Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(10):856-859 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI :** 10.36347/sajb.2015.v03i10.007

Research Article

Cellulase-assisted extraction of pleurotus eryngii polysaccharide

Qin Li, Shaoying Zhang *, Lifen Hu

College of Food Science, Shanxi Normal University, Linfen City, Shanxi province, China

***Corresponding author**
Shaoying Zhang
Email: zsynew@163.com

Abstract: The pleurotus eryngii polysaccharide was extracted through using cellulase as subsidiary condition. The optimized conditions were studied by one-factor experiment and orthogonal experiment. The result showed that the influential degree weakened as follows sequence: pH, enzyme quantity, time and temperature. The suitable condition for extraction of pleurotus eryngii polysaccharide was enzyme quantity 0.030%, pH 4.5, temperature 55℃, and 40 minutes with 5 g of pleurotus eryngii in 100 mL enzyme suspension. Under optimized condition, the pleurotus eryngii polysaccharide yield was 27.0 mg/Kg.
Keywords: polysaccharide, cellulase, pleurotus eryngii, extraction.

INTRODUCTION

Pleurotus eryngii, a new rare edible fungus variety, was successfully developed in recent years. It may be used as food or drug. Its sporophores is white and fleshy, texture is crisp and nutrition is abundant [1, 2]. Modern pharmacological research shows that pleurotus eryngii polysaccharide can enhance the body immunity and have certain functions such as anti-virus, anti-tumor, anti oxidant. Moreover, pleurotus eryngii polysaccharide may reduce the cholesterol content of body and prevent hardening of the arteries [3, 4, 5].

With the further research on the functional properties of polysaccharide, the source and extraction process of polysaccharide are paid more attention to. At present, the extraction method of polysaccharide includes ultrasonic-assisted method, microwave-assisted method, and so on [6, 7, 8, 9]. In this experiment, pleurotus eryngii polysaccharide was extracted through degradation of cell wall using cellulase. Meanwhile, the effects of enzyme quantity, temperature, pH and time on the polysaccharide yield of pleurotus eryngii were investigated. Furthermore, the extraction conditions were optimized through orthogonal experiment. This experiment might provide reference for the deep development of pleurotus eryngii products.

MATERIALS AND METHODS

Materials and Reagents

Pleurotus eryngii was originated of Linfen, China. Cellulase was purchased from Kelong Chemical Co., Ltd. (Chengdu, China). Anthrone, thiourea, glucose (analytical grade) were purchased from Xiya Reagent Co., Ltd. (Chengdu, China). Alfa Aesar Company (Tianjin, China) supplied other reagents.

1.2 Equipments and instruments

GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; RJ-TDL-40C Centrifuge, Ruijiang Analysis Instrument Co., Ltd., Wuxi, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China ; SHA-C Water-bathing Constant Temperature Vibrator, Jintan Ronghua Instrument Manufacture CO., LTD, China.

Extraction of pleurotus eryngii polysaccharide

Fresh pleurotus eryngii was cleaned and torn into strips, dried at 55 ℃ for 10 hours to constant weight. Afterward, it was smashed and sieved at 40 meshes. 5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of cellulase suspension with different concentration was added into the flask. The pH of cellulase suspension was adjusted. The mixtures were shaken for extracting polysaccharide with constant temperature shaking bath under certain temperature for some time. Subsequently, the mixtures were centrifuged at 3000 r/min for 30 min. The supernatant was appropriately diluted for determination of polysaccharide content.

Determination of polysaccharide content

The pleurotus eryngii polysaccharide content of extraction solution was determined by anthrone - sulfate method [10]. Firstly, anthrone reagent was prepared. 0.2 g of anthrone and 1g of thiourea were added into brown

flask. And 100 mL of concentrated sulfuric acid was added into the brown flask. The mixture as anthrone reagent was shaken up. Secondly, glucose standard curve was drawn. 0, 0.03, 0.06, 0.09, 0.12 and 0.15 mL of glucose standard solution with the concentration of 1 mg/mL were respectively added to 25 mL volumetric flasks. Subsequently, 1.0, 0.97, 0.94, 0.91, 0.88 and 0.85 mL of deionized water were respectively added into volumetric flasks in order. Afterward, each flask was added into 4 mL of anthrone reagent. The shaken reaction mixtures were incubated in a boiling water bath for 10 min. After cooling, the absorbance versus the prepared blanks was read at 620 nm, and glucose standard curve was drawn. Finally, the pleurotus eryngii polysaccharide content of extraction solution was determined according to above provided method through substituting glucose standard with extraction solution. The standard curve of glucose was shown in Figure 1. Pleurotus eryngii polysaccharide content was expressed as mg glucose equivalents per Kg dried pleurotus eryngii.

