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Short communication

Preparation and antioxidant activities of oligosaccharides from *Crassostrea gigas*

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ABSTRACT

Oligosaccharides were prepared from *Crassostrea gigas* by hydrolysis of polysaccharide in *C. gigas* with peroxide oxygen (H_2O_2). The hydrolysates were cleared of protein, filtered, ultrafiltered and precipitated with absolute ethanol to give *C. gigas* oligosaccharides (CGOs). Factors affecting CGO yields, i.e., reaction time, temperature, and H_2O_2 concentration, were optimised as follows: 2.96 h reaction time, 84.71 °C reaction temperature, and 2.46% H_2O_2 concentration. Under these conditions, the maximum yield of CGOs reached 10.61%. The CGOs were then partially characterised by Fourier transform infrared spectroscopy, UV spectroscopy, monosaccharide composition, and antioxidant activities. Results indicate that CGOs possessed strong hydroxyl radical activity, 2,2-diphenyl-β-picrylhydrazyl-radical-scavenging activity and reducing capacity at a concentration of 100 μg/mL.

1. Introduction

Free radicals are produced by many factors, such as physical effects, chemical reactions, and metabolic processes. Free radicals exert many pathological effects on living organisms, such as DNA damage, thereby causing carcinogenesis and inducing aging-related cellular degeneration (Liu, Ooi, & Chang, 1997). A variety of antioxidants can scavenge strong radicals (Liu et al., 1997). These antioxidants can be divided into synthetic chemicals, such as phenolic compounds and various naturally occurring substances, such as saccharides. Moreover, natural antioxidants from organism extracts have attracted increasing interest because of consumer concern about the safety of synthetic antioxidants in food (Yao, Cao, & Wu, 2013).

Crassostrea gigas, one major cultured shellfish in China, is nutritious and delicious and is thus considered a valuable food resource (Hou et al., 2014). Recent research found that *C. gigas* is rich in polysaccharides [4–8]. Gao et al. found that the water-soluble polysaccharide from *C. gigas* comprised mainly of →4)-α-D-Glc-(1→ with few →3,4)-β-D-Glc-(1→ and →2,4)-β-D-Glc-(1→ branched units) (Gao, Zhao, Wang, & Luan, 2014). *C. gigas* polysaccharides have been demonstrated to contain many functional activities, such as immunostimulatory (Jiang, Zhang, & Zhao, 2013), antimicrobial (Tian et al., 2013), antitumour (Chen et al., 2010), hepatoprotective (Shi et al., 2015) and antihypertensive (Wang et al., 2016) activities. However, data on the *C. gigas* oligosaccharides (CGOs) are limited.

At present, a number of methods for preparation of oligosaccharides from different organisms have been developed, e.g. peroxide oxygen (H_2O_2)-assisted preparation (Wu & Yu, 2015), microwave-assisted preparation (Li, Zhao, Lv, Li, & Yu, 2015) and enzymatic-assisted preparation (Tao et al., 2016). Nevertheless, the data regarding peroxide oxygen (H_2O_2)-assisted preparation of CGOs are limited.

In the present study, we prepared water-soluble CGOs from *C. gigas* by hydrolysis with H_2O_2, partially characterised the product and evaluated their antioxidant activities.

2. Materials and methods

2.1. Materials

Live *C. gigas* were purchased from a local farmers' market (Xinpu, China)·H_2O_2 (30%, v/v) was purchased from the Laiyang Kant Chemical Co., Ltd. (Laiyang, China). All other reagents used were of analytical grade.

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2.2. Preparation of CGOs

C. gigas meat, including the adductor muscle and mantle was stripped from the shells, shredded, dried in a hot air oven (JK-OOI-240A, China) at 60 °C to a constant weight, pulverised and sifted through a 40-mesh sieve.

The lipids in the dried powder were removed by the Soxhlet extraction method using light petroleum as the solvent. The lipid-removed powder was suspended in distilled water to give a suspension with a concentration of 2% (w/v)·H_2O_2 (2%, 2.5% and 3%, respectively) were added to the reactor containing 100 mL of the suspension, and the reactor was incubated in a thermostatic water bath at different temperatures (80, 85, and 90 °C, respectively) for designated time periods (2, 2.5 and 3 h, respectively).

The hydrolysates were filtered, proteins were removed using the Sevag method, concentrated (approximately 10%), precipitated using six volumes of absolute ethanol, filtered again and freeze-dried. The percentage yield of CGOs was calculated using Eq. (1) as follows:

$$\text{Yield} = 100\,W_2/W_1 \tag{1}$$

where W_1 and W_2 represent the weights of the recovered CGOs and the original *C. gigas* powder, respectively.

