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Preparation of polysaccharides from cyanobacteria *Nostoc commune* and their antioxidant activities

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ABSTRACT

In this study, water soluble polysaccharides were prepared from cyanobacteria *Nostoc commune* by water extraction. Factors affecting the polysaccharide yields were investigated, and the optimum extraction conditions were determined as follows: time, 4 h; temperature, 90 °C; the ratio of liquid to solid, 60:1 (v/w); and extraction times, 4. The extract was filtered, concentrated to ~10% (w/v), precipitated with 3 volumes of ethanol, freeze-dried, and ground to yield a water soluble power. The polysaccharide content of the product was 96.7%, and the yield was 9.18% (w/w). Fourier transform infrared spectra demonstrated that the product samples were mainly composed of polysaccharides. The polysaccharides showed high hydroxyl radical scavenging activity (92.71%) and reducing capacity (0.445) at the concentration of 10 mg/mL.

1. Introduction

Nostoc is a genus of the nitrogen-fixing cyanobacteria family of Nostocaceae and has the ability to use atmospheric nitrogen when combined nitrogen is not available (Morsy, Kuzuha, Takani, & Sakamoto, 2008). The species *Nostoc commune* is a prominent component of microbial populations worldwide, distributed from the tropics to the polar regions of the earth (Jensena et al., 2013). Traditionally, some species of *Nostoc*, including *N. commune*, have been used in China as a food source or as medicine to treat illness (Li et al., 2011).

N. commune forms macroscopic colonies in natural habitats with filaments embedded in extracellular polysaccharides (EPSs) (Pereira et al., 2009). It is believed that the EPSs of *N. commune* play a role in the stabilization of cells in the air-dried state, inhibiting the fusion of membrane vesicles during desiccation and acting as an immobilization matrix for secreted enzymes, which stay fully active after long-term air-dried storage (Jensena et al., 2013). Thus, *N. commune* can resist extreme desiccation and readily restore metabolic activity upon rehydration (Potts, 1994).

However, the main function attributed to the EPSs of *N. commune* is to play important roles in protecting the organism itself from a range of physical as well as biological stresses. The EPSs of *N. commune* have been studied considerably in recent years for their viscosities (Huang, Liu, Paulsen, & Klaveness, 1998), strong effect on the complement system, and reactive oxygen species scavenging ability (Tang, Hu, & Chen, 2007). However, the antioxidant activity of the EPSs of *N. commune* has not been frequently reported (Li et al., 2011).

In the present study, the extraction conditions of the EPSs of *N. commune* were optimized, the EPSs of *N. commune* were partially characterized, and the antioxidant activities, including reducing capacity and high hydroxyl radical scavenging activity (HRSA), were determined.

2. Materials and methods

2.1. Materials

N. commune was purchased from a local supermarket (Xinpu, China) and stored at 4 °C until use. All chemicals were reagent-grade.

2.2. Extraction of N. commune EPSs

The dried colonies were powdered and extracted with organic solvents in a Soxhlet apparatus (light petroleum, acetone and methanol). The materials were soaked in distilled to yield a suspension with varying ratios of liquid to solid (30, 40, 50, 60, 70, and 80, respectively, v/w). The reactor was maintained in a thermostatic water bath at different temperatures (75, 80, 85, 90, 95, and 100 °C, respectively) for varying times (1, 2, 3, 4, 5, and 6 h, respectively). After the first extraction, the suspensions were filtered through a Whatman GF/A filter paper. The filter residues were suspended in distilled water for the second extraction as described above. The extraction processes were performed for 6 times.

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2.3. Recovery of N. commune EPSs

The suspensions were filtered through a Whatman GF/A filter paper, concentrated to ~15% (w/v), proteins removed by Sevag method, precipitated with 3 volumes of absolute ethanol, filtered through Whatman GF/A filter paper again, and freeze-dried. The % yield of *N. commune* EPSs was calculated using Eq. (1).

$$\text{Yield} = 100 \times \frac{W_2}{W_1} \quad (1)$$

where W_1 and W_2 represent the weights of the recovered *N. commune* EPSs and the original *N. commune*, respectively.

2.4. Analytical methods

Ash, moisture, and total sugar contents of the samples were determined according to standard methods (Hou, 2004). 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 400–4000 cm^{-1}.

2.5. Antioxidant activity assays

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Andrews (1986). *N. commune* EPSs 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 absorbance, A_1 is the positive control absorbance, A_2 is the absorbance of the sample.

Reducing capacity was measured according to method described by Qiao et al. (2009) with slight modifications. Briefly, One milliliter of *N. commune* EPSs 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 the reaction mixture was shaken and its absorbance was detected at 700 nm against a blank (water instead of *N. commune* EPSs 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.

