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Short communication

Cellulase-assisted extraction of polysaccharides from *Cucurbita moschata* and their antibacterial activity

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ABSTRACT

In this study, cellulase-assisted extraction of water soluble polysaccharides from pumpkin (*Cucurbita moschata*) and their antibacterial activity were investigated. The polysaccharides yield was monitored during the extraction process. The optimum extraction conditions were determined as follows: time, 40 min; temperature, 55 °C; pH, 4.5; and cellulase amount, 4000 U/g. The extracts were centrifuged, filtered, proteins removed by Sevag method, concentrated to ∼15% (w/v), precipitated with 5 volumes of absolute ethanol, freeze-dried, and pulverized to yield a water soluble powder of pumpkin polysaccharides (PP). The sugar content of the product was 68.3%, and the yield was 17.34% (w/w), respectively. The PP had high antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* at the concentration of 100 mg/mL.

1. Introduction

Pumpkin (*Cucurbita moschata*) is an annual herbaceous plant of the family *Cucurbitaceae*. The fruit of pumpkin is one of the most important vegetables in traditional agricultural systems in the world (Maran, Mekala, & Manikandan, 2013). Pumpkin and its products are rich in nutrition, e.g. 100 g pumpkin powder contains 4.09 g fat, 21.06 g carbohydrate, and 301.57 mg calcium (Zhang & Guo, 2011).

Among the nutrients in pumpkin, the polysaccharides attracted more attention. Pumpkin polysaccharides (PP) are composed of galactose, glucose, arabinose, xylose, and glucuronic acid and are water insoluble but organic solvents soluble macromolecular compounds with important biological functions (Maran et al., 2013; Song et al., 2012; Yang, Zhao, & Lv, 2007). The PP have the biological effects of detoxification, anti-oxidation, reducing blood pressure, reducing blood lipids, and lowering cholesterol levels (Li et al., 2010; Nara, Yamaguchi, Maeda, & Koga, 2009; Wang, Zhang, & Dong, 2012; Yong, Ning, & Liu, 2006). However, the antibacterial activity of the PP was not frequently reported (Wan, Liu, Zhao, Zhou, & Liu, 2012a, 2012b).

The common extraction methods of polysaccharides from plant tissues are maceration, mechanical rabbling, heat reflux, ultrasound assistance, and acidic hydrolysis. These extraction methods require either long extraction time, or high extraction temperature, or expensive equipments, or environmental polution (Chen, Li, Liu, Yang, & Li, 2012; Maran et al., 2013; Ptichkina, Markina, & Rumyantseva, 2008). In contrast, an enzyme hydrolytic technology seems environmentally safe and more effective in terms of polysaccharides yield. However, enzyme-assistied extraction of the PP was also not frequently reported (Ptichkina et al., 2008).

In the present study, cellulase-assisted extraction of the PP was developed, the extraction conditions were optimised, and the antibacterial activity of the PP was determined.

2. Materials and methods

2.1. Materials

Fresh pumpkins (*C. moschata*), with similar maturity and weight, were purchased from a farmers market (Xinpu, China). The skin and seeds were peeled and the pulps were washed with tap water. The washed pulps were dried in the hot air oven (JK-OOI-240A, China) at 45 °C until a constant weight was observed. The dried pulps were then pulverized and sifted through a 60-mesh sieve to obtain the fine powder (moisture content ∼10% in dry basis). The powder was stored in dark bags and kept in dry environment until used.

2.2. Extraction of the PP

The dried pumpkin powder was extracted with organic solvents in a Soxhlet apparatus (light petroleum, acetone, and methanol).

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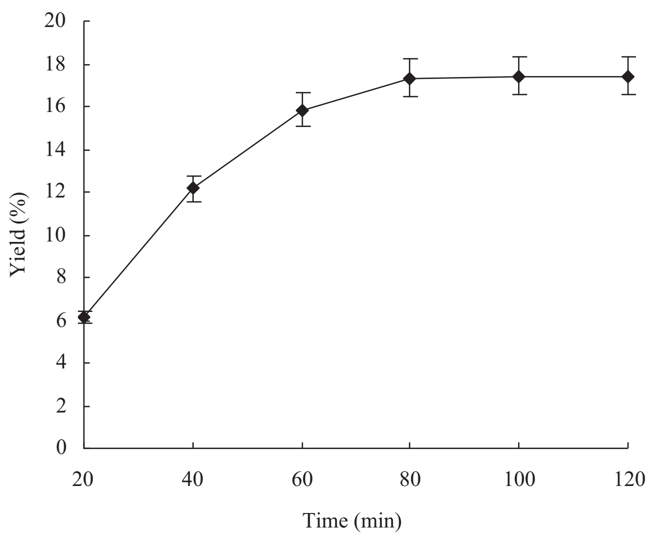

Fig. 1. Effect of extraction time on extraction of *Cucurbita moschata* polysaccharides with cellulase. Data are shown as mean ± SD (n = 3).

