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Extraction, preliminary characterization and antioxidant activity of Se-enriched Maitake polysaccharide

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ABSTRACT

A Se-enriched *Grifola frondosa* polysaccharide (Se-GP) was obtained from *G. frondosa* enriched with Se by spraying an Na_2SeO_3 solution during fruit body growth using a Box–Behnken design and compared to *G. frondosa* polysaccharide (GP) for preliminary characterization and determination of the antioxidant activity. Under optimal conditions, polysaccharide yields and both the Se-GP and GP contents do not differ; however, the Se content of Se-GP (17.52 μg/g) was 48.7 times that of GP. Three homogenous Se-GPs (Se-GP11, Se-GP22 and Se-GP33) or GPs (GP11, GP22 and GP33) were obtained via DEAE-52 and Sephacryl S-400 purification. Their molecular weight and polysaccharide content of these compounds were not obviously different, whereas the Se content of Se-GP11, Se-GP22 and Se-GP33 was 9.41, 6.59 and 16.25 times that of GP11, GP22 and GP33, respectively. The antioxidant activity of Se-GP for the DPPH, ABTS and hydroxyl radicals was higher than that of GP and was highest for the hydroxyl radical.

1. Introduction

Biochemical and nutritional researchers are paying increasing attention to polysaccharides due to their medicinal biological activities, especially their anti-oxidant, immunostimulatory, and anti-tumor properties (Li, Wang, Li, Li, & Wang, 2009a; Qiao et al., 2009; Sun & Liu, 2009; Sun et al., 2009; Yuan, Zhang, Fan, & Yang, 2008). The edible mushroom, *Grifola frondosa*, is a basidiomycete fungus belonging to the Polyporaceae family (Gu, Li, & Chao, 2006). Various bioactive compounds in this mushroom, such as polysaccharides, proteins, nonvolatile taste components, among others, have been explored (Cui et al., 2007; Gu et al., 2007; Tsai, Weng, Huang, Chen, & Mau, 2006). Polysaccharides obtained from the liquid-cultured mycelium and fruiting bodies of *G. frondosa* have demonstrated interesting biological activities, including anti-tumor, anti-oxidant, anti-hypertensive, anti-diabetic and anti-hyperliposis effects (Yang, Gu, Jeong, Jeong, & Song, 2007). Furthermore, selenium is an essential trace element that is important to the development and maintenance of a healthy body (Zhang et al., 2009). The selenium content of food varies widely between different world regions based on the selenium content of the soil. Selenium deficiencies may play an important role in the increasing number of cancer patients for several countries (Garland et al., 1995; Maihara et al., 2004). Mushrooms have been a part of the human diet for thousands of years. Various edible mushrooms are consumed for both enjoyment and for the health benefits of having relatively few calories and with a relatively high vegetables protein concentration. Compared to other crop plants, mushrooms also have proven to be good sources of many mineral elements, e.g., K, P, Zn and Cu. This finding would suggest that fungi possess an effective mechanism for absorbing certain trace elements from the growth medium (Lepšová & Mejstřík, 1988). Edible mushrooms are known to be selenium accumulators (Michelot, Siobud, Poirier, Dore, & Viel, 1995; Svoboda, Zimmermannová, & Kalač, 2000). According to our previous study, the total and organic selenium content of *G. frondosa* was 0.12 μg/g and 0.11 μg/g, respectively. Therefore, we sprayed Na_2SeO_3 during the growth stage of *G. frondosa* to capture selenium, and the total and organic selenium content of Se-enriched *G. frondosa* was 13.74 μg/g and 11.98 μg/g, respectively. The objectives of this study were to optimize the extraction conditions of Se-enriched *G. frondosa* polysaccharide (Se-GP) and to compared its preliminary characterization and antioxidant activities to *G. frondosa* polysaccharide (GP). To date, the antioxidant activities of polysaccharides containing selenium have received little attention. To the best of our knowledge, little research has been reported on the fruiting bodies of Se-enriched *G. frondosa*.