2.6. Statistical analysis

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 extraction of N. commune EPSs

The effect of time on the extraction of *N. commune* EPSs was determined over a period of 6 h (Fig. 1). The yields increased with time up to 6 h and then leveled off. Therefore, the optimal reaction time was 6 h.

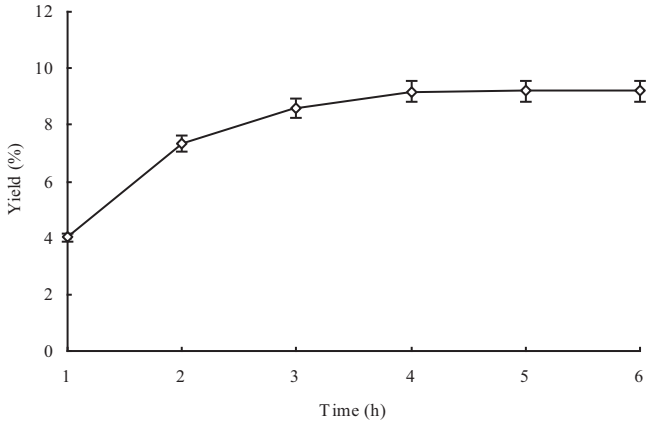

Fig. 1. Effect of time on extraction of *N. commune* EPSs. Data are shown as mean ± SD ($n = 3$).

3.2. Effect of temperature, the ratio of liquid to solid, and extraction times on extraction of N. commune EPSs

The reaction temperature, the ratio of liquid to solid, and extraction times affected extraction of *N. commune* EPSs. The temperatures studied were from 75 °C to 100 °C. The maximum yield (9.18%) was obtained at 90 °C (Fig. 2), ratio of liquid to solid 60:1 (v/w) (Fig. 3), and extraction 4 times (Fig. 4). Higher temperature increased the yields of *N. commune* EPSs due to the greater diffusion rate. On the other hand, the decrease in yields at 100 °C can be attributed to the evaporation of H_2O. Similarly, higher ratio of liquid to solid and more extraction times favored the yield of *N. commune* EPSs. In contrast to our findings, Sheng, Zhai, and Chen (2001) extracted *N. commune* EPSs at 98 °C for 3.8 h and 3 times.

3.3. Product characterization

The FTIR spectra of the product samples peaked at ~3396 cm^{-1} (O—H) (symmetrical deformation of —CH_3 and —CH_2), ~1072 cm^{-1} (stretching vibration of the C—O—C in glucose circle), and ~1619 cm^{-1} (special absorbance peaks of aldehyde in peach gum polysaccharides) (Fig. 5). These data also demonstrated that the product samples were mainly composed of polysaccharides. Ash, moisture, and total sugar contents were 0.8%, 1.1% and 96.7% (w/w), respectively. All product samples consisted of water-soluble white powders.

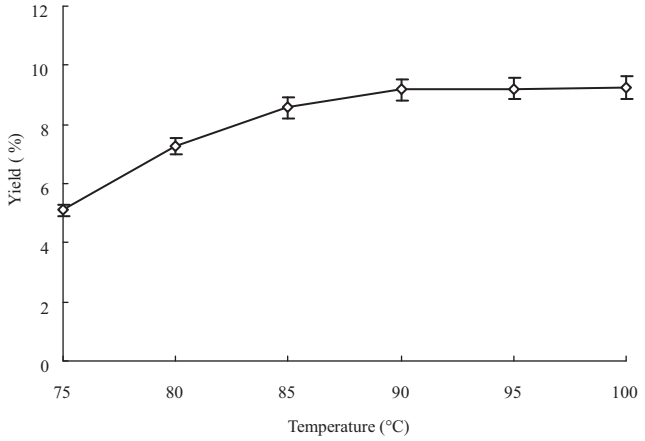

Fig. 2. Effect of temperature on extraction of *N. commune* EPSs. Data are shown as mean ± SD ($n = 3$).

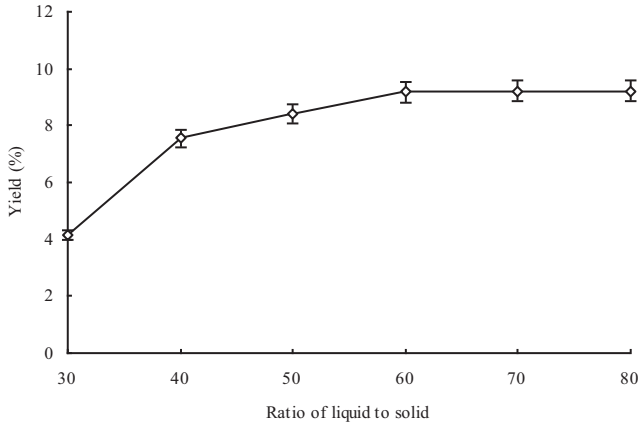

Fig. 3. Effect of the ratio of liquid to solid on extraction of *N. commune* EPSs. Data are shown as mean ± SD ($n = 3$).