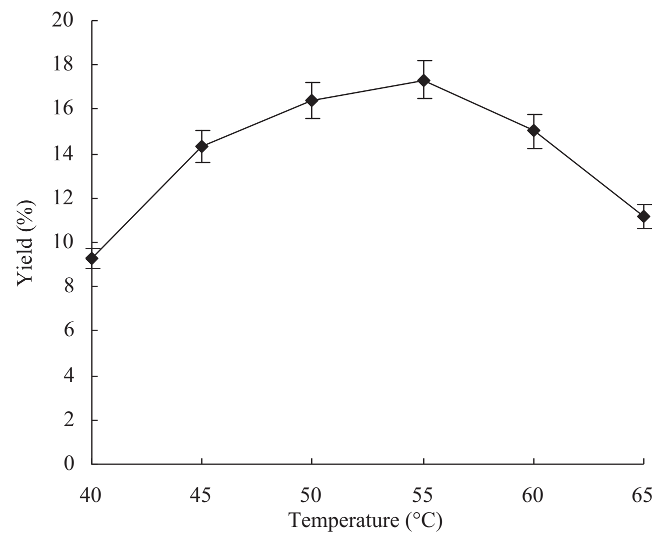

Fig. 2. Effect of temperature on extraction of *Cucurbita moschata* polysaccharides with cellulase. Data are shown as mean ± SD (n = 3).

The materials were soaked in distilled water to yield a suspension at a concentration of 1% (w/v). The pH of the suspension was adjusted to 3, 3.5, 4, 4.5, 5, and 5.5, respectively. To the above suspension, varying amounts of cellulase (1000, 2000, 3000, 4000, 5000, and 6000 U/g, respectively) were added. The reactor was maintained in a thermostatic water bath at different temperatures (40, 45, 50, 55, 60, and 65 °C, respectively).

2.3. Recovery of the PP

The hydrolysates were filtered through a Whatman GF/A filter paper, concentrated to ~15% (w/v), proteins removed by Sevag method, precipitated with 5 volumes of absolute ethanol, filtered through Whatman GF/A filter paper again, and freeze-dried. The % yield of PP was calculated using Eq. (1).

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

where W_1 and W_2 represent the weights of the recovered PP and the original pumpkin powder, respectively.

2.4. Analytical methods

The pH of the solution was recorded using a digital pH meter (Model: PHS-3C, CD Instruments, China). 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).

2.5. Antibacterial activity assay

Common pathogens like *Bacillus subtilis, Staphylococcus aureus, and Escherichia coli* were used to test the antibacterial activity of the PP. 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 disc was inoculated with 100 mg/mL PP. The sterile water inoculated disc was used as control. The antibacterial assessment was performed in duplicate and the average results were reported. The bacterial resistance of the PP samples was examined for a zone of inhibition of the disc (diameter 8 mm). The total diameter of the inhibition zone was measured.

2.6. Statistical analysis

All data are presented as mean ± SD 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 the PP

Reaction time affects the cellulase activity and is important for efficient hydrolysis. The effect of time on the extraction of the PP was determined over a period of 120 min (Fig. 1). The yields increased with time up to 80 min and then levelled off. Therefore, the optimal reaction time was 80 min.

3.2. Effect of temperature, pH, and cellulase amount on extraction of the PP

Temperature and pH of a reaction mixture also can influence cellulase activity and subsequently influence polysaccharides hydrolysis. In addition cellulase amount may also be important for efficient hydrolysis. Thus, the effects of different pH, temperature, and cellulase amount on extraction of the PP were investigated. The reaction was found to be optimal at 55 °C (Fig. 2), pH 4.5 (Fig. 3), and cellulase amount of 4000 U/g (Fig. 4). In contrast, a previous report has described optimal conditions for cellulase-assisted extraction of polysaccharides as pH 5.0, temperature of 56 °C, cellulase amount of 3500 U/g, and extraction time of 40 min. The varied reported optimal pH value, temperature, cellulase amount, and extraction time may be due to differences in substrates and enzyme sources.

