CURRENT TOPICS IN NUTRACEUTICAL RESEARCH Vol. 17, No. 4, pp. 445–450, 2019
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Research Article

Antioxidant Properties of Maillard Reaction Products Derived from Shrimp Shell Chitosan and Xylose, Fructose or Glucose

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Received July 28, 2018; Accepted March 8, 2019

Communicated By: Prof. Baodong Zheng

Under certain conditions, chitosans can react with different types of reducing sugars to form various final Maillard reaction products (MRPs). In this study, the optimal reaction conditions were selected, and three different final MRPs (xylose-MRPs, fructose-MRPs, glucose-MRPs) were analyzed. The structure of the three MRPs was analyzed using fluorescence, ultraviolet, and infrared spectroscopic methods, and then the antioxidant activities of the three MRPs were determined. The results showed that the three MRPs have similar structure and function, and the antioxidant activities between them have certain regularity. Antioxidant activities were ranked as follows: xylose-MRPs>fructose-MRPs>glucose-MRPs, and this order was inversely proportional to their reaction time, that is, shorter the reaction time, higher the antioxidant activity, while longer the reaction time, lower the antioxidant activity.

Keywords: Antioxidant activity, Chitosan, Maillard reaction products, Reducing sugars

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INTRODUCTION

The Maillard reaction is a non-enzymatic browning reaction between reducing sugars and amino groups of proteins which results in a variety of desirable compounds accompanied by the formation of aroma and taste-enhancing properties (Wei et al., 2018). These unique aromas are closely related to the raw material in the reaction system, such as roasted flavor of meat (Domínguez et al., 2014), and seafood flavor of marine products (Kubota et al., 2002; Zhang et al., 2006). The Maillard reaction has wide applications in food industry and occurs during thermal processing or storage of foods, and is also developed into an effective method for protein modification (Trevisan et al., 2016).

Chinese shrimp (*Fenneropenaeus chinensis*), also known as oriental shrimp, as an important marine aquaculture species cultivated in China, is primarily distributed in the Bohai Sea, Yellow Sea in China and in the western ocean region of North Korea. Chinese shrimp is one of the most important aquatic processing products of high consumption. However, the resulting by-products contain abundant proteins, minerals, phospholipids, and astaxanthin, comprising approximately 35–45% of the whole shrimp weight (Cai et al., 2016). The shrimp shells could be utilized as seasonings and other food additives (Liu et al., 2009).

Chitosan is a linear amino polysaccharide obtained by the deacetylation of chitin and is widely found in nature (Kumar et al., 2004). The reactive amino groups in the structure of chitosan support the chemical modification reactions more efficiently as compared with cellulose. Applications of chitosan are extensive, and its antiseptic and fresh-keeping function has been fully utilized in the food industry. However, due to its high viscosity and few antioxidant properties, its application has certain limitations (Chung et al., 2013). The use of chitosan and other reducing sugars to generate Maillard reaction can produce macromolecular melanoid substances. The application of these MRPs can break the original limitations, and can also increase the antioxidant properties of active chitosan (Jiang et al., 2013; Karnjanapratum et al., 2016). Maillard reaction between amino groups of chitosan and aldehydes or ketones groups of reducing sugars has been reported, and some Maillard reaction products (MRPs) are used as natural antioxidative, emulsifiers, and antimicrobial agents.

To exploit the potential functional properties of chitosan-based MRPs (Li et al., 2011), we prepared xylose, fructose and glucose-chitosan conjugates in present study. The antioxidant properties of the resulting MRPs were also investigated in order to gain more insight on Maillard reaction between these conjugates. The approach may have practical implications for the effective utilization of Chinese shrimp waste, and simultaneously, provide theoretical basis to produce various MRPs.

MATERIALS AND METHODS

Materials

Chitosan was obtained from the Chinese shrimp with the deacetylation degree of 95%. Glucose, xylose and frutcose were obtained from Shanghai Macklin Biochemical Co. Ltd. The rest of the reagents were purchased from Shanghai Chemical Reagent Company. All the reagents were of analytical grade. Distilled water was used for all the experiments.

Preparation of Different Types of MRPs

Preparation of xylose–chitosan MRPs

The xylose was dissolved in deionized water, and 1% solution was prepared. Then 1% solution chitosan prepared by dissolving in 0.5% acetic acid solution. The two solutions were mixed at 1:1 ratio. The pH was then adjusted to 8.0 followed by heating at 120°C in an oil bath to examine the effect of time on the overall system (Wu et al., 2014).

