the TEMPO-oxidized β-chitin (Scheme 1).

2.4. FT/IR spectroscopy and microscopic observation

Fourier transform infrared (FTIR) spectra of β-chitin and its oxidized products were obtained using a Nicolet FTIR spectrometer (Magna-IR 760 ESP, Nicolet Instrument Corp., Madison, WI).



Scheme 1. Scheme of the preparation of water-soluble TEMPO-mediated 6-carboxy β-chitin (T-chitin) in TEMPO/NaBr/NaClO mediated system.

Transmission electron microscopy (TEM) observation of samples was performed with a Philips CM200 TEM operated at 160 kV.

2.5. Determination of carboxylate contents

The carboxylate content of the water-soluble T-chitin was determined by the electrical conductivity titration method with minor modification (Fan et al., 2009; Saito & Isogai, 2004). To a dried sample (0.5 g) were added water (60 mL), and then 0.1 M HCl was added to the mixture to set the pH value in the range of 2.5–3.0. The data of electrical conductivity were recorded when 0.05 M NaOH solution was added. The carboxylate content of the sample was determined from the conductivity curve according to the following equation:

$$A = \frac{C_{NaOH} \times (V_2 - V_1)}{W}$$

where W is the weight of sample (g); V_1 and V_2 are the volume of NaOH consumed with the inflection point of conductivity curve respectively (mL); A is the carboxylate content (mmol/g).

2.6. Bile acid binding assay

Using the method of Muzzarelli et al. (2006) with minor modifications, the bile acid binding capacity of T-chitins was investigated *in vitro*. Sample (0.05 g) was mixed with 2 mL of 5 mg/mL bile acid, and the mixtures were adjusted to a total volume of 25 mL with distilled water. The mixtures were incubated for 2 h at 37 °C, and then filtered. The resulting samples (1.0 mL) were mixed with 1 mL 1% (w/v) furfural and 13 mL 45% (v/v) sulfuric acid, and then the mixtures were incubated for 20 min at 70 °C, and the absorbance was measured at 605 nm.

2.7. Hydroxyl radical scavenging ability

The scavenging ability of water-soluble T-chitin toward hydroxyl radicals was determined according to the method of Lee et al. (2005) with minor modifications. The reaction mixture contained $FeSO_4$ (5 mM), salicylic acid (5 mM), samples at different concentrations (0.5–10 mg/mL) and H_2O_2 (20 mM). After incubation for 1 h at 37 °C, absorbance was detected at 510 nm. The absorbance of the mixtures was measured at 510 nm against a

blank. The EC_{50} value (mg/mL) is the effective concentration at which hydroxyl radicals were scavenged by 50%.

2.8. DPPH radical scavenging ability

The scavenging effect of water-soluble T-chitin on DPPH radicals was measured using a modification of the method of Yamaguchi, Takamura, Matoba, and Terao (1998). A total of 2.5 mL of the 100% ethanol solution of DPPH (50 mg/L) was incubated with 2.5 mL samples at different concentrations (2.0–10.0 mg/mL). The reaction mixture was shaken thoroughly with vortex and incubated for 30 min at 33 °C, and the absorbance was measured at 517 nm against a blank. The percentage of DPPH radical scavenged was calculated as:

$$\text{Scavenging effect}\,(\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where A_{control} is the absorbance of the control (distilled water instead of samples).

2.9. Measurement of reducing power

The reducing power of water-soluble T-chitin was measured by the method of Oyaizu (1986). The reaction mixture contained different concentrations of T-chitin samples (2.5 mL), 0.2 mol/L sodium phosphate buffer (pH 6.6, 2.5 mL), and 1% (w/v) potassium ferricyanide (2.5 mL), and the mixtures were incubated for 20 min at 50 °C. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 4000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% (w/v) ferric chloride solution (0.5 mL). The absorbance values of the reaction mixtures were determined at 700 nm. The absorption indicated the intensity of the reducing ability, and increased absorbance of the reaction mixture indicated increased reducing power.

2.10. Metal ion chelating assay

The ferrous chelating ability of water-soluble T-chitin was determined according to the method of Carter (1971) with minor modifications. Each sample (2–10 mg/mL) was mixed with 9.25 mL of 100% methanol, 0.25 mL of 1.51 mM $FeSO_4$, and 0.5 mL of 2.4 mM ferrozine. After 20 min at 37 °C, the absorbance of the mixtures was determined at 562 nm against a blank. A lower absorbance indicates a higher chelating ability. The ability of T-chitin samples to chelate ferrous ion was calculated using the following equation:

$$\text{Chelating ability}\,(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100$$

where A_0 and A_1 are the optical density at 562 nm without and with samples, respectively.

