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Antioxidant activity of xanthan oligosaccharides prepared by different degradation methods

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ABSTRACT

Two kinds of water soluble xanthan oligosaccharides (XGOS-A and XGOS-B) with similar molecular weights were prepared by oxidative degradation of xanthan gum (XG) under acidic and alkaline condition, respectively. Antioxidant activity of XGOS-A and XGOS-B was evaluated by the scavenging of superoxide anion radical ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydrogen peroxide (H_2O_2), determination of ferrous ion chelating activity and reducing power. All the above antioxidant evaluation indicated that XGOS-A and XGOS-B exhibited good antioxidant activity in a concentration-dependent manner. The XGOS-B exhibited better antioxidant activity than XGOS-A. These results may be related to the different structure properties of XGOS-A and XGOS-B especially the different contents of pyruvate acid and reducing sugar.

1. Introduction

Xanthan gum (XG) is an anionic polysaccharide produced by the microorganism *Xanthomonas campestris*, whose primary structure consists of a pentasaccharide repeating units with a (1→4)-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyluronic acid-(1→2)-6-O-acetyl-α-D-mannopyranosyl side chains 3-linked to alternate glucose residues (Jansson, Kenne, & Lindberg, 1975). The most important properties of XG are high low-shear viscosity and strong shear-thinning character. The relatively low viscosity at high shear rate makes it easy to mix, pour, and swallow; its high viscosity at low shear rate gives it good suspension properties and lends stability to colloidal suspensions. XG is stable over a broad range of pH values and in foods containing salt or alcohol or with high enzymatic activity. Hence, XG is widely used as stabilizer and thickener in salad dressings, soups and gravies, convenience foods, frozen foods, desserts, toppings, dairy products and beverages (Katzbauer, 1998). Recently, the potential antioxidant activity of XG attracted more and more attention. It significantly inhibited Fe^{2+}-induced oxidation of soybean oil (Shimada, Fujikawa, Yahara, & Nakamura, 1994; Shimada, Muta, et al., 1994). Xanthan oligosaccharides prepared by using the xanthan-degrading enzyme showed hydroxyl radical scavenging ability (He, Zhang, Bai, Du, & Li, 2005). XG can be used as potential antioxidant for topical administration using a lipid model system (Trommer & Reinhard, 2005). And it was found to have a protective effect against $^{\bullet}OH$ radical-induced depolymerization of β-glucan by ascorbic acid (Paquet, Turgeon, & Lemieux, 2010). Degradation of XG is an important method to prepare the xanthan oligosaccharides with good water solubility and bioactivity (Christensen, Myhr, & Smidsrød, 1996; He et al., 2005). In this paper, two kinds of water-soluble xanthan oligosaccharides with similar molecular weights were prepared by oxidative degradation under acidic condition and alkaline condition, respectively. Their antioxidant activity has been investigated to find out the influence of different structure properties on the antioxidant activity of xanthan oligosaccharides.

2. Experimental

2.1. Materials

XG was purchased from Shanghai United Food Additives Co. (Shanghai, China). Luminol and DPPH were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). All other chemicals were analytical grade reagents, supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of xanthan oligosaccharides

Two kinds of xanthan oligosaccharides XGOS-A and XGOS-B were prepared by oxidative degradation of XG under acidic condition and alkaline condition, respectively. XG (3.0 g) was mixed

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with 200.0 mL distilled water. The system was heated up to 80 °C and stirred 30 min until a homogeneous solution was formed. The pH value of solution was adjusted to 1.0 (or 13.0) with 2.0 M HCl (or 2.0 M NaOH) solution, and H_2O_2 (50.0 mL, 30%, w/v) was added into the solution. After degradation for 5 days, the pH value of the solution was adjusted to 7.0 and then filtered by microporous filter (diameter 0.45 μm). The product XGOS-A (or XGOS-B) was finally collected by dialyzing against distilled water for 72 h using dialysis membranes with a 7000–14,000 Da molecular weight cut off and was dried in a vacuum freezing dryer.

2.3. Characterization of xanthan oligosaccharides

The structures of XG, XGOS-A and XGOS-B were confirmed by FT-IR spectroscopy, which was taken with KBr pellets on an EQUNOX55 FT-IR-Raman spectrophotometer with a revolution of 0.8 cm^{-1} in the range of 500–4000 cm^{-1}.

