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Response Surface Optimization of Crude Polysaccharides from Grey Oyster Mushroom (*Pleurotus sajor-caju* (Fr.) Singer) Using Pressurized Hot Water Extraction †

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Abstract: Grey oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer) is a popular edible mushroom in Thailand due to its high nutritional and medicinal benefits. This study aims to study the effects of temperature (100–140 °C), pressure (4–7 bar), and extraction time (20–60 min) on the extraction crude polysaccharides with environmentally friendly pressurized hot water. The extraction condition was optimized by the maximize yield using response surface method based on a central composite design (CCD). The temperature was the main effect on the increase of extracted yield. The optimum extraction condition was 140 °C, 10 bar, and 26.79 min with a corresponding yield of 31.31 ± 2.55%. Under these conditions, the total phenolic content of crude polysaccharides was 401 ± 8.24 mg GAE/g dry mushroom. In addition, the total glucan content was indicated as 34.50 ±1.79 g/100 g dry mushroom, which separated as 32.47 ± 1.95 mg/100 g of β-glucans and 2.04 ± 0.98 mg/100 g of α-glucans.

Keywords: grey oyster mushroom; bioactive substances; pressurized hot water; crude polysaccharides

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1. Introduction

Grey oyster mushroom (*Pleurotus sajor-caju* (Fr.) Singer) is a popular edible mushroom worldwide due to its high nutritional and medicinal benefits. It is sources of carbohydrate, protein, vitamins, and minerals [1]. The bioactive compounds in mushrooms, which is polysaccharides, especially of β-glucan, have been reported to possess immunomodulatory, antitumor, antiviral, wound healing, anti-obesity, and antidiabetics activities [2].

This study aims to investigate the effects of extraction temperature (100–140 °C), extraction pressure (4–7 bar), and extraction time (20–60 min) on yield of extracted crude polysaccharides with environmentally friendly pressurized hot water [3]. This technique regarded as a green and efficient technique to extract solid and semi-solid samples with water or other liquid solvents [3–5]. The extraction condition was optimized by the maximize yield using response surface method based on a central composite design (CCD). Under optimal condition, the total phenolic and total glucan content of crude polysaccharides was indicated as 34.50 ± 1.79 g/100 g dry mushroom, which separated as 32.47 ± 1.95 mg/100 g of β-glucans and 2.04 ± 0.98 mg/100 g of α-glucans.

2. Materials and Methods

2.1. Preparation of Sample

Grey oyster mushrooms (*Pleurotus sajor-caju* (Fr.) Singer) were harvested from mushroom farm in The Institute of biotechnology and genetic engineering, Chulalongkorn University. They were dried at 60 °C and stored in a desiccator at room temperature.

2.2. Extraction Method

The extraction of grey oyster mushrooms under pressurized hot water was performed in a 500-cm^3 batch reactor (Parr Instrument Company, Moline, IL, USA). Dried mushrooms of an approximately 10 ± 0.1000 g and distilled water of 300 mL were loaded into the reactor. Then, the reactor was heated according to the desired conditions of temperature of 100–140 °C, pressure of 4–7 bar and extraction time of 20–60 min. To stop the reaction, the reactor was cooled to room temperature by tap water. The obtained extract was 1st filtered filter cloth and 2nd filtered Whatman paper filters No. 1 and stored at 4 °C for recovery of polysaccharides.

2.3. Recovery of Crude Polysaccharides

The recovery of crude polysaccharides was done by precipitation with absolute ethanol. The extract solution was mixed with 2-fold volume of absolute ethanol and kept overnight at 4 °C. The mixture was then centrifuged at 1000 rpm for 10 min. The precipitate was dried in an oven at 40 °C for 24 h until constant weight was observed. The recovery yield of polysaccharides was calculated by Equation (1).

$$\% \text{ yield} = (\text{sediment weight after extract(g)}/\text{dry mushroom weight (g)}) \times 100 \quad (1)$$

2.4. Experimental Design and Data Analysis

The influence of temperature (A), pressure (B) and time (C) on crude polysaccharides yield was investigated by the central composite design (CCD) method. The optimal condition was located using The response surface method (RSM). Design-Expert® software (Stat-Ease, Inc., Minneapolis, MN, USA) was employed to design the experiment and to carried out the analysis of variance (ANOVA), regression model, and statistical analysis.

