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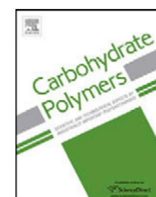

# Controllable antioxidative xylan–chitosan Maillard reaction products used for lipid food storage

Xiaoxia Li [a,b], Xiaowen Shi [a], Yong Jin [a], Fuyuan Ding [a], Yumin Du [a,*]

[a] Department of Environmental Science, School of Resource and Environmental Science, Wuhan University, Wuhan 430079, China
[b] School of Chemistry and Chemical Engineering, Wuhan Textile University, Wuhan 430073, China

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ABSTRACT

Controllable antioxidative xylan–chitosan Maillard reaction products (MRPs) were prepared by co-heating xylan and chitosan at different time periods and used for lipid food storage in lecithin model system and refrigerated pork meat. The results of antioxidant protective effect on lecithin liposome peroxidation induced by 2,2′-azobis(2-methylpropionamidine) dihydrochloride revealed that the MRPs heated for 120 min and 180 min showed much higher inhibitory activity than chitosan or MRP heated for 60 min. In the experiment of fresh pork protection, the MRPs heated for 60 and 120 min retarded the growth of spoilage organisms more effectively. Lipid oxidation potential of the meat, determined by thiobarbituric acid reactive substances, also showed that the samples treated by the MRPs heated for 60 and 120 min had higher acceptance than others. These results demonstrate that the MRPs of xylan and chitosan are promising controllable antioxidative preservatives for lipid food formulations, and the antioxidant behavior depends not only on the antioxidant substances, but also on the interaction of the food systems.

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## 1. Introduction

Hemicelluloses, representing about 20–35% of lignocellulosic biomass, have emerged as an immense renewable resource of biopolymers. Xylan-type polysaccharides are the main hemicellulose components of secondary cell walls and account for 50% of the biomass of annual and perennial plants, but their application potential has not yet been exploited commercially, compared to other polysaccharides, due to the source-dependent diversity, branching, and varying chemical composition (Ebringerova, Hromadkova, & Heinze, 2005). During the latest decade, xylan resourced from different plants have been explored by some research groups and used to produce many novel functional materials (Hansen & Plackett, 2008). For example, xylan from corn cobs is a promising polymer for drug delivery as it could be biodegraded by colon microflora but not be digested in the stomach (Oliveira et al., 2010). Also, many hydrophilic and hydrophobic xylan derivatives, which may have promising applications in plastic and papermaking industries, have been produced by chemical modifications (Fredon et al., 2002; Ren, Peng, & Sun, 2008). Besides, the relatively low molecular weight and structural heterogeneity may offer xylan some special functions, however, until now little information is available in literatures.

Chitosan, the deacetylated derivative of chitin, is a linear aminopolysaccharide. Maillard reaction between the amino groups of chitosan and the aldehydes or ketones of reducing sugars has been reported, and some Maillard reaction products (MRPs) are used as natural emulsifiers, antimicrobial, and antioxidative agents (Kato, 2002). Taking into account the potential exploitation of xylan and the functional properties of chitosan-based MRPs, we have prepared xylan–chitosan conjugates by heating the two biopolymers (Li, Shi, Wang, & Du, 2011). In this report, the antioxidant and antimicrobial activities of the xylan–chitosan MRPs, together with the color and fluorescence changes, were also investigated in order to gain more insight on the Maillard reaction between the two polysaccharides.

Meat and meat products are comparatively highly susceptible to rancidity due to microbial and oxidative spoilage. Thus, delaying lipid oxidation and preventing bacterial growth can have a significant contribution toward the extension of shelf life. In order to achieve these goals, food additives, especially natural products such as plants phenol extract, essential oil, and honey, have been used because of their antioxidative and antibacterial effects (Kerry, McCarthy, Kerry, Lynch, & Buckley, 2001). Chitosan exhibits antimicrobial activity against a range of food-borne microorganisms in several meat products and consequently has attracted attention as a potential natural food preservative (No, Meyers, Prinyawiwatkul, & Xu, 2007). Though it is documented that chitosan minimizes lipid oxidation in different food commodities,

* Corresponding author. Tel.: +86 27 68778501.
*E-mail address:* duyumin@whu.edu.cn (Y. Du).

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research on the use of chitosan as an antioxidant in muscle foods is still limited compared to other food products (Suman et al., 2010). Combination of chitosan with natural antioxidants is one way of extending shelf life of the meat products (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007; Kanatt, Chander, & Sharma, 2008). MRPs also exhibited promising antioxidative and antimicrobial effects and have been used to develop food preservatives. For example, the glucose–lysine MRPs showed higher antioxidant activity than plant phenolics such as cloves, ascorbic acid, and cinnamon in terms of decreased warmed-over-flavor and lipid peroxidation (Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007). Also, the addition of chitosan–glucose Maillard complex to lamb meat increased the shelf life by more than two weeks during chilled storage (Chander, Kanatt, & Sharma, 2008). Similarly, in the study of Chang, Chen, and Tan (2011), pork loins dipped in chitosan–glucose MRPs tended to retard lipid oxidation and microbial spoilage during refrigerated storage, however, little influence was observed on the change of the reactant concentration. We consider that the rapid reaction of small molecular glucose made the Maillard reaction into higher or even final stages in short time at high temperature and this may conceal the difference of the antioxidative ability of the chitosan–glucose MRPs. In our previous study, it has been shown that the xylan–chitosan MRPs with good antioxidant capacity and antimicrobial activity can be used as promising food preservatives (Li et al., 2011). In the present study, the controllable antioxidant activities of xylan–chitosan MRPs, including the inhibition of lipid peroxidation in lecithin liposome systems and the preservative effect on fresh pork during refrigerated storage, were investigated.

