The Biological Activities of Chitosan Oligosaccharide Derivatives prepared by Maillard Reaction

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Abstract: Two kinds of chitosan oligosaccharide derivatives (CG and CM) were prepared by Maillard reaction of chitosan oligosaccharide (COS) with glucose and maltose, respectively. The molar ratio of amino group of COS and carbonyl group of glucose (maltose) was 1:1. The structural changes of CG and CM were confirmed by fourier transform infrared (FT-IR) spectra, and their molecular weights were determined by gel permeation chromatography (GPC) method. The antioxidant and antibacterial activities of COS, CG and CM were also assessed. The FT-IR spectra of CG and CM showed the characteristic absorption peaks of COS. The antioxidant activity and the antibacterial activity of CG and CM against *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* were higher than those of COS, and CG prepared by COS and monosaccharide-glucose showed better antioxidant and antibacterial activity.

Key words: chitosan oligosaccharide; glucose; maltose; Maillard reaction; biological activities

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基于美拉德反应的低聚壳聚糖衍生物的生物活性研究

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摘要：以低聚壳聚糖作为氨基供体分别与提供羰基的葡萄糖和麦芽糖进行美拉德反应（氨基与羰基的物质量比均为 1:1），醇沉法提取低聚壳聚糖美拉德反应衍生物 CG 和 CM。对两种衍生物进行红外表征和分子量测定，并研究其抗氧化性能和抑菌性能。结果显示：两种衍生物均保留着低聚壳聚糖的特征吸收峰；其对 O_2^-、OH 及 DPPH 的清除能力以及还原能力和对大肠杆菌、铜绿假单胞菌和副溶血性弧菌的抑菌活性均得到显著提高，同时 CG 的抗氧化性和抑菌活性明显优于 CM。即与单糖进行美拉德反应制得的壳聚糖衍生物具有更好的生物活性。

关键词：低聚壳聚糖；葡萄糖；麦芽糖；美拉德反应；生物活性

Chitosan is a natural cationic polysaccharide obtained from the N-deacetylation chitin widely occurring in the nature[1]. Compared with chitosan, chitosan oligosaccharide (COS) has better water solubility. It has been reported that COS possessed various biological activities such as lowering of blood cholesterol, lowering of high blood pressure, protective effects against infections, controlling arthritis and enhancing antitumor [2]. Recently, antioxidant activity and antibacterial activity of COS and its derivatives have attracted much attention [3,4]. These researches showed that the antioxidant and antibacterial activity of chitosan and its derivatives mainly related to the content of active hydroxyl and amino groups in the polymer chains[5~7]. Compared with chitosan, the antioxidant and antibacterial activity of chitosan oligosaccharide (COS) and its derivatives will be much improved and may be more interesting because of less effect of hydrogen bonds [8~10].

Maillard reaction is a complex reaction between carbonyl and amino group. Many products of Maillard reaction has been found to exhibit antioxidant activity and antibacterial activity [11,12]. Since COS is likely to be involved in the Maillard reaction due to its amino groups, the aim of this investigation was to determine the antioxidant and antibacterial activities of COS Maillard

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reaction derivatives, and to evaluate the relationship between reducing sugar kinds and the biological activities of COS Maillard reaction derivatives. In present study, two kinds of COS Maillard reaction derivatives CM and CG were prepared by heating COS with glucose and maltose (molar ratio of amino group and carbonyl group was 1:1), respectively. The antioxidant and antibacterial activities of COS and its derivatives were investigated.

1 Materials and methods

1.1 Materials

Chitosan oligosaccharide (COS, the degree of deacetylation was 98%) was purchased from Zhejiang Jinke Biochemistry Co., Ltd. Luminol, DPPH were purchased from Sigma-Aldrich Chemical Co. All other chemical reagents were of analytical grade supplied by Sinopharm chemical reagents Co., Ltd.

1.2 Preparation of chitosan oligosaccharide derivatives

COS (20.0 g) was dissolved in water (200 mL), then glucose solution (22.3 g in 50 mL water) was added. The molar ratio of amino group of COS and carbonyl group of glucose in this reaction was 1:1. The solution was heated to 80 ℃ and stirred for 24 h. The reaction solution was precipitated by three-amount ethanol, and then was purified three times. The precipitation was collected and finally dried under vacuum at 60 ℃ for 24 h to obtain CG.