2.11. Statistical analysis

All of the analyses were performed in triplicate. Each experimental data point represents the mean from three independent experiments. The deviation from the mean at the 95% significance level was used to determine the differences in biological activity.

3. Results and discussion

3.1. Infrared spectra analyses

When a sufficient amount of NaClO is added to β-chitin/water slurries at pH 10–11 in the oxidation, β-chitin can be converted to the corresponding water-soluble polyuronic acid with partial depolymerization (Kato et al., 2004; Muzzarelli et al., 1999). Infrared spectroscopy has been used to determine the structure of chitin (Muzzarelli, 1988). Fig. 1 displays the FT-IR spectrum of β-chitin and T-chitin. As shown in Fig. 1, the characteristic absorption bands of β-chitin were 1654.56 cm^{-1} (amide I) and 1558.64 cm^{-1} (amide II). In the FT-IR spectra, the amide I band is split for α-chitin which was attributed to the occurrence of the intrasheet (C═O···H—N) and the intersheet hydrogen bonding (C═O···H—OCH_2), and the amide I band for β-chitin is a single peak because β-chitin has only intrasheet hydrogen bonding in its crystalline structure (Jang, Kong, Jeong, Lee, & Nah, 2004). After oxidation, two new bands at 1617.67 and 1408.89 cm^{-1} were observed, which were characteristic of the stretching vibration peaks of asymmetric and symmetric carboxy groups, respectively (Jin, Zhang, Yin, & Nishinari, 2006; Wang et al., 2011). The band of β-chitin at 2878.10 cm^{-1} may be due to the C—H stretching vibration at the C6 primary hydroxyls. The C—H stretching band decreased with TEMPO-mediated oxidation because the C6 primary hydroxyls are converted to C6 sodium carboxylate groups by oxidation. Therefore, the FT-IR showed the existence of carboxy groups in the derivative.

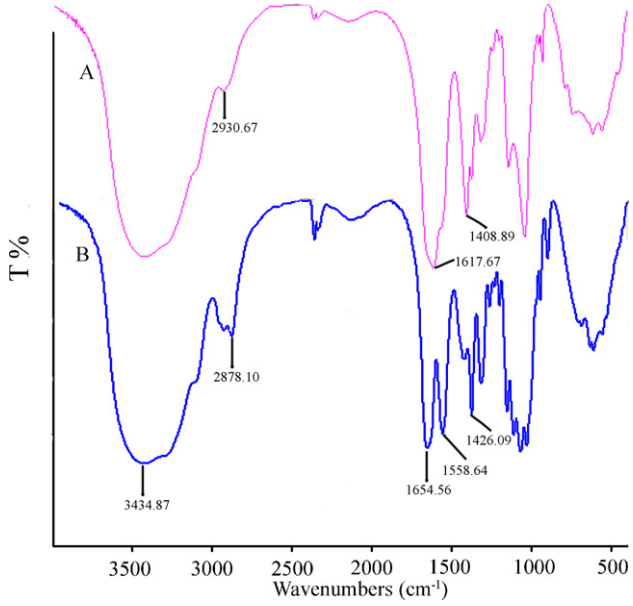


Fig. 1. IR spectra of TEMPO-mediated 6-carboxy β-chitin (T-chitin-2, carboxylate content 1.68 mmol/g) (A) and β-chitin (B).

Fig. 2 shows the TEM images of β-chitin and water-soluble T-chitin. As shown in Fig. 2, the group-like shape formed from many β-chitin molecules is much compacter than that of T-chitin. The result showed that after TEMPO/NaBr/NaClO oxidation, the inner structure of β-chitin becomes severely disrupted and its ability to form hydrogen bonds declines sharply, and hence β-chitin derivatives have much looser structures and the effect of their intramolecular hydrogen bonds is weaker.

