

Short communication

Preparation and antioxidant activity of *Lycium barbarum* oligosaccharides

Long-Fa Jiang*

School of Food Engineering, Huaihai Institute of Technology, 59 Cangwu Road, Xinpū 222005, China

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ABSTRACT

In this study, the *Lycium barbarum* oligosaccharides (LBO) were prepared by hydrolysis using hydrogen peroxide (H_2O_2). The yield of the LBO was monitored during the hydrolysis process. The hydrolysis conditions were optimized as follows: time, 4 h; temperature, 70 °C; and H_2O_2 concentration, 2.5% (v/v). The hydrolysates were filtered, concentrated to ~20% (w/v), precipitated with 6 volumes of absolute ethanol, freeze-dried, and ground to yield a water soluble and white powder. The sugar content of the product was 95.8%, and the yield was 21.05% (w/w), respectively. The LBO show higher hydroxyl radical scavenging activity (86.46%) than Vc (40.96) at the concentration of 100 µg/mL.

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1. Introduction

Free radicals, produced by physical factors, chemical reactions, and metabolic processes, have a wide variety of pathological effects, such as causing DNA damage, carcinogenesis, and cellular degeneration related to aging (Liu, Ooi, & Chang, 1997). This harmful action of the free radicals can be blocked by antioxidant substances, which can scavenge the free radicals (Li, Li, & Zhou, 2007). There are two basic categories of antioxidant, i.e. synthetic and natural ones and natural antioxidants from plant extracts have attracted increasing interest due to consumer concern about the safety of the synthetic antioxidants in food.

Lycium barbarum L., a traditional Chinese herb possessing vital biological activities, such as prevention of cancer, is widely used in Asian countries (Wang, Chang, Stephen Inbaraj, & Chen, 2010). Several major functional components such as carotenoids, flavonoids, and polysaccharides were believed to be closely associated with this biological activity (Lin, Wang, Chang, Stephen Inbaraj, & Chen, 2009). However, there is a paucity of data regarding the antioxidant activity of oligosaccharides from *L. barbarum* Linnaeus.

In the present study, the *L. barbarum* derived oligosaccharides (LBO) were prepared from *L. barbarum* polysaccharides by hydrolysis using hydrogen peroxide (H_2O_2) and were tested for their free radical scavenging activities.

2. Materials and methods

2.1. Materials

L. barbarum was purchased from a local supermarket (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 LBO with H_2O_2

L. barbarum was soaked in distilled water at ~60 °C for 24 h and homogenized in a blender (Guangzhou Aoyu of Electronic Science and Technology Co. Ltd., Guangzhou Province, China) to yield a suspension with 1% concentration (w/v). H_2O_2 was added into a reactor containing 500 mL of *L. barbarum* suspension to yield a suspension with 2.5% H_2O_2 , and the reactor was maintained in a thermostatic water bath at 70 °C for 4 h. The hydrolysates were filtered through a Whatman GF/A filter paper, concentrated to ~20% (w/v), precipitated with 6 volumes of absolute ethanol, filtered through Whatman GF/A filter paper again and freeze-dried. The yield of LBO was calculated using Eq. (1).

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

where W_1 and W_2 represent the weights of the recovered oligosaccharides and the original *L. barbarum*, respectively.

* Tel.: +86 0518 85895427; fax: +86 0518 85895428.

E-mail address: jianglongfa008@sina.com

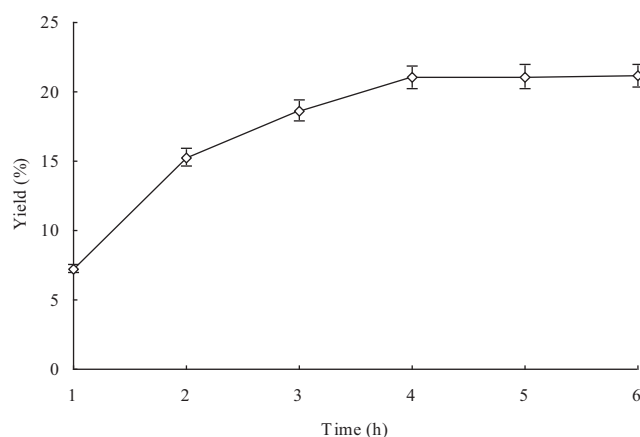


Fig. 1. Effect of time on hydrolysis of *L. barbarum* polysaccharides with H_2O_2 . Data are shown as mean $\pm$ SD ($n=3$).

2.3. Antioxidant activity assays

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Andrews (1986). Vc was used as control for this experiment. LBO hydroxyl scavenging activity was calculated as follows:

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

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.

2.4. Analytical methods

Ash, moisture, and total sugar 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. Statistical analysis

All data are presented as mean $\pm$ 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 *L. barbarum* polysaccharides with H_2O_2

The effect of reaction time on hydrolysis of *L. barbarum* polysaccharides by H_2O_2 was determined over a period of 6 h (Fig. 1). The yield sharply increased within 2 h (from 7.26% to 15.27%), gradually increased from 3 h to 4 h (from 15.27% to 18.64%) and leveled off after 4 h. Therefore, the optimal reaction time was adjudged to be 4 h.