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2. Materials and methods

2.1. Materials

Fruits from *G. frondosa* (Bacterial number: Qing gray 151, with total and organic Se contents of 0.12 μg/g and 0.11 μg/g, respectively) and Se-enriched *G. frondosa* (sprayed with 5.788 mg/bag of Na_2SeO_3 during the growth process to yield total and organic Se contents of 13.74 μg/g and 11.98 μg/g, respectively) were prepared. The fruit bodies were dried at 60 °C, crushed or ground into a powder and sieved through a 100 size mesh. DPPH, ABTS, and dextran standards (with MW of 50,000, 270,000,

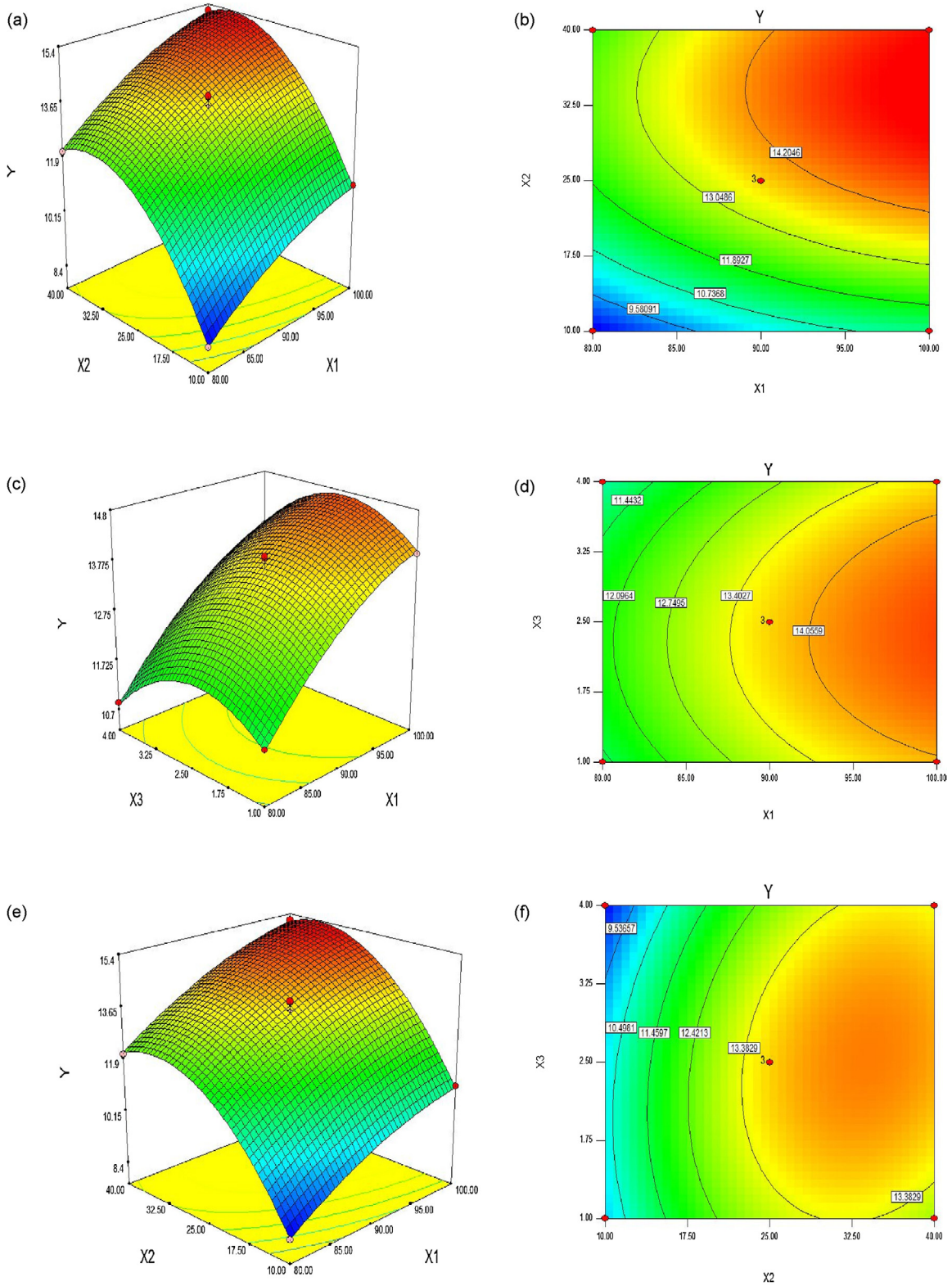

Fig. 1. Response surface plots (3-D) and contour plots (2-D) showing the effects of variables on the response *Y*. X_1: extraction temperature; X_2: ratio of water to raw material; X_3: extraction time.

