Effect of Extraction Methods on Polysaccharide of Clitocybe maxima Stipe

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Abstract: Clitocybe maxima (Gartn. ex Mey. Fr.) Quél. is a favorable edible fungi species. The proportion of its stipe is about 45% of entire fruit biomass, which is a low value defined byproduct. To increase its value added utilization, three extraction methods (as hot water, microwave-assisted and complex-enzyme-hydrolysis-assist) were conducted. The extraction effect on the polysaccharide of Clitocybe maxima stipe was compared and the processing conditions in extraction were optimized. The content of polysaccharide was determined with Phenol Sulfuric Acid Method. With three methods, the extraction yield and the polysaccharide amount were different greatly, which were 5.86 and 40.23% for hot water extraction, 9.4 and 52.62% for microwave-assist extraction, 10.26 and 53.13% for complex-enzyme-hydrolysis extraction, respectively. Results indicated that the complex-enzyme-hydrolysis-assist extraction was the optimal approach used for extracting polysaccharide from Clitocybe maxima stipe. It showed with the advantages in high efficiency, energy and running time saving.

Keywords: Clitocybe maxima, extraction methods, mushroom stipe, polysaccharide

INTRODUCTION

Clitocybe maxima (Gartn. ex Mey. : Fr.) Quél also known as “Da Bei San” or “Zhu Du Gu”, is one among the newly developing Rare Mushrooms (Deng et al., 2006; Rao, 2002). It has advantage of unique flavor, fresh and tender, rich nutrition, ad also very favorable to human body health, love by consumer (Zhu and Ma, 2007; Dong et al., 2010). It characters with a high proportion in stipe bio-mass, which contributes about 40-50% of entire fruit weight. The stipe is longer than 10 cm, with diameter of 1.5-2.5 cm (Chen et al., 2005). Although the stipe contains slightly lower protein and amino acid than its cap, it yet contains high in fibrous components. The nutritional characters impact the texture of stipe for edibility, even for processed dry products (Peng et al., 1994). Therefore, the mushroom stipe has been treated as bio-waste or byproducts with very low commercial value until now (Peng, 1998; Cai, 2009; Chen et al., 2010). Only from Zhangzhou, a city of Fujian, the daily yield of the stipe could reach 30-50 tons. It is urgent in that to develop the techniques in use of stipe with its characters of high nutrition and fibrous (Chen et al., 2009). The objectives of this study is to research on how to use the bioactive composition as polysaccharide from the mushroom stipe in order to raise the profit of cultivation with enhanced utilization of discarded mushroom stipe resources.

In this study, three extraction methods of hot water, microwave-assist and complex-enzyme-hydrolysis-assist were conducted to prepare polysaccharides from the mushroom stipe of C. maxima. To compare the effects of extraction methods, the extraction efficiency and polysaccharide content were detected. The optimal extraction method was found in based on increasing extraction yield, saving extraction time and energy.

MATERIALS AND METHODS

Materials and chemicals: The stipe of C. maxima was supplied by Nanjin Chengfa Mushroom Developing Company Co., LTD. in Zhangzhou, Fujian province. The fresh stipe was then washed, oven dried and ground through 20 mesh. Chemical of glucose, phenol, sulfuric acid, ethanol and acetone were all of analytical grade; cellulase, pectinase and protease with specific activity of 100,000 U/g was purchasing from Huzhou Lilai Biological technology Co., LTD.

Instrument and facility: Water bath (Type: DK-S26, Shanghai Jinhong laboratory equipment Co., LTD), freeze drier (Type: FD-1D-50, Beijing Boyikang laboratory equipment Co., LTD), electronic balance (Type: BS2000S, Sartorius Scientific Instruments (Beijing) Co., Limited), pH Meter (Type: PB-10, Sartorius Scientific Instruments (Beijing) Co., Limited), drying oven (Type: 101A, Shanghai Experimental Instrument Central Plant), rotary evaporator (Type: SENCO-GG17, Shanghai Shenke Science and Technology Co., Limited), high speed refrigerated centrifuge (Type: TGL-16, Hunan Xiangyi Instrument
Co., Limited), ultraviolet spectrophotometer (Type: 156P, Shanghai spectral instrument Co., LTD), microwave oven (Type: WD900CSL23, Glanze microwave oven electric appliance Co., LTD) were used.