3.2. Effect of temperature and H_2O_2 concentration on hydrolysis of *L. barbarum* polysaccharides

The reaction temperature and H_2O_2 concentration of a reaction mixture crucially affects hydrolysis of *L. barbarum* polysaccharides by H_2O_2 . The reaction was found to be optimal at 70 °C (Fig. 2) and 2.5% H_2O_2 (Fig. 3). The decrease in yield above 70 °C or 2.5% H_2O_2 can be attributed to the excessive hydrolysis

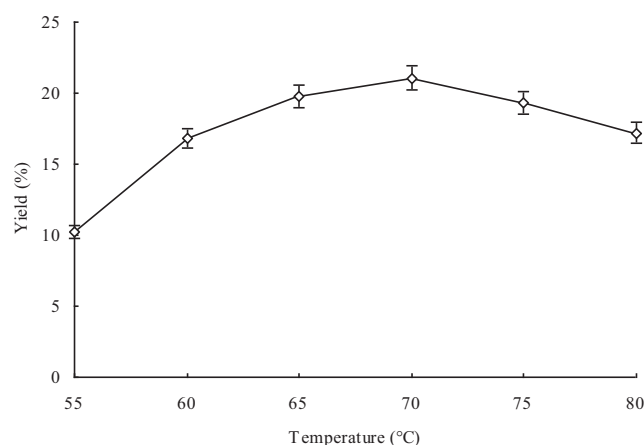


Fig. 2. Effect of temperature on hydrolysis of *L. barbarum* polysaccharides with H_2O_2 . Data are shown as mean $\pm$ SD ($n=3$).

of *L. barbarum* polysaccharides, which produced oligosaccharides with an extremely low degree of polymerization that made precipitation with ethanol difficult. In contrast to our findings, several studies reported that the optimal temperatures for chitosan extraction using 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, Cai, & Sun, 2012). The differences may be ascribed to the discrepancies in polysaccharide type, reaction time, and pH of reaction mixture.

3.3. Product characterization

Ash, moisture, and total sugar contents of the resulting product were 0.4%, 1.7% and 95.8% (w/w), respectively. The LBO yield was 21.05% (w/w). The DE of the resulting products was 7, indicating that the average degree of polymerization was ~ 14 . Interestingly, the water soluble powder showed white other than red probably due to the bleaching activity of H_2O_2 .

3.4. HRSA of LBO

Hydroxyl radicals ($HO^\bullet$) have the highest activity among reactive oxygen species and induce severe damage to biomolecules and thus has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Qiao et al., 2009). At a concentration of 100 mg/mL, the HRSA of the LBO was 86.46% (Fig. 4) and higher than that of Vc.

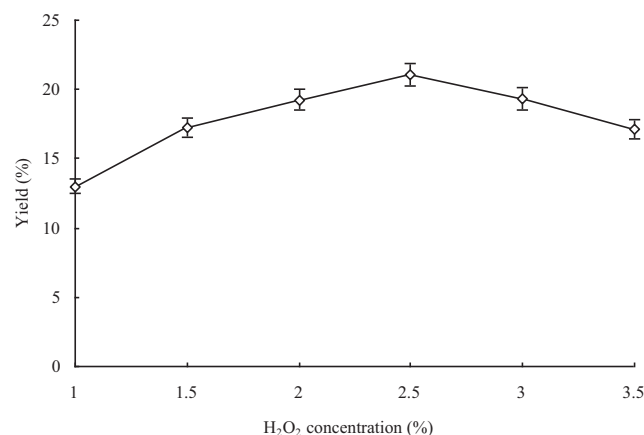


Fig. 3. Effect of H_2O_2 concentration on hydrolysis of *L. barbarum* polysaccharides. Data are shown as mean $\pm$ SD ($n=3$).

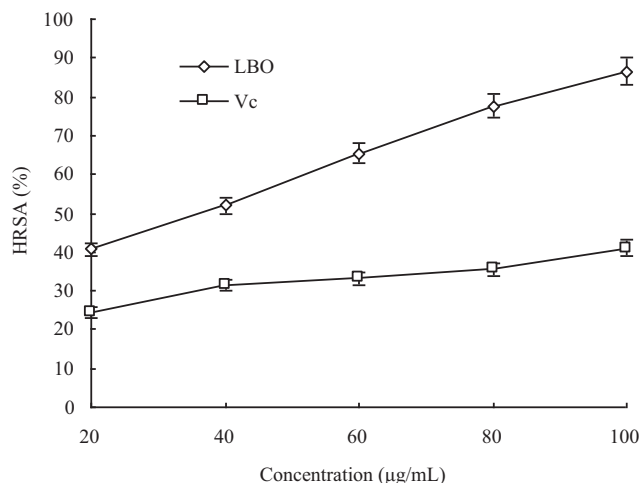


Fig. 4. Hydroxyl radical scavenging activity (HRSA) of *L. barbarum* oligopolysaccharides. Data are shown as mean $\pm$ SD ($n = 3$).

4. Conclusions

The LBO could be prepared from *L. barbarum* polysaccharides by hydrolysis using (H_2O_2). The hydrolysis process was monitored by the yield of the LBO. Higher temperature, higher H_2O_2 concentration, and prolonged time favored hydrolysis. However, too

high temperature and H_2O_2 concentration decreased the yield of the LBO. The LBO was partially characterized and their antioxidant activity was estimated. The average degree of polymerization of the LBO was ~ 14 . The results indicate that the LBO had higher HRSA than Vc, and was affected by the LBO concentration.

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