Table 1
Box–Behnken experimental design with there independent variables.

No.	X_1/extraction temperature (°C)	X_2/ratio of water to raw material	X_3/extraction time (h)	Y/yield (%)
1	80	10	2.5	8.40
2	100	10	2.5	11.06
3	80	40	2.5	12.13
4	100	40	2.5	15.17
5	80	25	1	11.35
6	100	25	1	13.93
7	80	25	4	10.84
8	100	25	4	13.62
9	90	10	1	9.92
10	90	40	1	12.98
11	90	10	4	8.55
12	90	40	4	13.47
13	90	25	2.5	13.85
14	90	25	2.5	13.88
15	90	25	2.5	13.50

670,000, 1,400,000 Da) were purchased from the Sigma Chemical Co. D-Glucose was obtained from Amresco Inc. All other reagents were of analytical grade.

2.2. Extraction and isolation of polysaccharides

Distilled water was used to extract 10 g of Se-enriched *G. frondosa* powder twice at the designated extraction temperature, ratio of water to material, and extraction time. The supernatant was collected, deproteinated using trichloroacetic acid (TCA), concentrated and precipitated with ethanol (1:4, v/v). After successively washing with ethanol, acetone and ether there times in order, the precipitate was lyophilized to yield the crude polysaccharide. The GP (with polysaccharide yield, content and Se content of 14.23%, 45.12% and 0.36 μg/g, respectively) was extracted under the optimal conditions. The selenium content was determined using hydride generation-atomic fluorescence spectrometry (HG-AFS) using a series of matrix-matched standard solutions prepared daily. The polysaccharide was determined via the phenol–sulfate method.

2.3. Experimental design

This study used a Box–Behnken statistical screening design with three independent variables (X_1, extraction temperature; X_2, ratio of water to material; and X_3, extraction time) with three levels to optimize the extraction conditions for the polysaccharide yield from Se-enriched *G. frondosa*. The Design-Expert (version 7.0) software package was used to analyze the experimental data. Any p values below 0.05 were considered to be statistically significant.

2.4. Characterization of Se-GP and GP

2.4.1. Purification of polysaccharide

Either Se-GP or GP was dissolved in distilled water and centrifuged before loading the supernatant onto a cellulose DEAE-52 column (1.6 cm × 50 cm). The crude polysaccharide was eluted by a program going from 0 M to 2 M NaCl and monitored for the presence of carbohydrates via a phenol–sulfate acid assay. Five fractions of Se-GP or GP, Se-GP1 or GP1 (distilled water), Se-GP2 or GP2 (0.05 M, NaCl), Se-GP3 or GP3 (0.1 M, NaCl), Se-GP4 or GP4 (0.15 M, NaCl), and Se-GP5 or GP5 (0.2 M, NaCl), were obtained. The distilled water, 0.05 M NaCl, and 0.1 M NaCl fractions were further fractionated on a Sephacryl S-400 column (1.6 cm × 50 cm) and eluted with 0.15 M NaCl. All of them were presented as a single symmetrical peak, named Se-GP11 and GP11, Se-GP22 and GP22, Se-GP33 and GP33.

2.4.2. Homogeneity and molecular weight of purified polysaccharide fractions

The homogeneity and molecular weights of the polysaccharide fractions were determined via high-performance gel-permeation chromatography (HPGPC) using an Aglient 1100 HPLC system (American) equipped with three columns connected in series, SB-805 HQ, SB-804 HQ, and SB-803 HQ (Shodex OHpak, Japan) (8 mm × 300 mm), with an RID-G1362A detector (American). A 20 μL of sample solution was injected for each run and eluted with a 0.02 M phosphate buffer solution at a flow rate of 0.4 mL/min. The column temperature was 25 °C. The standard curve was established using a series of dextrans with known MW.

