Antioxidant and antimicrobial activity of xylan–chitooligomer–zinc complex

Shuping Wu a, b, Yumin Du a, *, Yunzi Hu a, Xiaowen Shi a, *, Lina Zhang b

Corresponding authors. Tel.: +86 27 68778501.
E-mail addresses: duyumin@whu.edu.cn (Y. Du), shixwwhu@163.com (X. Shi).

A R T I C L E   I N F O

Article history:
Received 27 June 2012
Received in revised form 9 October 2012
Accepted 16 October 2012
Available online 12 November 2012

Keywords:
Hemicellulose
Xylan
Chitooligomer
Maillard reaction
Antioxidant
Antimicrobial
Complex

A B S T R A C T

In this study, a ternary complex based on natural polysaccharides was explored as a novel food preservative. Chitooligomer was obtained by enzyme hydrolysis of chitosan with immobilised neutral protease, and the degree of polymerisation (DP) was mainly from 2 to 5. Chitooligomer–zinc complex (CGZC) was first produced and then co-heated with xylan to prepare xylan–chitooligomer–zinc complex (XCGZC). XCGZC showed higher antioxidant and antibacterial activity than chitooligomer, chitooligomer–zinc and xylan–chitooligomer. The IC50 of XCGZC was 5.37 mg/mL, which was equal to the antioxidant ability of 3.28 mg/mL BHT. The diameter of the inhibition zone for XCGZC against Escherichia coli and Staphylococcus aureus was 17.2 ± 0.4 and 30.3 ± 0.6 mm vs. control of 6.0 mm. Besides, XCGZC had excellent antibacterial activity against Bacillus subtilis, Salmonella typhimurium, Bacillus megaterium. Therefore, XCGZC can be used as a novel promising preservative with antibacterial and antioxidant properties in the food industry.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Hemicelluloses (HCs) are the second most common polysaccharides found in vegetable fibres and cell walls of woods and annual plants, represent about 20–35% of lignocellulosic biomass (Saha, 2003). HCs are heteropolysaccharides composed of different sugar units. The major hemicelluloses being mannans, xylans, arabinans, and galactan (Maki-Arvela, Salmi, Holmbom, Willfor, & Murzin, 2011) are branched polymers of low molecular weight with a degree of polymerisation (DP) of 80–200 (Wrigstedt et al., 2010). Xylans are the most abundant hemicellulose and are not only existing in wood but also in various other plants such as grasses, cereal, and herbs (Ebringerova & Heinze, 2000). The 1,4-linked β-D-xylopyranosyl residues of xylan may be substituted at the 2'-OH or 3'-OH with glucuronic acid and 4-O-methyl glucuronic acid, arabinose, or a combination of acidic and neutral sugars (Dodd, Mackie, & Cann, 2011). During the last decade, there is an increasing interest in the application of xylan-type polysaccharides in the food area and grown field crops. On the other hand, great efforts have been devoted to develop high value-added products of xylan such as xylan aerogel foams (Venditti, Salam, Pawlak, & El-Tahlawy, 2011), xylan esters (Wrigstedt et al., 2010), xylan hydrogels (Yang, Zhou, & Fang, 2011). This includes functional modification of xylan with antioxidant or antibacterial activity used in the field of packaging films, food preservative as well as in biomedical areas. These studies are well documented in several research articles (Li, Shi, Wang, & Du, 2011; Melo-Silveira et al., 2012; Pristov, Mitrovic, & Spasojevic, 2011). Among these products, xylan and xylan derivatives complexing with chitosan (CS) or chitosan derivative have attracted much attention (Karaaslan, Tshabalala, & Buschle-Diller, 2012).

Chitooligomer (CG) is classified as either chitin or chitosan with a degree of DP < 20 and consisted of β-(1 → 4)-linked β-glucosamine (GlcN) and N-acetyl-β-glucosamine (GlcNAc) units, which is known to have distinct biological and physiological activities, such as antibacterial activity (Kulikov et al., 2012; Runarsson et al., 2010), antitumor (Kulikov et al., 2012; Maeda & Kimura, 2004; Xu et al., 2010) and immune enhancing effects (Dang et al., 2011). They are widely used in functional foods, pharmaceutics, cosmetic and water treatment, and so on. Chitooligomer can complex with metal ions and the product usually shows appealing biological activities. For instance, the chitooligomer zinc complex can inhibit the growth of the liver cancer cell line SMMC-7721 and was also found that the synergistic effect between the chitooligomer matrix and the planar construction of the zinc complex enhanced their anticancer activity (Wang et al., 2009).

