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Short communication

Preparation and antibacterial activity of the oligosaccharides derived from *Rhizoma Phragmites*

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ABSTRACT

In this study, we prepared *Rhizoma Phragmites* derived oligosaccharides (ROs) by hydrolysis with hydrogen peroxide (H_2O_2). The ROs yield was affected by reaction time, temperature, and H_2O_2 concentration. Too long reaction time and too high temperature decreased the ROs yield. Maximum ROs yield (11.26%) was obtained at reaction time 4 h, temperature 75 °C, and H_2O_2 concentration 3.5% (v/v). The oligosaccharides sample contained 93.16% sugar, of which the average degree was approximately 11, was water-soluble, and showed white. The ROs had the highest antibacterial activity against *Staphylococcus aureus*, followed by *Bacillus subtilis* and *Escherichia coli* at the concentration of 100 μg/mL.

1. Introduction

Phragmites communis is widely distributed throughout the world, and plays an important role in maintaining ecological balance and species diversity in wetland ecosystems (Liu, Jie, Liu, Li, & Guo, 2013). The fresh or dried *Rhizoma Phragmites* is a traditional Chinese herb. It can remove heat, promote the production of body fluids, ease the mind, relieve omitting, and induce excretion of urine (Yu, Jiang, Wang, & Lu, 2010).

R. Phragmites contains polysaccharides, triterpenes, betaine, etc. Among the components of *R. Phragmites*, the polysaccharides were well documented in recent years (Chao, Yang, Cai, Lu, & Qian, 2011; Fang, Wei, Liu, & Zhang, 1990; Han, Li, Gao, & Xia, 2012; Miao, Gu, Fang, & Miao, 2004; Nam et al., 2013; Shen, Ren, Zhang, & Yang, 2010; Yu et al., 2010). Yu et al. (2010) found that *R. Phragmites* polysaccharides (RPP) contain xylose, glucose, arabinose, and galacose with molar ratio of 1.00:59.03:2.09:1.96. RPP have antioxidant activity (Shen et al., 2010), immune activity (Fang et al., 1990), antitumor activity (Chao et al., 2011), hepatoprotective activity (Han et al., 2012), antiaging activity (Miao et al., 2004), and inhibitory effects on atopic dermatitis-like skin lesions in NC/Nga mice (Nam et al., 2013). However, the oligosaccharides derived from *R. Phragmites* have not been reported frequently.

Oligosaccharides can be prepared from its maternal polysaccharides by hydrolysis with different methods. At the same time, hydrogen peroxide (H_2O_2) is used to hydrolyse polysaccharides such as cellulose, starch, hemicellulose, and peach gum polysaccharides because H_2O_2 is easy to handle, readily available, and environmentally friendly (Chang, Tai, & Cheng, 2001; Qin, Du, & Xiao, 2002; Shao, Yang, & Zhong, 2003; Wu, Cai, & Sun, 2012). However, preparation of *R. Phragmites* derived oligosaccharides (ROs) was also not frequently reported.

Therefore, this study aims to develop H_2O_2-assisted preparation of the ROs. The extraction conditions were optimized, and the antibacterial activity of the ROs was determined.

2. Materials and methods

2.1. Materials

Dried *R. Phragmites* was purchased from a local pharmacy in Xinpu, China. H_2O_2 was purchased from the Laiyang Kant Chemical Co., Ltd. (Laiyang, China). Reagent-grade chemicals were used.

2.2. ROs preparation

The dried *R. Phragmites* was pulverized and sifted through a 60 mesh sieve to obtain a fine powder and stored in dark bags in a dry environment until use.

The dried *R. Phragmites* powder was extracted with organic solvents (light petroleum, acetone and methanol) in a Soxhlet apparatus, and then soaked in distilled water to yield a 1% (w/v) suspension. Different volumes of H_2O_2 (1.5%, 2%, 2.5%, 3%, 3.5%, and 4%, respectively) were poured into a reactor containing 500 mL of

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R. Phragmites suspension, and the reactor was maintained in a thermostatic water bath at different temperatures (60 °C, 65 °C, 70 °C, 75 °C, 80 °C, and 85 °C, respectively) for varying times (1 h, 2 h, 3 h, 4 h, 5 h, and 6 h, respectively). Aliquots of the reaction mixture were periodically collected and cooled below 10 °C to terminate the reaction.