Methods:
Extraction polysaccharide from mushroom stipe:
Extraction with hot water: (5.00 g) of mushroom stipe powder was accurately weighted and put into a baffled flask, 200 mL of distilled water was added, the flask was then put into the magnetic water bath mixer (600 r/min, 90°C), after 4 h, the suspension was centrifuged, the sediment was extracted twice with the same method, supernatants of the three centrifuge were merged and concentrated at 50°C by evaporation under reduced pressure. Three volumes of ethanol were added for precipitation of polysaccharide at 4°C overnight, the precipitation was obtained by centrifugation (5000 r/min, 10 min) was repeatedly washed with ethanol. The extraction was repeated three times.

Extraction assisted with microwave: (5.00 g) of stipe powder was accurately weighted and put into a baffled flask, 125 mL of distilled water was added. The flask was firstly heated for 8 min in a microwave oven (540 w) and then put into the magnetic water bath mixer (600 r/min, 90°C) for 2 h, the suspension was centrifuged and the following procedure was in same with the above method of hot water extraction.

Extraction assisted with complex enzyme hydrolysis: (5.00 g) of stipe powder was accurately weighted and mixed with 1% complex enzyme (cellulose: pectinase: protease = 1:1:1), distilled water was added at the solid-to-liquid ratio of 1.25, hydrolysis and extraction was conducted for 96 min at 47.0°C and pH 4.64. The suspension was centrifuged and the following procedure was in same with the above method of hot water extraction.

Standard curve: (0.5 g) glucose was accurately weighted and dissolved in distilled water to make the concentration of 1.0 mg/mL. The solution was diluted as concentration of 25, 50, 75, 100, 150 and 200 µg/mL, the volume for each concentration solution was 10.0 mL. (1.0 mL phenol and 5.0 mL sulfuric acid) were added into 1.0 mL of glucose solution for each concentration, mixed by vortex, determine the absorbency at 490 nm after reacting in a 30°C water bath for 20 min. (1.0 mL) distilled water was used as blank, standard curve was obtained with x-coordinate for concentration of glucose and y-coordinate for absorption value, drop-wise.

Determination on the purity of polysaccharide: Accurately imbibed 1 mL of solution in item 1.3.1, diluted to 100 mL with volumetric flasks, then the purity was calculated according to its OD value. Determination the yield of crude polysaccharide with equation as followed:

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\text{Yield} (\%) = \frac{W_1}{W_0} \times 100
\]

\[
W_1 = \text{The weight of extracted polysaccharide}
\]

\[
W_0 = \text{The weight of raw material}
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RESULTS AND DISCUSSION

Effect of extraction methods on the yield of polysaccharide: Hot water extraction method is traditional and conventional. Table 1 showed that the experimental data was ideal with standard deviation of 0.020 and the coefficient of variation in 0.29. The polysaccharide is polarity macromolecule compound which is easy to resolve in hot water when polar strong water was used as extraction resolution (Zhang and Han, 2005).

The extraction with hot water for polysaccharide of C. maxima stipe presented the advantages in facility requested simple, running cost low, impact to environment friendly and products safely use to humankind. Even though, the defect of it for the products observed in low yield (5.86%), low purity (40.23%) and turbid-appearance which is hard to be clarified.

Effect of microwave-assist extraction on polysaccharide yield: Table 2 showed that the average yield of extraction for three replicates was 9.24% with standard deviation of 0.017 and coefficient of variation in 0.18. Comparing with result of hot water extraction, the extraction yield with microwave-assist method was raised for about 57.8% and the time consumed for extraction was reduced to a half of it spent with hot water extraction. The results convince that Microwave-assist extraction method is an acknowledgeable technique to accelerate the speed of solvent extraction from solid by heating effect of microwave energy. It has the advantages of highly flexible in use and time saving with high extraction yield. In recent years, applied research for it on the extraction of nature products developed very rapidly, especially in its industrialization (Letellier and Budzinski, 1999; Cheng, 2002).

