Food Chemistry 137 (2013) 164–167

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Composition and distribution of the main active components in selenium-enriched fruit bodies of *Cordyceps militaris* link

Jing Z. Dong [a], J. Ding [a], Pei Z. Yu [a], Can Lei [a], Xiao J. Zheng [a], Y. Wang [b,*]

[a] Key Laboratory of Biologic Resources Protection and Utilization of Hubei Province, School of Biological Science and Technology, Hubei University for Nationalities, Enshi 445000, China
[b] Key Laboratory of Pant Germplasm Enhancement and Speciality Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form 28 September 2012
Accepted 22 October 2012
Available online 2 November 2012

Keywords:
Cordyceps militaris
Selenocompounds
Distribution
Active components
Minerals

ABSTRACT

Selenium-enriched *Cordyceps militaris* fruit bodies are industrially cultivated as functional food or medicinal food in China and southeast Asia. However, composition of selenium compounds and distribution of the main bioactive components are still unknown. In the selenium-enriched fruit bodies, the main soluble selenium compounds of low molecular weight were identified as SeMet (selenomethionine), and the main selenium compounds bound in proteins were identified as SeMet and SeCys (methylselenocysteine). Trace minerals as Se (selenium), Zn (zinc), Fe (iron) and the main active components as adenosine, cordycepin and carotenoids were mostly distributed in the terminal of fruit bodies, while P (phosphorus) and K (potassium) were evenly distributed in the fruit bodies. The results indicated that terminal of the fruit bodies should be the better materials for production of advanced functional food. So cultivation of relatively short and thick fruit bodies with bigger terminals deserves further research.

1. Introduction

Cordyceps militaris link, a kind of caterpillar-shaped entomopathogenic fungi in the class of Ascomycetes has been widely used as a folk medicine in east Asia as Korea, China, and Japan for revitalization of various systems of the body. *C. militaris* has many pharmacological functions such as anti-inflammatory (Das, Masuda, Sakurai, & Sakakibara, 2010), anti-oxidation and anti-aging (Khan, Tania, Zhang, & Chen, 2010), anti-tumor (Zhang et al., 2010), anti-proliferation (Chen, Luo, Li, Sun, & Zhang, 2004), anti-metastasis (Shih, Tsai, & Hsieh, 2007), anti-bacteria (Ahn, Park, Lee, Shin, & Choi, 2000), anti-leukaemia (Kodama, McCaffrey, Yusa, & Mitsuya, 2000), improving insulin secretion and anti-diabetes (Choi, Park, Choi, Jun, & Park, 2004), anti-fatigue (Jung, Kim, & Han, 2004), liver-protection (Won & Park, 2005) and so on. Adenosine was considered the main active component of *Cordyceps* TCM (The State Pharmacopoeia Commission of the China, 2005). Cordycepin present in *C. militaris* had been found to be a new anticancer compound (Wong et al., 2012). Cultivation of *C. militaris* is an important way to produce medicinal foods, so it is necessary to study quality control of *C. militaris* and related cultivation techniques.

Se (selenium) has been reported to have crucial significant physiological functions as antioxidation (Tara, Rayman, & Boskabadi, 2010), anticancer (Ramoutar & Brumaghim, 2010), immunity stimulation (Silva et al., 2010), inhibiting HIV (Yu et al., 2007) and so on. Compared to traditional *C. militaris*, selenium-enriched *C. militaris* contained much higher contents of some important active components as cordycepin, adenosine, carotenoids, polysaccharides and so on (Dong, Lei, Ai, & Wang, 2012; Dong, Liu, Lei, Zheng, & Wang, 2012). Nowadays, fruit bodies of selenium-enriched *C. militaris* are being industrially cultivated to produce medicinal food or important medicines as cordycein and adensine in China. However, the chemical composition of selenium compounds and distribution of the other main active components in the selenium-enriched fruit bodies are still not clear. In this study, composition and distribution of selenium compounds and the other main active components in *C. militaris* fruit bodies were studied to provide theoretical basis for cultivation and quality control of *C. militaris* production.

2. Materials and methods

2.1. Materials, chemicals and reagents

Selenium-enriched fruit bodies of *C. militaris* were prepared according to our published methods with some modifications (Dong, Liu, Lei et al., 2012; Dong, Lei, Ai et al., 2012). Namely, *C. militaris* was cultivated at the substrate with sodium selenate of 20 ppm, under pink light (LED lightening, 2/3 of 620–630 nm + 1/3 of 450–460 nm) and light intensity of 400Lx. When the club-shaped fruit bodies were covered with golden-yellow spores, the fruit bodies were harvested. The harvested fruit bodies were

* Corresponding author. Tel.: +86 27 87510771.
E-mail address: yingwang@wbgcas.cn (Y. Wang).

0308-8146/$ - see front matter Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.021

length-averagely cut into three parts: base part, middle part and terminal part, and then vacuum dried at 60 °C, respectively.

Standards of cordycepin and adinosine were purchased from the National Institute for Food and Drug Control (Beijing, China). SeCys, SeMet and proteinase K were from Sigma. Acetonitrile, sodium citrate, hexanesulfonic acid and acetic acid were of HPLC grade, and all the other chemicals were of analytical grade.

2.2. HPLC and LC–ICP–MS systems

HPLC system (Hangzhou Saizhi Sci & Tech Co., Ltd., China) consists of N2000 ChemStation, STI501 pump, STI 501 UV detector and Lichrospher C18 column (4.6 × 250 mm, Jiangsu Hanbon Sci & Tech Co., Ltd., China). HPLC–ICP–MS system consisting of an Agilent 1100 HPLC with C18 column (4.6 × 250 mm, Agilent), Agilent 7500s ICP–MS, concentric nebulizer and Scott-type double-pass spray chamber was employed for selenium-specific detection. The mobile phase for HPLC–ICP–MS were: 5 mmol/L sodium citrate:5 mmol/L hexanesulfonic acid = 95:5, flow rate of 1.0 mL/min, injection of 20 μL. An ultraviolet–visible spectrophotometer (Shanghai Youke Instrument and Meter Co., Ltd., China) was used for quantitation of total selenium and carotenoids.