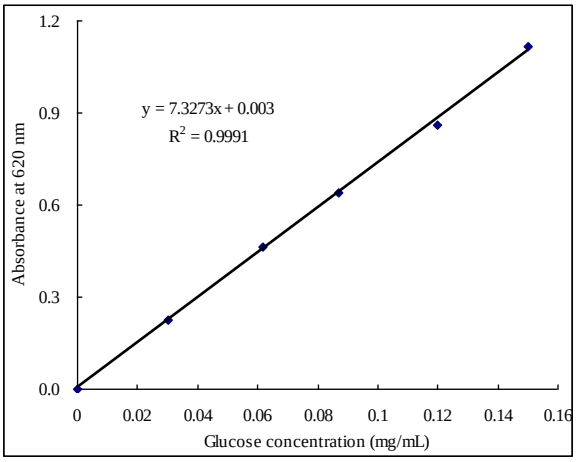

Fig-1: The standard curve of glucose

RESULT ANALYSIS AND DISCUSSION

Enzyme quantity

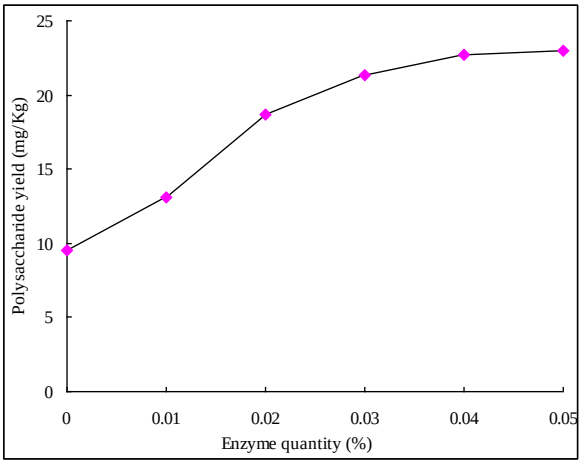

Fig- 2: Effect of enzyme quantity on pleurotus eryngii polysaccharide yield

5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of cellulase suspension with different concentration was added into the flask. The pH of cellulase suspension was adjusted to 5. The mixtures were shaken with shaking bath at 50 ℃ for 1 h. Subsequently, the mixtures were centrifuged at 3000 r/min for 30 min, and the polysaccharide content of supernatant was determined. As shown in Figure 2, with cellulase quantity enhancement from 0 to 0.04%, pleurotus eryngii polysaccharide yield increased. When enzyme increased to 0.04%, polysaccharide yield nearly reached to equilibrium and it was 136.5% higher than that of sample with no enzyme. As enzyme concentration further increased, pleurotus eryngii polysaccharide yield was almost unchanged.

PH:

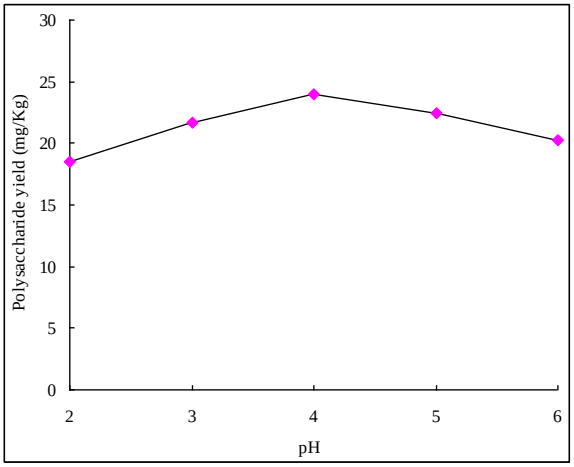

Fig-3: Effect of pH on pleurotus eryngii polysaccharide yield

5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of cellulase suspension with the concentration of 0.04% was added into the flask. The mixtures were shaken with shaking bath at different pH value under 50 ℃ for 1 h. Subsequently, the mixtures were centrifuged at 3000 r/min for 30 min, and the polysaccharide content of supernatant was determined. As described in Figure 3, with the increase of pH value, pleurotus eryngii polysaccharide yield first increased and then decreased. When the pH was 4.0, pleurotus eryngii polysaccharide yield achieved maximum for 24.0 mg/Kg. With the pH increasing further, it began to decrease. As a biological catalyst, enzyme needs to maintain a certain space-conformation [11]. As for catalyzing cellulose of pleurotus eryngii to degrade, probably pH 4.0 was the suitable condition. Once pH deviated 4.0, the conformation changed and catalytic activity decreased accordingly.

