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Cellulase-assisted extraction and antioxidant activity of the polysaccharides from garlic

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ABSTRACT

In the present study, the polysaccharides were prepared from garlic by using a cellulase-assisted extraction method and the antioxidant activity of garlic polysaccharides (GPs) was evaluated. To improve the yield of GPs, the influences of the several factors such as extraction time, temperature, pH, and cellulase amount on the extraction efficiency were studied. The optimal conditions for extraction of GPs were determined as follows: time, 80 min; temperature, 45 °C; pH, 5; cellulase amount, 8000 U/g. Under the optimised extraction conditions, the yield of GPS reached up to 35.34%. The GPs product exhibited strong antioxidant activity including hydroxyl radical scavenging activity, 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity, and reducing power. The results suggest that the GPs could be used as potential antioxidants.

1. Introduction

Traditionally, garlic (*Allium sativum*) has been widely used in many ancient civilizations and has been considered throughout much of history as a remedy for many human diseases (Shouk, Abdou, Shetty, Sarkar, & Eid, 2014). Garlic possesses various biological benefits, e.g. hypocholesterolemic (Stevinson, Pittler, & Ernst, 2000), hypoglycemic (Jala, Bagheri, Moghimi, & Rasuli, 2007), antioxidant (Banerjee, Mukherjee, & Maulik, 2003), anticancer (Milner, 2001), and antiobesity effects (Lee, Kim, Kim, & Kim, 2011).

The components of garlic are water, carbohydrates, protein, fat, dietary fiber, essential amino acids, vitamins, minerals, and various organosulfur compounds such as alliin, allicin, ajoene, diallyl disulfide, diallyl trisulfide, allyl methanethiosulfinate, and S-allylcysteine (Joo, Kim, Kim, & Kim, 2013). Among the components of garlic, the organosulfur compounds (Joo et al., 2013; Krumm et al., 2012) and oil (Abdultawab & Ayuob, 2013; Cheng et al., 2013) attract more attention. However, little information is regarding the biological activity of garlic polysaccharides (GPs).

The common extraction methods of polysaccharides from plant tissues including maceration, mechanical rabbling, heat reflux, ultrasound assistance, and acidic hydrolysis, require either long extraction time, or high extraction temperature, or expensive equipments, or environmental pollution. In contrast, an enzyme hydrolytic technology seems environmentally safe and more effective in terms of polysaccharides yield (Qian, 2014). However, enzyme-assisted extraction of the GPs was not frequently reported.

In this study, we prepared the GPs by using a cellulase-assisted extraction method, optimised the extraction conditions and evaluated the antioxidant activity of the GPs.

2. Materials and methods

2.1. Materials

Garlic was purchased from a local supermarket (Xinpu, China). Cellulase, with an enzymatic activity of 30,000 U/g, was purchased from Beijing Shengshi Jiaming Technology Development Co. Ltd. (Beijing, China). Reagent-grade chemicals were used.

2.2. GPs extraction

GPs extraction was carried out according to the method described by Qian (2014) with slight modifications. Garlic was sliced and then dried in a hot air oven (JK-OOI-240A, China) at 60 °C for 2 h. The dried garlic was then pulverized and sifted through a 60 mesh sieve to obtain a fine powder with approximately 8% moisture content (dry basis).

The garlic powder was extracted with organic solvents (light petroleum, acetone and methanol) in a Soxhlet apparatus to separate liposoluble components, and then suspended in distilled water to yield a 1% (w/v) suspension in a reactor. The pH of the suspension

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was adjusted to 5, and 8000 U/g of cellulase was added. The reactor was maintained in a thermostatic water bath at 45 °C for 80 min.

The extracts were filtered through a Whatman GF/A filter paper and concentrated to approximately 20% (w/v). The proteins were removed by using the Sevag method, and the extracts were precipitated by adding 3 volumes of absolute ethanol, followed by filtration using a Whatman GF/A filter paper, and freeze drying. The % GPs yield was calculated using Eq. (1) as follows:

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

where W_1 and W_2 represent the weights of the recovered GPs and the original dried garlic powder, respectively.

2.3. Antioxidant activity assays

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Yao, Cao, and Wu (2013) with a slight modification. Hydroxyl scavenging activity of GPs 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 the positive control absorbance and A_2 is the absorbance of the sample.

2-diphenyl-β-picrylhydrazyl radical scavenging activity (DRSA) was measured by the method described by Yao et al. (2013). Briefly, 0.2 mL of DPPH radical solution (400 μmol/L in dehydrated alcohol) was added to 1.0 mL of the GPs solution, and then 2.0 mL of distilled water was added. 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 (distilled water instead of the GPs and DPPH radical solution). Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. 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 (distilled water instead of the GPs solution), A_1 the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with distilled water instead of DPPH radical solution.