2.3. CGO characterisation

Total sugar, protein and reducing sugar contents were determined using the phenol–sulphuric acid colorimetric method, the Kjeldahl method and a method proposed by Hou (2004). Meanwhile, monosaccharide composition was analysed using the procedure reported by Sheng et al. (2007). The Fourier transform infrared (FTIR) spectra of the resulting CGO sample was recorded in KBr pellets by a Nicolet Nexus FTIR 470 spectrophotometer (Nicolet, USA) over a wavelength range of 400 cm^{-1} to 4000 cm^{-1}. The UV spectra were recorded on a UV spectrometer (Spectra Test, German).

2.4. Antioxidant activity assays

The hydroxyl radical ($HO^{\cdot}$) scavenging activity (HRSA) of the CGO sample was measured in accordance with the method of Qu, Li, Zhang, Zeng, and Fu (2016). The hydroxyl-radical-scavenging activity was calculated as follows:

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

where A_0 is the absorbance of the reagent blank absorbance, A_1 is the positive control absorbance and A_2 is the absorbance of the sample.

2-Diphenyl-β-picrylhydrazyl-radical-scavenging activity (DRSA) was determined in accordance with the method of Carmona-Jiménez, García-Moreno, Igartuburu, and Garcia Barroso (2014). The DPPH-free-radical-scavenging percentage was calculated by the following equation:

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

where A_0 is the absorbance of the control (water instead of CGOs solution), 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 DPPH solution.

Reducing capacity was measured in accordance with the method of Qiao et al. (2009). A higher absorbance indicates a better reducing capacity.

2.5. Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using Statgraphics Centurion XV version 15.1.02. A multifactor ANOVA with posterior multiple range test was used for determining statistical significance.

3. Results and discussion

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

The reaction conditions, i.e. time, temperature and H_2O_2 concentration, were optimised using a central composite design (CCD), which is shown along with the results in Table 1. The regression model was obtained by analysing the results of the experiment using a multifactor ANOVA as follows:

$$\begin{aligned} Y = {} & -895.96474 + 31.08210 \times X_1 + 18.61347 \times X_2 \\ & + 58.88526 \times X_3 - 0.021100 \times X_1X_2 - 0.50000 \times X_1X_3 \\ & - 0.23400 \times X_2X_3 - 2.02601 \times X_1^2 - 0.10278 \times X_2^2 \\ & - 7.63336 \times X_3^2 \end{aligned} \tag{4}$$

where Y is the CGO yield (%), X_1 is the time (h), X_2 is the temperature (°C) and X_3 is the H_2O_2 concentration.

ANOVA for the response surface quadratic model verified the statistical significance of Eq. (4). The results in Table 2 showed that the model obtained was highly significant, which was demonstrated by the values of F and P ($(P > F) < 0.0001$). The fit accuracy was verified by the high value of multiple correlation coefficient ($R^2 = 97.75\%$), which indicated that the response model explained the total variations by 97.75%. A regression model with an R^2 value > 0.9 is generally considered to reflect a high correlation (Haaland, 1989). Moreover, the value of the adjusted multiple correlation coefficient ($R^2_{Adj} = 95.73\%$) was sufficiently high to indicate the statistical significance of the model.

The interaction between reaction time and temperature and that between temperature and H_2O_2 concentration were significant ($p < 0.05$). However, the interaction between reaction time and H_2O_2 concentration was not significant ($p > 0.05$) (Table 2). According to the model, the optimum reaction conditions of time, temperature and H_2O_2 concentration maximum CGO yield were

Table 1
The central-composite design for optimizing extraction conditions.

Run	X1	X2	X3	Yield (%)
1	2.50	85.00	2.50	10.61
2	2.50	85.00	2.50	10.72
3	2.50	76.59	2.50	3.12
4	3.00	90.00	2.00	6.71
5	3.00	80.00	2.00	6.21
6	2.50	85.00	2.50	10.16
7	3.34	85.00	2.50	10.29
8	2.00	90.00	2.00	5.68
9	2.00	90.00	3.00	4.94
10	2.00	80.00	2.00	2.91
11	2.50	85.00	2.50	9.85
12	3.00	90.00	3.00	5.63
13	2.50	85.00	2.50	10.35
14	2.50	93.41	2.50	3.17
15	3.00	80.00	3.00	7.31
16	2.50	85.00	1.66	6.18
17	2.50	85.00	2.50	11.08
18	2.00	80.00	3.00	4.67
19	1.66	85.00	2.50	7.67
20	2.50	85.00	3.34	3.85

X_1 = time (min), X_2 = temperature (°C), X_3 = H_2O_2 concentration (%).