2.5. Antioxidant activity of Se-GP and GP

2.5.1. Determination of reducing power

The ferrous ion reducing power was determined using a slight modification to the method proposed by Oyaizu (1986). Samples with different concentrations (200–2000 μg/mL) were mixed with 2.5 mL of a phosphate buffer (pH 6.6, 0.2 M) and 2.5 mL of potassium ferricyanide (1%, w/v) and allowed to sit for 5 min at 50 °C. Then, 2.5 mL of 10% trichloroacetic acid was added to terminate the reaction before centrifuging at 4000 × g for 10 min. A 2.5 mL sample of the supernatant was collected and mixed with 2.5 mL of distilled water and 2.5 mL of $FeCl_3$ (0.1%, w/v). After incubating at room temperature for 15 min, the absorbance of the mixture was measured at 700 nm using ascorbic acid as a positive control.

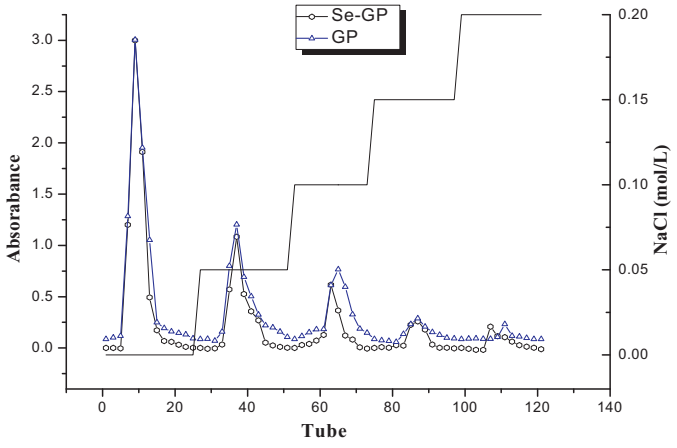

Fig. 2. Five fractions of Se-GP or GP obtained from DEAE. Se-GP is polysaccharide that extraction from Se-enriched *Grifola frondosa*. GP is polysaccharide that extraction from *Grifola frondosa*.

