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Preparation, composition analysis and antioxidant activities of konjac oligo-glucomannan

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ABSTRACT

Konjac oligo-glucomannan (KOG) was prepared by degradation of konjac glucomannan (KG) using β-mannanase. The hydrolysis process was monitored by the viscosity of the enzymatic hydrolysates. Factors affecting the enzymatic hydrolysis of KG were investigated, and the optimum hydrolysis conditions were as follows: time 2 h; temperature 50 °C; pH 6.0; and enzymatic concentration 150 U/g. Under these optimized conditions, minimum viscosity (31.9 mPa·s) of the hydrolasate was obtained. The average degree of polymerization (DP) of the resulting KOG was approximately equal to 5.2. The results of Fourier transform infrared (FTIR) spectra of KG and KOG indicated that KG was successfully degraded. In addition, their antioxidant activities were evaluated by determination of hydroxyl radical (•OH) and 1,1-diphenyl-2-picrylhrazyl radical (•DPPH) scavenging activity, and determination of reducing power. The results showed that KOG exhibited significant antioxidant activities. Taken together, this study suggested that KOG could potentially be used as a natural antioxidant.

1. Introduction

Amorphophallus konjac K. Koch is a perennial herbaceous herb. It grows in mountain or hilly areas in subtropical regions mainly in the South East of Asia (Zhang, Xie, & Gan, 2005). Konjac glucomannan (KG) is an essential polysaccharide which is the main component of the roots and tubers of the *Amorphophallus konjac* plant. It has the structure of a linear random copolymer of 1,4-linked-β-D-mannopyranose and β-D-glucopyranose units in a molar ratio of 1.6:1, with approximately 1 in 19 sugar units being acetylated at the side C-6 position (Chen et al., 2013; Iglesias-Otero, Borderías, & Tovar, 2010; Jian, Zeng, Xiong, & Pang, 2011; Jian et al., 2013). Unlike many other biopolymers, its molecular weight distribution was fairly narrow, and the molecular chains were extending, linear, semi-flexible, little rigid and without branching (Wen, Cao, Yin, Wang, & Zhao, 2009).

KG cannot be hydrolyzed by digestive enzymes in the human upper gastrointestinal tract and is therefore regarded as an indigestible dietary fiber, which has been demonstrated to be effective in weight reduction, regulation of lipid metabolism, improvement of glucose metabolism and cholesterol reduction (Chua, Baldwin, Hocking, & Chan, 2010; Xiong et al., 2009; Zhou et al., 2013). Konjac is recognized as a safe material according to the FDA (Pérols, Piffaut, Scher, Ramet, & Poncelet, 1997), and it is also used as a functional healthcare drug for diabetics and adiposis in China.

Additional to the health-promoting benefits of KG, it is widely used in food, beverage and pharmaceutical industries for thicking, texturing, gelling and water imbibing (Chua et al., 2010; Liu, Wang, & Ding, 2013; Zhang et al., 2005). KG can promote synergistic effects when combined with both protein and starch, thereby forming different textures. In the presence of KG, mechanical strength in compression of collagen hydrogel scaffold was enhanced (Weska, Achilli, Beppu, & Mantovani, 2012), where thermodynamic incompatibility occurred between denatured whey protein and KG (Tobin, Fitzsimons, Chaurin, Kelly, & Fenelon, 2012). More recently, it was also demonstrated that KG has good cryoprotective effect on myofibrillar protein from grass carp (*Ctenopharyngodon idella*) during frozen storage (Xiong et al., 2009). At the same time, KG also was applied to control rheology and structure of potato starch (Khanna & Tester, 2006; Silva, Birkenhake, Scholten, Sagis, & van der., 2013), cassava starch (Shanavas, Moorthy, Sajeev, Misra, & Sundazeem, 2010), and corn starch (Yoshimura, Takaya, & Nishinari, 1998), revealing that the behavior of KG is similar to a physical barrier to prevent amylopectin chain association (Khanna & Tester, 2006).

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Recent researches found that its degradation products with different molecular weight have particular biological functions, such as anti-tumor (Vuksan et al., 2000), immuno regulation (Onishi et al., 2005), and cytothesis (Yeh, Lin, & Chen, 2010). These findings promote researchers to pay more and more attention to the research and development of konjac degradation products (Suzuki et al., 2009). Presently, several strategies have been developed to obtain oligosaccharides by the depolymerization of Konjac Glucomannan (Courtois, 2009), such as acid degradation, oxidative degradation, radiation-induced degradation (Relleve et al., 2005), microwave-induced degradation (Zhou, Yao, & Wang, 2006), and physical methods (Pang et al., 2012). The traditional methods used to degrade polysaccharides are usually time-consuming. In the previous researches, it was found that γ-irradiation could effectively degrade Konjac Glucomannan, but the molecular weight distribution of the products was wide. Besides, the molecular weight was always higher than 400,000 Da in the safe irradiation dose (Xu, Sun, Yang, Ding, & Pang, 2007). Enzymatic hydrolysis has been most widely used in the degradation of polysaccharide because of the characteristics, safety and in room temperature of 25 ± 1 °C (Albrecht et al., 2011; Jian et al., 2013).