Molecular weights of XGOS-A and XGOS-B were determined by gel permeation chromatography (GPC). The GPC was performed on a Waters-515 Chromatograph equipped with Waters 2410 refractive index detector and Ultrahydrogel 500 and 120. Elution was carried out using 0.07% Na_2SO_4 solution as the mobile phase at a flow rate of 0.5 mL/min. The temperatures of the column and detector were both maintained at 40 °C during the determination process. The reference standard was glucan (molecular weight: 473,000 Da, 188,000 Da, 76,900 Da, 43,200 Da, and 10,500 Da, respectively).

The pyruvate acid contents of XGOS-A and XGOS-B were assayed by ultraviolet spectrophotometry method described by Lou (Lou & Gao, 2005). A standard curve of absorbance against concentration of pyruvate acid at wavelength 320 nm was constructed to calculate the pyruvate acid content of XGOS-A and XGOS-B.

The reducing sugar contents of XGOS-A and XGOS-B were assayed by dinitrosalicylic acid (DNS) method described by Miller (Miller, 1959). A standard curve of absorbance against concentration of glucose at wavelength 520 nm was constructed to calculate the reducing sugar content of XGOS-A and XGOS-B.

2.4. Antioxidant activity of xanthan oligosaccharides

2.4.1. Superoxide anion radical scavenging assay

Superoxide anion radical scavenging activity was evaluated by chemiluminescent method on a bio-chemical luminometer (IFFDM-D, Xi'an, China). Superoxide anion radical was produced by a luminol-enhanced autoxidation of pyrogallol. The chemiluminescent reaction was processed in a Na_2CO_3–$NaHCO_3$ (pH = 10.20, 0.05 M) buffer solution at ambient temperature. The samples were dissolved in Na_2CO_3–$NaHCO_3$ buffer solution to prepare scavenger solutions at different concentrations (1.0–10.0 mg/mL). The scavenging effect of XGOS-A and XGOS-B against superoxide anion radical was evaluated according to their quenching effects on the chemiluminescence (CL) signal of luminal–pyrogallol system. The scavenging effect against superoxide anion radical was calculated using the following equation: scavenging effect (%) = $(CL_0 - CL_i)/CL_0 \times 100$, where CL_0 and CL_i represent chemiluminescence peak areas of the blank group and test group, respectively. The free radical produced in the system was proved to be superoxide anion radical tasted by superoxide dismutase (SOD), catalase and mannitol (Sun, Xie, & Xu, 2004).

2.4.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was processed according to a similar program described above. Hydroxyl radical was produced in a Fe(II)–H_2O_2–luminol system (Halliwell, Gutteridge, & Aruoma, 1987). The chemiluminescent reaction was processed in a KH_2PO_4–NaOH (pH = 7.40, 0.05 mol/L) buffer solution. Scavenging activity of the samples was evaluated according to their quenching effects on the chemiluminescence signal of the system. The capability of scavenging against hydroxyl radical was calculated as: scavenging effect (%) = $(CL_0 - CL_i)/CL_0 \times 100$, where CL_0 and CL_i represent chemiluminescence peak areas of the blank group and test group, respectively. The free radical produced in the system was proved to be hydroxyl radical tested by superoxide dismutase, catalase and mannitol.

2.4.3. DPPH radical scavenging assay

The DPPH radical scavenging effect of the samples was measured using the modified method of Yamaguchi et al. (Yamaguchi, Takamura, Matoba, & Terao, 1998). 2.0 mL of ethanolic solution of DPPH (0.1 mmol/L) was incubated with test samples at different concentrations (0.1–2.0 mg/mL, 2.0 mL), respectively. The reaction mixture was shaken well and incubated for 30 min at 33 °C and the absorbance of the resulting solution was read at 517 nm against a blank. The DPPH radical scavenging effect was measured as a decrease in the absorbance of DPPH and was calculated using the following equation: scavenging effect (%) = $(1 - A_{\text{sample 517 nm}}/A_{\text{control 517 nm}}) \times 100$.

2.4.4. Hydrogen peroxide scavenging assay

The activity of xanthan oligosaccharides to scavenge H_2O_2 was determined according to the method of Ruch et al. (Ruch, Cheng, & Klauning, 1989). A solution of H_2O_2 (40 mM) was prepared in Na_2HPO_4–NaH_2PO_4 buffer solution (pH = 7.40, 0.2 mol/L). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm. Different concentrations of samples (0.1–2.0 mg/mL) in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The activity of all samples to scavenge H_2O_2 was calculated using the following equation: scavenging effect (%) = $(1 - A_{\text{sample 230 nm}}/A_{\text{control 230 nm}}) \times 100$.

