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Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of lentinan

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ABSTRACT

Dynamic high pressure microfluidization (DHPM) was applied to assist the lentinan extraction. Response surface methodology (RSM), based on Box–Behnken design, was employed to optimize the DHPM-assisted extraction conditions of lentinan. Three main independent variables (DHPM pressure, ratio of water to raw material, extraction temperature) were taken into consideration. A yield of 7.200% was obtained under a modified condition (ratio of water to raw material of 65 mL/g, DHPM pressure of 147 MPa, extraction temperature of 83 °C), which matched well with the predicted value of the model. The molecular weight of the DHPM-assisted extract and hot water extract was 913,329 and 965,361 Da, respectively. Compared to the traditional hot water extraction, the lentinan extracted by DHPM assisting had better scavenging capacity of hydroxyl radical, superoxide anion free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and nitrite. It could be concluded that the DHPM was a promising method to enhance the yield and antioxidant activity of lentinan during extraction.

1. Introduction

Mushrooms have been used for traditional foods and medicines in Asia. Generally, mushrooms are rich in carbohydrates, minerals, vitamins and low in fat [1].

Lentinus edodes, the second most cultivated edible mushroom in the world, comprising of about 25% of the worldwide production [2], is widely cultivated in China, Japan and other Asian countries due to its nutritional values and medical application. Studies have demonstrated that lentinan exhibited multiple bioactivities, including anti-oxidation, anti-cancer, anti-aging, immunoregulation, anti-tumor, antiviral, reducing blood fat and resisting arteriosclerosis [3]. Nowadays, lentinan has attracted great attention of biologist and pharmaceutist because of its high bioactivity, safety and wide source. However, to the best of our knowledge, there are few reports regarding the extraction process of lentinan. Hot water extraction is the main and conventional method for polysaccharide. It usually needs longer time and higher temperature but with lower extraction efficiency. Recent researches have suggested that ultra high pressure-assisted extraction could potentially enhance the yield and bioactivity of bioactive compounds [4–7].

DHPM is an emerging dynamic high pressure technology. Unlike high hydrostatic pressure, the combined forces of high-velocity impact, high-frequency vibration, instantaneous pressure drop, intense shear, cavitation, and ultra-high pressures up to 200 MPa were used [8]. Studies of DHPM were focused on the modification on the biomacromolecule like protein [9], dietary fiber [10], and enzyme [11]. Our research group was the first one who applied DHPM to extract maize pollen polysaccharide, and we found that DHPM-assisted extraction could efficiently improve the yield of maize pollen polysaccharide [12], suggesting its great potential in extracting polysaccharides.

Recently, RSM has been widely used to optimize extraction process of bioactive compounds, such as polysaccharides [13,14] and anthocyanins [15]. It has been demonstrated that RSM is an effective statistical technique for optimizing complex process because it allows more efficient and easier arrangement and interpretation of experiments compared to other methods [16,17].

In this work, we first optimized the conditions for extracting the lentinan. We then applied DHPM technology in the lentinan extraction. The in vitro antioxidant activities of lentinan prepared by DHPM-assisted and hot water extraction were compared side

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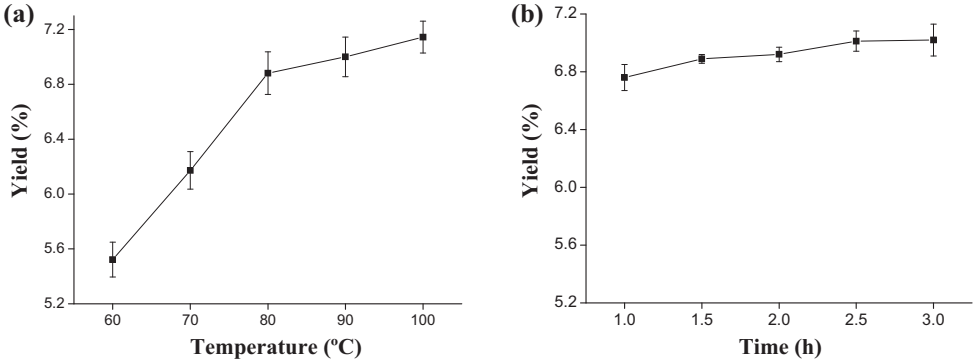

Fig. 1. Effect of extraction temperature (a) and time (b) on the yield of lentinan.

by side. The DHPM-assisted extraction significantly improved all aspects of the polysaccharide, suggesting its great potential in the food extraction industry.

