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Preparation and characterisation of the oligosaccharides derived from Chinese water chestnut polysaccharides

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ABSTRACT

Hydrogen peroxide (H_2O_2) is a strong oxidant that cleaves glycosidic bonds in polysaccharides. In this study, the oligosaccharides were prepared by removing the starch from Chinese water chestnuts through hydrolysis using α-amylase and then hydrolysing the remaining polysaccharides with H_2O_2, during which the oligosaccharide yield was monitored. The yield of oligosaccharide was affected by reaction time, temperature, and H_2O_2 concentration. Extended reaction times, high temperatures, and high H_2O_2 concentrations decreased oligosaccharide yield. Under optimum conditions (i.e., reaction time of 4 h, reaction temperature of 80 °C, and 2.5% H_2O_2 concentration), the maximum oligosaccharide yield was 3.91%. The oligosaccharides derived from Chinese water chestnuts polysaccharides exhibited strong hydroxyl and 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity when applied at a concentration of 100 μg/mL. The results indicate that the oligosaccharides derived from Chinese water chestnuts polysaccharides possessed good antioxidant properties and can be developed as a new dietary supplement and functional food.

1. Introduction

Chinese water chestnut (*Eleocharis dulcis*) belongs to the sedge family, which grows in marshes or ponds. This herbal plant is a very popular food amongst Asians because of its unique taste. The fruit possesses several health benefits, such as antimicrobial effects and antioxidant activity. It is also used to inhibit inflammation and treat pharyngitis and laryngitis (Zhan et al., 2014).

Previous reports have shown that Chinese water chestnut contains large quantities of starch (Mei et al., 2013; Singh, Bawa, Riar, & Saxena, 2009; Torres, Moreira, Chenlo, & Morel, 2013; Yadav, Guleria, & Yadav, 2013) and other polysaccharides composed of galactose and glucose at a ratio of 1:10 (Wang, Wang, & Li, 2012). However, the oligosaccharides derived from Chinese water chestnut have not been reported.

In this study, oligosaccharides from Chinese water chestnut were extracted by hydrolysing polysaccharides with hydrogen peroxide (H_2O_2), a strong oxidant that cleaves glycosidic bonds in polysaccharides (Tian, Liu, Hu, & Zhao, 2004). Optimum hydrolysis conditions and the antioxidant activities of Chinese water chestnut oligosaccharides (CWCOs) were subsequently investigated.

2. Materials and methods

2.1. Materials

Chinese water chestnut was purchased from a farmer's market (Xinpu, China). H_2O_2 (30%, v/v) was purchased from Laiyang Kant Chemical Co., Ltd. (Laiyang, China). α-amylase with 4000 U/mg activity was purchased from Fuchen Chemical Reagents Co. (Tianjin, China). All other chemicals used were of reagent grade.

2.2. Preparation of CWCOs

Chinese water chestnuts were washed with tap water, peeled, sliced, and dried in a hot air oven (JK-OOI-240A, China) at 60 °C to a constant weight. The dried fruit was then pulverised and sifted through a 60-mesh sieve.

The dried powder was suspended in tap water to yield a suspension with a concentration of 1% (w/v). The suspension was

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incubated at 90 °C for 5 min and cooled to 50 °C. About 20 mg of α-amylase was added to a reactor containing 500 mL of the suspension; this reactor was maintained in a thermostatic water bath at 50 °C for 4 h. An aliquot of the suspension was withdrawn and tested for the presence of starch using iodine solution. These experimental procedures were repeated to guarantee that starch was completely removed from the suspension. Varying concentrations of H_2O_2 (1%, 1.5%, 2%, 2.5%, 3%, and 3.5%) were added to the suspension, and the reactor was incubated in a thermostatic water bath at different temperatures (65 °C, 70 °C, 75 °C, 80 °C, 85 °C, and 90 °C) for designated time periods (1, 2, 3, 4, 5, and 6 h). Aliquots of the suspension were withdrawn periodically and cooled below 10 °C to terminate the reaction.

The hydrolysates were filtered and concentrated to approximately 20%. Proteins were removed using the Sevag method, precipitated using six volumes of absolute ethanol, filtered, and freeze dried. The percentage yield of CWCOs obtained was calculated using Eq. (1).

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

where W_1 and W_2 represent the weights of the recovered CWCOs and the original Chinese water chestnuts, respectively.