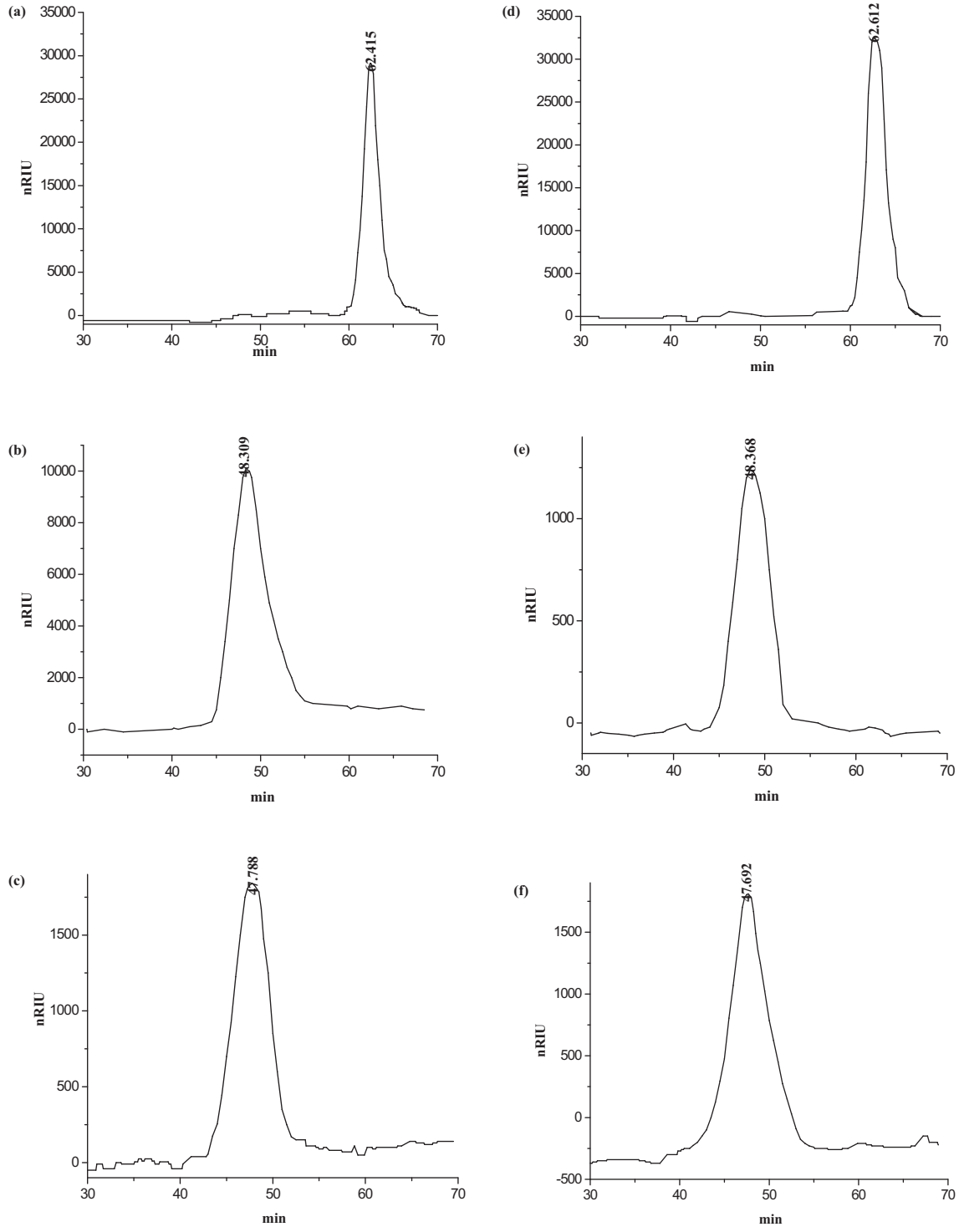

Fig. 3. HPLC profile of three fractions of Se-GP or GP, Se-GP11 (a), Se-GP22 (b), Se-GP33 (c) or GP11 (d), GP22 (e), GP33 (f). Se-GP11, Se-GP22, Se-GP33 of Se-GP were obtained from DEAE-52 elution with distilled water, 0.05 M NaCl, 0.1 M NaCl, and then purified by Sephacryl S-400. GP11, GP22, GP33 of GP were obtained from DEAE-52 elution with distilled water, 0.05 M NaCl, 0.1 M NaCl, and then purified by Sephacryl S-400.

2.5.2. Hydroxyl radical scavenging assay

The scavenging of hydroxyl radicals was measured according to Yuan et al. (2010). A 2.0 mL $FeSO_4$ (6 mM) solution was mixed with 2.0 mL of the samples extracts, and 2.0 mL H_2O_2 (6 mM) was added to start the reaction. After incubating for 10 min at room temperature, 2.0 mL of salicylic acid (6 mM) was added and incubated for 30 min. The absorbance was measured at 510 nm. All tests were performed in triplicate, and the hydroxyl radical scavenging activity was calculated using to the following equation:

$$\text{Scavenging ability}\,(\%) = \left[1 - \frac{A_i - A_j}{A_0}\right] \times 100 \quad (1)$$

Table 2
Fit statistics for Y.

	Master model	Predictive model
Mean	12.18	12.18
R^2	0.9982	0.9982
Adjust-R^2	0.9950	0.9950
CV	1.20	1.20

where A_0 was the absorbance control, A_i was the absorbance in the presence of the sample, and A_j was the absorbance of the blank reagent.

2.5.3. DPPH radical scavenging assay

The radical scavenging capabilities of GP and Se-GP were determined using the DPPH radical method according to Scherer and Godoy (2009). Several 2 mL polysaccharide samples with different concentrations were added to 2 mL of a 2×10^{-4} mol/L ethanol DPPH solution. The mixture was shaken and incubated at room temperature for 30 min, and the absorbance was measured at 517 nm. All tests were performed in triplicate, and the radical scavenging activity was calculated as follow:

$$\text{Scavenging capacity}\,(\%) = \left[1 - \frac{A_i - A_j}{A_0}\right] \times 100 \quad (2)$$

where A_0 was the absorbance of the blank sample, A_i was the absorbance in the presence of sample, and A_j was the absorbance of the blank reagent.

2.5.4. ABTS radical scavenging assay

The ABTS assay was performed according to the method described by Li et al. (Sun et al., 2009) with a slight modification. The ABTS solution was diluted with a phosphate buffer to an absorbance of 0.70 ± 0.02 at 734 nm and preserved in darkness at constant temperature for 12–16 h. Several 0.2 mL samples with gradient concentrations in water were added to 3.8 mL of the diluted ABTS solution. The absorbance was determined at 734 nm after 6 min. All tests were performed in triplicate. The radical scavenging activity was calculated using (2).

