Carbohydrate Polymers 118 (2015) 41–43

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Preparation of the oligosaccharides derived from *Flammulina velutipes* and their antioxidant activities

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ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 21 October 2014
Accepted 28 October 2014
Available online 20 November 2014

Keywords:
Oligosaccharides
Flammulina velutipes
Hydrogen peroxide

ABSTRACT

The oligosaccharides were prepared from *Flammulina velutipes* by hydrolysis of *F. velutipes* polysaccharides with hydrogen peroxide (H_2O_2). The yields of *F. velutipes* derived oligosaccharides (FVOs) were monitored during the hydrolysis process. FVOs yields were affected by three factors, i.e. reaction temperature, H_2O_2 concentration, and time, which were optimized by using an orthogonal design experiments as follows: reaction temperature 70 °C, H_2O_2 concentration 3%, and reaction time 6 h. Under these optimum conditions, the maximal yield of the oligosaccharides reached 17.10%, which was higher than that of hot water extraction method. The oligosaccharides were partially characterized by Fourier transform infrared spectrum, monosaccharide composition, and antioxidant activity. The results indicate that the oligosaccharides derived from *F. velutipes* showed strong hydroxyl radical activity and reducing capacity at the concentration of 100 μg/mL.

1. Introduction

The dynamic balance between the antioxidant defense and prooxidant conditions reflects the antioxidant status in humans. When this balance is broken by some factors, e.g. aging, impaired physiological functioning may occur, resulting in diseases and accelerated aging (Tiwari, 2004). Many antioxidants including both synthetic chemicals such as phenolic compounds and various naturally occurring substances such as saccharides are found to be strong radical scavengers (Yao, Cao, & Wu, 2013). Moreover, natural antioxidants from plants have attracted increasing interest due to consumer concern about the side-effects of some synthetic antioxidants in food.

Flammulina velutipes is a commercially important edible mushroom cultivated widely in many countries, especially in China and Japan (Zhang, Xiao, Deng, He, & Sun, 2012). It has been reported that both the fruiting bodies and the fungal mycelia of *F. velutipes* contain bioactive polysaccharides with beneficial immunomodulatory, anti-tumor and biological activity on hepatocytes, anti-proliferation activity, and antioxidant activity (Pang et al., 2007; Sheng et al., 2007; Shi, Yang, Guan, Zhang, & Zhang, 2012; Yang et al., 2012; Zhang et al., 2013). However, so far, the oligosaccharides derived from *F. velutipes* have not been reported.

Therefore, it is of our interests to prepare the oligosaccharides from *F. velutipes* polysaccharides by hydrolysis with hydrogen peroxide (H_2O_2). The optimum hydrolysis conditions and the antioxidant activities of the *F. velutipes* derived oligosaccharides (FVOs) were investigated.

2. Materials and methods

2.1. Materials

F. velutipes was purchased from a farmers' market (Xinpu, China). H_2O_2 (30%, v/v) was purchased from the Laiyang Kant Chemical Co., Ltd. (Laiyang, China). All other chemicals were reagent-grade.

2.2. Preparation of FVOs

F. velutipes were washed with tap water, dried in a hot air oven (JK-OOI-240A, China) at 60 °C to a constant weight, pulverized, and sifted through a 60-mesh sieve.

The dried powder was suspended in tap water to yield a suspension at a concentration of 1%. Hydrolysis was performed under the conditions specified in Table 1. Aliquots of the suspension were

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http://dx.doi.org/10.1016/j.carbpol.2014.10.074
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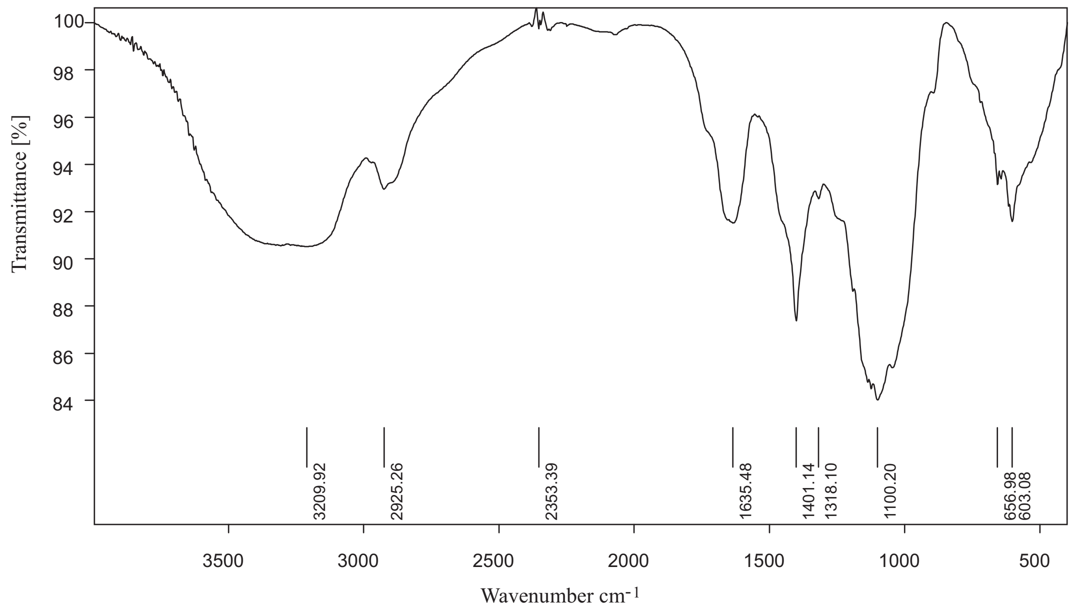