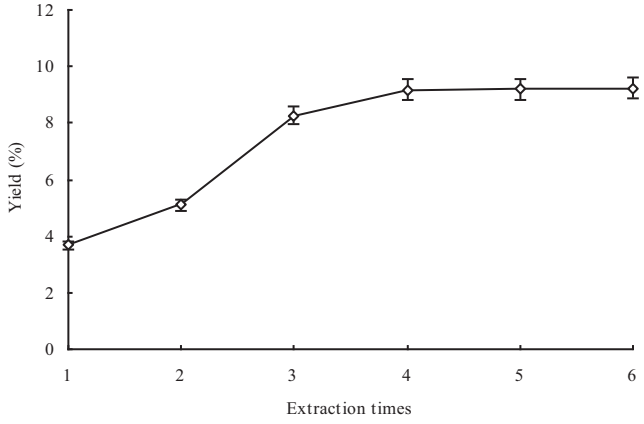

Fig. 4. Effect of extraction times on extraction of *N. commune* EPSs. Data are shown as mean ± SD ($n = 3$).

3.4. HRSA and reducing capacity of N. commune EPSs

Hydroxyl radicals (HO•) have the highest activity among reactive oxygen species and induce severe damage to biomolecules. HO• has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Qiao et al., 2009). The HRSA of *N. commune* EPSs was presented in Fig. 6. The scavenging effect increased with concentration up to 10 mg/mL. At a concentration of 10 mg/mL, the HRSA was 92.71%. Apparently, *N. commune* EPSs showed high HO• scavenging activity.

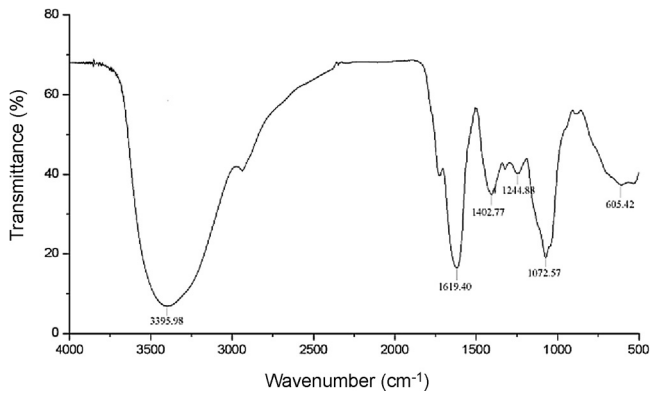

Fig. 5. FT-IR spectra of peach gum polysaccharides.

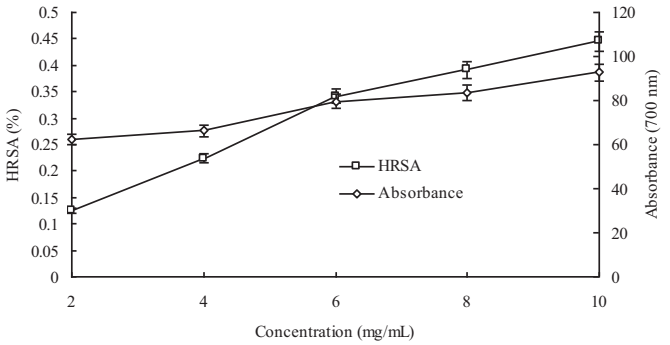

Fig. 6. Hydroxyl radical scavenging activity (HRSA) and reducing capacity of *N. commune* EPSs. Data are shown as mean ± SD ($n = 3$).

Reducing capacity is also essential to assess the antioxidant activity (Duh, Du, & Yen, 1999). Fig. 6 shows the reducing capacity of *N. commune* EPSs using the $K_3Fe(CN)_6$ reduction method. The reducing capacity of *N. commune* EPSs increased with the sample concentration. At the concentration of 10 mg/mL, the reducing capacity (absorbance at 700 nm) of *N. commune* EPSs was 0.445. It has been reported that there was a direct correlation between antioxidant activity and reducing capacity (Qiao et al., 2009). The results indicate that there could be a direct correlation between antioxidant activity and reducing capacity in *N. commune* EPSs.

4. Conclusions

In this study, water soluble *N. commune* EPSs were prepared by water extraction. The extraction process was monitored by the yield of the polysaccharides. Higher temperature and ration of liquid to solid, more extraction times, and prolonged time favored polysaccharides extraction. The polysaccharides were partially characterized and their antioxidant activity was estimated. The polysaccharides had high antioxidant activity and reducing capacity.

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