2.6. Statistical analysis

Data were reported as the means ± SD (standard deviation) after three determinations. SPSS version 16.0 software was used for all statistical calculations (SPSS Inc., Chicago, USA). An ANOVA was used to determine the differences between the sample results. All values with $p < 0.05$ were considered significantly different.

3. Results and discussion

3.1. Box–Behnken design

Response surface methodology (RSM) is a collection of mathematical and statistical techniques first established by Box and Wilson (Box & Wlson, 1951). This methodology has been widely applied for developing, improving, and optimizing conditions in food and pharmaceutical studies (Luo & Chen, 2010; Xia et al., 2011). Table 1 shows the effects of the three variables (temperature (X_1), ratio of water to raw material (X_2) and time (X_3)) studied during the experimental and the results of 15 runs using a BBD design. The measured response was the polysaccharide yield. The maximum polysaccharide yield (15.17%) was recorded for a temperature of 100 °C, ratio of water to the raw material of 1:40, and time of 2.5 h. The experimental data were analyzed using an analysis of variance, and the results are given in Table 2. Values of "prob > F" below 0.05 indicate that the terms are significant. For this model, X_1 ($p < 0.01$), X_2 ($p < 0.01$), X_3 ($p < 0.01$), and X_2X_3 ($p < 0.05$) were significant.

The above results suggest that changing the three terms significantly affects polysaccharide yield (Fig. 1 a–f). The model is expressed by the following equation:

$$Y = 13.74 + 1.38X_1 + 1.98X_2 - 0.21X_3 + 0.095X_1X_2 + 0.05X_1X_3 + 0.46X_2X_3 - 0.42X_1^2 - 1.63X_2^2 - 0.88X_2^3 \quad (3)$$

The R^2 of (3) (0.9982) indicates that only 0.18% of the total variations were not explained by the model. The high adjusted R^2 (0.9950) and low CV (1.20) confirm that the model was highly significant. At the same time, the model proved sufficient for predictions within the experiment range with a prediction determination coefficient (predicted R^2, 0.9920). The "lack of fit F-value" was 0.13, which implied the lack of fit was not significant relative to the pure error, and there was a 93.44% chance that this "lack of fit F-value" could occur due to noise. The precision was adequate (56.362) and indicates that this model can be used to navigate design space and optimize the temperature, ratio of water to raw material and time for the polysaccharide yield. The software predicted that the optimum temperature, ratio of water to raw material and time were 100 °C, 1:34.72, 2.62, respectively, and the polysaccharide yield, polysaccharide content and Se content under these condition were 15.37%, 48.68% and 17.52 μg/g, respectively. These results agree well with the predicted polysaccharide yield, which was 15.37%.

3.2. Homogeneity and molecular weight of purified polysaccharide fractions

As shown in Fig. 2, Se-GP or GP were fractionated with a DEAE-cellulose column to obtain five fractions: Se-GP1 or GP1 (distilled water), Se-GP2 or GP2 (0.05 M, NaCl), Se-GP3 or GP3 (0.1 M, NaCl), Se-GP4 or GP4 (0.15 M, NaCl), Se-GP5 or GP5 (0.2 M, NaCl), respectively. The three main Se-GP or GP fractions (distilled water, 0.05 M NaCl and 0.1 M NaCl) were further purified on Sephacryl S-400, and their profiles showed a single and symmetrical peak and the HPGPC profile shown in Fig. 3 (a–f), which indicates each purified fraction was a homogeneous polysaccharide. The HPGPC results revealed that the MW of Se-GP11, Se-GP22, Se-GP33 or GP11, GP22, GP33 were 7.2×10^3 Da (polysaccharide and Se content, 96.16%