3.2. Carboxylate content and in vitro bile acid binding capacity

The carboxylate content, yield and *in vitro* bile acid binding capacities of T-chitin are shown in Table 1. As the amount of NaClO added was increased, the carboxylate content and yield of the water-soluble T-chitin increased. When the amount of NaClO added was 20 mmol/g of chitin, the yield of the water-soluble T-chitin reached 74.55%, and the corresponding carboxylate groups were further formed up to about 2.08 mmol/g. It is reported that no

Table 1
The reaction conditions, yield and the bile acid binding capacity of TEMPO-mediated 6-carboxy β-chitin (T-chitin) with different carboxylate content.

Product	NaClO addition amount (mmol/g chitin)	Carboxylate content (mmol/g chitin)	Yield (%)	Bile acid binding capacity (mg/g)
β-Chitin	–	–	–	15.85
T-chitin-1	8	1.33	34.43	18.35
T-chitin-2	12	1.68	54.20	41.18
T-chitin-3	16	1.80	61.25	23.72
T-chitin-4	20	2.08	74.55	21.61

N-deacetylation occurs on the TEMPO-oxidized α-chitins, irrespective of the amount of NaClO added in the oxidation (Fan, Saito, & Isogai, 2009).

Binding of bile acids and subsequent excretion in feces has been recognized as a significant mechanism to eliminate excess cholesterol (Muzzarelli, 1996; Parvathy, Susheelamma, Tharanathan, & Gaonkar, 2005; Zhang, Zhang, & Cheung, 2003). Therefore, the high binding capacities of bile acids are closely involved in cholesterol-lowering effects in the body. The binding capacity of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 against bile acid was 18.35 mg/g, 41.18 mg/g, 23.72 mg/g, and 21.61 mg/g, which were 1.16-fold, 2.60-fold, 1.50-fold, and 1.36-fold higher than that of β-chitin, respectively (Table 1). It was noticed that T-chitin-2 displayed much better binding capacity than any other sample. These data suggested the possible application of T-chitin as a cholesterol-lowering adjuvant.

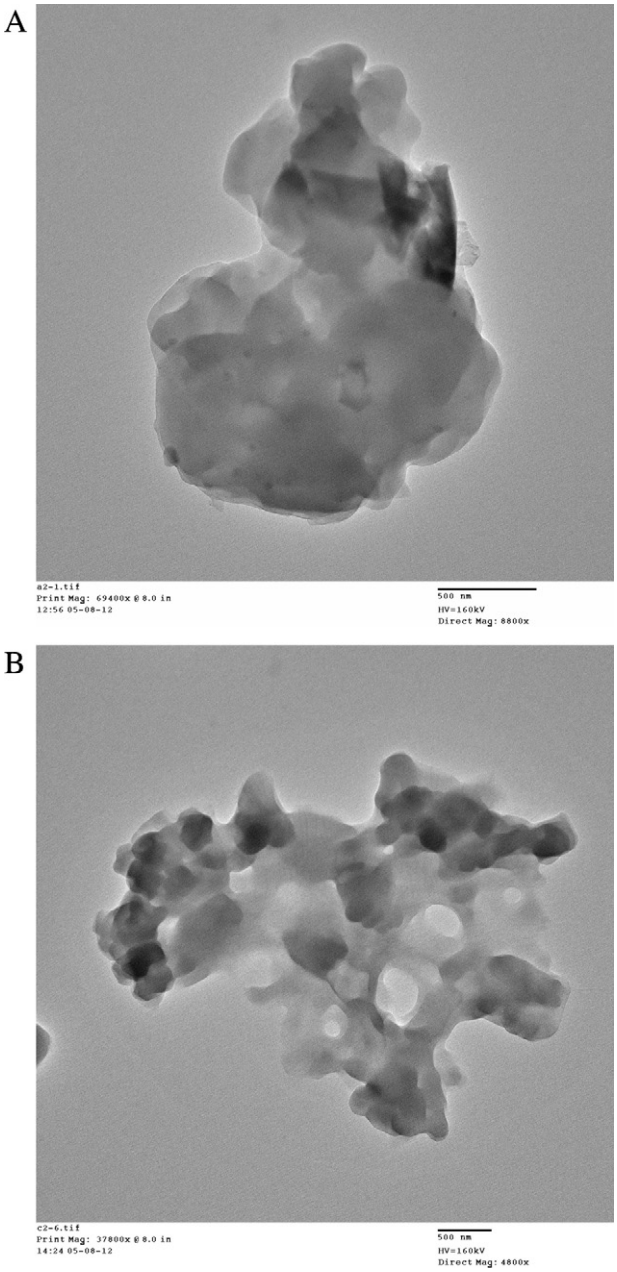


Fig. 2. Transmission electron micrographs of β-chitin (A) and TEMPO-mediated 6-carboxy β-chitin (T-chitin-2, carboxylate content 1.68 mmol/g) (B).

