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Original Research Article

Determination of cordycepin content of Cordyceps militaris recombinant rice by high performance liquid chromatography

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Abstract

Purpose: *To assess the suitability of high performance liquid chromatography (HPLC) for the determination of cordycepin content of Cordyceps militaris recombinant rice*
Methods: *Cordyceps militaris recombinant rice was made by mixing brown rice with artificial Chinese caterpillar fungus culture medium powder using twin-screw extrusion technology. Cordycepin content was determined by reversed-phase HPLC with water:acetonitrile (95:5, v/v) as mobile phase, detection wavelength of 260 nm, and flow rate of 1.0 mL/min.*
Results: *Cordycepin contents showed good linearity in the range of 1 - 50.0 µg/mL (r^2 = 0.9996), and while recovery ranged from 103.2 to 109.9 %. Relative standard deviation (RSD), precision and repeatability RSD was 2.38, 0.76 and 1.46 %, respectively.*
Conclusion: *The HPLC method is simple, fast, accurate and reproducible. It is suitable for determination of cordycepin content of artificial Chinese caterpillar fungus culture medium and brown rice recombinant rice.*

Keywords: *Recombinant rice, Cordycepin, Chinese caterpillar fungus, Aweto, Cordyceps militaris*

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INTRODUCTION

Cordyceps militaris, also called North Aweto, is commonly known as Chinese caterpillar fungus. Aweto possesses significant medicinal value, which has led to increases in its domestic and foreign demand [1,2]. However, production of natural *Cordyceps militaris* is decreasing yearly due to poor growth conditions, thereby resulting in shortfalls in supply and inability to meet increasing demand [3,4]. At present, Aweto substitutes are made by artificial cultivation of the fruiting body of *Cordyceps militaris*, using a fast-growing biotechnological approach [5,6]. Research has shown that the solid medium of Chinese caterpillar fungus also contains cordycepin, sometimes even at higher levels than the fruiting body [7,8].

Chinese caterpillar fungus has many important bioactive substances, such as nucleosides (for example, cordycepin), polysaccharides and cordycepic acid (mannitol) [9,10]. Cordycepin can be used to improve immune function, and for treating cancer, leukemia, chronic bronchitis, pulmonary heart disease, hyperlipidemia,

hyperglycemia, and also as anti-aging agent [11]. Cordyceps polysaccharides may be used as antitumor, anti-radiation and immune-boosting compounds. Studies have shown that cordyceps D-mannitol has diuretic, anti-asthmatic, expectorant, and antioxidant properties [12].

In this study, *Cordyceps militaris* recombinant rice was made by twin screw extruder [16] with cordyceps culture medium and brown rice as raw materials, and a reversed-phase HPLC method was developed for the analysis of cordycepin content of the recombinant rice[3,14].

EXPERIMENTAL

Chemicals and samples

Chromatographic grade acetonitrile was purchased from Tianjin Kermel Chemical Reagent Company Ltd., China.

Cordycepin standard ($C_{10}H_{13}N_5O_3$) was obtained from Meilun Biological. Chinese Caterpillar fungus culture medium was a product of Hubei Academy of Agricultural Sciences (Wuhan, China). Experimental water was ultra-pure water, and the other reagents used in the experiment were all analytically pure.

Chromatographic conditions

HPLC analysis was carried out in high performance liquid chromatograph equipment (Agilent Technologies) with a 250 mm eighteen alkyl bonded phase silica column of internal diameter 4.6 mm.

Mobile phase was acetonitrile:water in a volume ratio of 5:95, and flow rate was 1 mL/min. Sample injection volume was 10 µL. Analysis was done at a column temperature of 35 ^{o}C and wavelength of 260 nm [15].

Codycepin standard curve

A 100 µg/mL standard solution of cordycepin was prepared by dissolving 10mg of cordycepin in 100 mL mobile phase (5:95 v/v acetonitrile: water) with shaking. Serial dilutions of the standard were prepared and subjected to HPLC analysis to determine cordycepin concentrations. Peak times, peak heights, and peak areas of the standard cordycepin concentrations were determined. Data obtained were used for plotting standard curve and for deriving linear regression equation.

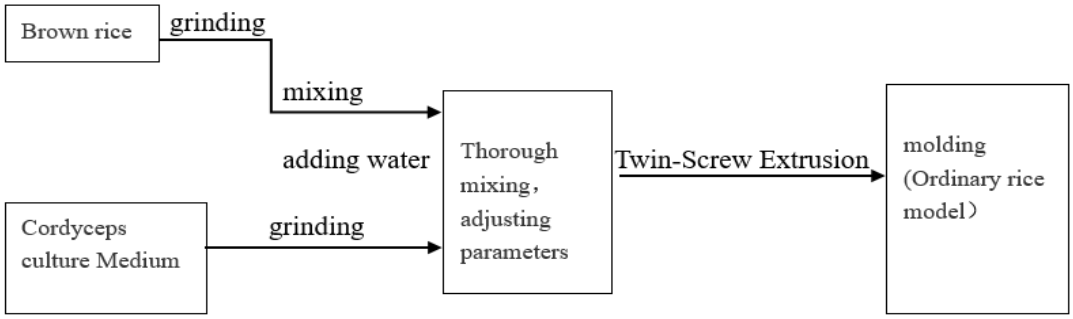
Production process for cordyceps recombinant rice

The procedure used for production of recombinant rice is summarised in Scheme 1 [16].