The extraction with Microwave-assist method revealed in higher efficiency than it in hot water method. That was concomitant with a reduced running time to a half of it spend for hot water extraction. Comparing with the polysaccharides extracted with hot
The physiochemical linkages in between the components was wrapped in the cell wall of *C. maxima* also reduced. The reason lays in that the polysaccharide (Sanehez-Avila extraction composition to solvent diffusion interface molecular in a short time, accelerates diffusion of movement and collision efficiency, activates the vibration and swing of the molecular, intensifies the during microwave heating, which induces rotation, changed with the alternate of electric field direction the reason that the orientation of polar molecule was polysaccharides molecule released. Another may lies in microwave that can rupture the cell wall and help for method may due to, one is the heating function of hot water extraction method and time-consuming was improved for extraction with Microwave-assist higher yield and more purified polysaccharide. The water, the method of Microwave-assist obtained a higher yield and more purified polysaccharide. The improvement for extraction with Microwave-assist method may due to, one is the heating function of microwave that can rupture the cell wall and help for polysaccharides molecule released. Another may lies in the reason that the orientation of polar molecule was changed with the alternate of electric field direction during microwave heating, which induces rotation, vibration and swing of the molecular, intensifies the movement and collision efficiency, activates the molecular in a short time, accelerates diffusion of extraction composition to solvent diffusion interface (Sanehez-Avila *et al.*, 2009; Feng *et al.*, 2006).

**Effect of complex enzyme hydrolysis assist extraction on polysaccharide yield:** Table 3 showed that the yield of polysaccharides in the average of three replications was 10.26% with a standard deviation in 0.020 and coefficient of variation in 0.29. After the stipe powder of *C. maxima* hydrolyzed with complex enzymes of cellulase, pectinase and protease, the yield of crude polysaccharide was raised for about 75% and the time consumed for extraction reduced to 1/3 of it for hot water extraction. Enzyme-assist extraction revealed obviously in its advantages that over the conventional hot water extraction. It is an innovative research field in polysaccharide extraction, introduced in recent years.

Complex-enzyme-hydrolysis-assist method can be operated under low bath temperature (47°C), the yield of polysaccharide was significantly improved to 1.11 times of microwave-assist extraction or 1.75 times of hot water extraction method and time-consuming was also reduced. The reason lays in that the polysaccharide was wrapped in the cell wall of *C. maxima* stipe with the physiochemical linkages in between the components of pectin, cellulose and protein etc. With multi- enzymatic hydrolysis, it breaks down the linkages and helps to release the polysaccharide and raise extraction rate. Meanwhile, the enzyme-hydrolysis-assist extraction exhibits its advantages in mildness for extraction conditions and highly specificity for application. It would not usually damage the molecular three-dimensional structure, so that to maintain the bioactivity of mushroom polysaccharide. It also helps to improve the purity of polysaccharide. This result is consisting with that of previous experiment in which the polysaccharide was extracted from the fruit body of mushroom *C. maxima* (Yang *et al.*, 2011).

**Comparing extraction efficiency of crude polysaccharide among methods:** Table 4 indicated that complex-enzyme-hydrolysis-assist method not only shorten the time consuming of extraction largely but also has advantage in the aspect of extraction yield and purity of polysaccharide. From the comprehensive view of time saving, energy saving and environmental protection, the complex-enzyme-hydrolysis-assist method is an adequate extraction method to prepare polysaccharide from the stipe of mushroom *Clitocybe maxima* with advantage of simpleness, convenience and efficiency.

**CONCLUSION**

With comparison between three extraction methods (i.e., traditional hot water, microwave-assist and complex-enzyme-hydrolysis-assist) used for polysaccharide of *C. maxima* stipe, the complex-enzyme-hydrolysis-assist extraction exhibits the best with the highest in extraction efficiency and the shortest in its running time. The results suggest that it is considered to be more fitted for industrialization.

The study in finding of an effective extraction method for *C. maxima* stipe polysaccharide would enhance the further study in developing of new approach for a comprehensive utilization of *C. maxima* stipe. It is intention to promote the processing for mushroom byproducts such as stipe polysaccharides.

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