## 2. Materials and methods

### 2.1. Materials

Chitosan was obtained from Qingdao Yunzhou Ltd. (China) with the deacetylation degree of 95%. Xylan isolated by alkaline extraction of corn cobs was obtained from Shanghai Hanhong Ltd. (China) and the properties were investigated in our last study (Li et al., 2011).

### 2.2. Preparation of xylan–chitosan MRPs

Chitosan was dissolved in 0.5% (v/v) aqueous acetic acid to obtain 1% (w/v) solution, and filtered to remove insoluble residues. Xylan 1% (w/v) was then dissolved in the chitosan solution and refluxed at 100 °C in an oil bath. After 0 min, 60 min, 120 min, and 180 min, the reaction mixture was cooled to an ice bath. The solutions with different heating time were used directly and coded as XC-0, XC-60, XC-120, XC-180, respectively. Pure xylan and chitosan with the concentration of 1% (w/v) in 0.5% (v/v) aqueous acetic acid were prepared as controls and coded as xylan and CS respectively.

### 2.3. X-ray diffraction of the MRPs

Diffractograms were recorded in reflection mode in the angular range of 5–40° ($2\theta$) with a D8 Advance X-ray Diffractometer. The Cu K$\alpha$ radiation generated at 40 kV and 40 mA was monochromatized using a 20 μm Ni filter. Solutions were tested after being freeze-dried and without grinding, while xylan powder was measured directly.

### 2.4. Molecular weight ($M_W$) measurements

The $M_w$ profiles of the MRPs were estimated by gel permeation chromatography (GPC) using a TSK-G3000PW gel filtration column and monitored by RI 150 refractive index detector. 0.2 M $CH_3COOH$/0.1 M $CH_3COONa$ was used as the eluent, and the flow rate was maintained at 1.0 mL/min. Three-fold dilutions of the samples were tested. Pullulan standards (Shodex Standard P-82, Japan) were used for a calibration curve. All data provided by the GPC system were collected and analyzed using the Jiangshen Workstation software package (Dalian, China).

### 2.5. Reducing power of the MRPs

Measurement of reducing power was the same as our previous report: 1.0 mL of the solution was mixed with 1.0 mL water and 1.0 mL of 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. Trichloroacetic acid (TCA, 10%, 2.5 mL) was added to the mixture and centrifuged at 10,000 × $g$ for 5 min. Then the supernatant (2 mL) was mixed with 2 mL of water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was measured, and higher value indicated greater reducing power. There were three parallel samples in each group of the measurements.

### 2.6. Inhibition of liposome lipid peroxidation

For the antioxidant protection of test compounds, 2 mM 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) was added into the lecithin liposome (10.0 mg/mL in 0.01 M, pH 7.4 phosphate buffered saline) to initiate lipid oxidation, and the result was measured by the TBA assay modified from Yin, Hwang, and Chan (2002). Briefly, 1 mL of sample was mixed with 0.5 mL of 30% TCA, and then centrifuged at 5000 × $g$ for 5 min. 1 mL of supernatant was mixed with 1 mL of 0.02 M 2-thiobarbituric acid solution (TBA) and heated in water bath at 90–100 °C for 30 min. The absorbance of the pink supernatant was read at 532 nm, and called as $A_s$. The blank was made with 1.0 mL deionized water substitution for 1.0 mL sample, named as $A_c$. In order to exclude the interference of aldehydes produced in the Maillard systems, the conjugates were measured directly by the above assay without the addition of AAPH and lecithin, and the absorbance was named as $A_m$. There were three replicate samples in each group for the measurements, and the inhibition percentage (%) was calculated as formula:

$$\text{Inhibition percentage (\%)} = \left[1 - \frac{A_s - A_m}{A_c}\right] \times 100$$

### 2.7. Treatment of pork meat with the MRPs

Fresh porcine longissimus muscle, which was obtained from a local meat processing company, was cut into cubes of 1 $cm^3$, and dipped in the chitosan or MRP solutions for 10 min. Samples without any dipping treatment was used as control. The treated meats were then gently drained on a tissue paper, placed in plastic bags, and stored in the refrigerator at 4 °C for 20 days.

### 2.8. Microbiological analysis and pH of meat

Total viable counts (TVC) were determined by the pour-plate method. A 10-g sample and 90 mL of sterilized distilled water were homogenized by a meat grinder. Several ten-fold serial dilutions were diluted with sterilized saline. 1 mL of each serial dilution was mixed evenly with 15 mL of liquefied agar. After solidification, it was incubated at 35–37 °C for 48 h. Microbiological data were transformed as $\log_{10}$ colony forming units (cfu) per gram of sample. The pH was measured using a pH meter by adding nine parts of deionized distilled water into one part of homogenized sample. All counts were performed in duplicate.

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