Thu Dau Mot University
Journal of Science
ISSN 2615 - 9635
journal homepage: ejs.tdmu.edu.vn

The optimization of *cordyceps militaris* extraction process from fruiting body

*by **Nguyen Thi Ngoc Nhi** (Thu Dau Mot University)*

***Article Info**: Received April 24th, 2023, Accepted May 24th,2023, Available online June 15th,2023*
Corresponding author: *nhintn@tdmu.edu.vn*
https://doi.org/10.37550/tdmu.EJS/2023.02.405

ABSTRACT

Cordyceps has long been considered as a valuable medicinal herb known to possess numerous biological activities, including anti-microbial, anti-cancer, anti-metastasis and immunomodulatory effects. *With its benefits, many studies on optimizing the cultivation and production of C. militaris have been carried out. In addition, extraction methods have also been improved to intense efficiency extract the medicinal substances contained in this rare fungi. In this study, the aim was to optimize the process of C. militaris extraction from fruiting bodies based on 17 experimental data using water extraction method. The factors that affects to the extraction productivity such as: extraction temperature, extraction time and water/fungi ratio were investigated within a certain range. The experiments were arranged according to the Box-Behnken design, and then the results was optimized by Design expert software (version 13). In the optimal condition, the maximum productivity can be up to 32.23% with the extraction temperature is at 98^{o}C, the water/fungi ratio is 18:1 and the extraction time is 4 hours.*

***Keywords*:** cordyceps militaris, extraction process, optimization, water extract

1. Introduction

Cordyceps militaris is one of the *Cordyceps* genus fungi. It is commonly found in temperate and tropical forests, such as those in Korea, China, Japan, Vietnam. It can be grow naturally on culture that containing necessary nutrients and substrates. *Cordyceps* is used as a traditional medicine for a long time (Zhu *et al.*, 1998; Ng *et al.*, 2005). The pharmacological effects of *Cordyceps* have been proven through many scientific studies.

Cordycepin (also known as 3'-deoxyadenosine) from this fungus showed anti-microbial, anti-cancer, anti-metastasis, immunomodulatory activities (Das *et al.*, 2010). The compound CM-hs-CPS2 contained in the extract of *C. militari*s has the ability to antioxidant activity (Wu *et a.l*, 2011). Two types of polysaccharides, CPS-1 and CPS-2, increase the ability to recover from ethanol-induced liver damage due to the antioxidant capacity of fungi polysaccharides (Yan *et al.*, 2008). Acidic polysaccharide (APS) extracted from the fungus *C. militaris* has the potential to be applied in the treatment of influenza A. This substance contributes to the regulation of the immune activity of macrophages (Ohta *et al.*, 2007). Extracted *C. militaris* protein has the ability to inhibit the fungus *Fusarium oxysporum* and exhibited cytotoxicity against human breast and bladder cancer cells (Park *et al.*, 2009). Cordycepin also showed resistance to Clostridium (Ahn *et al.*, 2000) and its application as a substance immunomodulators are used in the treatment of diseases of the immune system (Shin *et al.*, 2009).

There were so many methods for *Cordyceps* extract, might be using a single extract method or simultaneous extract method. Sornchaithawatwong et al was extracted cordycepin from *C. militaris* by using extraction with a mixture of ethanol and water in 2022. Otherwise, Zhang et al. (2012) was optimized a synergistic extraction method, using water extraction plus ultrasonic extraction. Water extraction method has been applied to many fungi, but the efficiency is not high. In our study, we focus on optimizing the water extraction to save chemical, reduce the risk of organic (ethanol) residue in the extract and safer for consumer's health.

2. Materials and method

2.1. Material

C. militaris is grown on a medium containing brown rice, sugar and other organic nutrients at Thu Dau Mot University. When the mycelium is about 5-6 mm high, fresh fruiting body is collected and sublimated with a freeze drying machine of Bon Mua Biotech. The dried powder of the fungus is then extracted with water according to the selected parameters. Collect entire extract and evaporated solvent to obtain the *C. militaris* biomass extract. This extract is freeze-dried one more time to form a dry extract.

