

Optimizing the polysaccharide extraction from the Vietnamese Lingzhi (*Ganoderma lucidum*) via enzymatic method

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Abstract: *Ganoderma lucidum* is commonly used in Vietnam and other countries since more than 4000 years ago with excellent beneficial health effects. Lingzhi has potential applications as a functional product with polysaccharide, and many other healthy components. This polysaccharide obtained good application in human. Lingzhi becomes a common mushroom in Vietnam, growing almost all year. The fruit of this mushroom contains high healthy polysaccharide. The polysaccharide extraction was surveyed and the enzymatic method of the extraction was optimized. The experiment was conducted according to a central composite design, with the following variables: Lingzhi-to-water ratio (L/W, 1:20–10:1), solvent temperature (ST, 35–55°C), enzyme concentration (EC, 0 – 0.8mL/250mL ~ 0 – 3.2%), and extraction time (ET, 1–7 hrs). *Lingzhi* were ground and mixed with the solvent, treated with enzyme, filtered and evaluated for polysaccharide contents. The polysaccharide extraction parameters were optimized. The result showed that the best adequate extraction conditions were L/W, 1:50; ST, 50°C; EC, 0.63mL/250mL ~ 0.25 %; and ET, 2.34 hrs. At such condition, 5.24% polysaccharide was extracted. The method is simple and can apply in the industry.

Keywords: *Ganoderma lucidum*, polysaccharide, Lingzhi, Enzyme, Optimizing Extraction

1. Introduction

Polysaccharide is one of the most important compounds in Lingzhi mushroom. Various polysaccharides have been extracted from the fruit body of lingzhi. *G. lucidum* polysaccharides (GL-PSs) are reported to exhibit a broad range of bioactivities, including anti-inflammatory, hypoglycemic, antiulcer, antitumorigenic, and immunostimulating effects (Miyazaki and Nishijima 1981; Hikino et al. 1985; Tomoda et al. 1986; Bao et al. 2001; Wachtel-Galor, Buswell et al. 2004). There are two major kinds: GL-A: Gal:Glu:Rham: Xyl (3,2:2,7;1,8:1,0) M= 23000 Da, and GL-B: Glu:Rham: Xyl (6,8:2,0;1,0) M= 25000 Da. To analyzing the structure of polysaccharide the main compounds of polysaccharide are glucose (Bao et al. 2001; Wang et al. 2002), xylose, mannose, galactose, and fructose... (Lee, Lee, and Lee 1999; Bao et al. 2002)

The aim of this research was focusing on the optimality of

the polysaccharide extraction from lingzhi fruit in enzymatic method.

2. Materials and Methods

2.1. Materials

Lingzhi was collected from Cu Chi farm (Ho Chi Minh City). The collected lingzhi fruit was dried and ground well before using to extraction.

2.2. Survey the Optimized Polysaccharide Extraction by Enzymatic Method

- Survey the polysaccharide extraction with enzymatic method with different extraction temperature (35, 40, 45, 50, 55°C), different enzyme concentration (0, 0.4, 0.5, 0.6, 0.7, 0.8 mL), in different extraction time (1.0, 2.5, 4.0, 5.5, 7.0 hrs).

- The experiment was conducted according to a central composite design, with the following variables: enzyme concentration (0.5 - 0.7 mL, center value is 0.6mL) and extraction time (1 - 4hrs, center value is 2.5hrs).

2.3. Spectrophotometer Analysis

Color changes of the treated samples were measured at 490 nm with a UV/Vis spectrophotometer (Harivaindaran, Rebecca, Chandran, 2008).

2.4. Statistical Analysis

All measurements were done in triplicate, except the center value was repeated 5 times. Using Modde 5 software to perform optimization. Analyses of Variance (ANOVA) were conducted by using SPSS Version 13.0 for Windows (SPSS). Turkey tests were performed to test the significant differences between the mean values for treatments ($p < 0.05$).

3. Results and Discussion

3.1. Preliminary Survey the Extracted Polysaccharides Content from Lingzhi Fruit

The water was chosen for polysaccharides extraction (Nguyen Thi Minh Tu, 2009). In the initial researches the ratio of solvent and raw material was 1:50 gave the best result for extraction with 2.14% of efficiency. At the boiling temperature the best extraction time was 60 min, which gave the efficiency at 1.90%. The extraction efficiency in the boiling water was higher than in the ethanol 96° at this solvent boiling temperature. (Nguyen Tuan Anh *et al.*, 2007).