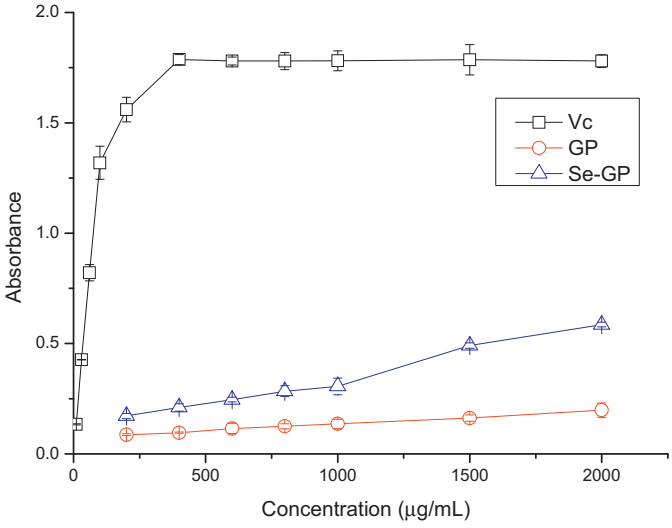

Fig. 4. The reducing power of Se-GP and GP. Se-GP is polysaccharide that extraction from Se-enriched *Grifola frondosa*. GP is polysaccharide that extraction from *Grifola frondosa*. Each value was the mean and standard deviation of three determinations.

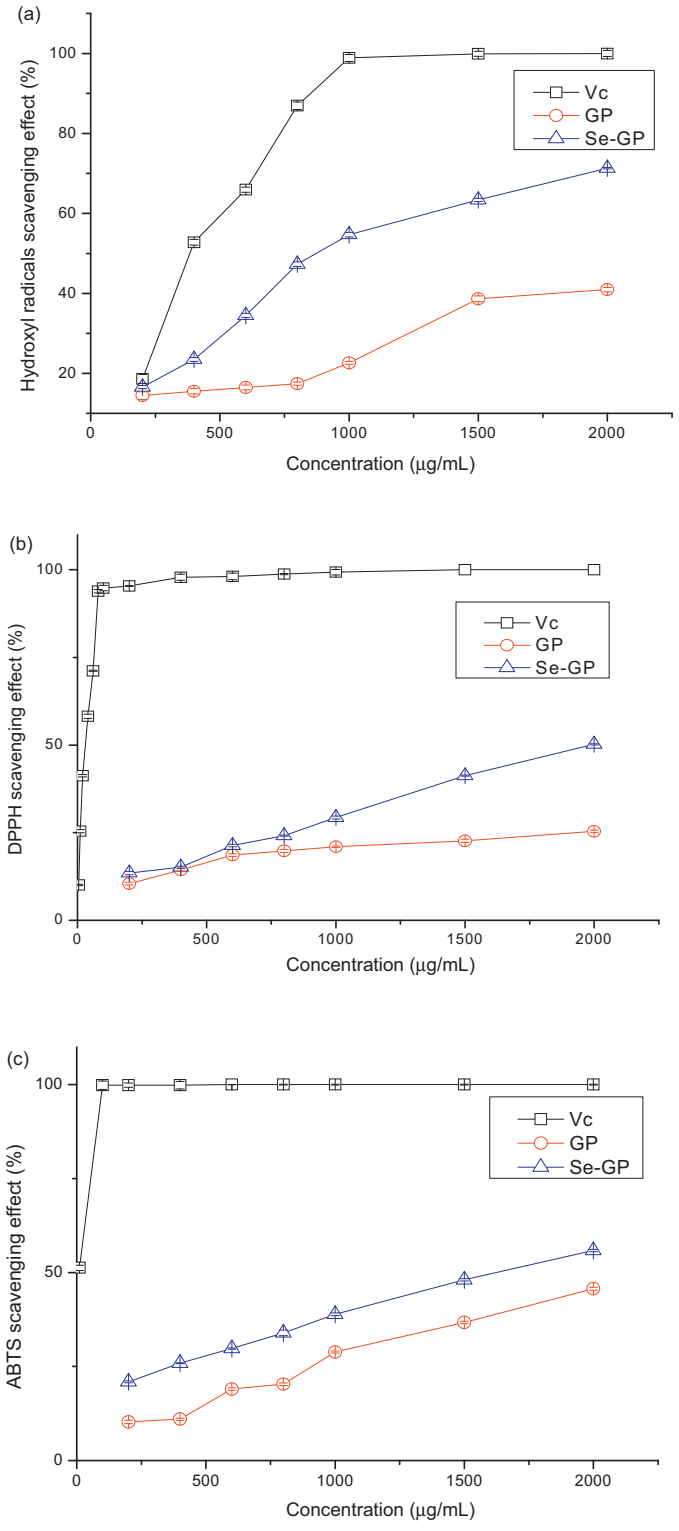

Fig. 5. The scavenging activity of Se-GP and GP on hydroxyl radicals (a), DPPH (b) and ABTS (c). Se-GP is polysaccharide that extraction from Se-enriched *Grifola frondosa*. GP is polysaccharide that extraction from *Grifola frondosa*. Each value was the mean and standard deviation of three determinations.