Maillard reaction is a chemical reaction involving the condensation between a carbonyl group of reducing sugars, aldehydes or ketones, and an amino group of amino acids, proteins or any nitrogenous compound, which has been utilised in many food processing and manufacturing procedures. Some Maillard reaction products (MRPs) show excellent antioxidant (Chang, Chen, & Tan, 2011) and antimicrobial effects (SantAnna, Malheiros, & Brandelli, 2011). As far as we know, the Maillard reaction between the amino groups of chitooligomer and the reducing ends of xylan has not been reported.
In our previous report, the interaction of xylan with chitosan based on the Maillard reaction was investigated (Li et al., 2011). It was clarified that the xylan–chitosan conjugates possessed excellent antioxidant activity depending on the heating time. However, the conjugates had no prominent antimicrobial activity against microorganisms. The purpose of this study was to develop a natural food preservative with both antioxidant and antibacterial properties. This food preservative was ternary complex composed of chitooligomer, xylan and zinc. Chitooligomer–zinc complex (CGZC) was prepared by compositing chitooligomer with zinc ions, and then co-heated with xylan, to prepare xylan–chitooligomer–zinc complex (XCGZC). The antioxidant and antibacterial activities were mainly from MRPs of XCGZ and CG chelating with zinc ions. The experimental configurations for XCGZC preparation were optimised and the mechanism of MRPs production was discussed in this study. We believe this work will gain more insight into the development of hemicellulose in various food formulations.

2. Materials and methods

2.1. Materials

Xylan isolated by alkaline extraction of corn cobs was purchased from Shanghai Hanhong Ltd. (China). Chitooligomer was obtained by enzyme hydrolysis of chitosan with immobilised neutral protease. The neutral protease derived from Bacillus subtilis 1.398 was a product of Ningxia XiaSheng Industry Co. Ltd. (China). The polymerisation degree of chitooligomer was between 2–5 studied by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). 2,5-dihydroxybenzoic acid (DHB) was purchased from Sigma–Aldrich. All other chemicals used were of analytical grade obtained from Sinopharm Chemical Reagent Co. Ltd.

2.2. Preparation of chitooligomer by enzymatic hydrolysis

Chitosan (10 g) was dissolved in 250 mL 1% (v/v) acetic acid and filtered to remove insoluble residue. The solution was then adjusted to pH 5.4 using NaOH and kept at 50 °C with shaking at 110 rpm. 0.02% (w/v) enzyme dissolved in 0.1 M acetate buffer (pH 5.4) was added to initiate the reaction. 50 mL of the mixture was taken out after 1 h and heated at 100 °C for 10 min to remove the enzyme. The hydrolysate was filtered and the filtrate was neutralised with 10% NaOH to pH 9.0. The precipitate was collected by filtration and washed thoroughly with ethanol.

2.3. Preparation of chitooligomer–zinc complex (CGZC)

Chitooligomer (4 g) and ZnSO4·7H2O (0.667 g) was dissolved in 100 mL acetic acid (1%, v/v) in a beaker. The pH was adjusted to 5.0 with aqueous solution of sodium acetate (5 g/L). The solution was then stirred using a magnetic stirrer at room temperature for 5–6 h. Following the coordination equilibrium, the mixture was poured into 200 mL ethanol–aceton solvent (v/v = 1). The resulting precipitate was obtained by vacuum filtration. The product was repeatedly washed with ethanol and finally dried under vacuum to constant weight.