2.2. Method

Preliminary experiments were conducted to investigate the influence of each factor on extraction efficiency. Based on the experimental results, each factor was chosen 3 levels of impact and the experiments were using Box-Behnken design. Experimenting on the combined effects of three factors such as: extraction temperature (X_1), water/fungus ratio (X_2) and extraction time (X_3) to find out the optimal conditions for the extraction process. Each factor is placed at one of three equally spaced values, usually coded as 0 (center values), -1 and +1 (marginal value equidistant from central value). The extraction temperature in the experiment was limited to 80-100^oC, the water/fungi ratio was from

10-15, and the extraction time ranged from 3-5 hours. There were 17 experiments, each experiment was repeated 3 times. Experimental data were processed by Design expert software (version 13). The response variable (predicted productivity) is a second order polynomial model as below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j$$

which y is predicted productivity; β_0 is the regression coefficient for main; X_i is independent factor that effects on y; β_i is the regression coefficient level 1 that describes the influence of X_i on y; β_{ii} is the interactive regression coefficient describing the effect of X_i on y; β_{ij} is the interactive regression coefficient describing the simultaneous effect of X_i and X_j on y, k is factors surveying in experimental design (k = 3).

The experimental productivity of *C. militaris* extraction process is calculated as H (%) = $(m_{dr}/m_i)*100$ (g/g) which m_{dr} is mass of dry extract and m_i is mass of initial fruiting bodies of fungi.

3. Results and discussion

To estimate the total effect of three factors on the *C. militaris* extracted productivity, each factor is survey on 3 parameter levels (Table 1) and the result of experiments showed in Table 2.

TABLE 1. Coded level of factors use in study

Factor	Symbol	Range		
Extraction temperature	X_1	10	15	20
Water/fungi ratio	X_2	80	90	100
Extraction time	X_3	4	5	6

TABLE 2. The effect of three factors on extraction efficiency

Run	Extraction temperature	Water/fungi ratio	Extraction time	Average performance
1	90	10	5	25.07
2	90	20	3	27.37
3	80	20	4	27.25
4	100	10	4	25.62
5	90	15	4	30.18
6	80	15	5	28.50
7	90	10	3	23.99
8	100	15	5	31.96
9	80	10	4	24.72
10	100	20	4	31.77
11	90	15	4	30.10
12	80	15	3	26.51

13	100	15	3	30.43
14	90	15	4	30.05
15	90	15	4	30.50
16	90	15	4	30.37
17	90	20	5	30.68

According to multiple analysis of 17 data entries, Design expert (version 13) software provided a linear regression equation:

Productivity (H%) = $30.17 + 1.6X_1 + 2.21X_2 + 0.989X_3 + 0.906X_1X_2 + 0.556X_2X_3 - 2.74X_2^2 - 0.735X_3^2$ with R^2 value is 0.992.

The ANOVA analysis shows in Table 3 as below:

TABLE 3. ANOVA for reduced quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	106.97	8	13.37	127.67	< 0.0001
X_1	20.47	1	20.47	195.43	< 0.0001
X_2	38.98	1	38.98	372.21	< 0.0001
X_3	7.82	1	7.82	74.67	< 0.0001
$X_1.X_2$	3.28	1	3.28	31.34	0.0005
$X_1.X_3$	0.054	1	0.054	0.520	0.4915
$X_2.X_3$	1.24	1	1.24	11.8	0.0089
$X_2.X_2$	31.79	1	31.79	303.53	< 0.0001
$X_3.X_3$	2.28	1	2.28	21.77	0.0016
Residual	0.838	8	0.105		
Lack of Fit	0.697	4	0.174	4.93	0.756
Pure Error	0.141	4	0.035		
Cor Total	107.81	16			

Based on Table 3, the model F-value of 127.67 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, X_1X_2, X_2X_3, X_2^2, X_3^2 are significant model terms while X_1^2 and X_1X_3 does not meet the need of p-value, so we do not show it on the table. The lack of fit F-value of 4.93 implies there is a 7.56% chance that a lack of fit F-value this large could occur due to noise.

According to ANOVA analysis, all 3 factors have an impact on the efficiency the *Cordyceps* extracting process. In which, the ratio of water/fungi has the greatest influence compared to the other two factors. It was demonstrated in the table above that the F value of the water/fungi ratio is higher than the F value of the extraction time and temperature. Therefore, the change of the water/fungi ratio will lead to a large change in the extraction yield of *C. militaris.*

Besides, we also found that the combined effect of extraction time and extraction temperature on the yield was not significant. Therefore, in terms of the synergistic effect between the pairs of factors, there is a reciprocal interaction between the extraction temperature and the water/mushroom ratio as well as between the water/fungus ratio and the extraction time. These interactions influence the efficiency of *Cordyceps* extraction.