Preparation of fructose-chitosan MRPs

Fructose (0.5 g) was dissolved in deionized water and 1% solution was prepared. Then 1% solution of chitosan (0.5 g) was prepared by dissolving it in 0.5% acetic acid solution, and finally these two solutions were mixed at 1:1 ratio. The pH of the solution was adjusted to 11, and heated in an oil bath at 110°C to examine the effect of time on the entire system (Dong et al., 2014).

Preparation of glucose-chitosan MRPs

The chitosan was dissolved in 0.5% acetic acid solution, and 1% solution was prepared. Similarly, 1% glucose solution was prepared, and the above two solutions were mixed at a ratio of 1:1 and the pH of resulting mixture was adjusted to 4.0. Afterward, it was heated in an oil bath at 120°C, and the reaction time was examined (Guo et al., 2013; Bártek et al., 2013).

Absorbance Analysis

The absorbance of MRPs was measured at 420 nm spectro photometrically (Nanjing Jiancheng Bioengineering), and the effect of time on the accumulation of MRPs was investigated.

Fluorescence Spectroscopy

For this, a certain concentration of sample solution was prepared, and then the sample solution was diluted until the concentration reached 1 mg/mL. The experimental parameters for the measurement of sample solution were fluorescence spectrum excitation wavelength at 347 nm, and scanning emission spectrum range at 370~550 nm (Li et al., 2013).

Ultraviolet Full-band Scanning

For this, certain concentration of sample solution was prepared, and then the sample solution was diluted until the concentration reached 1 mg/mL. UV scanning spectrum was obtained within the wavelength range of 200–800 nm (Liu et al., 2014).

Fourier Infrared Spectroscopy

For this, 25 mg of the sample and 225 mg of potassium bromide were mixed, milled, and pressed into thin slices followed by wavelength scan at 4000~400 cm^{-1}. During this analysis, potassium bromide was taken as blank and the infrared spectrum of the sample was obtained (Cornelly et al., 2002).

Determination of Antioxidant Capacity

Reducing force measurement

Reducing force measurement was determined according to Wang et al. (2019) with slight modifications. About 1 mL of each sample was prepared at different concentrations of 1, 3, 5, 7, 9, and 10 mg/mL. The resulting samples were mixed with 1 mL of 0.2 mol/L phosphate buffer, 1 mL of 1% potassium ferricyanide solution, and then the mixed solution was placed at 50°C. The reaction was carried out for 20 min. Subsequently, 1.0 mL of 10% trichloroacetic acid solution was added, and centrifuged at 3000 rad/s for 10 min, and 2.5 mL of the supernatant was taken and further 2.5 mL of distilled water and 1.2 mL of 0.1% ferric chloride solution were sequentially added. The absorbance was measured at 700 nm using distilled water as a reference.

DPPH free radical scavenging capacity determination

DPPH free radical scavenging activity was determined according to Wang et al. (2019) with slight modifications. About 2 mL of each sample was prepared at different concentrations of 1, 3, 5, 7, 9, and 10 mg/mL. To each concentration, 2.5 mL of 100 μl/L DPPH-methanol solution was added and allowed to react at room temperature for 30 min followed by measuring the absorbance (A1) at 517 nm. Then sample was replaced with deionized water and DPPH, the absorbance under the same conditions were denoted as A0 and A2, respectively.

DPPH free radical scavenging rate:

$$I\% = (A0 - A1 + A2)/A0 \times 100\%$$

ABTS free radical scavenging capacity determination

ABTS free radical scavenging activity was determined according to Zheng et al. (2016) with slight modifications. About 0.2 mL of each sample was prepared at different concentrations of 1, 3, 5, 7, 9, and 10 mg/mL and then 4 mL of 7 mmol/L ABTS solution was added in sequence, shaken, and reacted at room temperature for 6 min. Distilled water was used instead of the sample (A0), and the same treatment was used as a blank control, and the absorbance was measured at a wavelength of 734 nm using distilled water as a reference (A1), Deionized water was used instead of the ABTS (A2).

