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

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A B S T R A C T

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 hydrolysate 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-picrylhyrazyl 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.

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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 (Perols, Piffaut, Scher, Ramet, & Poncetel, 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 thickening, 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).
Recent researches found that its degradation products with different molecular weight have particular biological functions, such as anti-tumor (Vukson et al., 2000), immuno regulation (Onish et al., 2005), and cytosis (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 (Rellevé et al., 2005), microwave-induced degradation (Zhou, Yao, & Wong, 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 its 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-picrylhydrazyl 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-glucan (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 nanofiltered using a polymer membrane (MW cut-off limit = 8000 Da) to remove undegraded KG and was then lyophilized in a freeze-dryer.

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): $\text{DP}=\text{TRS}/\text{DRS}$.

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 400–4000 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 1.0–10–phenanthroline hydrate (2.5 mM), 1.0 mL FeSO₄ (2.5 mM) and 0.5 mL H₂O₂ (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.

### Table 1: The variables investigated and their levels.

<table>
<thead>
<tr>
<th>Variables investigated</th>
<th>Levels of each variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Hydrolysis time (h)</td>
<td>2 3 4</td>
</tr>
<tr>
<td>B: Hydrolysis temperature (°C)</td>
<td>45 50 55</td>
</tr>
<tr>
<td>C: pH</td>
<td>5.5 6.0 6.5</td>
</tr>
<tr>
<td>D: Enzymatic concentration (U/g)</td>
<td>100 150 200</td>
</tr>
</tbody>
</table>
The reducing power of extracts was determined by the method of Yuan et al. (2006) with some modifications. 2.0 mL test samples were mixed with 2.0 mL of phosphate buffer (0.2 mM, pH 6.6) and 2.0 mL of 1% potassium ferricyanide (w/v). The mixtures were incubated for 20 min at 50 °C. After incubation, 2.0 mL of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation at 3000 rpm for 10 min. The upper layer (2 mL) was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride. The reaction mixture was shaken well and incubated for 10 min, and the absorbance of the resultant solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the samples.

3. Results and discussion

3.1. The DP of KOG

From the above experiment, it can be concluded that the value of total sugar is 50 mg/mL, and the value of reducing sugar is 9.531 mg/mL, thus the value of DP is 5.2. Sato, Sawabe, Kishimura, Hayashi, and Saeki (2000) reported that the overall DP (the mean value of total fractions) of alginate oligosaccharide (AO) prepared by degrading sodium alginate using alginate lyase, was 6.1. Xue et al. (2001) reported that low-molecular-mass polysaccharide from Laminaria japonica had higher antioxidative activity than did high-molecular-mass polysaccharide. Wang, Lai, Chen, and Chen (2008) found that glucomannno-oligosaccharides (GMO) produced antioxidative effects by increasing radical scavenging ability and eliminating lipid peroxide formation. Furthermore, fermentation of GMO with a DP of 5 produced great inhibitory effects on thio-barbituric acid-reactive substances formation. These findings explain that with the proper molecular weight, the antioxidant activity of polysaccharide and its derivatives would be enhanced.

3.2. Effect of single factors on KG hydrolysis

3.2.1. Effect of time on KG hydrolysis

The results of enzymatic hydrolysis of konjac powder for different times were shown in Fig. 1(A). The reaction time was set at 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h, while the other parameters were set as temperature 50 °C, pH 6.0, and enzyme concentration 150 U/g. A rapid decrease in intrinsic viscosity was observed within 2 h, and a slower decrease appeared in the range from 2 to 3 h, while no further decrease was observed after 3 h. The optimum reaction time was thus 3 h. Since the loss in viscosity of konjac powder after degrading enzymes treatment reflects the effect of enzymatic scission at sites along the KG polymer chain.

Additionally, as shown in Fig. 1(A), the results demonstrated that enzyme treatment resulted in a substantial loss in viscosity of the konjac powder, in other word, depolymerization. Such depolymerization took place very quickly during the initial 2 h. The decreased hydrolytic efficiency of β-mannanase was probably due to the decreased substrate concentration, the increased product concentration, and the inactivation of β-mannanase with time.

3.2.2. Effect of temperature on KG hydrolysis

The results of enzymatic hydrolysis of konjac powder at different temperatures were shown in Fig. 1(B). The temperature was set at 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C, while other hydrolysis variables were set as pH 6.0, time 3 h and enzymatic concentration 150 U/g. From 40 °C to 50 °C, intrinsic viscosity of the products decreased, which was contrary to the trend from 50 °C to 60 °C. Generally, the reaction rate increases with temperature while the stability of the enzymes declines, and at high temperature, catalytic activity will be lost rapidly before significant conversion is reached (Garcia, Sanchez, Martinez, & Aracil, 1999; Foresti & Ferreira, 2007). Temperature may affect the hydrolysis efficiency in a positive way or vice versa. A rise in temperature will increase the reaction rate as
explained by the transition state theory (Serri, Kamaruddin, & Long, 2006). Normally, a high temperature used in the reaction causes the denaturation of enzyme. Thus the optimum temperature for hydrolysis of konjac powder by the enzyme was 50 °C.