2.4. Preparation of xylan–chitooligomer–zinc complex (XCGZC)

CGZC was dissolved in 1% (v/v) glacial acetic acid at a concentration of 1% (w/v) and filtered to remove insoluble residue. Xylan (4%, w/v) was added to the CGZC solution and the pH of the mixture was adjusted to 9.0 using sodium hydroxide (1 M). The mixture was refluxed in an oil bath at 100 °C for 5 h so as to occur the Maillard browning reaction. The heated solution was immediately cooled in an ice-water bath. After that, the precipitate with a brown colour was collected by adding 200 mL ethanol–acetone mixed solvent (v/v = 1) into the heated solutions. The precipitate was repeatedly washed with ethanol and finally dried under vacuum to constant weight. In order to study the Maillard reaction between xylan and CGZC, aliquots (8 ml) were withdrawn at 0, 60, 120, 180, 240 and 300 min and placed in an ice-water bath. For comparison, xylan, chitooligomer, xylan–chitooligomer and chitooligomer–zinc complex were respectively heated under the same experimental conditions as controls.

2.5. MALDI-TOF-MS measurement

Chitooligomer (1 mg/ml) in 20% aqueous ethanol was mixed with the same volume of DHB (15 mg/ml) in 50% aqueous ethanol. The mixture (0.5 μL) was loaded on a sample probe and allowed to dry at ambient temperature and pressure to obtain a homogenous sample surface of small crystals. The crystals were treated with a small volume of ethanol (0.4 μL) and dried in ambient air. Positive ion MALDI-TOF-MS was performed using a VOYAGER-DE-STR time-of-flight mass spectrometer (Applied Biosystems ABI, USA) equipped with a delayed-extraction system, with flight paths of 1.3 m for the linear mode and 2.0 m for the reflectron mode. The spectrometer was equipped with a nitrogen laser with 3 ns pulse width at 337 nm for evaporation and ionisation of sample. The sample was accelerated at 20 kV for the linear and reflectron mode in the ion source, with a delay ranging from 50 to 200 ns. For PSD experiment, after ions of interest had been separated from undesired ones by the timed ion selector, the ions could be focused on the detector by adjusting the potential of the reflection in a stepwise manner. The segments of the fragment ion spectra thus obtained were stitched together by software to create a complete PSD spectrum (Ahn & Yoo, 1999).

2.6. Structure analysis of complexes

CGZC and XCGZC were freeze-dried and grounded to powder. FT-IR spectra were recorded with KBr discs in the range of 4000–400 cm−1 on Nicolet-170 SX spectrophotometer. X-ray diffraction was recorded by a Rigaku Kmax-r AX diffractometer with scanning scope of 5–40° and a scanning speed of 2°/min, using Cu Kα radiation.

2.7. Spectrophotometric analysis

The UV absorbance and browning of the sample was measured according to the literature (Kanatt, Chander, & Sharma, 2008; Li et al., 2011). The heated solutions were appropriately diluted with distilled water for spectrophotometric analysis and antioxidant activity. UV absorbance and browning intensity were measured at 294 and 420 nm, respectively. The fluorescence intensity was measured at an excitation wavelength of 343 nm and an emission wavelength of 415 nm on a Hitachi-F4500 fluorescence spectrophotometer.

2.8. Scavenging of free radicals

The DPPH radical scavenging activity of heated solutions previously heated at different time was estimated according to the methods described by (Brand-Williams, Cuvelier, & Berzet, 1995) with slightly modifications. An aliquot (0.1 ml) of the heated solutions was added to 2.0 ml of 0.1 mM DPPH in ethanol. After being mixed vigorously and being left to stand for 30 min at room
temperature in the dark, the absorption was measured using Unico UV-2000 at 517 nm. The percentage of DPPH radical scavenging activity was calculated as follows:

\[
\text{Scavenging activity} (\%) = \left(1 - \frac{A_A - A_r}{A_0}\right) \times 100
\]

where \(A_0\) was the absorbance of 0.1 mM DPPH, \(A_A\) was the absorbance of 0.1 mM DPPH with the solutions, and \(A_r\) was the absorbance of ethanol with solutions. The antioxidant activity of each sample against time was expressed in terms of IC\(_{50}\) (concentration in micrograms per millilitre required to inhibit DPPH radical formation by 50%) calculated from the log-dose inhibition curve. Butylated hydroxy toluene (BHT) in ethanol was used for comparison. There were three replicate samples in each group for the measurements of free radical scavenging.