ABTS free radical scavenging rate:

$$I\% = (A0 - A1 + A2)/A0 \times 100\%$$

Determination of hydroxyl radical scavenging ability

Hydroxyl radical scavenging ability was determined according to Li et al. (2018) with slight modifications. Different concentrations of sample solution were prepared with in the concentration gradient ranging from 1, 3, 5, 7, 9, to 10 mg/mL. From each gradient, 1 mL was taken and to this solution, 1.0 mL of 9 mmol/L ferrous sulfate solution, 1 mL of 9 mmol/L water salicylic acid and 1 mL of 9 mmol/L hydrogen peroxide were added and incubated in a shaker at 37°C for 30 min. Using distilled water as a reference, the absorbance was measured at 510 nm, denoted as A1, and the sample was replaced by deionized water as the solution to be tested. The absorbance was measured under the same conditions, and recorded as A0. Instead of other reagents, absorbance measurement under the same conditions, denoted as A2.

Hydroxyl radical scavenging rate:

$$I\% = (A0 - A1 + A2)/A0 \times 100\%$$

Data Analysis

All experiments were carried out in triplicate, and the mean values with standard deviation errors were reported. Mean separation and significance were analyzed using the IBM SPSS software. A $P < 0.05$ was considered statistically significant.

RESULTS

Absorbance Analysis of MRPs

Absorbance analysis of xylose–chitosan MRPs

As shown in Figure 1A, when the absorbance of xylose-MRPs was measured at 420 nm, the accumulation of MRPs increased with an increase in reaction time, which showed a monotonous increasing trend. When the reaction time reached 60 min, the accumulation of MRPs reached the maximum, and as the reaction time continued to prolong, the properties of MRPs changed. As the temperature decreased, a self-gelation phenomenon occurred. Therefore, the highest accumulation of xylose-MRPs was at 60 min.

Absorbance analysis of fructose-chitosan MRPs

As shown in Figure 1B, when the fructose-MRPs were measured at 420 nm, the accumulation of MRPs has been fluctuating with an increase in reaction time, which was roughly decreasing initially, then increasing and then further decreasing. The results of the absorbance of the final product of MRPs in each reaction time show that when the reaction time reached 95 min, the accumulation of MRPs was the highest, which was much higher than the accumulation of MRPs at other wavelengths, and at reaction time beyond 105 min. Afterward, the entire Maillard system was degraded, and the final product was burnt and turned to paste and the further reaction was terminated. Therefore, in the whole system, the optimal accumulation time of fructose-MRPs was 95 min.

Absorbance analysis of glucose-chitosan MRPs

As shown in Figure 1C, as the reaction time increased, the accumulation of MRPs increased gradually. From 100 min to 120 min, the accumulation rate of MRPs was the fastest, especially when the reaction time interval reached 2 h. The accumulation of MRPs was the highest after two 2-h intervals, and the entire product was scorched and the glucose-MRPs were destroyed. It is concluded that at 120 min, the accumulation of glucose-MRPs was highest which was selected as the optimal experimental condition.

Fluorescence Spectroscopy

The Maillard reaction, after completion, was accompanied by a substance with fluorescent properties, which can be detected at a specific wavelength (excitation wavelength 340–370 nm, emission wavelength 420–440 nm). The Maillard reaction formed a product in the primary stage without fluorescence, but before the brown pigment was produced in the advanced reaction stage, the substance formed in the primary reaction stage can be crosslinked with an adjacent protein or amino acid to form a polymer having fluorescent properties. The Maillard reaction between chitosan and three reducing sugars was carried out. Their reaction products could theoretically suit the following conditions: at the excitation wavelength of 347 nm, the strongest fluorescence emission wavelength is in the range of 420–427 nm and a peak appears. This phenomenon marked the formation of a substance with fluorescent properties of MRPs. At 420~427 nm, all three products showed peaks, with their obvious peak size (Fig. 2). The differences were ranked from large to small: xylose-MRPs>glucose-MRPs>fructose-MRPs. This phenomenon demonstrated the extent to which the Maillard reaction occurs.