3.3. Product characterisation

The ash, moisture, protein, and total sugar contents were 0.9%, 1.3%, 21.7%, and 68.3% (w/w), respectively. The DE of the resulting products was 1.97, indicating that the average degree of

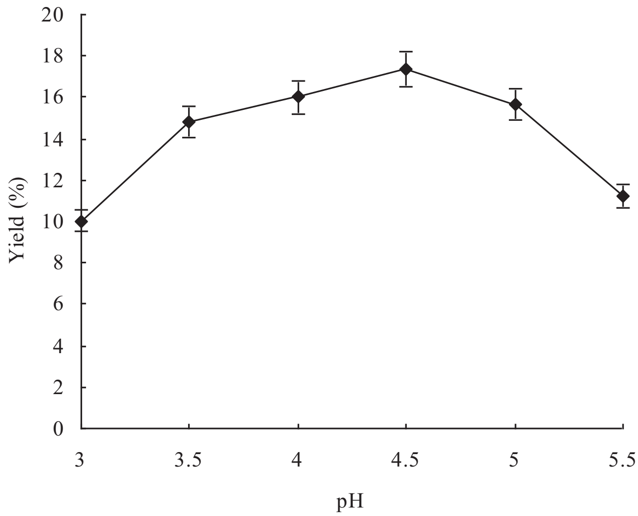

Fig. 3. Effect of pH on extraction of *Cucurbita moschata* polysaccharides with cellulase. Data are shown as mean ± SD ($n = 3$).

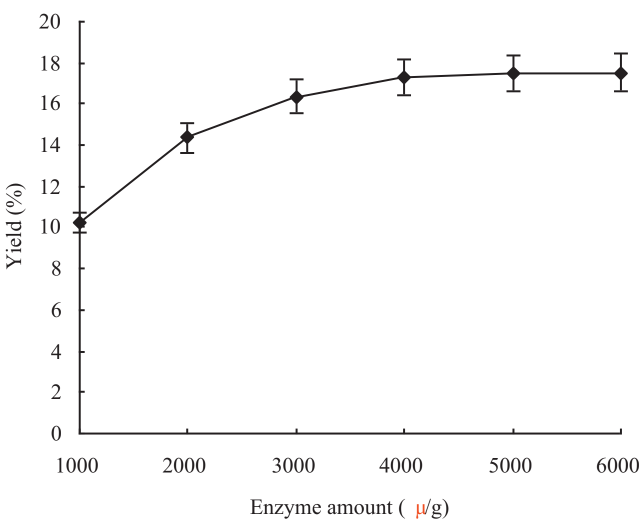

Fig. 4. Effect of cellulase amount on extraction of *Cucurbita moschata* polysaccharides. Data are shown as mean ± SD ($n = 3$).

polymerisation was ∼50. All product samples consisted of water-soluble powders.

3.4. Antibacterial activity of the PP

The antibacterial activity of the PP is shown in Fig. 5. The inhibition zone diameter was 9.78 mm, 13.21 mm, and 11.36 mm for *B. subtilis, S. aureus, and E. coli*, respectively by PP, indicating that the PP had high antibacterial activity. Similarly, Wan et al. observed that the PP could effectively inhibit the growth of *E. coli* (2012).

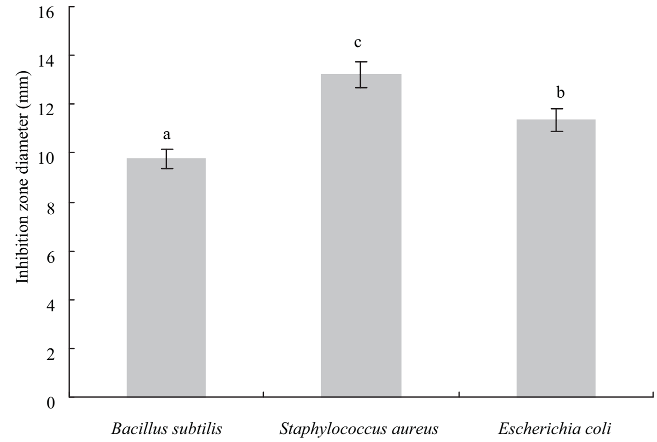

Fig. 5. Antibacterial activity of *Cucurbita moschata* polysaccharides. Different letters on the bars indicate the significant differences ($P < 0.05$). Data are shown as mean ± SD ($n = 3$).

4. Conclusions

In this study, water soluble PP were prepared by cellulase-assisted extraction. The extraction process was monitored by the yield of the PP. The PP yield was affected by temperature, pH, cellulase amount, and reaction time. The PP was partially characterized and their antibacterial activity was determined. The results show that the PP high antibacterial activity.

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