3.2.3. Effect of pH on KG hydrolysis

The results of enzymatic hydrolysis of konjac powder at different pH were shown in Fig. 1(C). The hydrolysis process was carried out at pH of 5.0, 5.5, 6.0, 6.5 and 7.0, while the other reaction conditions were set as temperature 50 °C, time 3 h and enzymatic concentration 150 U/g. When the pH was in the range of 5.0–6.0, intrinsic viscosity of the products decreased with increasing pH. When it was 6.0–7.0, the opposite trend was observed. Thus, the optimum pH for the hydrolysis of konjac powder was 6.0. β-mannanases from various bacteria were active and stable rather in acidic pH range, but in terms of the hydrolysis of KG, the optimal pH of the enzyme was around 7–8. This was affected by the buffer compositions (Nakajima & Matsuura, 1997), so the activity and stability of the enzyme are different.

3.2.4. Effect of enzyme concentration on KG hydrolysis

The results of enzymatic hydrolysis of konjac powder with different enzyme concentrations were shown in Fig. 1(D). Enzyme concentration was set at 10, 50, 100, 150, 200, 250 U/g, while other hydrolysis variables were set as temperature 50 °C, pH 6.0, and time 3 h. The increase of enzyme concentration led to a decrease in the intrinsic viscosity of products. As the reaction went on, no further decrease was observed when the enzyme concentration exceeded 150 U/g. The results may be due to greater hydrolysis of the konjac powder with increasing β-mannanase addition. With consideration of the cost, the optimum enzyme concentration was thus 150 U/g.

3.3. Orthogonal experimental results

The best condition for preparing KOG was studied by the orthogonal test. Based on the results of single factor experiment, in order to further analyze the influence of various factors on the viscosity of the products, four controllable variables, reaction time, temperature, pH and concentration were chosen as factors to carry out $L_9 (3^4)$ orthogonal experiments. $K$ means the sum of the corresponding level value under each factor; $R$ means range which represents the influence of the each factor, which is expressed by $R = K_{\text{max}}/3 - K_{\text{min}}/3$.

Obviously, the influential order of each variable on the degradation of KG was $C>B>D>A$ by comparing $R$ values in Table 2. Owing to the value of $R$ for $C$ and $B$ factors being high, it was shown that the contribution of $C$ and $B$ factors for degradation were significant. Thus, pH and temperature have the greatest influence on degradation of KG. Furthermore, according to the values of $R$ for the other two factors, it could be found that the reaction time and enzyme concentration have a weak influence on the degradation as compared with pH and temperature. For each variance, the optimum level of each variable was $A_1B_1C_3D_2$. With consideration of the cost, the optimum reaction condition was obtained as follows: reaction time 2 h, reaction temperature 50 °C, pH 6.0, and enzyme concentration 150 U/g.

3.4. FTIR analysis

FTIR is of importance in the study of the molecular structure. The intensity and the position of the bands are sensitive to the conformations of macromolecules on the molecular level. Fig. 2 shows the FTIR spectra of KOG and KG, respectively. In FTIR spectra, the characteristic absorption peak of KOG was observed at 1600–400 cm$^{-1}$, while the major bands of KG was observed at 3000–1000 cm$^{-1}$. It can be seen that the IR spectrum of the KG displayed a broad intense peak at around 3404 cm$^{-1}$ characteristic of stretching of hydroxyl groups (Xie et al., 2010). The stretching peak of C=H of methyl at 2926.6 cm$^{-1}$ (KOG) and of the carbonyl at 1723 cm$^{-1}$ (KG) was assigned to the acetyl groups in the KG. The strong absorption band at 1649 cm$^{-1}$ (KG) and 1578 cm$^{-1}$ (KOG) were attributed to the C=O asymmetric stretching vibrations of the carboxylate (−COO$^-$) groups (Xie et al., 2013). The bands in the region of 1421 cm$^{-1}$ (KOG) and 1419 cm$^{-1}$ (KG) were due to hydroxyl stretching vibration. The bands in the region of 1043 cm$^{-1}$ and 1014 cm$^{-1}$ (KOG) and 1027 cm$^{-1}$ (KG) were ascribed to C−O stretching vibration. The band in the region of 649 cm$^{-1}$ (KOG) was due to bending vibration of the C−C−C(O) and, at the same time, it has a substituent in α.