Ultraviolet Analysis

As shown in Figure 3, the three products had similar properties, and all three products depicted the maximum absorption peaks at a wavelength of 279.2–281.8 nm, indicating the formation of Schiff

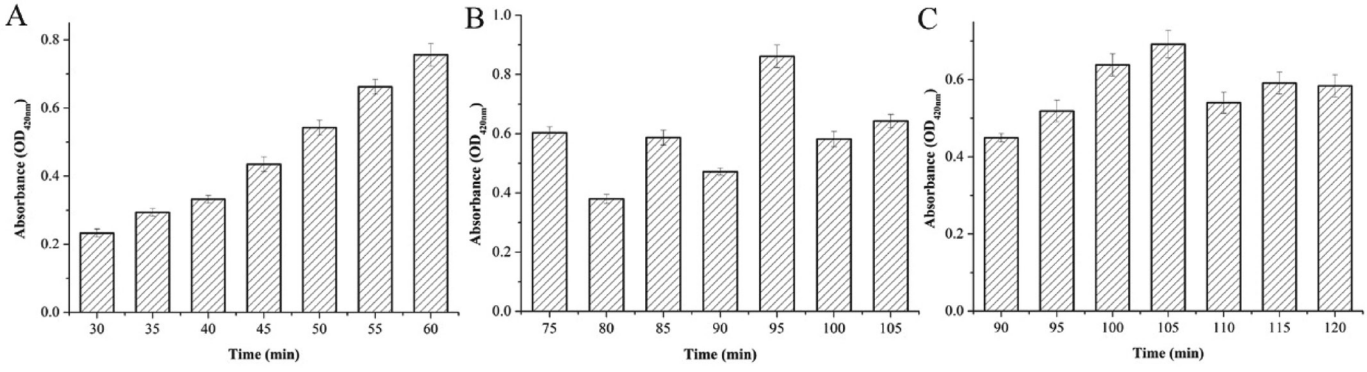

FIGURE 1 | The relationship between accumulation of products and duration of different reactions. (A) Xy-MRPs; (B) Fru-MRPs; (C) Glu-MRPs.

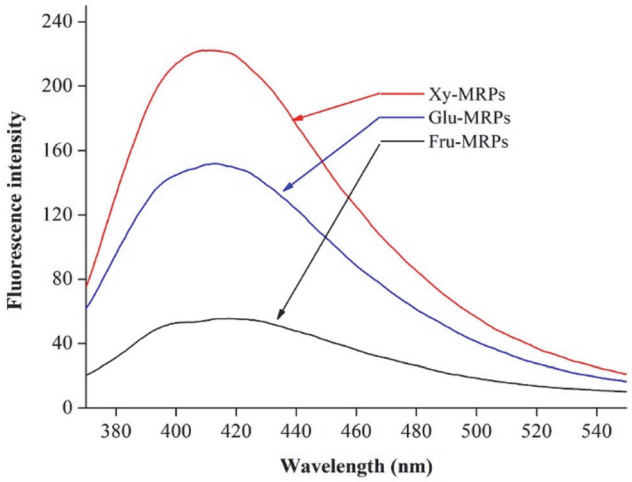

FIGURE 2 | Fluorescence spectrum of the three different types of MRPs.

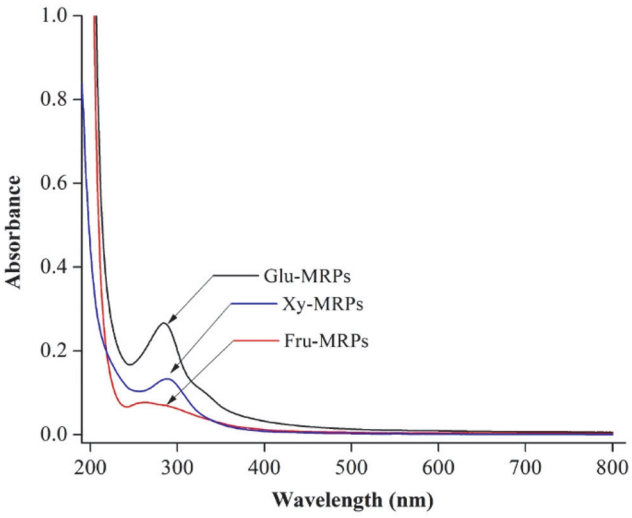

FIGURE 3 | Ultraviolet scanning spectrum of the three different types of MRPs.

base. The difference in the intensity of the absorption peak may be related to the difference in the reactants. A previous report also showed that in the Maillard reaction of different reducing sugars with chitosan, the absorption peak of the reaction products was at 280 nm which was in accordance with our data.