It is reported that the bile acid-binding activity is related to ionic interaction, and charge–charge interactions are the major force between the anionic bile acid and the bile acid sequestrants (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997). For example, the capacity of 6-oxychitosan for cholic acid decreases with increasing degree of oxidation as a result of cationicity loss (Yoo et al., 2005). Moreover, the lipid uptake takes place by hydrophobic interactions with the insoluble salts formed by sequestrants upon contact with bile (Muzzarelli et al., 2006). Compared with the native β-chitin, the introduction of carboxy groups improved the water solubility of T-chitin. Therefore, the solubility improvement should be considered for enhancing the biological activity such as bile acid-binding capacity (Wang et al., 2011). When negative charge in T-chitin structure increased above a certain level with the introduction of carboxy groups, the significant decrease in the bile acid binding capacity could be caused by ionic repulsion due to its anionic characteristics. Furthermore, factors such as ionic and hydroxyl group interactions and entrapping in the polymer matrix have been suggested to contribute to the overall bile acid-binding capacity of polymers (Chang, Lee, Yoo, & Lee, 2006; Shin et al., 2005).

3.3. Scavenging effect on hydroxyl radicals

Hydroxyl radical is the most reactive oxygen species formed in biological systems, and can easily penetrate through cell membranes damaging almost every molecule found in living cells (Gulcin, 2006). Therefore, removing hydroxyl radical is very important for protection of biological systems (Liu et al., 2010; Yuan, Zhang, Fan, & Yang, 2008). As shown in Fig. 3, the oxidized product was found to possess the hydroxyl radical scavenging activity, which increased as its concentration increased to certain extent and then appeared to reach a plateau. EC_{50} values (mg/mL) of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 1.17, 0.67, 0.74, and 0.94, respectively. These EC_{50} values were also comparable to N,O-carboxymethyl chitosan (4.23–5.64 mg/mL) with scavenging abilities of hydroxyl radicals reported previously (Zhao, Huang, Hu, Mao, & Mei, 2011). Obviously, T-chitin products from squid pens were an effective scavenger for hydroxyl radicals. Wang et al. (2011) reported that at 6 mg/mL, the hydroxyl scavenging abilities of the oxidized polysaccharides by the TEMPO/NaBr/NaClO oxidation reached 70%, while the native polysaccharides showed no antioxidant activity. Chen, Tsai, Huang, and Chen (2009) reported that the antioxidative capacities of sulfate of low-molecular-weight agar (LMAG) may be due to scavenging free radicals by electron-transfer (ET) to form stable macromolecular radicals through the

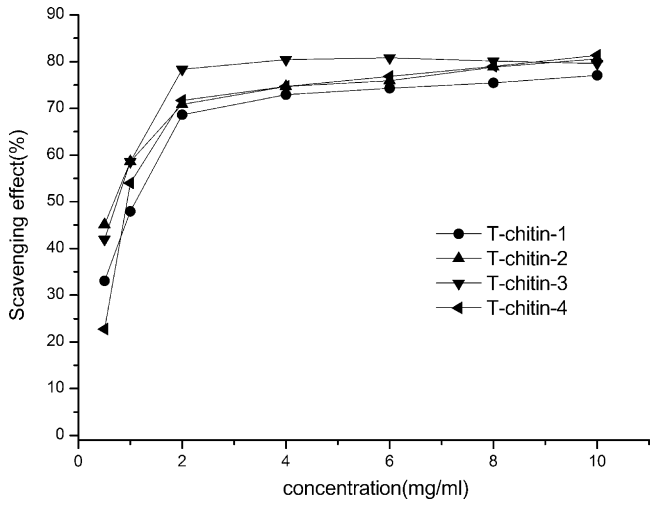


Fig. 3. Scavenging effects of TEMPO-mediated 6-carboxy β-chitin (T-chitin) with different carboxylate content toward hydroxyl radicals. The carboxylate content of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 1.33, 1.68, 1.80, and 2.08 mmol/g, respectively.