Conditions for formation of cordyceps recombinant rice were: content of cordyceps in culture medium: 20 %; total moisture content: 40 %; screw speed; 250 rpm; barrel temperature: 80 ^{o}C.

Preparation of rice extract

Rice sample (0.5 g) was uniformly ground and placed in a 100-mL volumetric flask containing about 80 mL of water. It was then subjected to ultrasonic extraction for 3 h, after which 1 mL was centrifuged at the speed of 4000 rpm. The supernatant fluid was filtered through a 0.45 µm microporous membrane (Tianjin Jinteng Laboratory Equipment Co., Ltd.), and the filtrate was used for HPLC analysis. Analysis was repeated six times, and relative standard deviation was obtained. To test for recovery, 5 samples of the cordyceps culture medium were each spiked with 10mL of 5 ug/mL cordycepin, and the recovery of added codycepin via HPLC was determined. Average peak area and RSD were calculated.



Scheme 1: Outline of the production of recombinant rice

RESULTS

The peak time, peak area and peak height of the six concentrations of cordycepin standard solution are shown in Table 1. The standard curve was linear, with a good linear correlation (regression equation was y = 67.397x - 3.6872 and regression coefficient of R^2 = 0.9992). Cordycepin concentrations of cordyceps culture medium and cordyceps recombinant rice were 14.13 and 2.56 μg/mL, respectively (Table 2). As shown in Table 3, RSD values for precision and repeatability were 0.76 and 1.46 % respectively. Recovery of added codycepin ranged from 103.15 to 109.90 %, with RSD of 2.38 %.

DISCUSSION

The standard curve of cordycepin had very good linearity (R^2 > 0.99). This shows that it is suitable for determination of cordycepin in the range of 0 - 50 ug/mL.

Table 1: Peak time, peak area and peak height of standard cordycepin chromatogram

Concentration of cordycepin standard solution	Peak time (min)	Peak area	Peak height
1	6.592	70	5.4
2	6.590	133	10.2
5	6.585	332.6	25.8
10	5.567	709.2	54.7
20	6.589	1280.1	96.6
50	6.589	3383.9	257

Table 2: Content of cordycepin in cordyceps recombinant rice

Material	Peak time (min)	Peak area	Cordycepin concentration (μg/mL)
Brown Rice	6.463	Not detectable	
Cordyceps culture medium	6.583	968.13635	14.13
Cordyceps recombinant rice	6.582	177.38225	2.56

Table 3: Precision, repeatability and recovery of the proposed method

Precision test	Number	1	2	3	4	5	6
	Peak time (min)	5.645	5.569	5.959	5.486	5.969	5.981
	Peak area	1054.3	1054.6	1038.7	1043.2	1047.9	1062.8
	Mean peak area			1050.25			
	RSD			0.76%			
Repeatability test	Number	1	2	3	4	5	6
	Peak time (min)	6.091	6.097	6.142	6.168	6.166	6.176
	Peak area	251.9	248	257.8	257.8	254.7	250.2
	Mean peak area			253.40			
	RSD			1.46%			
Recovery test	Number	1	2	3	4	5	
	Peak time (min)	6.009	6.041	6.051	6.066	6.085	
	Peak area	1083.1	1074.5	1042.2	1031.4	1017.9	
	Mean peak area			1049.82			
	RSD			2.38%			
Recovery of standard addition	Original cordycepin content (μg)	1417.24	1418.65	1420.07	1419.78	1419.5	
	Standard Addition (μg)	50	50	50	50	50	
	Actual concentration (μg/mL)	14.67	14.69	14.7	14.7	14.69	
	Measured concentration	16.13	16	15.52	15.36	15.16	
	Recovery	109.90%	108.93%	105.56%	104.49%	103.15%	
	Mean recovery			106.41%			
	RSD			2.38%			

Results obtained in the determination of codycepin concentrations in culture medium and recombinant rice indicated that the high temperature extrusion method did not affect their codycepin concentrations, nor did it affect the stability of codycepin. The RSD of the precision test for the determination of cordycepin was 0.76 % (i.e., < 10 %), which indicates that the measured value was very close to the actual value. It also implies that the HPLC method has a high degree of precision.

In the repeatability test, RSD was 1.46 % (i.e., < 10 %), indicating that standard deviation between the individual values of the same sample was very small. Thus reproducibility of the results is good.

Ideally, recovery should be close to 100 %. However, due to the nature of samples and reagents, interference from impurities and operating errors, standard recovery in the range of range of 90 - 110 % is acceptable. In this study, mean recovery was 106.41 %, illustrating that recovery of added cordycepin was accurate and in accordance with analytical requirements.

CONCLUSION

The findings of this study demonstrate that analysis of cordycepin in recombinant rice with reversed-phase high performance liquid chromatographic method is feasible, fast, reliable and reproducible. Thus, the method is suitable for the determination of cordycepin content of artificial Chinese caterpillar fungus culture medium and recombinant brown rice.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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