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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 $P < 0.05$.