2.9. Measurement of reducing power

The reducing power of the complexes was determined according to the method of (Kanatt et al., 2008). An aliquot of the sample (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. Trifluoroacetate (10%, 2.5 mL) was added and the mixture was centrifuged at 10,000 g for 5 min. The supernatant (2 mL) was mixed with 2 mL of water and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm. There were three replicate samples in each group for reducing power.

2.10. Antibacterial activity

The antibacterial activity of XCGZC at different concentrations against Gram positive bacteria including Staphylococcus aureus, B. subtilis and Bacillus magatarius and Gram negative bacteria involving Escherichia coli and Salmonella typhimurium was determined using the disc diffusion method. Cell suspension (10\(^7\) cfu/mL, 50 \(\mu\)L) was added onto agar plates before paper discs (diameter of 6.0 mm) containing test solutions were placed on plates. Inhibition zones were observed and measured after incubation at 37 °C for 36 h.

2.11. Statistical analysis

All experiments were carried out in triplicate, and average values with standard deviation errors were reported. Mean separation and significance were analysed using the IBM SPSS software. A statistical difference at \(p < 0.05\) was considered significant.

3. Results and discussion

3.1. Properties of xylan and chitooligomer

The sugar composition of xylan was determined by our previous experiment through precolumn derivation HPLC method (Li et al., 2011), which mainly contained arabinose and xylose as neutral sugars. This kind of xylan isolated from the corn cobs was a water-soluble branched polysaccharide with low molecular weight. The substitution degree of arabinoses on the hemicellulose backbone was 0.26 and the content of uronic acid in the xylan was 1.4 ± 0.2%. In our study, chitooligomer with good water solubility was used. Fig. 1 showed the MALDI-TOF-MS spectrum of chitooligomer, which revealed that the degree of polymerisation of chitooligomer was between 2 and 5. Chitooligomer contained intensive quasi-molecular ions \([M + Na]^+\) and \([M + K]^+\) ion by PSD experiments using the MALDI-TOF mass spectrometer. The peak at \(m/z\) 378 (\([M + Na]^+) was attributed to the sodium form of...
GlcN–GlcNAc, as well as the precursor [M + K]+ at m/z 394 due to the potassium from of GlcN–GlcNAc. As shown in the MALDI spectra, the chitooligomer was mainly composed of the (GlcN)2, GlcN–GlcNAc, (GlcN)3, (GlcN)4–GlcNAc and (GlcN)5. This indicated that the neutral protease can split the β-1,4-glycosidic linkages of GlcN–GlcN and also selectively cleave GlcNAc–GlcN linkage. It was consistent with the conclusion of (Li et al., 2005).

3.2. Structure of complexes

The X-ray diffraction spectra of xylan, chitooligomer, XCGZC and CGZC were shown in Fig. 2. The diffraction spectrum of xylan exhibited a wide range of crystalline peaks at 18.5°, while chitooligomer showed two major peaks at 9.34° and 19.9°. After chitooligomer was chelated with ZnSO4, the CGZC showed two major peaks at 31.78° and 36.32°, revealing the formation of a new crystalline phase. The peak at 9.34° was disappeared, while the peak at 19.9° was shifted to low angle direction. When the XCGZC was heated for 5 h at 100 °C, the crystallisation peak strength of XCGZC weakened and the peak width increased. Therefore, Maillard reaction can reduce the crystallinity of XCGZC for the MRPs of xylan and CGZC.