In recent years, there has been increasing interest in finding natural antioxidants since the synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are suspected of being responsible for liver damage and carcinogenesis (Witschi, 1986; Grice, 1988). A multitude of natural antioxidants have already been isolated from different kinds of plant materials such as seeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Yuan et al., 2006). Sun, Yao, Zhou, and Mao (2008) reported that the *N*-carboxymethyl chitosan oligosaccharides (*N*-CMCOSs) have antioxidant activity, and different degrees of substitution of *N*-CMCOSs have different ability in the scavenging of 1,1-diphenyl-2-picrylhrazyl radical (•DPPH) radical, superoxide anion and determination of reducing power. However, there are few reports regarding the antioxidant activities of degradation products from KG. The objective of this research was to study and optimize enzymatic hydrolysis conditions of KG using β-mannanase. The composition and antioxidant activities of the oligosaccharides were evaluated.

2. Materials and methods

2.1. Materials

KG flour, with purity 90%, was obtained from Hubei Johnson Konjac Co., Ltd (Hubei, China). Furthermore, β-mannanase, with activity of 50,000 U/g, was purchased from Beijing Challenge Bio-Technology Co., Ltd (Beijing, China). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of konjac oligo-glucomannan (KOG)

Konjac powder was dispersed in 150 mL of 0.2 M acetate buffer to obtain a 5% (w/v) suspension, and then mixed with β-mannanase (enzymatic concentration ranging from 150 to 250 U/g) to start the reaction. The mixture was incubated at pH 5.0–7.0 for reaction times ranging from 1 to 6 h, while the temperature of the water bath was kept steady at a given temperature (reaction temperature ranged from 40 to 60 °C). The reaction was stopped by boiling the samples for 10 min, KOG thus obtained was concentrated with a rotary evaporator, and then mixed with 95% ethyl alcohol. The KOG, which had been collected as a precipitate by centrifugation at 4000 rpm for 20 min, was resuspended in 95% ethyl alcohol three times. After being redissolved in distilled water, KOG was nanofiltrated using a polymer membrane (MW cut-off limit = 8000 Da) to remove undegradated KG and was then lyophilized in a freeze-dryer.

Table 1
The variables investigated and their levels.

Variables investigated	Levels of each variable		
	1	2	3
A: Hydrolysis time (h)	2	3	4
B: Hydrolysis temperature (°C)	45	50	55
C: pH	5.5	6.0	6.5
D: Enzymatic concentration (U/g)	100	150	200

2.2.2. Determination of the degree of polymerization (DP)

The total reducing sugar is the amount of reducing sugar after KG degradation, while direct reducing sugar (DRS) is the amount of reducing sugar before degradation. The ratio of TRS to DRS is thus the degree of polymerization (DP), and can be used to monitor effectiveness of enzymatic reaction. TRS was detected by sulfuric acid-phenol method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), DRS was determined by the DNS colorimetry method (Sumner & Sisler, 1944). The DP was calculated by the following formula (Chen et al., 2013): DP = TRS/DRS.

2.2.3. Orthogonal test

Owing to the degradation effect being the highest under optimum conditions, it was very important for enzymatic degradation to look for these optimum parameters. However, study of the effect of changing single factor on enzymatic degradation was not enough to judge what parameter was optimum because other factors were fixed under this condition. So the optimum parameters should be obtained by using orthogonal test design. Reference to the design theory of orthogonal test, four controllable variables, hydrolysis time (A), hydrolysis temperature (B), pH (C) and enzymatic concentration (D), were selected for optimization. Three levels of each factor were investigated. The selected factors and levels were given in Table 1. The viscosity of the degradation products was measured under the above-mentioned factors and levels, and a further orthogonal analysis was carried out.

2.2.4. Fourier transform infrared (FTIR) spectrum analysis

IR spectra of KG and KOG were recorded on a Thermo Nicolet 5700 Fourier transform infrared (FTIR) spectrometer (Thermo Electron, Madison, WI, USA) in the wavenumber range 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 128 co-added scans, using the KBr disc method.

2.2.5. Determination of antioxidant activities

The scavenging activity of hydroxyl radicals was measured according to the method described by Yuan et al. (2005) with some modifications. 1.0 mL test samples were mixed with 1.0 mL of phosphate buffer (0.4 mM, pH 7.4), 1.0 mL 1,10-phenanthroline hydrate (2.5 mM), 1.0 mL $FeSO_4$ (2.5 mM) and 0.5 mL H_2O_2 (20 mM, 1%, v/v). The mixtures were incubated for 60 min at 37 °C, the absorbances of the mixtures were measured at 536 nm against a reagent blank after the reaction.

The DPPH scavenging activity of the samples was measured using the modified method of Sun et al. (2008). 4.0 mL of 95% ethanol solution of DPPH (0.1 mM) was incubated with test samples (1.0 mL). The reaction mixture was shaken well and incubated for 30 min at 33 °C in the dark and the absorbance of the resulting solution was read at 517 nm against a blank, the radical scavenging activity was measured as a decrease in the absorbance of DPPH.