So for the control sample, the condition of polysaccharide extraction was: 1:50 of the ratio of solvent and raw material, in 60 min. of the extraction time at boiling temperature.

3.2. Survey the Optimized Polysaccharide Extraction Condition

The extraction time was 2.5 - 4hrs with the highest received polysaccharide (2.84 - 2.93%, respectively), and there was no big difference between the two rates. So that, the extraction time of 2.5hrs was chosen for the other studies. If the extraction time was shorter it could be not enough to all polysaccharide extraction, but if longer, the polysaccharide could be destroyed and reduced.

In the polysaccharide extraction the optimum concentration of using cellulase was 0.24% (~0.6mL/250mL) with the highest received polysaccharide (3.38%). The lower concentration of using enzyme was not enough to all polysaccharide extraction; but the higher using cellulase not affect much on the quantity of the extracted polysaccharide.

The optimum extraction temperature was 50°C with the highest received polysaccharide (4.37%). The lower temperature was not enough to all polysaccharide extraction; and the higher extraction temperature was affected on the quality of polysaccharide and decreased the amount of received polysaccharide. If the extraction temperature were increased, the using enzyme would be inactive. That's why although the efficiency of the polysaccharide extraction was higher when the extraction temperature was rising, but in the enzymatic method, the temperature is only raising until the enzyme is still active.

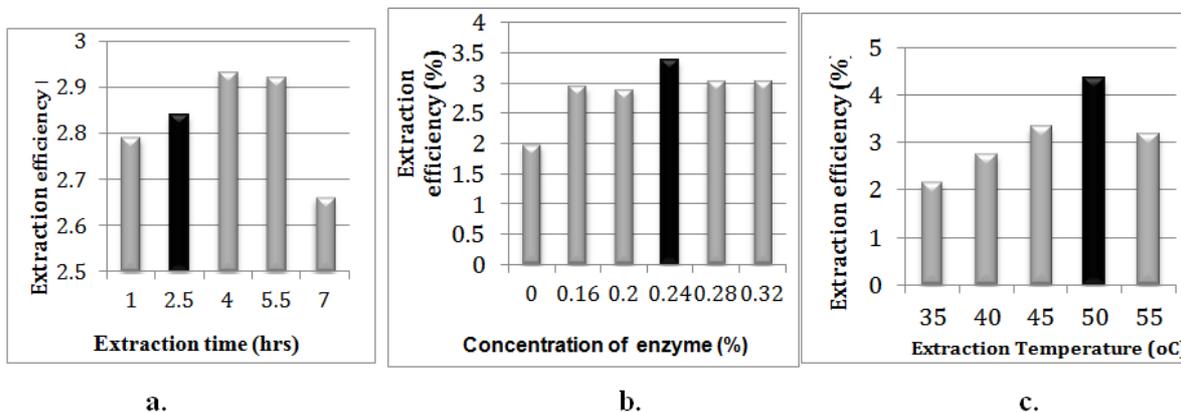


Fig. 1. The factor that effect on the polysaccharide extraction. a. Effect of the extraction time, b. Effect of the concentration of enzyme, c. Effect of the extraction temperature.

The enzymatic method gave the best result when cellulose was used with 0.6mL (~0.24%) of concentration, at 50°C in 2.5hrs. In this condition, the amount of polysaccharide was 4.37%.

3.3. Survey the Optimizing of Concentration of Using Enzyme and Extraction Time

The experiment was conducted according to a central composite design, with the following variables: enzyme

concentration (0.5 - 0.7 mL, center value is 0.6mL) and extraction time (1 - 4hrs, center value is 2.5hrs)

The optimal result was treated by Modde 5 (Fig.2) with $Q^2 = 0.629 > 0.5$ and $R^2 = 0.933 > 0.8$. The results are absolutely suitable with the optimal model. The regression equation of polysaccharide extracted efficiency depending on the enzyme concentration and extraction time was as following:

$$Y = 3.923 + 0.243 X_1 - 0.363 X_1^2 - 0.505 X_2^2$$

(X_1 is the enzyme concentration, X_2 is the extraction time)