CM was prepared by heating COS (20.0 g) with maltose (21.2 g in 50 mL water) according to the similar procedure. The molar ratio of amino group of COS and carbonyl group of maltose was also 1:1.

1.3 Characterization

The structures of COS, CG and CM were confirmed by FT-IR-Laman spectrophotometer (EQUNOX55).

Molecular weight distributions of products were detected by gel permeation chromatography method. The determination was performed on a Waters-515 Chromatograph equipped with Waters 2410 refractive index detector and Ultrahydrogel 500. Elution was carried out using 0.1 mol/L sodium nitrate 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 ℃ during the determination process.

1.4 Superoxide anion scavenging activity

Superoxide anion scavenging activity was determined using chemiluminescence technology. The assay was carried out on a chemical luminometer. The chemiluminescent reaction was processed in a Na_2CO_3-$NaHCO_3$ (pH=10.20, 0.05 mol/L) buffer solution. Scavenging activity of the samples was evaluated according to their quenching effects on the chemiluminescence signal of the luminal-pyrogallol system. The capability of scavenging against superoxide anion was calculated as: scavenging effect (%) = $(CL_0-CL_1)/CL_0\times100\%$, where CL_0 and CL_1 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 tested by superoxide dismutase, catalase and mannitol [13].

1.5 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was processed as a similar program described above. 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 (%) = $(CL_0-CL_1)/CL_0\times100\%$, where CL_0 and CL_1 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 [14].

1.6 DPPH radical scavenging activity

DPPH scavenging activity of the samples was measured using the method of Yamaguchi et al [15].2.0 mL of DPPH solution (0.1 mmol/L in ethanol) was incubated with 2.0 mL of varying concentrations of test samples. The reaction mixture was shaken well and incubated for 30 min at 33 ℃ 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 and was calculated using the following equation: scavenging effect (%) = $(1-A_{samples}/A_{control})\times100\%$.

1.7 Measurement of reducing power

Reducing power of the samples was determined by the method of Oyaizu [16]. Different concentrations of the samples solutions (2.0 mL) were mixed with 2.5 mL sodium phosphate buffer (pH=6.60, 0.2 mol/L) and 2.5 mL potassium ferricyanide (1 % *m/V*). The mixtures were

incubated for 20 min at 50 ℃, and then 2.5 mL trichloroacetic acid (10 % *m/V*) was added to the mixtures, followed by centrifugation at 2000 rpm for 10 min. The supernatant (2.0 mL) was mixed with 2.5 mL distilled water and 0.5 mL ferric chloride solution (0.1% *m/V*) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

1.8 Antibacterial activity

The microorganism strains used in this study were two common bacteria *Escherichia coli* ATCC 43889, *Staphylococcus aureus* AB91093 and two food-borne pathogens *Pseudomonas aeruginosa* ATCC 27853, *Vibrio parahaemolyticus* ATCC 33847, all obtained from college of food technology research center, Shanghai Ocean University.

The test microorganisms were maintained in Tryptone Soya Broth at 37℃ for 12 h. The microorganisms were diluted to 10^6 cfu/mL, and then 0.1 mL microorganism was added into a sterile petri dish containing solidified nutrient agar. After setting for a while, a cup-borer (6 mm diametre) was used to make a uniform cups in each petri dish. The cups were filled with 0.2 mL 2.0 g/L COS, CG and CM solution, respectively, and allowed to diffuse for 30 minutes. The petri dishes were incubated at 37 ℃ for 24 hours, then the zone of inhibition was measured with a vernier caliper. Nisin (2.0 g/L) were used as the positive control, sterile water as blank control.

All analyses were performed in triplicate. Data of antioxidant and antibacterial evaluation were expressed by average value.

2 Results and discussion

2.1 Characterization

The structure changes of CG and CM were showed in Figure 1. Both FT-IR spectra of CG and CM showed the characteristic absorption peaks of COS [17]. Absorption peaks of COS at 1622 cm^{-1}, 1516 cm^{-1} and 1381 cm^{-1} are contributed to amide Ⅰ(C=O), free amino(-NH_2) and amide Ⅱ, respectively [18]. The absorption peak of CM and CG at 1516 cm^{-1} was lower than that of COS, which indicated that the content of free amino groups in COS derivatives decreased after Maillard reaction.