2.4.5. Ferrous ion chelating activity assay

Chelating activity was determined according to the method of Chan et al. (Chan, Lim, & Chew, 2007). 0.1 mL of ferrous chloride (2.0 mmol/L) was mixed with test samples at different concentrations (0.2–4.0 mg/mL, 3.5 mL), respectively. Then the reaction was initiated by 0.2 mL ferrozine (5.0 mmol/L). After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. The activity of all samples to chelate ferrous ion was calculated using the following equation: chelating activity (%) = $(1 - A_{\text{sample 562 nm}}/A_{\text{control 562 nm}}) \times 100$.

2.4.6. Reducing power determination

The reducing power was determined by the method of Yen and Chen (Yen & Chen, 1995). Different concentrations of test samples (2.0 mL) were mixed with 2.5 mL sodium phosphate buffer (pH = 6.60, 0.2 M) and 2.5 mL potassium ferricyanide (1%, w/v), respectively. The mixtures were incubated for 20 min at 50 °C, cooled down in ice water, and then 2.5 mL trichloroacetic acid (10%, w/v) was added to the mixtures, followed by centrifugation at 3000 rpm for 10 min. 2.0 mL supernatant was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride solution (0.1%, w/v) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.5. Statistical analysis

All analyses were performed in triplicate. Data of antioxidant evaluation were expressed as mean ± standard error of the mean. SPSS 11 (SPSS Inc., Chicago, USA) was used to evaluate the significant difference at $P < 0.05$.

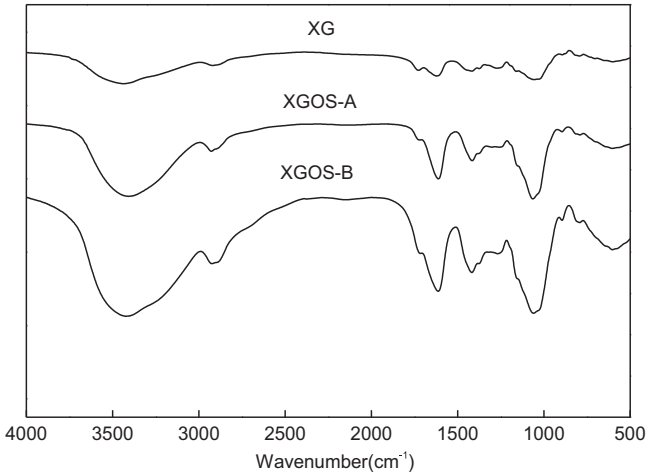

Fig. 1. FT-IR spectra of XG, XGOS-A and XGOS-B.

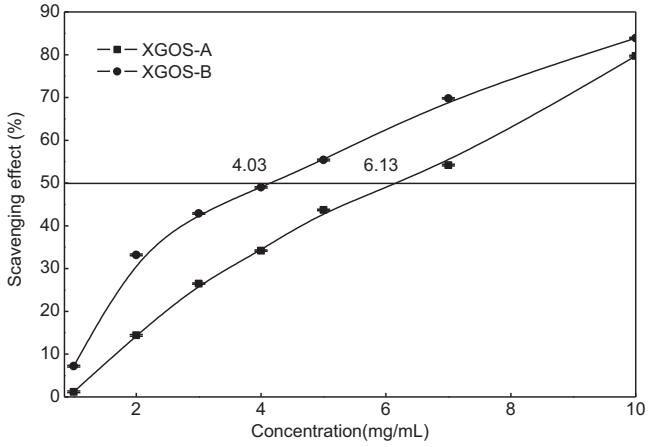

Fig. 2. Scavenging effect of XGOS-A and XGOS-B on superoxide anion radical.