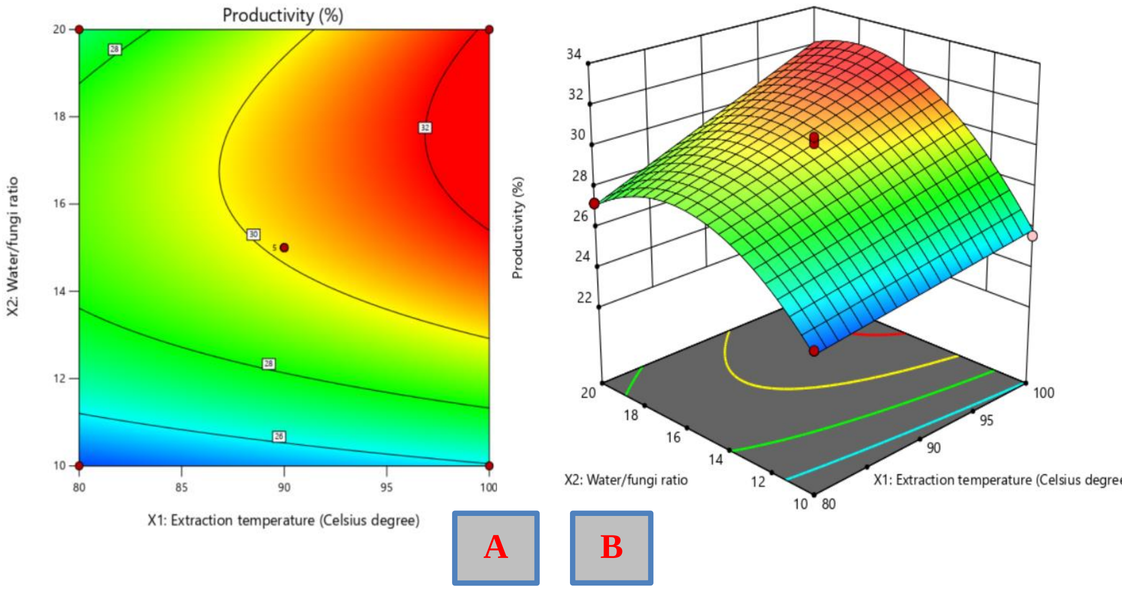

Figure 1. Extraction temperature and water/fungi ratio co-impact on extraction efficiency *(A: 2D contour; B: 3D surface)*

The X and Y-axes in the model graph in figure 1 represent extraction temperature and water/fungi ratio respectively. It pointed out that the highest productivity area (more than 30%) is equivalent to X value range from 95 to 100^{o}C and Y range 15 to 20 (extraction time is 4).