Temperature

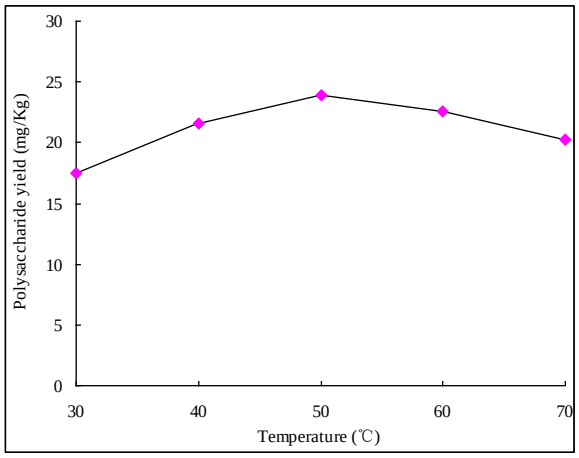

Fig-4: Effect of temperature on pleurotus eryngii polysaccharide yield

5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of cellulase suspension with the concentration of 0.04% was added into the flask. The pH of cellulase suspension was adjusted to 4. The mixtures were shaken with shaking bath at different temperature for 1 h. Subsequently, the mixtures were centrifuged at 3000 r/min for 30 min, and the polysaccharide content of supernatant was determined. As shown in Fig.4, with temperature increase, pleurotus eryngii polysaccharide yield first increased and then decreased. When the temperature rose to 50 ℃, it achieved the maximum for 23.9 mg/Kg. As the temperature further rise, polysaccharide yield began to decrease. Enzyme was a kind of biological active protein, and each enzymatic reaction has an optimum temperature. At optimal temperature, enzyme activity reaches the peak, and catalytic efficiency is also the highest [12]. However, in the excess of higher or lower temperature, enzyme activity became down.

Time

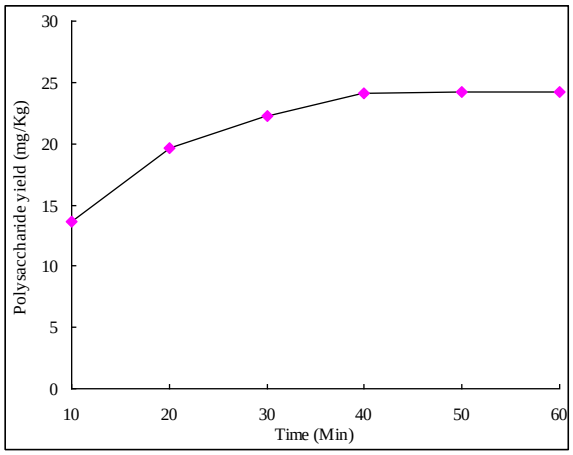

Fig-5: Effect of time on pleurotus eryngii polysaccharide yield

5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of cellulase suspension with the concentration of 0.04% was added into the flask. The mixtures were shaken with shaking bath at pH 4 under 50 ℃ for some time. Subsequently, the mixtures were centrifuged at 3000 r/min for 30 min, and the polysaccharide content of supernatant was determined. Pleurotus eryngii polysaccharide yield initially increased with extraction time extension. At 40 minute, polysaccharide yield nearly reached to equilibrium, and it was 77.1% higher than that of 10 minute. As extraction time further prolonged, polysaccharide yield almost unchanged.

Orthogonal experiment

Table1: Result of orthogonal design $L_9(3^4)$

NO.	A Enzyme quantity %	B pH	C Temperature ℃	D Time Min	Polysaccharide yield mg/Kg
1	1(0.025)	1(3.5)	1(45)	1(35)	24.5
2	1	2(4.0)	2(50)	2(40)	25.8
3	1	3(4.5)	3(55)	3(45)	26.3
4	2(0.030)	1	2	3	25.6
5	2	2	3	1	24.7
6	2	3	1	2	26.6
7	3(0.035)	1	3	2	26.8
8	3	2	1	3	25.4
9	3	3	2	1	24.5
k_1	25.53	25.63	25.50	24.57	
k_2	25.63	25.30	25.30	26.40	
k_3	25.57	25.80	25.93	25.77	
R	0.08	0.09	0.03	0.04	

According to orthogonal experiment (in table1), the extraction condition of $A_2B_1C_3D_2$ was the best, namely enzyme quantity 0.035%, pH 3.5, temperature 55℃, and 40 minutes, and the pleurotus eryngii polysaccharide yield was 26.8 mg/Kg. Analyzing k value ,the optimizing combination is $A_2B_3C_3D_2$, namely enzyme quantity 0.030%, pH 4.5, temperature 55℃, and 40 minutes. By test, the pleurotus eryngii polysaccharide yield was 27.0 mg/Kg. According to range R, in the process of extracting pleurotus eryngii polysaccharide, the influential factors were B > A > D > C, namely pH > enzyme quantity > time> temperature.