The molecular weights of COS, CG and CM were detected to be 8190 Da, 12430 Da and 11750 Da, respectively. The results indicated that molecular weights of COS derivatives increased after Maillard reaction.

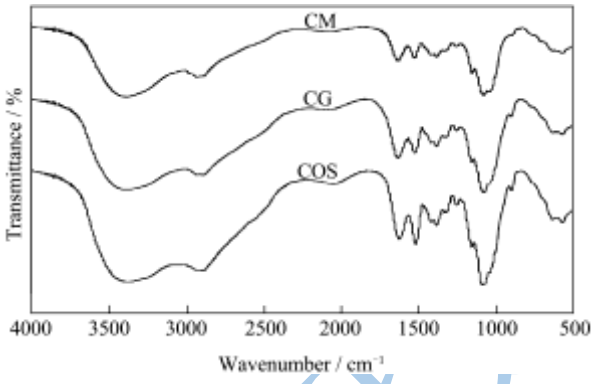

Fig.1 FTIR spectra of COS, CG and CM

2.2 Superoxide anion scavenging activity

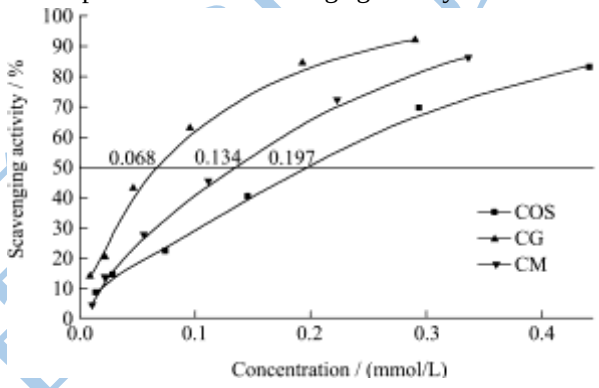

Fig.2. Scavenging activity of COS, CG and CM on superoxide anion

The superoxide anion is a highly toxic species that is generated by numerous biological and photochemical reactions [19]. The superoxide anion scavenging activity of COS, CG and CM was showed in Figure 2. The scavenging activity increased with their concentration. IC_{50}s of CG, CM and COS were 0.068, 0.134 and 0.197 mmol/L, respectively. The scavenging activity on superoxide anion was in the order of CG> CM> COS. Research showed that superoxide anion scavenging capacity of COS may be owing to hydroxyl and amino groups in its molecular [20].The content of amino groups in COS derivatives decreased after Maillard reaction. Compared with COS, CG and CM showed unexpected higher scavenging activity against superoxide anion. The cause of this phenomenon is not known warranting further investigation.

2.3 Hydroxyl radical scavenging activity

The hydroxyl radical is considered the most reactive free radical in biological tissues and can caused huge damage to DNA in cells [21]. Figure 3 showed that

hydroxyl radical scavenging activity of COS, CG and CM. Their scavenging activity was in the order of CG> CM> COS. It can be indicated that hydroxyl radical scavenging activity of COS derivatives increased after Maillard reaction and CG showed a better scavenging activity than CM.

