Advance Journal of Food Science and Technology 5(3): 370-373, 2013
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: December 17, 2012 Accepted: January 19, 2013 Published: March 15, 2013

Effect of Extraction Methods on Polysaccharide of *Clitocybe maxima* Stipe

Junchen Chen, Pufu Lai, Hengsheng Shen, Hengguang Zhen and Rutao Fang
Research Institute of Agri-engneering Technology, Fujian Academy of Agricultural Sciences, Fuzhou, 350003, P.R. China

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 microware-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 4o-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

Corresponding Author: Junchen Chen, Research Institute of Agri-engneering Technology Fujian Academy of Agricultural Sciences, Fuzhou, 350003, P.R. China, Tel.: 18950257193

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 distill 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 distill 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), distill 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 dissolve in distill 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) distill 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:

$$\text{Yield } (\%) = W_1/W_0 \times 100 \quad (1)$$

W_1 = The weight of extracted polysaccharide
W_0 = The weight of raw material

RESULTS AND DISCUSSION

Standard curve: Figure 1 showed that equation of linear regression for glucose was Y = 6.962X - 0.0053, correlation coefficient R^2 = 0.9985. It indicated that there was a good linear relation between the concentration of glucose and absorbance, which is correspond the Lambert-Beer law.

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 three replicates in 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

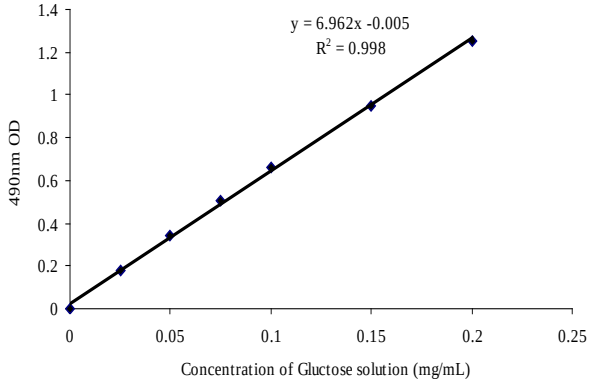

Fig. 1: Example of a figure caption

Table 1: Yield of polysaccharide by hot water extraction (%)

Replicate	1	2	3	Mean	RSD	CV
Yield (%)	5.83	5.85	5.87	5.85	0.020	0.29

Table 2: Yield of polysaccharides by microwave-assist extraction (%)

Replicate	1	2	3	Mean	RSD	CV
Yield (%)	9.23	9.26	9.23	9.24	0.017	0.18

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 replicates 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

Table 3: Yield of polysaccharides by complex enzyme hydrolysis method (%)

Replicate	1	2	3	Mean	RSD	CV
Yield (%)	10.25	10.24	10.29	10.26	0.023	0.22

Table 4: Comparison for extraction efficiency among various methods

Extraction methods	Extraction time (min)	Yield (%)	Purity (%)
Hot water	240	5.86	40.23
Microwave-assist	128	9.24	52.62
Complex-enzyme-hydrolysis-assist	96	10.26	53.13

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.

ACKNOWLEDGMENT

The authors thank the Scientific Research Fund from Fujian Provincial Department of Science and

Technology (2011R1017-3) and the Research Fund from Fujian Academy of Agricultural Sciences for Young Scientists (2011QB-5).

REFERENCES

Cai, Y., 2009. Research on the anniversary cultivation and processing technology of mushroom *Clitocybe maxima*. Thesis of Chinese Academy of Agricultural Sciences (in Chinese).

Chen, J., H. Shen, B. Tang and J. Yang, 2005. Techniques of high-yield cultivation and processing for *Clitocybe maxima*. Edible Fungi China, 24: 32-33 (in Chinese).

Chen, J., H. Shen, Y. Li, B. Tang and Li Wu, 2009. An processing method for nutrient biscuit with the stipe of mushroom *Clitocybe maxima* as dietary fiber. China Patent, ZL 200910305965.0 (in Chinese).

Chen, J., Y. Li, H. Shen, B. Tang and Y. Cai, 2010. Technology and nutritional analysis of kudzu and mushroom starch-noodle made by *Clitocybe maxima* Stalk. Proceeding of 5th Symposium of Nationwide Research Institutes Association on the Agricultural Products Processing and Chinese Processing Technique and Industrial Development of Agriculture Product. Nanjing, China, pp: 275-281 (in Chinese).

Cheng, M., 2002. Application of micro-wave extraction technology. Chinese Tradit. Patent Med., 24: 134-135 (in Chinese).

Deng, W., T. Li, Z. Chen, L. Wu, W. Yang and C. Zhang, 2006. A critical note on the scientific name of cultivated edible fungus zhudugu. Acad. J. Edible Fungi, 13: 71-74 (in Chinese).

Dong, H., D. Cai and Y. Li, 2010. Research status and prospect of mushroom Panus giganteus. Edible Fungi China, 29: 3-6 (in Chinese).

Feng, L., H. Wu, H. Yao, C. Xie and F. Zhao, 2006. Optimization process of microwave assisted extraction the polysaccharide from the stem of mushroom *lentinus edodes*. Forest By-Prod. Spec. China, 84: 30-31 (in Chinese).

Letellier, M. and H. Budzinski, 1999. Microwaveassisted extraction of organic compounds. Analusis, 27: 259-271.

Peng, Z., 1998. Research on the biological characteristics and domestication of mushroom *Clitocybe maxima*. Thesis of Zhejiang Agricultural University (in Chinese).

Peng, Z., M. Gong and C. Shou, 1994. An evaluation of the nutritive value of a high proteinous mushroom *Clitocybe maxima*. Acta Agric. Zhejiangensis, 6: 247-252 (in Chinese).

Rao, H., 2002. The biological nature of mushroom *Clitocybe maxima* and high-yield culture technique. Edible Fung, 4: 13 (in Chinese).

Sanehez-Avila, N., F. Priego-Capote and J. Ruiz-Jimrnez, 2009. Fastand selective determination of triterpenie compounds in olive leaves by liquid ehmmatogaphy-tandem nlags spectrometry with multiple reaction monitoring after microwave-assisted extraction. Talanta, 78: 40-48.

Yang, X., Y. Chen and Z. Li, 2011. Compare study of the ultrasonic extraction and the enzymolysis method of polysaccharid in *Clitocybe maxima*. J. PuTian Univ., 18: 28-32 (in Chinese).

Zhang, H. and W. Han, 2005. Comparison study of extraction polysaccharide of lentinus edodes microwave-assisted and traditional hot-water method. Food Res. Dev., 26: 68-71 (in Chinese).

Zhu, Y. and F. Ma, 2007. The culture technique of mushroom *Clitocybe maxima*. Edible Fung, 4: 38 (in Chinese).