Infrared Spectroscopy

Figure 4 shows the results of the infrared spectroscopic analysis of the MRPs at 400~4000 nm. The infrared absorption spectra of the three MRPs showed similar trend. The positions of the absorption peaks in the infrared spectra of the three MRPs were similar (Fig. 4). The absorption strength was found to be different, which indicates that the structure of MRPs formed by the Maillard reaction of different reducing sugars and chitosan was also different. The peaks mainly appeared at 900–2000 nm. Among these absorption peaks, the absorption peak at 900 nm proved the occurrence of Maillard reaction; while the range 1200–1400 nm represented the C–O and C–N tensile changes of the amide I and II bands, and the range of

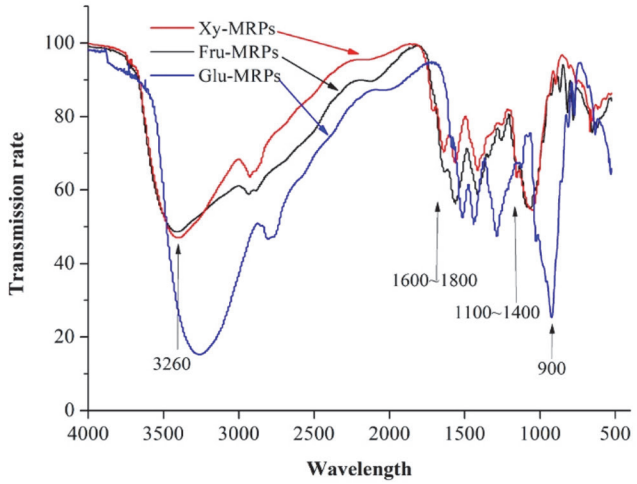

FIGURE 4 | External red scanning spectrum of three different types of MRPs.

1600–1800 nm was mainly referring to C–N stretching N–H. All three products have an absorption peak at 3261.01 nm, which is related to the expansion and contraction of N–H, O–H and C–H. At the same time, it can be seen from their infrared spectra that the secondary structure of the MRPs generated by the Maillard reaction has a certain influence.

Antioxidant Capacity of MRPs

Reducing force measurements

As the concentration of the three different MRPs increased (Fig. 5A), their respective reducing powers also increased. It can be seen among the three different MRPs, as the concentration increased, it did not change the order of reducing power. The order of their reducing power was xylose-MRPs>fructose-MRPs>glucose-MRPs, and this phenomenon was inversely proportional to the reaction time. The reducing power MRPs formed at longer reaction time was less than MRPs formed at shorter reaction time. It is speculated that the effect of high temperature on MRPs was more obvious with the extension of reaction time, which may lead to the destruction of the structure of MRPs.

DPPH free radical scavenging activity

As the concentration of three different MRPs increased, the apparent clearance of DPPH free radicals was also improved significantly (Fig. 5B). Among the three MRPs, the DPPH free radical scavenging ability was stronger. The glucose-MRPs and xylose-MRPs were obtained up to 80% or more, but fructose-MRPs were relatively weak. At the same concentration, the maximum range was 70%, while, glucose-MRPs were relatively stronger than xylose-MRPs with the following order: glucose-MRPs>xylose-MRPs>fructose-MRPs.

ABTS free radical scavenging activity

With increasing concentrations of MRPs, a significant increase in the clearance rate of ABTS free radicals was observed (Fig. 5C). Among the three MRPs, the strong ability (70%) to remove ABTS free radicals was observed in glucose-MRPs and fructose-MRPs, but xylose-MRPs were significantly weaker than the other two MRPs. Whereas at the same concentration,