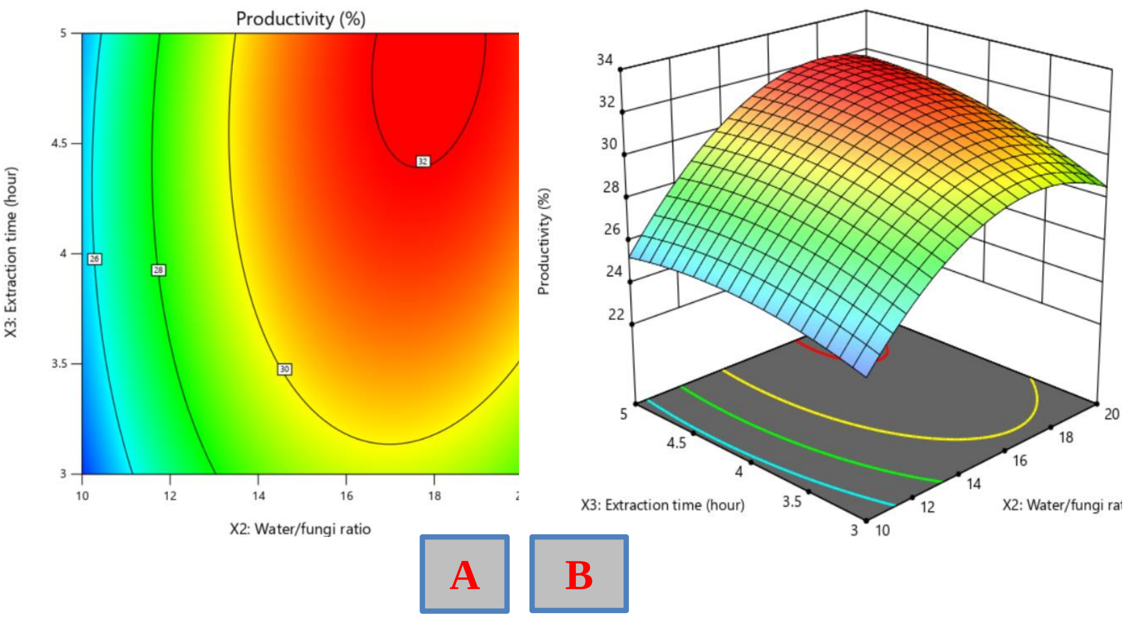

Figure 2. *Extraction time and water/fungi ratio co-impact on extraction efficiency (A: 2D contour; B: 3D surface)*

In figure 2, when water/fungi ratio value range 15 to 20 and extraction time from 4 to 5 hours (extraction temperature is 95^{o}C), its co-effect on the efficiency can be up to 33%.

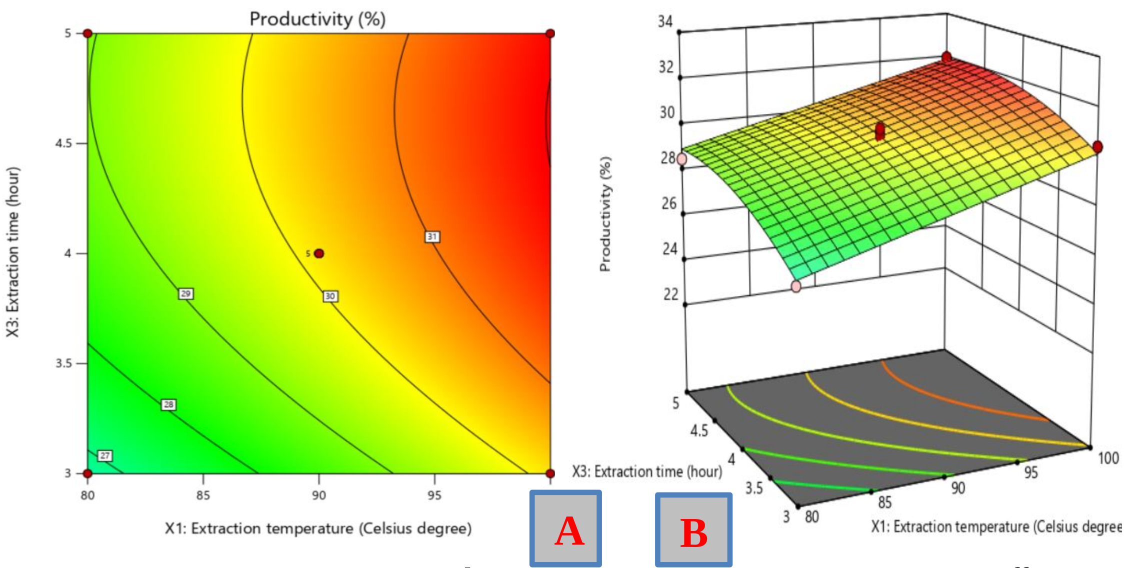

Figure 3. Extraction temperature and extraction time co-impact on extraction efficiency (A: 2D contour; B: 3D surface)

Due to what graphs show in figure 3, when the temperature is 95 to 100°C and time range 4 to 5 hours, the highest performance zone can get over 30%.

According to the linear regression equation above and the combined effect between factors on the extraction yield, the optimal condition was chosen. Comparison between predicted and experimental values is shown in Table 4:

TABLE 4. Comparison between predicted and experimental values

Independent variables			**Dependent variables**	**Optimized value**	
X_1 (°C)	X_2	X_3 (hour)		Experimental	Predicted
98	18:1	4	H	31.98	32.23

The experimental result show in table 4 is the average of three determinations. The different between experimental and predicted result is insignificant. From our research result, the extraction performance we got is higher than the extraction efficiency (10.862%) of the endogenous fungus *Fusarium oxysporum* using water extraction (Li *et al*, 2012). Moreover, extraction productivity of this study is similar to the other study results, such as *Termitomyces mummiformis* (32%), *Termitomyces heimii* (28%), *Termitomyces microcapus* (20%), *Pleurotus sajor-caju* (32%) and *Pleurotus djamor* (21%) (Puttaraju *et al*, 2006). It proves that model is suitable and feasible in real.