Reducing power was measured according to method described by Qiao et al. (2009) with some modifications. Briefly, One milliliter of the GPs 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 the GPs solution) 10 min later. Absorbance of the reaction mixture indicates the reduction capability of sample.

$$\text{reducing power} = (A_1 - A_2) \quad (3)$$

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. 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).

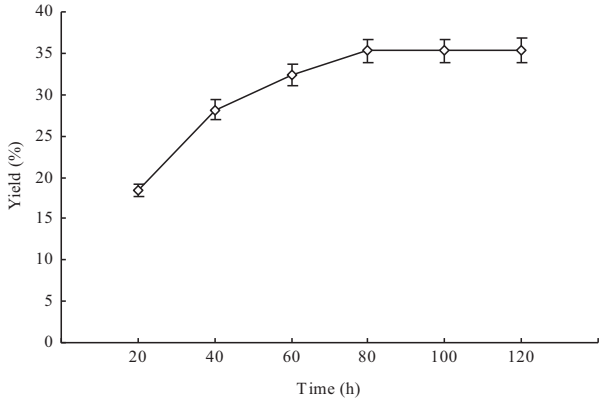

Fig. 1. Effect of reaction time on extraction of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

2.5. Statistical analysis

All experiments were performed in duplicate and analyses of all samples were run in triplicate. All data are presented as mean ± S.D. Statistical analysis was performed using Statgraphics Centurion XV version 15.1.02. The results obtained were analyzed using one-way analysis of variance (ANOVA) for mean differences among the samples. P-values of <0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Effect of time, temperature, pH, and cellulase amount on GPs extraction

Reaction time, temperature, and pH affect the cellulase activity and are important for efficient extraction of the GPs, while cellulase amount may also be important for efficient extraction of the GPs. Therefore, the effects of different reaction time, temperature, pH, and cellulase amount on extraction of the GPs were investigated. The reaction was found to be optimal at time 80 min (Fig. 1), 45 °C (Fig. 2), pH 5 (Fig. 3), and cellulase amount of 8000 U/g (Fig. 4). In contrast, a previous report has described optimal conditions for cellulase-assisted extraction of polysaccharides as time 40 min, 55 °C, pH 4.5, and cellulase amount of 4000 U/g (Qian, 2014). The different reported optimal reaction time, temperature, pH value, and cellulase amount could be ascribed to the differences in substrates and enzyme sources.

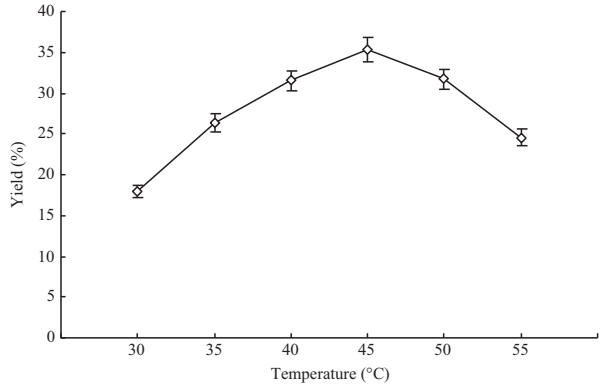

Fig. 2. Effect of temperature on extraction of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

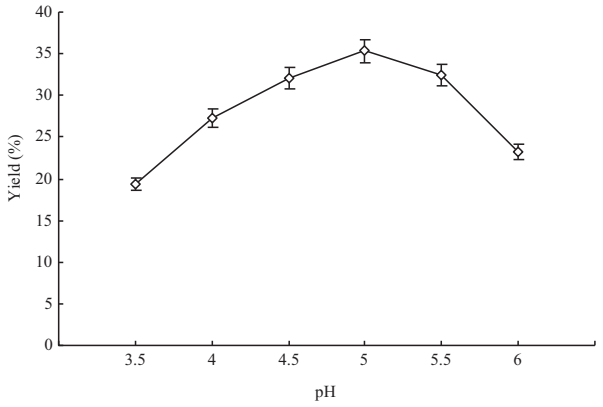

Fig. 3. Effect of pH on extraction of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

3.2. Product characterization

The ash, moisture, and total sugar contents in the GPs product were 1.7%, 3.9% and 92.81% (w/w), respectively. The GPs product samples were water-soluble and showed light yellow.