Fig. 1. Fourier transform infrared spectrum of *Flammulina velutipes* oligosaccharides.

Table 1
Analysis of L9 (3)^3 test results of hydrolysis conditions with H_2O_2.

No.	A, temperature (°C)	B, H_2O_2 concentration (%)	C, time (h)	Yield (%)
1	65	2	4	11.82
2	65	2.5	5	14.96
3	65	3	6	17.10
4	70	2.5	4	9.90
5	70	3	6	12.70
6	70	2	3	11.86
7	75	3	4	11.68
8	75	2	6	15.80
9	75	2.5	3	16.14
K_1	11.49	13.16	11.13	
K_2	14.63	13.67	14.49	
K_3	14.54	13.83	15.03	
R	3.14	0.67	3.9	

withdrawn periodically and cooled below 10 °C to terminate the reaction.

The hydrolysates were filtered, concentrated (approximately 15%) using a rotary evaporator at 50 °C under vacuum, proteins removed using the Sevag method, precipitated using 5 volumes of absolute ethanol, filtered again, and freeze-dried. The percentage yield of FVOs was calculated using Eq. (1).

$$\text{Yield} = 100\,W_2/W_1 \quad (1)$$

where W_1 and W_2 represent the weights of the recovered FVOs and the dried *F. velutipes* powder, respectively.

2.3. Analytical methods

Ash, moisture, protein, and total sugar contents of the samples were determined according to standard methods (Hou, 2004). Monosaccharide composition analysis was conducted according to Sheng et al. (2007). The Fourier transform infrared (FTIR) spectra of representative hydrolysate samples were obtained in KBr pellets by using a Nicolet Nexus FTIR 470 spectrophotometer over a wavelength range of 400 cm^{-1}–4000 cm^{-1}.

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Andrews (1986). FVOs hydroxyl scavenging activity was calculated as follows:

$$\text{HRSA}(\%) = \frac{A_1 - A_2}{A_1 - A_0} \times 100 \quad (2)$$

where: A_0 is the absorbance of the reagent blank, A_1 is the positive control absorbance, A_2 is the absorbance of the sample.

Reducing capacity was measured according to method described by Yao et al. (2013) with some modifications. Briefly, 1 mL of FVOs solution, 1.0 mL phosphate buffer (0.2 M, pH 6.6), and 1.0 mL potassium ferricyanide (1%, w/v) were mixed and incubated at 50 °C for 20 min. After cooling down, 1.0 mL trichloroacetic acid (10%, w/v) and 0.2 mL fresh ferric trichloride ($FeCl_3$, 0.1%, w/v) were added to the reaction mixture. Then reaction mixture was shaken and its absorbance was detected at 700 nm against a blank (water instead of FVOs solution) 10 min later. Absorbance of the reaction mixture indicates the reduction capability of sample.

$$\text{Reducing capacity} = (A_1 - A_2) \quad (3)$$

where A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of $FeCl_3$ solution.

2.4. Statistical analysis

All data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used for comparing the groups. A p value of <0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Effect of time, temperature, and H_2O_2 concentration on the yield of FVOs

Reaction temperature, H_2O_2 concentration, and time are important for efficient hydrolysis of the *F. velutipes* polysaccharides and thus affect the yields of FVOs. Based on the results of single factor experiments (data not shown), an orthogonal test was used to optimize the hydrolysis conditions. Table 1 shows FVOs yield obtained under the experimental conditions evaluated. Reaction time had