The *R. Phragmites* suspension was hydrolysed under the optimized hydrolysis conditions, i.e. reaction time 4 h, temperature 75 °C, and H_2O_2 concentration 3.5% (v/v). The hydrolysates were filtered through a Whatman GF/A filter paper and concentrated to approximately 15% (w/v). The proteins were removed by using the Sevag method, and the hydrolysates were precipitated by adding 5 volumes of absolute ethanol, followed by filtration using a Whatman GF/A filter paper, and freeze drying. The % ROs yield was calculated using Eq. (1) as follows:

$$\text{Yield} = 100\,W_2/W_1, \tag{1}$$

where W_1 and W_2 represent the weights of the recovered ROs and the original dried *R. Phragmites* powder, respectively.

2.3. Antibacterial activity assay

Antibacterial activity assay was performed according to the method described by Qian (2014), with a slight modification. Common pathogens like *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* were used to test the antibacterial activity of the ROs. The test strain was sub-cultured and incubated for 1 h at 37 °C. After the incubation, the bacterial culture was swabbed on the previously prepared and solidified Muller Hinton Agar plates. Sterile paper disks were placed on the Petri dish (diameter 8 mm) and each disk was inoculated with 100 mg/mL ROs. The sterile water inoculated disk was used as control. The bacterial resistance of the ROs samples was examined for a zone of inhibition of the disk. The total diameter of the inhibition zone was measured.

2.4. Analytical methods

Ash, moisture, total sugar and protein contents of the samples were determined according to standard methods (Hou, 2004). The reducing sugars were estimated by the Somogyi method and expressed as a dextrose equivalent (DE) value (Nelson, 1944). The DE values were used to evaluate the average degree of polymerization of the ROs.

2.5. Statistical analysis

All the experiments were carried out three times and each in three replicates. All data are presented as mean ± S.D. Statistical analysis was performed using Statgraphics Centurion XV Version 15.1.02. A multifactor ANOVA with posterior multiple range test was used to find significant differences of two groups.

3. Results and discussion

3.1. Effect of time on hydrolysis of RPP with H_2O_2

Reaction time is important for efficient hydrolysis of RPP with H_2O_2. The effect of reaction time on the hydrolysis of RPP by H_2O_2 was investigated over a period of 6 h (Fig. 1). Maximum ROs yield was obtained after 4 h. Extension of reaction time further decreased ROs yield. The decrease in ROs yield after 4 h can be attributed to the excessive hydrolysis of H_2O_2, which produced oligosaccharides with an extremely low degree of polymerization that made precipitation with ethanol difficult. Therefore, the optimal reaction time was judged to be 4 h.

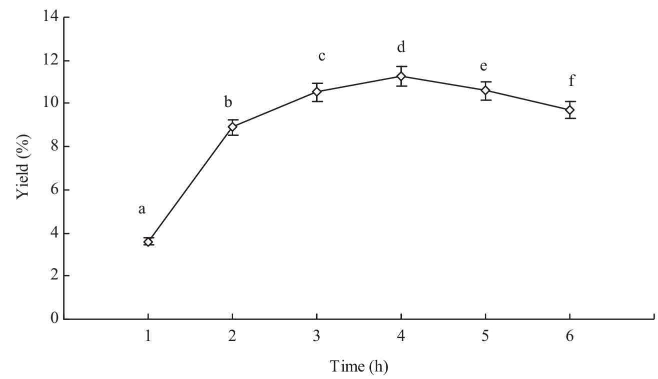

Fig. 1. Effect of time on the yield of ROs. Bars represent the standard deviation ($n = 3$). Different letters on the bars indicate significant differences ($P < 0.05$).

3.2. Effect of temperature on hydrolysis of RPP with H_2O_2

Reaction temperature crucially affects RPP hydrolysis with H_2O_2. The temperatures investigated were performed up to 85 °C. Maximum ROs yield was obtained at 75 °C (Fig. 2). Increasing reaction temperature further also decreased ROs yield. The decrease in RP above 75 °C can also be attributed to the excessive hydrolysis of H_2O_2 at high temperatures. In contrast, a number of studies reported that the optimum temperatures for chitosan hydrolysis with H_2O_2 are from 40 °C to 60 °C (Tian, Liu, Hu, & Zhao, 2004) and 70 °C (Huang, Wang, Huang, Zhuo, & Guo, 2007), whereas that for curdlan hydrolysis using H_2O_2 is 60 °C (Wu et al., 2012), and that for peach gum polysaccharides hydrolysis with H_2O_2 is 55 °C (Yao, Cao, Pan, & Wu, 2013). The differences in optimal temperatures for polysaccharides hydrolysis with H_2O_2 may be ascribed to the discrepancies in polysaccharide type, reaction time, and pH of reaction mixture (Yao, Cao, Pan, & Wu, 2013).