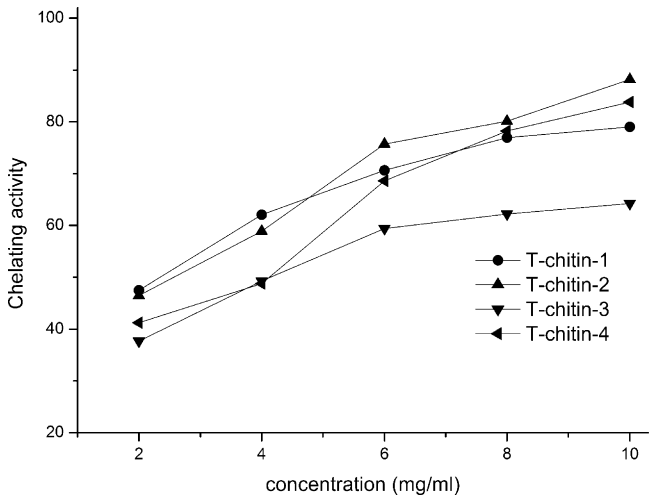


Fig. 5. Chelating effects of TEMPO-mediated 6-carboxy β-chitin (T-chitin) with different carboxylate content on ferrous ions. The carboxylate content of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 1.33, 1.68, 1.80, and 2.08 mmol/g, respectively.

sulfate group, so the antioxidant activity presented by the oxidized TEMPO-C products are in the same way as the ET from the carboxylate group of the modified polymer to the free radical ($R^{\bullet}$) to form a stable free-radical ion. The oxidized TEMPO-C product loses an electron from carboxylate groups to form stable radicals.

3.4. Scavenging effect on DPPH radicals

DPPH is a stable nitrogen-centered and lipophilic free radical that is widely used in evaluating the antioxidant activities. Fig. 4 shows the DPPH radical scavenging activity of various T-chitin at various concentrations. As shown in Fig. 4, the scavenging ability of various T-chitin on DPPH radicals was in the range of 11.0–26.1% at 10 mg/mL. It is well accepted that DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability (Chen & Ho, 1995). The introduction of hydrophilic carboxy groups on β-chitin decreased the intramolecular hydrogen bonds resulting in the exposure of more hydroxyl groups, and this led to greater 'availability' of the hydroxyl groups in the β-chitin molecules, which may enhance the interaction between T-chitin molecules and the radicals. In addition, T-chitin had much greater water solubility, which made hydroxyl groups more available to interact with radicals in the testing systems. As the NaClO addition amount increased, more C6 primary hydroxyls converted to carboxylate groups and decrease the total hydroxyl group number. The scavenging abilities of T-chitin with a higher degree of substitution (DS) are poorer than that with a lower DS.

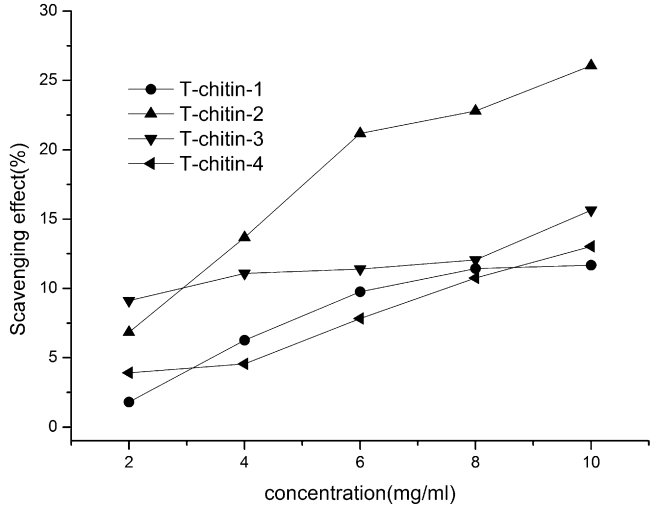


Fig. 4. Scavenging effects of TEMPO-mediated 6-carboxy β-chitin (T-chitin) with different carboxylate content toward DPPH radicals. The carboxylate content of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 1.33, 1.68, 1.80, and 2.08 mmol/g, respectively.