2. Materials and methods

2.1. Materials

The fruit body of *L. edodes* was purchased in the farmer's market of Nanchang, China. Then it was smashed after oven-drying at 50 °C for 2 days, and sieved through a 100 mesh screen. The powder was packed by a plastic bag in a drier for use.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma (St. Louis, USA), and Vc was purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals were of analytical grade.

2.2. Extraction of lentinan

Dynamic high pressure microfluidization-assisted extract (DHPME) was prepared as follows: five grams of the dried powder was immersed in distilled water in a 500 mL beaker, and then it was homogenized in an ordinary homogenizer (Shanghai Donghua Homogenizer Company, China) at 30 MPa once. After that, the emulsion was treated by a microfluidization homogenizer (M-110EH, Microfluidics Company, USA) at a selected pressure twice. Then the solution was extracted by hot water bath at a selected temperature for a certain time, then cooled and centrifuged at 4000 rpm for 10 min by a centrifuge (TDL-5A, Shanghai Anting Instrument, China). The supernatant was concentrated and a threefold volume of absolute ethanol was added into the concentrated solution for precipitating polysaccharides at 4 °C overnight.

The precipitate was redissolved in distilled water and the polysaccharide content (m) was determined by the phenol-sulfuric acid method according to Hou [18]. The yield of the polysaccharide was calculated as follows:

$$\text{Yield}\ \ (\%) = \left[\frac{m}{\text{materials weight}\ \ (5\ \ \text{g})}\right] \times 100 \qquad (1)$$

Equal volume of 75% (v/v) ethanol was added to the redissolved solution to remove the oligosaccharide, and then centrifuged and the supernatant was precipitated again by threefold volume of absolute ethanol, centrifuged, and the procedure was repeated twice. The precipitate was washed by absolute ethanol twice and ether once, and then dissolved in distilled water, centrifuged at 10,000 rpm for 30 min, then equal volume acetone was added to the supernatant, centrifuged (4000 rpm, 10 min), and the cloudy part was dissolved in distilled water and dialyzed for two days by running water and one day by distilled water. Then the solution was freeze-dried and stored for later analysis [19–21].

Traditional hot water extract (HWE) was prepared the same as above but without DHPM treatment.

2.3. Experiment design

The effect of extraction temperature and time on the yield of lentinan was shown in Fig. 1. The extraction time showed little effect on the yield of lentinan, therefore, it was not used for the optimization. A Box–Behnken design with three variables was used for the optimization. Based on the preliminary experiments, the pressure (X_1), the ratio of water to raw material (X_2) and extraction temperature (X_3) were chosen as key variables and were prescribed into three levels coded +1, 0 and −1 for high, medium and low, as shown in Table 1. Each experiment was performed in triplicate and the averages of lentinan yield were taken as response. To predict the optimal point, a quadratic equation was used for this model.

$$Y = \beta_0 + \sum_{i-1}^{k}\beta_i X_1 + \sum_{i=1}^{k}\beta_{ii}X_i^2 + \sum_{i>j}^{k}\beta_{ij}X_iX_j \qquad (2)$$

where Y is the estimated response; β_0 is the constant, β_i, β_{ii}, and β_{ij} are the regression coefficients for linearity, square, and interaction, respectively; and X_i and X_j are the independent variables. And k equals to the number of the tested factors ($k = 3$).

2.4. Molecular weight and carbohydrate content determination

The molecular weights of lentinan samples were determined by gel-permeation chromatography (GPC) based on the method of Lai et al. [22]. The total carbohydrate content was measured by the phenol–sulfuric acid method [18].

2.5. Assay for antioxidant activities

2.5.1. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities of samples extracted by DHPM-assisting and traditional hot water were determined according to Fan [23] with some modifications. 2 mL different concentrations (0.5–5.0 mg/mL) of the samples, 2 mL $FeSO_4$ (9 mM),

Table 1
Independent variables and their levels used in the experimental design.

Level	Factor		
	Pressure (X_1) (MPa)	Ratio of water to raw material (X_2) (mL/g)	Temperature (X_3) (°C)
−1	120	50	70
0	140	60	80
1	160	70	90