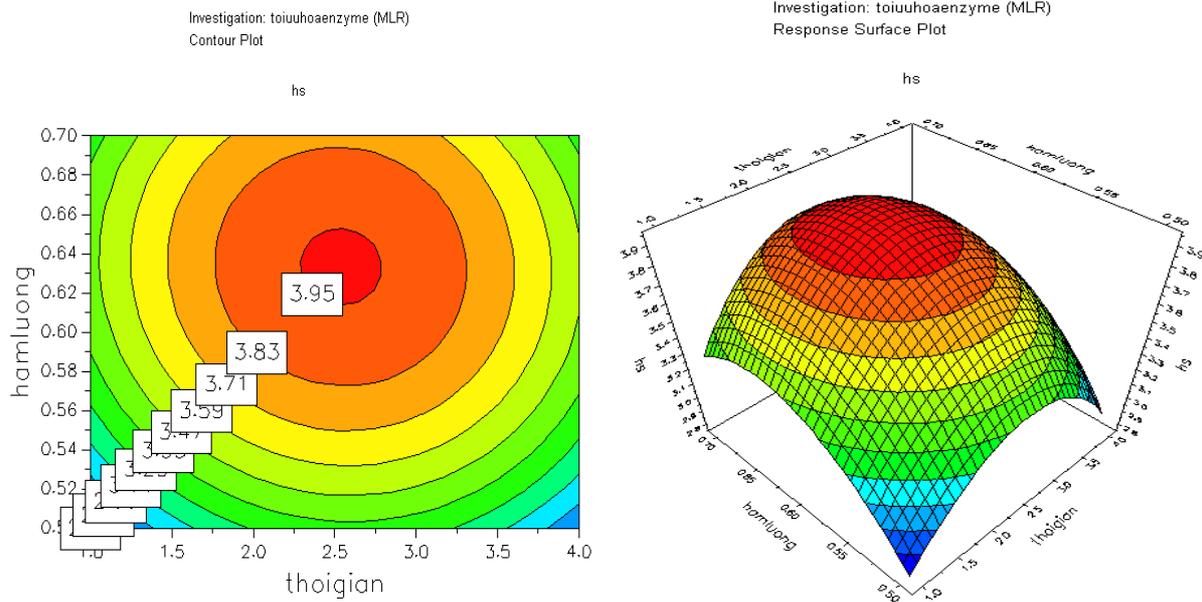


Fig. 2. Contour pilot and response surface pilot of survey the optimization of the enzyme concentration and extraction time

Table 1. Results of optimizing the concentration of enzyme and extraction time to the extracted polysaccharide from Lingzhi fruit.

Samples	Enzyme concentration (mL/250mL)	Extraction time (hrs)	Absorption A (490nm)	Quantity of polysaccharide	Extraction efficiency (%)
M1	0.5	1	0.459	145.9229532	2.91846
M2	0.7	1	0.502	160.7383757	3.21477
M3	0.5	4	0.462	146.9565874	2.93913
M4	0.7	4	0.488	155.9147498	3.11829
M5	0.4586	2.5	0.425	134.2084331	2.68417
M6	0.7414	2.5	0.576	186.2346842	3.72469
M7	0.6	0.379	0.441	139.7211485	2.79442
M8	0.6	4.621	0.478	152.4693027	3.04939
M9	0.6	2.5	0.591	191.4028548	3.82806
M10	0.6	2.5	0.618	200.7055619	4.01411
M11	0.6	2.5	0.629	204.4955537	4.08991
M12	0.6	2.5	0.597	193.4701231	3.8694
M13	0.6	2.5	0.589	190.7137654	3.81428

Optimal results obtained after empirically verifiable were: the enzyme concentration was 0.25 % (~0.634mL/250mL), extraction time was 2.34 hrs, and predictable efficiency was 5.24 %.

50°C, the extraction concentration was 2.34 hrs, the using cellulose concentration was 0.25%; then the extraction efficiency was 5.24%.

4. Conclusion

The lingzhi fruits, which were harvested in Cu Chi farm of Vietnam, were surveyed to find the highest quantity of polysaccharide extraction method. The optimal parameters of the extraction condition with distilled water were: the ratio of raw material and solvent was 1:50; extraction time was 60 min at boiling temperature; so the extraction efficiency was 2.14%. The optimal parameters of the polysaccharide extraction by enzymatic method were: the ratio of raw material and solvent was 1:50; extraction temperature was

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