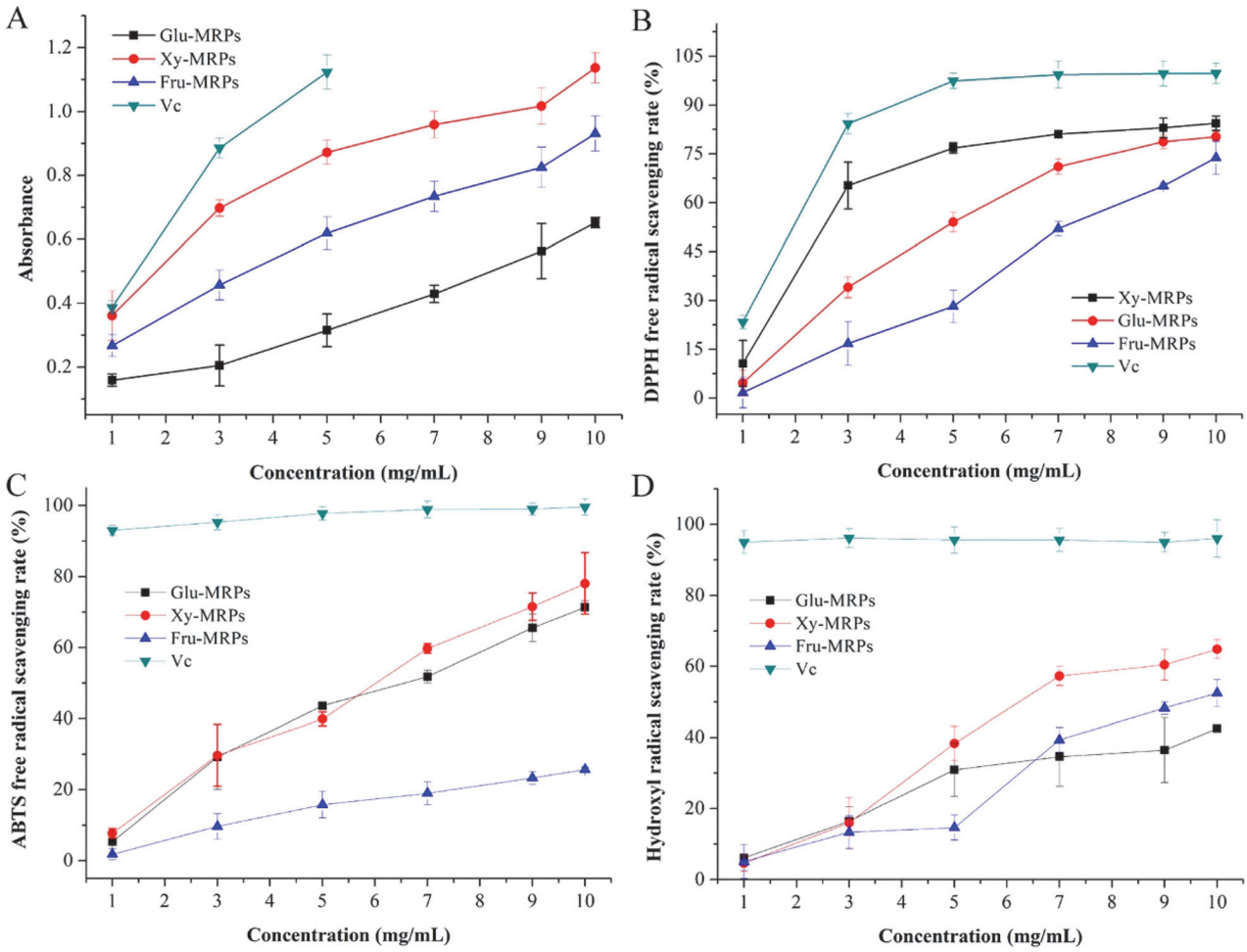

FIGURE 5 | Antioxidant activities of MRPs at different concentrations. (A) reduction power; (B) DPPH radical scavenging activity; (C) ABTS radical scavenging activity; (D) hydroxyl radicals scavenging ability. Vc was positive control. Values are expressed mean ± SD.

xylose-MRPs can only reach 25%, while, glucose-MRPs were also relatively stronger than xylose-MRPs and the overall order was glucose-MRPs>fructose-MRPs>xylose-MRPs.

Hydroxyl radical scavenging results

As the concentration of three different MRPs increased, the apparent clearance of hydroxyl radicals was also significantly improved (Fig. 5D). Among the three MRPs, their ability to scavenge hydroxyl radicals was not much different. The overall ranking of their ability to scavenge hydroxyl radicals was in a following order: xylose-MRPs>fructose-MRPs>glucose-MRPs, which was the same as the order of reducing power. The prediction may be due to the prolongation of reaction time and high temperature. These conditions influenced the structure or properties of the three MRPs, leading to this phenomenon.

CONCLUSIONS

In this study, the activity of chitosan was increased by the Maillard reaction. The Maillard reaction between different reducing sugars and chitosan was closely related to the reaction time. The three different final MRPs obtained showed higher antioxidant activities, and their antioxidant activities were inversely proportional to the reaction time, which indicates that longer the reaction time, higher the antioxidant activity of the final product. The antioxidant activities of the MRPs is ranked as xylose-MRPs>fructose-MRPs>glucose-MRPs.

ACKNOWLEDGMENTS

This study was supported by the Major Projects of Science and Technology in Anhui Province (17030701025, 17030701058, and 18030701158).

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