2.3. Analytical methods

Ash, moisture, 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 400–4000 cm^{-1}.

The hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Andrews (1986). The CWCOs HRSA 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 absorbance of the positive control, and A_2 is the absorbance of the sample.

2,2-Diphenyl-β-picrylhydrazyl radical scavenging activity (DRSA) was measured according to the method described by Qiao et al. (2009). Briefly, around 0.2 mL of DPPH free radical (DPPH.; 400 μmol/L in dehydrated alcohol) was added to 1.0 mL of the CWCOs solution. Then, 2.0 mL of H_2O was added to the solution. The mixture was shaken and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm against a blank standard (H_2O instead of CWCO and DPPH. solution). Lower absorbances of the reaction mixture indicate higher free-radical scavenging activities. The scavenging percentage was calculated by the following equation:

$$\text{DRSA}(\%) = \frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100 \quad (3)$$

where A_0 is the absorbance of the control (H_2O instead of CWCO solution), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample obtained under conditions identical to those in A_1 but using H_2O instead of DPPH· solution.

2.4. Statistical analysis

All experiments were carried out in triplicate. All of the data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare data between groups. A p value of <0.05 was considered statistically significant.

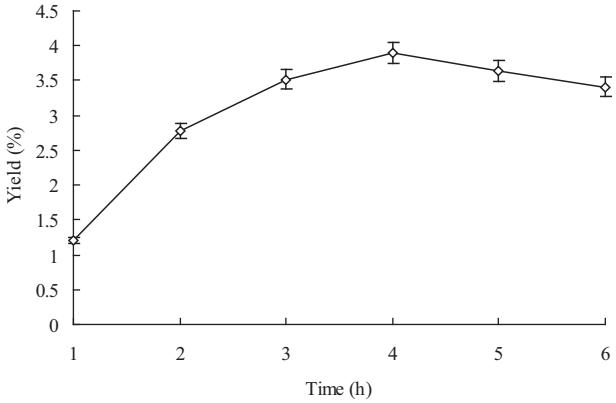

Fig. 1. Effect of time on the yield of Chinese water chestnut oligosaccharides. Bars represent the standard deviation. Data are shown as mean ± SD (n = 3).

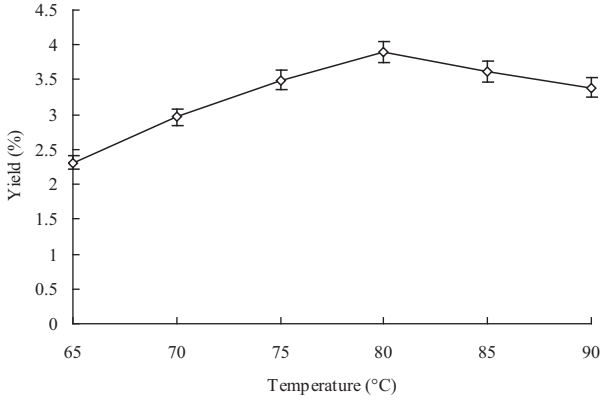

Fig. 2. Effect of temperature on the yield of Chinese water chestnut oligosaccharides. Bars represent the standard deviation. Data are shown as mean ± SD (n = 3).

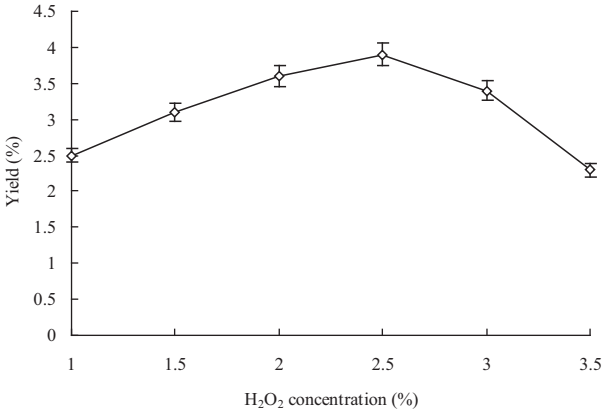

Fig. 3. Effect of H_2O_2 concentration on the yield of Chinese water chestnut oligosaccharides. Bars represent the standard deviation. Data are shown as mean ± SD (n = 3).

3. Results and discussion

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

The reaction time, temperature, and H_2O_2 concentration are important parameters to ensure efficient hydrolysis of the polysac-