3.3. Effect of H_2O_2 concentration on hydrolysis of RPP with H_2O_2

The effect of the H_2O_2 concentration on hydrolysis of RPP with H_2O_2 is shown in Fig. 3. The maximum ROs yield was achieved at a H_2O_2 concentration of 3% (v/v). Increasing H_2O_2 concentration further did not increase ROs yield. It is interesting that rather high ROs yields were obtained at varying H_2O_2 concentrations, e.g. ROs yields reached 8.23% at the lowest H_2O_2 concentration (1%, v/v).

3.4. Product characterization

The resulting product sample was water-soluble and white powder. The ash, moisture, and total sugar contents of the resulting product were 0.5%, 1.2%, and 93.16% (w/w), respectively. The DE of

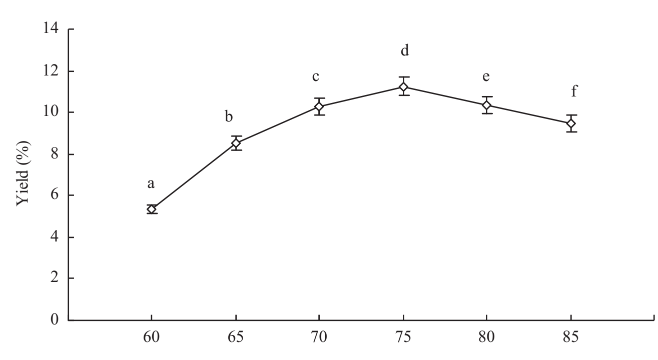

Fig. 2. Effect of temperature on the yield of ROs. Bars represent the standard deviation ($n = 3$). Different letters on the bars indicate significant differences ($P < 0.05$).

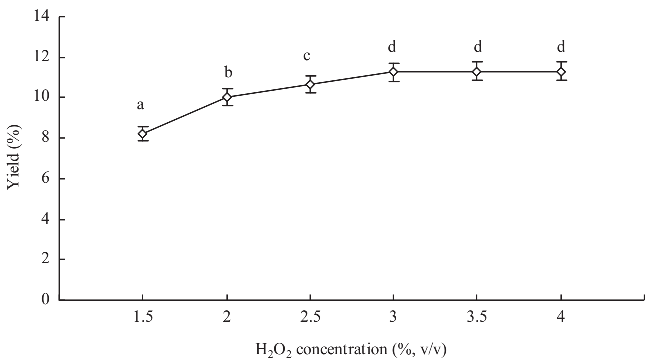

Fig. 3. Effect of H_2O_2 concentration on the yield of ROs. Bars represent the standard deviation ($n = 3$). Different letters on the bars indicate significant differences ($P < 0.05$).

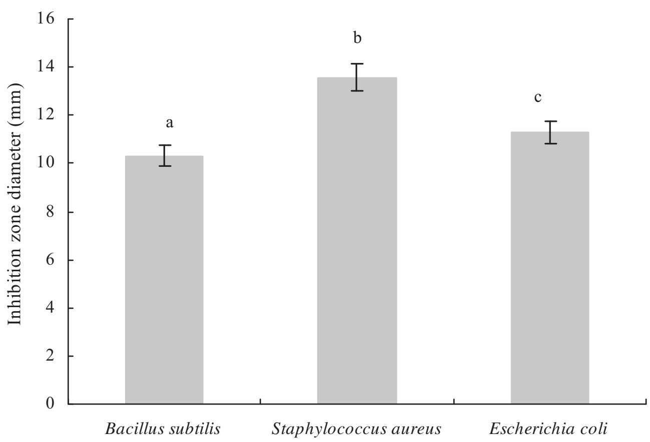

Fig. 4. Antibacterial activity of ROs. Bars represent the standard deviation ($n = 3$). Different letters on the bars indicate significant differences ($P < 0.05$).

the resulting products was 9.08, indicating that the average degree of polymerization was ~11.

3.5. Antibacterial activity of ROs

The antibacterial activity of ROs is shown in Fig. 4. The inhibition zone diameter was 13.57 mm, 11.29 mm, and 10.31 mm for *S. aureus*, *E. coli*, and *B. subtilis*, respectively by ROs, indicating that ROs had high antibacterial activity. Similarly, Yao, Geng, Wang, Zheng, and Li (2011) found that RPP was extremely sensitive to *Saccharmoyces cerevisiae*, highly sensitive to *S. aureus*, and moderately sensitive to *Aspergillum niger* and *B. subtilis*.

4. Conclusions

ROs could be prepared from RPP by hydrolysis with H_2O_2, and ROs yield was affected by reaction time, temperature, and H_2O_2 concentration. The average degree of polymerization of ROs was ~11. The results indicate that ROs had rather high antibacterial activity against *S. aureus*, *E. coli*, and *B. subtilis*. Thus, the ROS can be developed into a novel antibacterial product for use on food preservation.

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