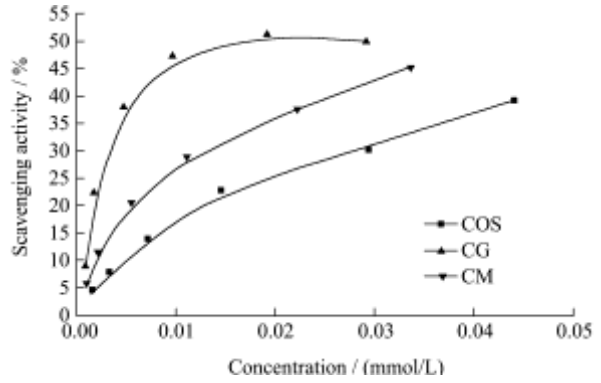

Fig.3 Scavenging activity of COS, CG and CM on hydroxyl radical

2.4 DPPH radical scavenging activity

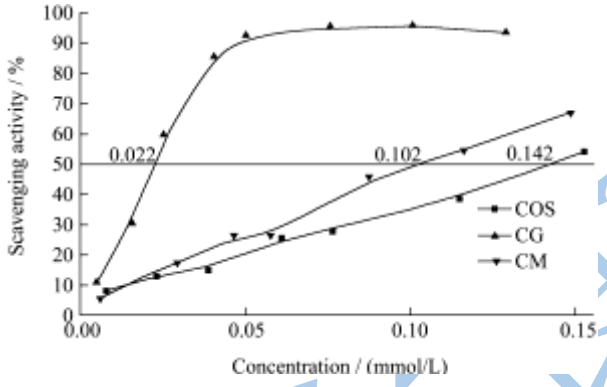

Fig.4 Scavenging activity of COS, CG and CM on DPPH radical

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 is well accepted that the DPPH radical scavenging by antioxidants is due to their hydrogen-donating ability [22]. The scavenging activity of COS and its derivatives were showed in Figure 4. CG, CM and COS exhibited scavenging activity against DPPH free radicals, with IC_{50} values of 0.022, 0.102 and 0.142 mmol/L, respectively. DPPH free radical scavenging capacity of COS and its derivatives was in the order of CG ＞ CM＞ COS.

2.5 Measurement of reducing power

Reducing power, an important index to characterize the sample's capacity of donating electron, can be measured by transformation breaking of bivalent iron ion (Fe^{2+}) to ferri ion (Fe^{3+}). Research showed that antioxidant activity and reducing power were closely related. The reducing power of COS, CG and CM was showed in Figure 5. At the concentration of 0.20 mmol/L, the absorption values of CG, CM and COS were 1.383, 0.449 and 0.326, respectively. The reducing power of COS and its derivatives was in the order of CG＞ CM＞ COS.

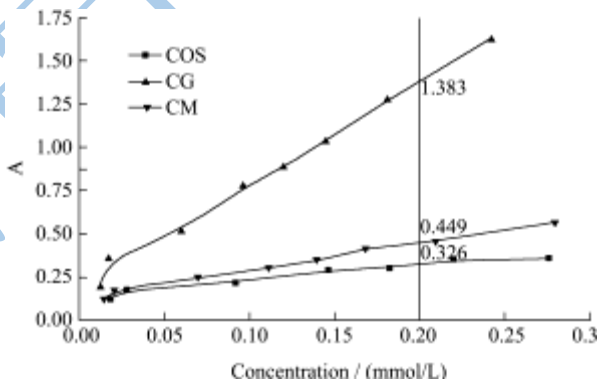

Fig.5 Reducing power of COS, CG and CM

2.6 Antibacterial activity

Table 1 The inhibiting effect of COS, CG and CM against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*

Bacteriostat	Antibacterial circle diameter/mm			
	Escherichia coli(G^-)	*Staphylococcus aureus*(G^+)	*Pseudomonas aeruginosa*(G^-)	*Vibrio parahaemolyticus*(G^-)
Sterile water	6.0	6.0	6.0	6.0
Nisin	13.1	17.5	13.7	11.4
COS	9.0	9.5	9.1	8.2
CG	12.0	13.0	12.2	10.6
CM	10.7	7.1	10.6	9.6

The antibacterial activities of COS, CG and CM are shown in Table 1. The results showed that COS, CG and CM exhibited antibacterial activities. The inhibitory effect of COS and its derivatives against *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* was in the order of CG＞ CM＞ COS, but inhibiting effect of COS and its derivatives on *Staphylococcus aureus* was in the order of CG＞ COS＞ CM.

3 Conclusion

In this paper, CG and CM were prepared by heating COS with glucose and maltose, respectively. The antioxidant activity of COS, CG and CM was investigated by scavenging of $O_2^{\cdot-}$, ·OH and DPPH free radicals scavenging capacity and the determination of reducing power, and their antibacterial activities were assessed by inhibitory effect on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*, respectively. The results showed that the antioxidant and antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* of COS and its derivatives was in the order: CG＞ CM＞ COS. The antioxidant activity of CG and CM was higher than that of COS. The antibacterial activity of COS and its derivatives against *Staphylococcus aureus* was in the order of CG＞ COS＞ CM, which might be related to the specificity of strains (such as the difference of cell wall between G^+ and G^-) and different inhibitory mechanisms of COS and its derivatives against microorganisms [23]. The result indicated that Maillard reaction can be an effective method to prepare COS derivatives with better antioxidant and antibacterial activity. CG prepared by COS reacted with glucose showed better biological activity than CM. The mechanism of antioxidant and antibacterial activity of COS Maillard reaction derivatives need further researches. This research will provide a good approach for preparation of natural, safe and effective antioxidant and preservative.

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