FT-IR spectroscopy has been shown to be a powerful tool for the study of the physicochemical properties of polysaccharides. Fig. 3 showed the FT-IR spectra of CG, CGZC, xylan and XCGZC. In the spectrum of CG, the strong peak at 1410 cm⁻¹ was corresponding to νC–N. The peak of amide I band in CG was at 1637 cm⁻¹. The characteristic peak at 1559 cm⁻¹ was derived from carbonyl groups formed during the opening of anhydride as it routed with the amino group of CS (Wang, Du, & Liu, 2004). The FT-IR absorption of xylan at 1641 cm⁻¹ was basically associated with adsorbed water. The bands between 1175 and 1000 cm⁻¹ were typical of xylan (Mazza & Buranov, 2010), and a broad intense signal at 1043 cm⁻¹ was reflected the stretching and bending vibrations of C–O, C–C or C–OH. The characteristic β-glycosidic linkage between the sugar units gave the sharp band at 897 cm⁻¹, corresponding to the C1 group frequency (Li et al., 2011). The FT-IR spectrum of CGZC exhibited many differences from that of CG. The wide peak at 3398 cm⁻¹, corresponding to the stretching vibration of –NH₂ group and –OH group, shifted to lower wave number at 3378 cm⁻¹, as can be observed in Fig. 3. The absorption band at 1615 cm⁻¹ contributed to δNH was weakened and the band at 1410 cm⁻¹ corresponding to νC–N was disappeared. Several new absorb peaks were observed at 1380–1250 cm⁻¹ in the spectrum of CGZC. The CGZC complex formation could be described based on Lewis acid–base theory: Zn²⁺ ions acting as the acid are the acceptor of a pair of electrons given by CG acting as the base (Wang et al., 2004). The reaction was described as follows:

\[ \text{Chito-NH}_2 + \text{H}_3\text{O}^+ \rightarrow \text{Chito-NH}_3^+ + \text{H}_2\text{O} \]

\[ \text{Chito-NH}_3^+ + \text{Zn}^{2+} + \text{H}_2\text{O} \rightarrow (\text{Chito-Zn})^{2+} + \text{H}_3\text{O}^+ \]

Fig. 4. Spectrophotometric analysis of sample as a function of reaction time. (a) UV absorbance at 294 nm; (b) Browning at 420 nm; (c) Fluorescent intensity; Em:415 nm, Ex:343 nm. (— xylan; — chitooligomer; — xylan-chitooligomer; — chitooligomer–zinc; — xylan–chitooligomer–zinc).
The bands near 1109 and 618 cm⁻¹ were contributed to the stretching vibration of S–O bond (Wang, Zhao, Gao, & Yu, 1999). The absorption band at around 1650 cm⁻¹ had been assigned to the C=O linkage derived from the Schiff base (Umemura & Kawai, 2008). Therefore, the absorption band at 1644 cm⁻¹ may represent the overlap of the C=O group and C≡N linkage, suggesting that the formation of MRPs between xylan and CGZC via the Schiff base as expected.

3.3. Browning and fluorescence

Maillard reaction was a process involving the interaction of reducing sugars and amino compounds (Chung, Kuo, & Chen, 2005). Another type called caramelization occurs when polyhydroxycarbonyl compounds (sugar, polyhydroxycarboxylic acids) are heated to relatively high temperatures in the absence of amino compounds (Hodge, 1953). The browning intensity of xylan, chitooligomer–zinc complex, xylan–chitooligomer and xylan–chitooligomer–zinc cooled solutions was respectively measured at 420 nm using Unico UV-2000 spectrophotometer. In increase in browning as observed by absorbance at 294 and 420 nm was seen in the chitooligomer, xylan–chitooligomer, chitooligomer–zinc and xylan–chitooligomer–zinc [Fig. 4(a) and (b)]. Xylan did not show any UV absorbance and no significant colour change when heated alone, indicating that there was no caramelization occurred under the experimental conditions. To some extent, the UV-absorbance value indicates the existence of intermediate compounds in the nonenzymatic browning reactions (Hodge, 1953), whereas the absorbance values at 420 nm may be related to the content in brown polymers. High UV absorbance at 294 nm was indicative of the formation of intermediate compounds of Maillard reaction (Kanatt et al., 2008). Chitooligomers easily turned brown during shelf life due to Maillard reaction taken place in Chitooligomers itself and the structure and properties for browning of Chitooligomers had been changed in the process. The proposed mechanism was described in the original research article (Zeng et al., 2007). Chitooligomer–zinc had certain UV absorption as the heating time prolonged that was caused by browning occurred in chitooligomer under heating circumstances. There were three major stages (initial stage, intermediate stage, final stage) on sugar–amine browning reactions in model systems (Hodge, 1953). The colour did not change in initial stage, in which sugar–amine was condensed and amadori rearrangement happened. During the intermediate stage, the colour of the solution had a weaken change or become yellow, with strong absorption in near-ultraviolet. In the final stage, the highly coloured compounds are formed from aldol condensation and aldehyde–amine polymerisation, together with the formation of heterocyclic nitrogen compounds.