CONCLUSION

The pleurotus eryngii polysaccharide yield first increased and then decreased with pH enlargement or temperature rising, and it increased with enzyme quantity enhancement or time extension. The influential degree weakened as follows sequence: pH, enzyme quantity, time and temperature. The suitable condition for extraction of pleurotus eryngii polysaccharide was enzyme quantity 0.030%, pH 4.5, temperature 55℃, and 40 minutes with 5 g of pleurotus eryngii in 100 mL enzyme suspension. Under optimized condition, the pleurotus eryngii polysaccharide yield was 27.0 mg/Kg.

Acknowledgments

This work was supported by the project of Natural Science Foundation of Shanxi under grant no. 2012021025-3, and by the project of Natural Science Foundation of Shanxi Normal University under Grant no. ZR1311.

REFERENCES

1. Li XB, Feng T, Zhou F, Zhou S, Liu YF, Li W, *et al.;* Effects of drying methods on the tasty compounds of Pleurotus eryngii. Food Chemistry, 2015; 166(1): 358-364.
2. Lin JT, Liu CW, Chen YC, Hu CC, Juang LD, Shiesh CC, *et al.;* Chemical composition, antioxidant and anti-inflammatory properties for ethanolic extracts from Pleurotus eryngii fruiting bodies harvested at different time. LWT - Food Science and Technology, 2014; 55(1): 374-382.
3. Chen JJ, Yong YY, Xing MC, Gu YF, Zhang Z, Zhang SZ, *et al.;* Characterization of polysaccharides with marked inhibitory effect on lipid accumulation in Pleurotus eryngii. Carbohydrate Polymers, 2013; 97(2): 604-613.
4. Ma G, Yang W, Mariga AM, Fang Y, Ma N, Pei F *et al.;* Purification, characterization and antitumor activity of polysaccharides from Pleurotus eryngii residue. Carbohydrate Polymers, 2014; 114(19):297-305.
5. Jung HY, Bae IY, Lee SY, Lee HG; Effect of the degree of sulfation on the physicochemical and biological properties of Pleurotus eryngii polysaccharides.Food Hydrocolloids, 2011; 25(5): 1291-1295.
6. Liu Y, Qiang ML, Sun ZG, Du YQ; Optimization of ultrasonic extraction of polysaccharides from Hovenia dulcis peduncles and their antioxidant potential. International Journal of Biological Macromolecules, 2015; 80: 350-357.
7. Wu Z, Li H, Yang Y, Tan HJ; Ultrasonic extraction optimization of L. macranthoides polysaccharides and its physicochemical properties. International Journal of Biological Macromolecules, 2015; 74:224-231.
8. Chen C, Shao Y, Tao YD, Wen HX; Optimization of dynamic microwave-assisted extraction of Armillaria polysaccharides using RSM, and their biological activity. LWT - Food Science and Technology, 2015; 64(2):1263-1269.
9. Cheng ZY, Song HY, Yang YJ, Liu Y, Liu ZG, Hu HB, *et al.;* Optimization of microwave-assisted enzymatic extraction of polysaccharides from the fruit of Schisandra chinensis Baill. International Journal of Biological Macromolecules, 2015; 76:161-168.
10. Li QY, Jv HF, Zhai C; Study on the Extraction Technology of Polysaccharide from Schisandra. Food science, 2004; 25(5):105-108.
11. Ou YJ, Luo Q, Ai L, Li JE; Digestive Enzyme distribution in Adult Oplegnathus fasciatus and Effects of Ph on the Enzymatic Activity. Amino acids & biotic resources, 2010; 34(3): 1-4.
12. David ES, Diana JO, Keith RM, Roger AS; Protease-catalyzed condensation of peptides as a potential means to reduce the bitter taste of hydrophobic peptides found in protein hydrolysates. Enzyme and Microbial Technology, 1998; 22(2):101-110.