3. Results and discussion

3.1. Characterization of xanthan oligosaccharides

Structure change of XG and degraded xanthan oligosaccharides were confirmed by FT-IR spectra. As shown in Fig. 1, there are characteristic absorption peaks of XG in the FT-IR spectra of XGOS-A and XGOS-B (Gils, Ray, & Sahoo, 2009; Hamcerencu, Desbrieres, Popa, Khoukh, & Riess, 2007; Su, Ji, Lan, & Dong, 2003). Peaks appearing at 2918 cm^{-1} and 1055 cm^{-1} were assigned to —CH_2 and acetal groups, respectively. The vibration peaks of —OH appeared at 3426 cm^{-1} in XG, 3408 cm^{-1} in XGOS-A and 3423 cm^{-1} in XGOS-B, respectively. The vibration peaks of —OH shifted to low wave numbers compared with XG, which indicated that the intermolecular interaction between xanthan oligosaccharides was relative stronger than that between XG (Ning, 1989). Moreover, the peak at 1625 cm^{-1} could be assigned to —C=O of pyruvate acid (Su et al., 2003) and the peak of XGOS-B at 1625 cm^{-1} was stronger than that of XGOS-A. This result indicated the higher content of pyruvate acid in XGOS-B.

According to the standard curve of pyruvate acid, the pyruvate acid contents of XGOS-A and XGOS-B were estimated to be 2.3% and 4.8%, respectively.

Gel permeation chromatography results indicated that XGOS-A and XGOS-B had the similar average molecular weights of 7500 Da and 7330 Da, respectively.

According to the standard curve of glucose, the reducing sugar contents of XGOS-A and XGOS-B were estimated to be 16.0% and 21.1%, respectively.

3.2. Antioxidant activity of XGOS-A and XGOS-B

Antioxidant activity of polysaccharide is attracting much attention (Jin et al., 2012; Raza et al., 2012). Hydroxyl groups and characteristic functional groups in the polymer chain are the main source of antioxidant activities of polysaccharides. Chitosan derivatives have good antioxidant activities because the active hydroxyl and amino groups in the chain (Sun, Zhu, Xie, & Yin, 2011; Xie, Xu, & Liu, 2001). Degraded κ-carrageenan oligosaccharides have good antioxidant activities because the high content of hydroxyl groups and the "hydroxyl activating" function of carboxyl groups (Sun, Tao, Xie, & Xu, 2010). Xanthan gum is composed of pentasaccharide-repeating units, containing D-glucose, D-mannose, D-glucuronic acid, acetal-linked pyruvate acid and D-acetyl groups. The hydroxyl groups or reducing sugar and pyruvate acid can enhance antioxidant activities of xanthan gum and its derivatives. XG has shown various antioxidant activities in different systems (He et al., 2005; Paquet et al., 2010; Shimada, Fujikawa, et al., 1994; Shimada, Muta, et al., 1994; Trommer & Reinhard, 2005). After degradation, the antioxidant activities of polysaccharides will be enhanced because of an increase in the number of exposed "activated hydroxyl groups" (Sun et al., 2010, 2011). In order to evaluate antioxidant activities of XG, it has been oxidatively degraded and assayed by scavenging superoxide anion radical, hydroxyl radical, DPPH radical, and hydrogen peroxide, the determination of ferrous ion chelating activity and reducing power.

3.2.1. Superoxide anion radical scavenging effect

The superoxide anion radical which is generated in numerous biological reactions, is a highly toxic radical species. Although superoxide anion radicals cannot directly initiate lipid oxidation, they serve as potential precursors of highly reactive oxygen species, such as hydroxyl radical and singlet oxygen (Banerjee, Dasgupta, & De, 2005; Kanatt, Chander, & Sharma, 2007). Fig. 2 shows the scavenging effect of XGOS-A and XGOS-B on superoxide anion radical. The scavenging effect increased with the increasing concentrations of XGOS-A and XGOS-B. The 50% inhibitory concentrations (IC_{50}s) of XGOS-A and XGOS-B were 6.13 and 4.03 mg/mL, respectively. The results indicated that XGOS-B had higher activity upon the elimination of superoxide anion radical compared with XGOS-A at all tested concentrations. Ascorbic acid and pyruvate acid were used as a control and their IC_{50} were 0.15 mg/mL and 0.86 mg/mL, respectively.

3.2.2. Hydroxyl radical scavenging effect

The hydroxyl radical is considered the most reactive free radical in biological tissues. It easily reacts with molecules such as amino acids, proteins, and DNA, thus resulting in cell damage. It was also believed to be an active initiator for peroxidation of lipids (Yang, Liu, Han, & Sun, 2006). Fig. 3 shows that the scavenging effect of XGOS-A and XGOS-B on hydroxyl radical. The scavenging effect increased with the increasing concentrations of XGOS-A and XGOS-B. The IC_{50}s of XGOS-A and XGOS-B were 9.40 and 2.50 mg/mL, respectively. The results indicated that XGOS-B had higher activity upon the elimination of hydroxyl radical compared with XGOS-A at all tested concentrations. Ascorbic acid and pyruvate acid were used as a control and their IC_{50} were 1.50 mg/mL and 1.80 mg/mL, respectively.