The development of colour and UV absorbance was an important and obvious characteristic of the Maillard reaction. However, many studies had been carried out on the development of fluorescence (Ferrer, Alegria, Farre, Clemente, & Calvo, 2005; Morales & Jimenez-Perez, 2001). Fluorescence products have been used to measure the level of MRPs formed. The fluorescence intensity of xylan–chitooligomer–zinc, xylan–chitooligomer and chitooligomer–zinc increased with the reaction time according to Fig. 4(c). When chitooligomer–zinc and xylan were heated together, the fluorescence intensity increased rapidly to 632.2 at 300 min, revealing fluorophores formed during the Maillard reaction between chitooligomer–zinc and xylan. Obviously, the fluorescence intensity of xylan–chitooligomer–zinc was higher than that of chitooligomer–zinc in the same reaction time, revealing that there was more fluorophores of xylan–chitooligomer–zinc than those of chitooligomer–zinc as the heating time prolonged. The relative fluorescence intensity of xylan was relatively weak while prolonging heating time. When chitooligomer was heated alone, the fluorescence intensity increased rapidly to a maximum at 120 min, and then, decreased during long time intensity in our experiment, probably due to the formation of different fluorescent chemical structures. In the beginning, a predominant fluorescence chemical structure did not exist. With prolonged heating, some of MRPs were involved in the formation of melanoidins. Fluorophores formed during the Maillard reaction in the model system were the precursors of the brown pigments (Morales & van Boekel, 1997).