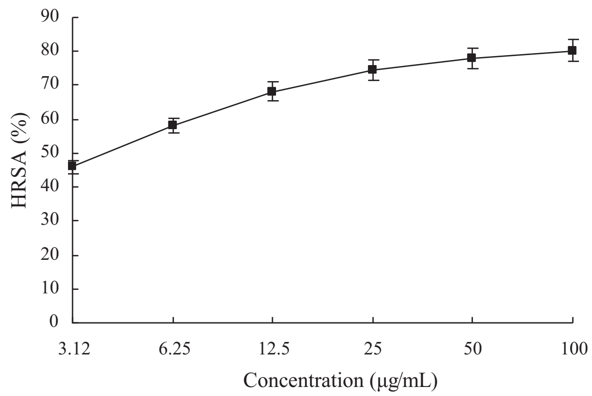

Fig. 2. Hydroxyl radical scavenging activity (HRSA) of *Flammulina velutipes* oligosaccharides. Data are shown as mean ± SD ($n = 3$).

the highest *R* value (3.9) and exerted the greatest effect on the FVOs yield. The effects of the variables on FVOs yields followed the order: extraction time > temperature > H_2O_2 concentration. As shown in Table 1, the optimum hydrolysis conditions were reaction time 6 h, temperature 70 °C, H_2O_2 concentration 3%. Under these optimum conditions, the maximal yield of the oligosaccharides reached 17.10%, which was higher than that of hot water extraction method (Zhang et al., 2013). In contrast, the optimal conditions for hydrolysis of *Laminaria japonica* polysaccharides with H_2O_2 were reaction time 24 h, 75 °C, and H_2O_2 concentration 4% (Wu, 2014); the optimum reaction time, temperature, and H_2O_2 concentration for hydrolysis of *Rhizoma Phragmites* polysaccharides by using H_2O_2 were 40 h, 75 °C, and 3.5% of H_2O_2 (Qian, 2014); Jiang (2014) reported that the optimum conditions for the hydrolysis of *Lycium barbarum* polysaccharides with H_2O_2 were reaction time 4 h, 70 °C, and H_2O_2 concentration 2.5%. The different reported optimum conditions could be due to the different polysaccharides sources.

3.2. Product characterization

Ash, moisture, protein, and total sugar contents in the products were 2.93%, 2.14%, 0.81%, and 93.27%, respectively. Analysis of sugar composition performed on the FVOs by GC indicated that they consist of fucose, mannose, glucose, and galactose. The products were white and water soluble powders. The FT-IR spectra of the FVOs showed peaks at 2925–3209 cm^{-1} (O—H), 1635 cm^{-1} (C=O), 1401 cm^{-1} (symmetrical deformation of $-CH_3$ and $-CH_2$), 1100 cm^{-1} (furanoside), and 857 cm^{-1} (the special absorb peaks of β-glucoside bond) (Fig. 1).

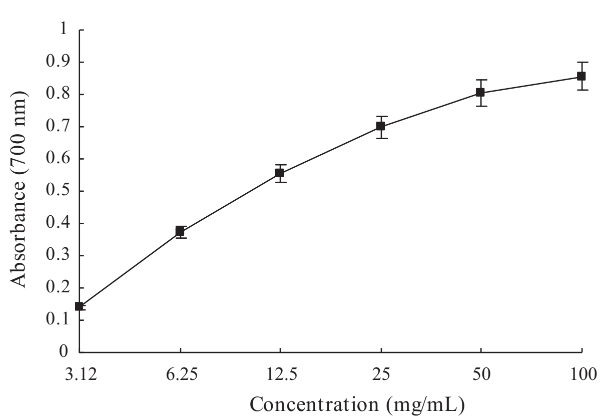

Fig. 3. Reducing capacity of *Flammulina velutipes* oligosaccharides. Data are shown as mean ± SD ($n = 3$).

3.3. HRSA and reducing capacity of FVOs

Among the reactive oxygen species, the hydroxyl radical is the most reactive and could induce severe damage to adjacent biomolecules. Therefore hydroxyl radical has been widely used as a tool for the evaluation of the radical scavenging activities of antioxidants (Yao et al., 2013). The scavenging activities of FVOs increased with concentration increasing up to 100 μg/mL, indicating a concentration-dependent manner (Fig. 2). At the concentration of 100 μg/mL, HRSA of FVOs reached up to 80.24% (Fig. 2). The reducing capacity of a material could be a significant indicator of its potential antioxidant activity (Fan, Li, Deng, & Ai, 2012). Fig. 3 shows the reductive activities of FVOs using the $K_3Fe(CN)_6$ reduction method. The reducing capacity of FVOs increased with the increase of sample concentration. At the concentration of 100 mg/mL, the reducing capacity (absorbance at 700 nm) of FVOs was 0.856. These data on reducing capacity of the tested FVOs indicated that it could play a role in the antioxidant observed.

4. Conclusions

In the present study, water soluble FVOs from *F. velutipes* were prepared by hydrolysis with H_2O_2. The hydrolysis conditions were optimized by orthogonal test. Preliminary structural characterizations were conducted using chemical properties and FTIR spectrum. The FVOs are natural antioxidants and may be potential functional food ingredients.

Acknowledgment

This research was supported by A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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