3.2.3. DPPH radical scavenging effect

DPPH radical is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreased significantly on exposure to proton radical scavengers. Further it

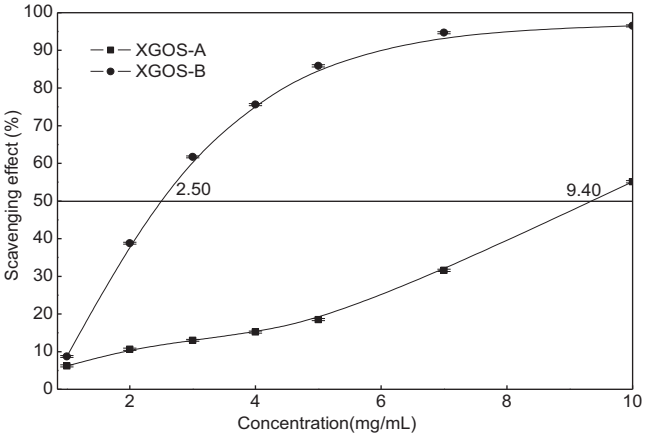

Fig. 3. Scavenging effect of XGOS-A and XGOS-B on hydroxyl radical.

is well accepted that the DPPH radical scavenging by antioxidants is due to their hydrogen-donating ability. In this experiment, the purple color of the reaction mixture changes to yellow and its absorbance at 517 nm decreases in the presence of antioxidant samples (Chen & Ho, 1995). Fig. 4 depicts the DPPH radical scavenging effect of XGOS-A and XGOS-B. Scavenging effect of DPPH radical increased with the increasing of concentrations of XGOS-A and XGOS-B. The IC_{50} of XGOS-B was 1.16 mg/mL, while IC_{50} of XGOS-A could not be observed within the range of experimental condition. It indicated that scavenging effect of XGOS-B on DPPH radical was also better than that of XGOS-A. Ascorbic acid and pyruvate acid were used as a control and their IC_{50} were 0.04 mg/mL and 0.26 mg/mL, respectively.

3.2.4. Hydrogen peroxide scavenging effect

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radical in the cells. H_2O_2 can cross cell membranes rapidly and once inside the cell, it can potentially react with Fe^{2+} or Cu^{2+} to form hydroxyl radicals and this may be the origin of many of its toxic effects in neuronal cells. It is, therefore, advantageous for cells to control the amount of H_2O_2 that is allowed to accumulate. The scavenging activity of xanthan oligosaccharides (0.1–2.0 mg/mL) on H_2O_2 was shown in Fig. 5. The IC_{50} of XGOS-B was 0.80 mg/mL, while IC_{50} of XGOS-A was 1.46 mg/mL. Ascorbic acid and pyruvate acid were used as a control and their IC_{50} were 0.24 mg/mL and 0.36 mg/mL, respectively.

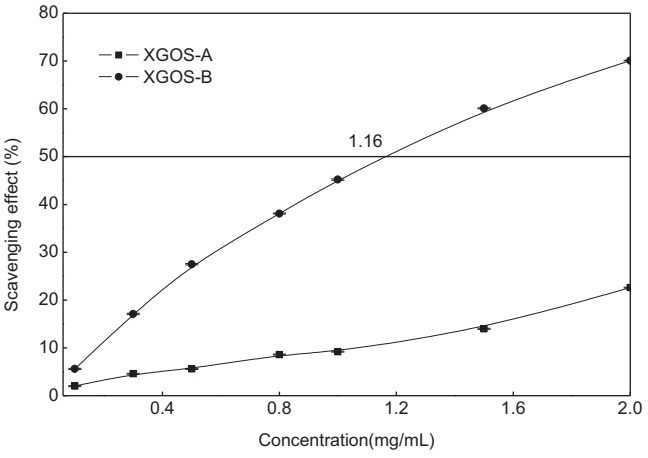

Fig. 4. Scavenging effect of XGOS-A and XGOS-B on DPPH radical.