3.4. DPPH radical scavenging activity and reducing power

Fig. 5(a) showed DPPH scavenging activity of xylan, chitooligomer, chitooligomer–zinc, and xylan–chitooligomer–zinc solutions. The scavenging activity of xylan alone did not have scavenging potential. DPPH scavenging activity of chitooligomer increased to a maximum value at 120 min as the heating time prolonged. The active hydroxyl and amino groups in the polymer chains of chitooligomer can react with a free DPPH radical to form stable macromolecule radical, and the NH₂ groups can form ammonium groups NH₃⁺ by absorbing a hydrogen ion from solution. And the content of active hydroxyl and amino groups in their polymer chains may affect the antioxidant activity (Sun, Zhou, Xie, & Mao, 2005). Another type called caramelization occurs when polyhydroxycarbonyl compounds (sugar, polyhydroxycarboxylic acids) are heated to relatively high temperatures in the absence of amino compounds (Hodge, 1953). The browning intensity of xylan, chitooligomer, chitooligomer–zinc complex, xylan–chitooligomer and xylan–chitooligomer–zinc cooled solutions was respectively measured at 420 nm using Unico UV-2000 spectrophotometer. Increase in browning as observed by absorbance at 294 and 420 nm was seen in the chitooligomer, xylan–chitooligomer, chitooligomer–zinc and xylan–chitooligomer–zinc [Fig. 4(a) and (b)]. Xylan did not show any UV absorbance and no significant colour change when heated alone, indicating that there was no caramelization occurred under the experimental conditions. To some extent, the UV-absorbance value indicates the existence of intermediate compounds in the nonenzymatic browning reactions (Hodge, 1953), whereas the absorbance values at 420 nm may be related to the content in brown polymers. High UV absorbance at 294 nm was indicative of the formation of intermediate compounds of Maillard reaction (Kanatt et al., 2008). Chitooligomers easily turned brown during shelf life due to Maillard reaction taken place in Chitooligomers itself and the structure and properties for browning of Chitooligomers had been changed in the process. The proposed mechanism was described in the original research article (Zeng et al., 2007). Chitooligomer–zinc had certain UV absorption as the heating time prolonged that was caused by browning occurred in chitooligomer under heating circumstances. There were three major stages (initial stage, intermediate stage, final stage) on sugar–amine browning reactions in model systems (Hodge, 1953). The colour did not change in initial stage, in which sugar–amine was condensed and amadori rearrangement happened. During the intermediate stage, the colour of the solution had a weaken change or become yellow, with strong absorption in near-ultraviolet. In the final stage, the highly coloured compounds are formed from aldol condensation and aldehyde–amine polymerisation, together with the formation of heterocyclic nitrogen compounds. The development of colour and UV absorbance was an important and obvious characteristic of the Maillard reaction. However, many studies had been carried out on the development of fluorescence (Ferrer, Alegria, Farre, Clemente, & Calvo, 2005; Morales & Jimenez-Perez, 2001). Fluorescence products have been used to measure the level of MRPs formed. The fluorescence intensity of xylan–chitooligomer–zinc, xylan–chitooligomer and chitooligomer–zinc increased with the reaction time according to Fig. 4(c). When chitooligomer–zinc and xylan were heated together, the fluorescence intensity increased rapidly to 632.2 at 300 min, revealing fluorophores formed during the Maillard reaction between chitooligomer–zinc and xylan. Obviously, the fluorescence intensity of xylan–chitooligomer–zinc was higher than that of chitooligomer–zinc in the same reaction time, revealing that there was more fluorophores of xylan–chitooligomer–zinc than those of chitooligomer–zinc as the heating time prolonged. The relative fluorescence intensity of xylan was relatively weak while prolonging heating time. When chitooligomer was heated alone, the fluorescence intensity increased rapidly to a maximum at 120 min, and then, decreased during long time intensity in our experiment, probably due to the formation of different fluorescent chemical structures. In the beginning, a predominant fluorescence chemical structure did not exist. With prolonged heating, some of MRPs were involved in the formation of melanoidins. Fluorophores formed during the Maillard reaction in the model system were the precursors of the brown pigments (Morales & van Boekel, 1997).
After that, the scavenging activity of chitooligomer decreased during long time heating in our experiment. The possible reason may be sugar–amine condensation and amadori rearrangement in the chitooligomer provided DPPH scavenging activity during the initial stage. Sugar dehydration, sugar fragmentation and amino acid degradation in the intermediate stage made the scavenging activity of CG reach maximum. In the final stage, aldol condensation, aldehyde–amine polymerisation and formation of heterocyclic nitrogen compounds reduced the content of active hydroxyl and amino groups in their chitooligomer chains, simultaneously may affect the antioxidant activity. Our results indicated that xylan–chitooligomer–zinc complex showed higher scavenging activity than other xylan–chitooligomer and chitooligomer–zinc complex. The scavenging activity of xylan–chitooligomer reached a maximum at 180 min and then decreased with the time prolonging. This phenomenon may be due to the content of melanoidins and reductones of MRPs of xylan–chitooligomer during the final stage. The scavenging activity of xylan–chitooligomer–zinc reached maximum 57.4%, when the melanoidins of MRPs of xylan–chitooligomer–zinc arrived to be saturated during 240 min. The IC_{50} of XCGZC was 5.37 mg/mL, which can be described that the 300 min heated xylan–chitooligomer–zinc solutions were equal to the antioxidant ability of 3.28 mg/mL BHT, and was endowed with good antioxidant capacity. The good radical scavenging activity of the complex can be attributed to the advanced MRPs melanoidins, which showed high antioxidant capacity through a chain-breaking, oxygen-scavenging and metal-chelating mechanism without showing cytotoxic effects (Borrelli et al., 2003).

The reducing power of xylan, chitooligomer, xylan–chitooligomer, chitooligomer–zinc, xylan–chitooligomer–zinc solutions was shown in Fig. 5(b). The reducing power of chitooligomer solution did not have significant changes. The tendency of reducing capacity for xylan–chitooligomer solutions was similar to that of scavenging activity. The solution of xylan–chitooligomer–zinc and chitooligomer–zinc showed an increasing reducing power when prolonging the heating time, but there was no reducing power when heating xylan alone. Furthermore, the reducing capacity of xylan–chitooligomer–zinc was higher than that of chitooligomer–zinc under the same experimental conditions which was consistent with the results of browning and fluorescent intensity described above. Some studies have indicated that the antioxidant effects was related to the development of reductones (Shon, Kim, & Sung, 2003), which were terminators of free radical chain reactions.