3.5. Chelating effect on ferrous ions

Ferrous ion chelating effects of T-chitin were determined by measuring the decrease in the absorbance at 562 nm of the iron (α)-ferrozine complex. As shown in Fig. 5, the chelating effects of all five T-chitin increased with increasing concentrations and exhibited high chelating ability. When the concentration of T-chitin was 8 mg/mL, the chelating effects of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 76.98%, 80.15%, 62.24%, and 78.23%, respectively. This might be due to the fact that the binding of the carboxy group on T-chitin improved the ferrous ion-chelating effect. The most effective pro-oxidants present in food systems are ferrous ions (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988). The previous reports showed that factors affecting the ion-chelating ability of chitosan are rather complex. Qin (1993) suggested that ferrous ion chelation might involve the binding of Fe^{2+} with the hydroxyl group on C6 and amino group on C2 of the chitosan molecule. The intramolecular hydrogen bonds in β-chitin inhibit its reactivity with hydroxyl groups. After TEMPO-mediated oxidation, the inner structure of β-chitin becomes severely disrupted and its ability to form hydrogen bonds declines sharply, hence activating the hydroxyl groups contributed to improving the ion-chelating ability of T-chitin. The high ferrous ion-chelating ability of T-chitin may be beneficial if they are formulated into foods.

3.6. Reducing power

Fig. 6 depicts the reducing power of different T-chitin using the potassium ferricyanide reduction method. As shown in Fig. 6, the reducing power of T-chitin increased with increasing concentrations. Moreover, at 10 mg/mL, the reducing power of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 0.104, 0.093, 0.090, and 0.058, respectively. Huang et al. (2012) reported that at 2 mg/mL, the reducing power of Low molecular weight (LMW) chitosans

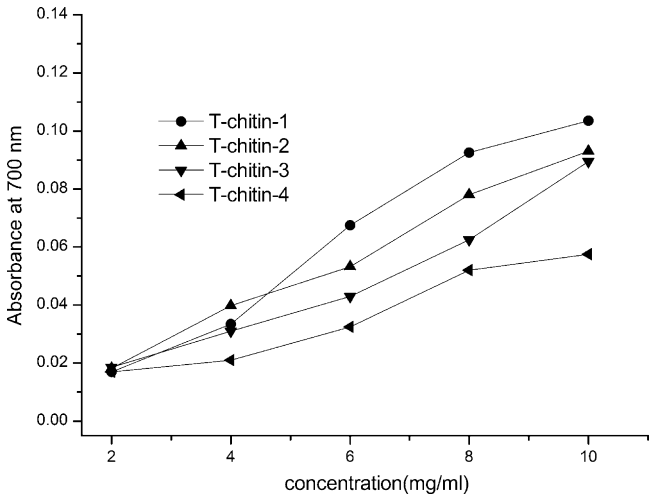


Fig. 6. Reducing power of TEMPO-mediated 6-carboxy β-chitin (T-chitin) with different carboxylate content. The carboxylate content of T-chitin-1, T-chitin-2, T-chitin-3, and T-chitin-4 were 1.33, 1.68, 1.80, and 2.08 mmol/g, respectively.

were more than 0.50 respectively. Compared with LMW chitosans, it seems that T-chitin was not effective in reducing power. The reducing properties are generally associated with the presence of reductones (Duh, Du, & Yen, 1999), which have been shown to exert antioxidant action by breaking the free radical chain through donation of a hydrogen atom (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The reducing power of T-chitin suggested that it was likely to contribute unsignificantly toward the observed antioxidant effect.

4. Conclusion

When sufficient amounts of NaClO were used, β-chitins are converted to water-soluble T-chitin by TEMPO/NaBr/NaClO oxidation of the C6 primary hydroxyls to carboxy groups. Our results revealed that the bile acid binding capacities of T-chitin by introducing carboxylate groups were better than that of the native β-chitin sample and the highest bile acid binding capacity of T-chitin reached 41.18 mg/g. The bile acid binding capacity of T-chitin decreases with increasing degree of oxidation, *i.e.*, loss of cationicity. EC_{50} values of T-chitins against hydroxyl radicals were below 1.2 mg/mL compared to a previously reported value (0.93–3.36 mg/mL) of LMW chitosans (Huang et al., 2012). This study demonstrates that T-chitins behave as effective radical scavengers, especially in terms of their ferrous ion chelating effect and scavenging ability toward hydroxyl radicals. On the basis of the results obtained, T-chitin with presumed antioxidant properties and bile acid binding capacity may be used as a source of antioxidant, and a possible food supplement or ingredient in the pharmaceutical industry.

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