3.3. HRSA and DRSA of the GPs

Among the reactive oxygen species, the hydroxyl radical is the most reactive and could induce severe damage to adjacent biomolecules. The DPPH radical is a stable radical that can readily undergo scavenging by an antioxidant. Both hydroxyl radical and DPPH radical have been widely used as a tool for the evaluation of the radical scavenging activities of antioxidants (Qiao et al., 2009). Both HRSA and DRSA were presented in Fig. 5. The scavenging effects of GPs increased with concentration increasing up to 10 mg/mL, indicating a concentration-dependent manner. At the concentration of 10 mg/mL, HRSA and DRSA were 61.25% and 72.68%, respectively, indicating that the GPs have strong radical scavenging activity.

3.4. Reducing power of the GPs

Reducing power of a material is also an index for evaluation of its antioxidant activity. The reducing natures are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Chen, Zhang, Jiang, Mu, & Miao, 2012). The reductive activities of the GPs using the $K_3Fe(CN)_6$ reduction method are shown in Fig. 6. The reducing power of the GPs increased with the increase of sample concentration, also indicating a concentration-dependent manner. The reducing power (absorbance at 700 nm) of the GPs was 0.53 at the concentration of 10 mg/mL.

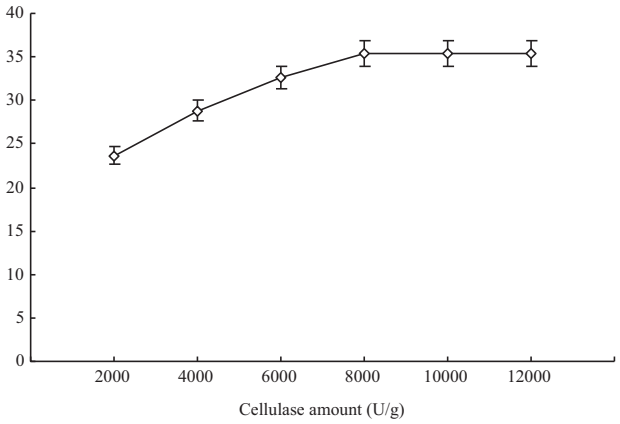

Fig. 4. Effect of cellulose amount on extraction of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

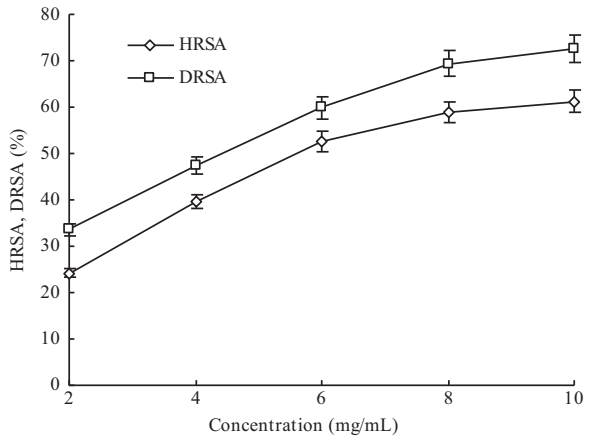

Fig. 5. Hydroxyl radical scavenging activity (HRSA) and 2, 2-diphenyl-β-picrylhydrazyl radical scavenging activity (DRSA) of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

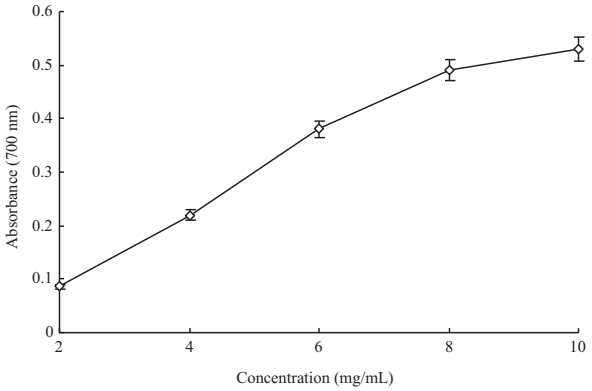

Fig. 6. Reducing capacity of garlic polysaccharides. Bars represent the standard deviation. Data are shown as mean ± S.D. (n = 3).

4. Conclusions

The GPs can be effectively prepared by using a cellulase-assisted extraction method. Maximum GPs yield was obtained at time, 80 min; temperature, 45 °C, pH 5, and cellulase amount, 8000 U/g. The results show that the GPs exhibited a strong hydroxyl radical scavenging, DPPH radical scavenging, and reducing power and could be explored as a novel and potential natural antioxidant agent for use in functional foods.

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