and 0.5981 μg/g, respectively), 8.2×10^5 Da (polysaccharide and Se content, 94.14% and 1.5491 μg/g, respectively), 1.34×10^6 Da (polysaccharide and Se content, 97.79% and 4.5036 μg/g, respectively) and 6.9×10^3 Da (polysaccharide and Se content, 94.12% and 0.0653 μg/g, respectively), 8.0×10^5 Da (polysaccharide and Se content, 96.91% and 0.2351 μg/g, respectively), 1.12×10^6 Da (polysaccharide and Se content, 95.17% and 0.2772 μg/g, respectively), respectively. These values indicated there is no difference in the MW, polysaccharide composition and polysaccharide content of Se-GP and GP except for their Se content.

3.3. Antioxidant activity

3.3.1. Reducing power

The reducing power is a significant potential activity index, and higher absorbance values indicate stronger reducing power. The reducing power of Se-GP, GP and ascorbic acid are compared in Fig. 4. The maximum reducing powers for Se-GP, GP and ascorbic acid were obtained at 2000 μg/mL (absorbance = 0.586), 2000 μg/mL (absorbance = 0.198) and 400 μg/mL (absorbance = 1.787), respectively. These results revealed that Se-GP may possess more electrons to react with the Fe^{3+} (approximately three times that of GP). However, the reducing power of Se-GP was much weaker than that of ascorbic acid.

3.3.2. Scavenging activity of Se-GP and GP for hydroxyl radicals

As shown in Fig. 5 (a), Se-GP was found to have improved scavenging for hydroxyl radicals than GP at concentrations from 200 to 2000 μg/mL. The scavenging of hydroxyl radicals by Se-GP and GP is dose-dependent manner and was 71.32% and 40.95%, respectively, at a dose of 2000 μg/mL.

3.3.3. Scavenging activity of Se-GP and GP for DPPH radicals

DPPH is a free radical compound that has been used widely to test the free radical scavenging of various samples (Li, Chen, Wang, Tian, & Zhang, 2009b). A comparison of the radical scavenging capacity of GP and Se-GP is shown in Fig. 5(b). Higher the concentrations have higher antioxidant activities. The antioxidant activity follows the same sequence at each concentration: GP < Se-GP. The inhibition rate of Se-GP is twice the GP at a concentration of 2000 μg/mL, which was 50.20% and 25.41%, respectively.

3.3.4. Scavenging activity of Se-GP and GP for ABTS radicals

The results of the ABTS scavenging assay are described in Fig. 5(c). Similar to the DPPH and hydroxyl radicals, Se-GP had a higher scavenging capacity than GP at concentrations from 200 to 2000 μg/mL. The scavenging activity reached 55.87% and 45.68% for Se-GP and GP, respectively, at 2000 μg/mL.

4. Conclusions

BBD proved to be a valuable tool for optimizing technology for polysaccharide extraction. The optimal extraction conditions for Se-GP were a temperature of 100 °C, ratio of water to raw material of 1:34.72 and a time of 2.62 h. Under those conditions, the polysaccharide yield, polysaccharide content and Se content were 15.37%, 48.68% and 17.52 μg/g, respectively. After purifying using DEAE-52 and Sephacryl S-400, three Se-GP or GP fractions were obtained and no difference between the polysaccharide content and MW of Se-GP and GP was detected, except for the Se content. The Se contents of Se-GP11, Se-GP22 and Se-GP33 were 9.41, 6.59 and 16.25 times greater than those GP11, GP22 and GP33, respectively. The antioxidant experiments suggested that the scavenging capacity of Se-GP was greater than that of GP for ABTS, DPPH and hydroxyl radicals. For ABTS, DPPH and hydroxyl radicals, Se-GP demonstrated the best scavenging capabilities for hydroxyl radicals and reached 71.32% at a dose of 2000 μg/mL. These results suggest that Se-GPs have potent antioxidant properties and should be explored as a potential Se supplements and antioxidant health foods. Future studies will determine the monosaccharide composition and characterize the difference between GP and Se-GP.

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