Table 2

<table>
<thead>
<tr>
<th>Experimental no.</th>
<th>Time (h) (A)</th>
<th>Temperature (°C) (B)</th>
<th>pH (C)</th>
<th>Concentration (U/g) (D)</th>
<th>Viscosity (mPa s)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
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<td>1</td>
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<td>2</td>
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<tr>
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<tr>
<td>$K_3$</td>
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<td>109.4</td>
<td>101.6</td>
<td>101.8</td>
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<tr>
<td>$R$</td>
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<td>3.13</td>
<td>3.43</td>
<td>2.03</td>
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</table>

Fig. 2. FTIR spectra of the KOG and KG.
place of the bond. The absorption band at 620 cm\(^{-1}\) was due to bending vibration of the \(\text{C} - \text{CO} - \text{C}\), while there is no substituent in \(\alpha\) place of the bond.

As far as the position and intensity of various bands of the KG and KOG were concerned, the major bands centered at 1800–1200 cm\(^{-1}\) showed significant change, which shifted to a little lower wave number when the KG was degraded and, at the same time, their intensity was decreased. The peak at 1723 cm\(^{-1}\) disappeared in the spectra of KOG, these significant changes suggested the KG degradation occurred.

3.5. Antioxidant activities of KOG

3.5.1. Hydroxyl radical scavenging activity

It is known that the hydroxyl radical is a powerful oxidant that can react with nearly all the biomacromolecules functioning in living cells (Santanam, Ramachandran, & Parthasarathy, 1998; Xie et al., 2012) and that oxidative stress can mediate a wide variety of degenerative processes and diseases (Dhalla, Temsah, & Netticadan, 2000; Sayre, Smith, & Perry, 2001). Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules (Yuan et al., 2005). The scavenging effects of samples on hydroxyl radicals were shown in Fig. 3A and B. Scavenging activity of hydroxyl radical increased with the increasing of concentrations of KOG (A) and Vc (B). Scavenging effect on hydroxyl radical of KOG was between 40.0% and 60.0% at 6.0 mg/mL, but the effect on hydroxyl radical of Vc was between 40.0% and 60.0% at 0.4 mg/mL, KOG showed certain effects on scavenging the hydroxyl radicals, but lower than that of Vc. For hydroxyl radical, there are two types of antioxidation mechanism: one suppresses the generation of the hydroxyl radical and the other scavenges the hydroxyl radicals generated (Qi et al., 2005). In this study, in another assay system, KOG can scavenge the hydroxyl radicals because of the reducing hemiacetal hydroxyl
in the chains, which can make highly oxidizing free radicals reduced.

3.5.2. DPPH free-radical scavenging activity

DPPH radical is a stable lipophilic free radical which has been generally used for estimating antioxidant activity of food and medicine materials (Li, Shi, Wang, & Le, 2007). Fig. 3C and D depicted the DPPH radical scavenging effect of KOG (C) and Vc (D). Scavenging activity of DPPH radical increased with the increasing of concentrations of KOG and Vc. Moreover, as shown in Fig. 3C, 50% inhibition concentrations (IC50) of KOG was 3.816 mg/mL. DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. Further it is well accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability. In the present study, Vc showed a relatively high inhibitory effect compared to KOG at low concentration, which may be attributable to its strong hydrogen-donating ability.

3.5.3. The reducing power of KOG

The antioxidant activity has been reported to have a direct, positive correlation with the reducing power (Yuan et al., 2006). Fig. 3E and F showed the reducing powers of KOG (E) and Vc (F) using the potassium ferricyanide reduction method. The reductive potential of the KOG and Vc increased with increasing concentration. Vc showed a relatively high inhibitory effect compared to KOG at low concentration. At a concentration of 2.0 mg/mL, the absorbance of KOG was 0.17. Sun et al. (2008) found that the absorbance of chitosan oligosaccharide (COS) was 1.29 at the same concentration. A direct correlation between antioxidant activities and reducing power of certain plant extracts has been reported. The reducing power properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radicals’ chain by donating a hydrogen atom (Yuan et al., 2006; Sun et al., 2008). This result may be related to the fact that molecules with different electron-donating groups affected the activity of electron-donating, which led to the different reducing power.

4. Conclusion

It can be concluded that konjac oligo-glucosaminan (KOG) was successfully prepared by degradation of konjac glucosaminan (KG) using β-mannanase. A minimum intrinsic viscosity of KOG was obtained under the optimum conditions of time 2 h, temperature 50 °C, pH 6.0, and enzyme concentration 150 U/g. Through the FTIR spectra, KOG had similar structural characteristics with KG and, KOG retained the characteristics of functional groups of KG. In addition, KOG exhibited antioxidant activities to some extent, but they were significantly weak compared to Vc. Some researchers reported that with the decrease of molecular weight, the antioxidant activity of chitosan and its derivatives will be enhanced (Sun et al., 2008). However, there is little research on the relationship between the antioxidant activity and DP of the KOG. This study suggested KOG with small molecular weight can be successfully prepared by enzymatic hydrolysis, and KOG could potentially be used as a natural antioxidant.

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