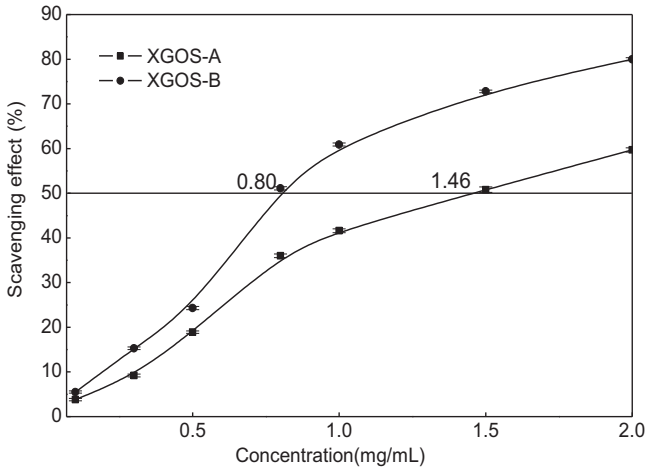

Fig. 5. Scavenging effect of XGOS-A and XGOS-B on hydrogen peroxide.

3.2.5. Ferrous ion chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and the activity of many enzymes. However, iron is an extremely reactive metal and will catalyze oxidative changes in lipid, protein, and other cellular components (Decker & Welch, 1990). Fig. 6 shows the chelating effect of XGOS-A and XGOS-B on ferrous ions. The chelating effect increased with increased concentration of XGOS-A and XGOS-B. The 50% chelating concentrations (the concentration at which chelating activity reached 50% effect) of XGOS-A and XGOS-B on ferrous ions were 3.75 mg/mL and 2.53 mg/mL, respectively. The cheating activity of XGOS-A is relatively lower compared with XGOS-B. EDTA was used as a control and its 50% chelating concentration was 0.54 mg/mL. Pyruvate acid showed no ferrous iron chelating activity in this experiment.

3.2.6. Reducing power

Reducing power assay has also been used to evaluate the ability of antioxidants to donate electrons (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003). Antioxidant compounds cause the reduction of ferric (Fe^{3+}) form to the ferrous (Fe^{2+}) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Therefore, reducing power can be determined by measuring the formation of Perls' Prussian blue at 700 nm. In this experiment, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric

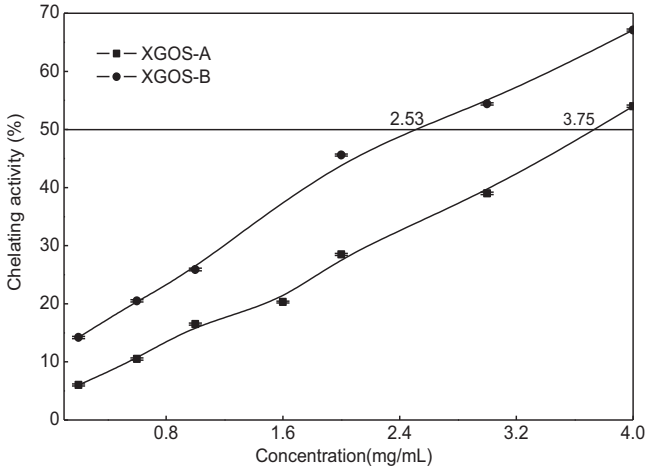

Fig. 6. Ferrous ion chelating activity of XGOS-A and XGOS-B.

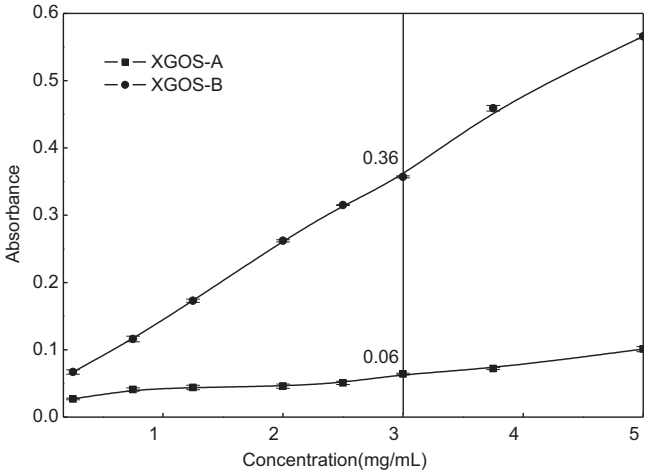

Fig. 7. Reducing power of XGOS-A and XGOS-B.