3. Results and Discussion

3.1. Effect of Factors on the Extraction Yield

Table 1 shows the analysis of variance (ANOVA), temperature and time was obsered as the sinificant factors on the extraction yield due to a *p*-value less than 0.0001. In addition, the interaction of temperature and pressure (AB) and pressure and time (BC) was also presented their effect on the yield. As shown in Figure 1, the % yield increased with the increasing of temperature and pressure. However, it dramatically decreased with the long extraction time. This is due to the degradation of polysaccharides [6,7]

Table 1. The analysis of variance (ANOVA) table of the effect of reaction condition on the extraction yield.

Source	Sum of Squares	df	Mean Square	*p*-Value
Model	677.12	10	67.71	<0.0001
A-Temperature	282.61	1	282.61	<0.0001
B-Pressure	1.24	1	1.24	0.6329
C-Time	32.11	1	32.11	0.0232
AB	43.99	1	43.99	0.0094
AC	5.34	1	5.34	0.3268
BC	81.38	1	81.38	0.0009

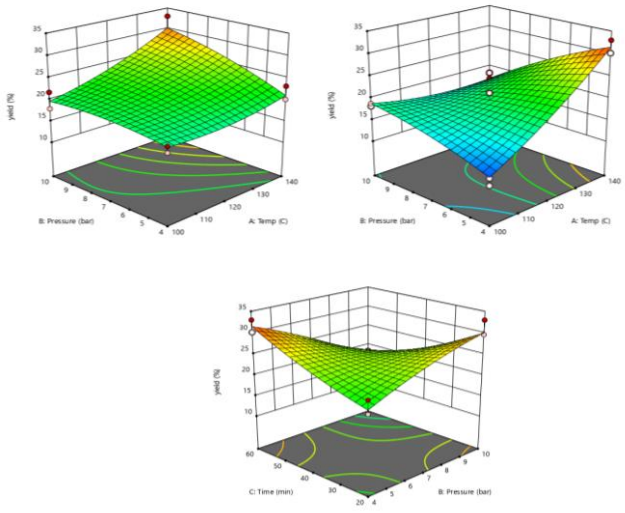

Figure 1. (**a**) Effect of interaction of temperature and pressure and (**b**) effect of interaction of pressure and time on extraction yield.

3.2. Optimal Condition of the Highest Yield

The optimum conditions obtained was carried out with the assistance of the optimization function embedded in the Design-Expert® software as shown in Table 2. Under this optimal condition, the predicted % yield of 27.3307 was obtained. The experimental runs under the suggested optimal conditions in triplicate provided the actual value of 31.31 ± 2.55. As β-glucan is an importantbioactive compounds in mushrooms, the total glucant was also analyed using the mushroom and yeast beta glucan assay kit (Megazyme International, Wicklow, Ireland). The total glucan content from obtained polysaccharide was indicated as 34.50 ± 1.79 g/100 g dry mushroom, which separated as 32.47 ± 1.95 mg/100 g of β-glucans and 2.04 ± 0.98 mg/100 g of α-glucans.

Table 2. Optimal conditions for SCGs extraction with pressurized hot water and the predicted and the actual values of response.

Operating Parameter	Optimal Condition	% Yield		Total Glucan (mg/100 g)	
		Predicted Value	Actual Value	β-Glucans	α-Glucans
Temperature (°C)	120	27.3307	31.31 ± 2.55	32.47 ± 1.95	2.04 ± 0.98
Pressure (bar)	10				
Extraction time (min)	26.79			34.50 ±1.79	

4. Conclusions

Crude polysaccharides of grey oyster mushrooms were successfully extracted by pressurized hot water. The temperature was the main effect on the increasing of extracted yield. The optimum extraction condition was 140 °C, 10 bar, and 26.79 min with a corresponding yield of 31.31 ± 2.55%. Under these conditions, the total phenolic content of crude polysaccharides was 401 ± 8.24 mg GAE/g dry mushroom. In addition, the total glucan content was indicated as 34.50 ± 1.79 g/100 g dried mushroom, which separated as 32.47 ± 1.95 mg/100 g of β-glucans and 2.04 ± 0.98 mg/100 g of α-glucans.

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Conflicts of Interest: The authors declare no conflict of interest.

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