### 3.5. Antimicrobial assessment

Table 1 showed the antibacterial activity of the samples against E. coli and S. aureus. There was no antimicrobial activity against E. coli and S. aureus for xylan, with or without heating. Chitooligomer solution without heating could inhibit the growth of E. coli. In contrast, for S. aureus, the antimicrobial activity was relatively weak. This phenomenon was consistent with the study of (No, Young Park, Ho Lee, & Meyers, 2002; Zheng & Zhu, 2003).

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reaction time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Xylan</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>Xylan</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>Chitooligomer</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Chitooligomer</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Xylan–chitooligomer</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>Chitooligomer–zinc</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>Xylan–chitooligomer–zinc</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Chitooligomer–zinc</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Xylan–chitooligomer–zinc</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Xylan–chitooligomer–zinc</td>
<td>6.5 ± 0.1</td>
</tr>
</tbody>
</table>

* Against E. coli.
* Against S. aureus.

Fig. 6. The gradient colour of Maillard reaction in the xylan–chitooligomer–zinc (A), and antimicrobial activity of xylan–chitooligomer–zinc at different reaction time against S. aureus (B).
The chitooligomer solution with increased heating time showed no activity against the microorganism. The possible reason might be that cationic amino groups of chitooligomer were screened through reacting with the reductive sugar and free amino groups in chitooligomer, and aldol condensation, aldehyde–amine polymerisation and formation of heterocyclic nitrogen compounds reduced the content of active amino groups in their CG chains. Xylan–chitooligomer did not have distinct antibacterial activity. The antimicrobial activity of chitooligomer–zinc gradually increased with prolonged heating time. The diameter of the inhibition zone of chitooligomer–zinc against E. coli and S. aureus was 9.6 ± 0.3 and 15.0 ± 0.5 mm after the solutions heated for 300 min, respectively. The inhibition zone against E. coli and S. aureus reaches 17.2 ± 0.4 mm and 30.3 ± 0.6 mm at the reaction time of 300 min, suggesting the primary reason of xylan–chitooligomer–zinc solution exerting higher antibacterial activity was mainly due to chelating with zinc ions of chitooligomer. Fig. 6 showed the gradient colour change of Maillard reaction in the xylan–chitooligomer–zinc, and antimicrobial activity of xylan–chitooligomer–zinc at different reaction time against S. aureus using the disc diffusion method. In the initial stage, it was colourless for the xylan–chitooligomer–zinc solution. Correspondingly, the solution had no antibacterial activity against S. aureus. During the intermediate stage, the colour of the solution gradually became yellow, and showed certain antimicrobial activity. With the time prolonged, a dark brown colour was observed in the solution and the antibacterial activity against S. aureus was rapidly increased.

Table 2 gave the antimicrobial activity of different concentration of XCGZC against microorganisms. Among all the bacteria tested, S. aureus was visibly inhibited by XCGZC. With the concentration of XCGZC at 10 mg/mL, the diameter of the inhibition zone against S. aureus is around 35 ± 0.3 mm, indicating that XCGZC had intensive antimicrobial activities against S. aureus.

4. Conclusion

XCGZC was developed as a novel food preservative as the solution of xylan and chitooligomer–zinc complex (the mass ratio is 1:1) was heated for 300 min at 100 °C. It has shown superior antioxidant activity as compared to chitooligomer–zinc complex and chitooligomer. XCGZC has also been found wide spectrum antimicrobial activities against all of the microorganisms used in the test. Among all the bacteria tested, S. aureus was visibly inhibited by XCGZC. Thus, XCGZC was endowed with antioxidant as well as antibacterial activities and can be used as a promising candidate material in the food industry.

Acknowledgements

This work was supported by a Grant from the Major State Basic Research Development Program of China (973 Program) (No. 2010CB732204), New Century Excellent Talents in University (NECT-10-0618), and Hubei Qianjiang Huashan Aquatic Food and Product Co., Ltd.