reducing power (Ercan & Ekrem, 2001). Fig. 7 depicts the reducing power of XGOS-A and XGOS-B. The absorbance of the solutions of XGOS-A and XGOS-B increased with the increasing of their concentrations. At a concentration of 3.0 mg/mL, the absorbances of the solution of XGOS-A and XGOS-B were 0.06 and 0.36, respectively. The results showed that the reducing power of XGOS-B was stronger than that of XGOS-A. Ascorbic acid and pyruvate acid were used as a control, and at the same concentration, the absorbance of the solution of ascorbic acid was estimated to be 1.06, but pyruvate acid showed no reducing power in this experiment.

4. Conclusions

Two kinds of water-soluble xanthan oligosaccharides with similar molecular weights showed different antioxidant activity. XGOS-B prepared under alkaline condition exhibited significantly stronger antioxidant activity in all above assays. The results identified clearly that antioxidant activity of xanthan oligosaccharides was closely correlated to the degradation method. It is known that active hydroxyl of polysaccharides may take part in free radicals scavenging and contribute to the antioxidant activity (Zha et al., 2009). The initial xanthan gum showed no antioxidant activity in these antioxidant evaluation systems due to its poor water solubility and strong intermolecular action. Since degradation leads to more active hydroxyl group being exposed, xanthan oligosaccharides showed good water solubility and hence exhibited significant antioxidant activity.

Pyruvate acid and reducing sugar content might also involve in antioxidant activity of xanthan oligosaccharides and result in the different antioxidant activity of XGOS-A and XGOS-B. Pyruvate acid has been reported to have the antioxidant activity (Varma, Hegde, & Henein, 2003) and showed antioxidant activity in $\bullet O_2^-$, •OH, DPPH and H_2O_2 scavenging activity assay. XG can inhibit autoxidation of soybean oil in emulsion by binding Fe^{2+} through a pyruvate residue. And The Fe^{2+}-binding activity corresponded to the content of pyruvate acid in xanthan. Depyruvated xanthan did not inhibit effectively the autoxidation of soybean oil (Shimada, Fujikawa, Yahara, & Nakamura, 1994; Shimada, Muta, et al., 1994). Pyruvate acid can inhibit oxygen radical production by neutralizing hydrogen peroxide (O'Donnell-Tormey, Nathan, Lanks, DeBoer, & de la Harpe, 1987; Stanko et al., 1995).

Pyruvate acid of xanthan oligosaccharide is more likely to be retained in XGOS-B prepared under alkaline condition during the degradation (Bradshaw, Nisbet, Kerr, & Sutherland, 1983; Tako & Nakamura, 1984). In this work, XGOS-B contained pyruvate acid 4.8% while XGOS-A contained pyruvate acid 2.3%. This suggested that the higher antioxidant activity of XGOS-B might be partly due to the higher pyruvate acid content. The fact that pyruvate acid showed no activity in ferrous ion chelating may be owed to that pyruvate acid itself cannot bind Fe^{2+} effectively.

The reducing sugar, reported to be one of the factors possessing antioxidant activity of medicinal plant (Arzamastsev et al., 1999; Thetsrimuang, Khammuang, & Sarnthima, 2011), could be also related to the antioxidant activity of xanthan oligosaccharides. The reducing sugar contents of XGOS-A and XGOS-B were determined to be 16.0% and 21.1%, respectively. The reducing capacity of a compound is a significant indicator of its potential antioxidant activity. The higher reducing sugar content meant a higher potential antioxidant capability.

The above result indicated that XGOS-B prepared under alkaline condition showed better antioxidant activity than XGOS-A prepared under acidic condition. The oxidative degradation of xanthan under alkaline condition could be an effective degradation method to prepare xanthan oligosaccharides with better antioxidant activity. Although the antioxidant activity of natural polysaccharides and their derivatives is relatively lower compared with that of recognized antioxidants such as ascorbic acid (Huang, Zhao, Hu, Mao, & Mei, 2012; Ying, Xiong, Wang, Sun, & Liu, 2011; Yuan et al., 2006), the researches of natural polysaccharides have attracted a lot of attention because of their abundance in resources and non-toxicity. With regard to their natural occurrence and biocompatibility, the antioxidant activity of degraded xanthan oligosaccharides will be helpful to expand their applications in biomedicine.

Acknowledgments

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