4. Conclusion

Followed to the statistical experimental data, the optimal water-extract condition for fruiting bodies *C. militaris* extraction were selected as extraction temperature (98°C), the water/fungi ratio (18:1), the extraction time (4 hours) and the maximum efficiency was predicted to be 32.23%. Based on this study, we are strongly believed in its application on industrial production.

References

Ahn, Y. J., Park, S. J., Lee, S. G., Shin, S. C. & Choi, D. H. (2000). Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp.. *Journal of Agricultural and Food Chemistry*, *48*, 2744-2748.

Das, S., Fujihara, S., Masuda, M., & Sakurai, A. (2010). Efficient production of anticancer agent cordycepin by repeated batch culture of *Cordyceps militaris* mutant. *Proceedings of International Conference on Engineering, Science and Applications*, 20-22.

Li, P., Lu, S., Shan, T., Mou, Y., Li, Y., Sun, W. & Zhou, L. (2012). Extraction optimization of water-extracted mycelial polysaccharide from endophytic fungus *Fusarium oxysporum* Dzf17 by response surface methodology. *International Journal of Molecular Sciences*, *13*(5) 5441-5453.

Ng, T. B. & Wang, H. X. (2005). Pharmacological actions of Cordyceps, a prized folk medicine, *Journal of Pharmacy and Pharmacology*, *57*, 1509-1519.

Ohta, Y., Lee, J. B., Hayashi, K., Fujita, A., Park, D. K. & Hayashi, T. (2007). *In vivo* antiinfluenza virus activity of an immunomodulatory acidic polysaccharide isolated from *Cordyceps militaris* grown on germinated soybeans. *Journal of Agricultural and Food Chemistry*, *55*, 10194-10199.

Park, B. T., NA, K. H., Jung, E. C., Park, J. W., & Kim, H. H. (2009). Antifungal and anticancer activities of a protein from the mushroom *Cordyceps militaris*. *Korean Journal of Physiology and Pharmacology*, *13*, 49-54.

Puttaraju, N. G., Venkateshaiah, S. U., Dharmesh, S. M., Urs, S. M. N., & Somasundaram, R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*, *54*(26), 9764-9772.

Shin, S. M., Lee, S. W., Kwon, J. H., Moon, S. H., Lee, S. J., Lee, C. K., Cho, K. H., Ha, N. J., & Kim, K. J. (2009). Cordycepin suppresses expression of diabetes regulating genes by inhibition of lipopolysaccharide-induced inflammation in macrophages. *Immune Network*, *9*(3), 98-105.

Sornchaithawatwong, C., Kunthakudee, N., Sunsandee, N., & Ramakul, P. (2022). Selective extraction of cordycepin from *Cordyceps militaris*–optimisation, kinetics and equilibrium studies. *Indian Chemical Engineer*, *64*(1), 1-13.

Wu, F., Yan, H., Ma, X., Jia, J., Zhang, G., Guo, X. & Gui, Z. (2011). Structural characterization and antioxidant activity of purified polysaccharide from cultured *Cordyceps militaris*. *African Journal of Microbiology Research*, *5*(18), 2743-2751.

Yan, H., Zhu, D., Xu, D., Wu, J., & Bian, X. (2008). A study on *Cordyceps militaris* polysaccharide purification, composition and activity analysis. *African Journal of Biotechnology*, *7*(22), 4004-4009.

Zhang, H., Wang, J. W., Dong, S. Z., Xu, F. X., & Wang, S. H. (2012). The optimization of extraction of cordycepin from fruiting body of *Cordyceps militaris* (L.) Link. *In Advanced Materials Research* (Vol. 393, pp. 1024-1028). Trans Tech Publications Ltd.

Zhu, J. S., Halpern, G. M. & Jones, K. (1998). The scientific rediscovery of an ancient chinese herbal medicine: *Cordyceps sinensis*: Part I. *Journal of Alternative and Complementary Medicine*, *4*, 289-303.