2.3. Isolation and identification of selenium compounds from the fruit bodies

Preparation of lipid fraction: dried fruit bodies (approximately 30 g) was ground, and added with chloroform–methanol (2:1, 60 mL), and then shaken vigorously and filtered. The procedure was repeated three cycles. The extracts were combined and vacuum dried at 60 °C as lipid fraction.

Preparation of LMW (low-molecular-weight) fraction: perchloric acid (0.2 mol L^{-1}, 10 mL) was added to 0.2 g lipid-free samples (the residue after preparation of lipid fraction). The mixture was ultrasonicated for 2 h, centrifuged at 5000 rpm for10 min, and filtered. The solution obtained was the low-molecular-weight fraction (LMW) and was further analysed by HPLC–ICP–MS.

Protein isolation and enzymatic hydrolysis: sodium hydroxide solution (0.1 mol L^{-1}, 10 mL) was added into 1.0 g lipid-free sample. After agitation (Vortex) the mixture was centrifuged (10 min, 5000 rpm), the solid residue was again treated with sodium hydroxide solution for three cycles. The combined solutions (20 mL) were neutralised to pH 7.0 with 4 mol L^{-1} phosphoric acid, and acetone was added to make the final concentration to 80%. The sample was kept at −20 °C for 30 min, and the precipitate (proteins) obtained by centrifugation (10 min, 5000 rpm) was washed with acetone, and vacuum-dried at room temperature. The precipitate was solubilised in acetic acid-ammonium acetate buffer (50 mmol L^{-1}, pH 4.5) containing 5% sodium dodecylsulfate (water bath 60 °C, 5 min) and filtered through a hydrophilic low-protein-binding polysulfone filter (0.45 μm). The filtrates were used as protein samples. The proteins obtained above were solubilised in 5 mL phosphate buffer (pH 7.5) and incubated with 1.0 ml of proteinase K (20 mg mL^{-1}, 37 °C, overnight), then acetone was added to final concentration of 80%, and the mixture was centrifuged at 5000 rpm for 10 min. The supernatant was vacuum dried at 60 °C, and the residue containing selenoamide acids was solubilised in the mobile phase for HPLC–ICP–MS analysis.

2.4. Chemical specific image of selenium and main minerals

Chemical specific images of Se, Zn (zinc), Fe (iron), P (phosphorus), and K (potassium) were conducted by Synchrotron-Based X-ray Absorption Spectroscopy at beamline 20-ID (PNC/XOR) of the APS. The fruit bodies were collected for synchrotron radiation analysis which was conducted according to the published methods (Pickering, Prince, Salt, & George, 2000; Shen et al., 2002).

2.5. Determination of selenium, cordycepin, adenosine and carotenoids

Distribution of the other components as total selenium, cordycepin, adenosine and carotenoids in different parts of fruit bodies were analysed respectively. Contents of carotenoids were determined according to the published method (Santamaria, Reyes-Duarte, & Bárzana, 2000). Contents of cordycepin and adenosine were analysed according to our established methods (Dong, Liu, Lei et al., 2012). Total selenium of the samples was analysed according to the method by Liu and Gu (2009).

3. Results

3.1. Composition of selenocompounds in the fruit bodies

Total selenium of the lipid fraction was analysed and on no occasion was selenium detected (data not shown). Selenium compound might be distributed in the lipid-free fractions, so selenium compounds in the lipid-free fractions were extracted and isolated for HPLC–ICP–MS analysis.

The main soluble LMW selenocompounds in the lipid-free fraction of fruit bodies was identified as SeMet (selenomethionine), and then selenite and selenate, while Se-Cys was detected as the micro-selenocompound. (Fig. 1A). The organic selenium in the form of SeMet was determined to be more than 5 folds to inorganic selenium (data not shown). SeMet had been found to be the principal seleno-amino acid in Se-enriched plants (Brown & Shrift, 1982), which was in agreement with this study.

Selenium was detected to be in the forms of SeMet and SeCys (methylselenocysteine) in protein fractions (Fig. 1B). It can be concluded that as a selenium supplementation food, selenium-enriched *C. militaris* fruit bodies provide selenium source mainly in the form of SeMet and SeCys.

3.2. Distribution of selenium and other components in the fruit bodies

Except for even distribution of P and K, the trace minerals as Se, Zn, and Fe were specifically concentrated in the terminal of fruit bodies (Table 1, Fig. 2), and very fewer in the central and basal parts of fruit bodies. The contents of cordycepin and adenosine of different parts of fruit bodies are significantly different: top (0.536 ± 0.072%, 0.037 ± 0.005%) > middle (0.301 ± 0.08%, 0.021 ± 0.004%), base (0.312 ± 0.058%, 0.018 ± 0.004%). Carotenoids and organoselenium in different parts of fruit bodies were also significantly different: top (0.115 ± 0.008%, 42.13 ± 5.61 ppm) > middle (0.079 ± 0.006%, 16.12 ± 1.75 ppm) > base (0.061 ± 0.024%, 23.28 ± 1.66 ppm) (Table 1). The main reason might be that the top of the fruit bodies was the growth center (Fig. 3), which made Se, Zn and Fe concentrate into apexes. In plants, organoselenium was exclusively distributed in the young leaves, and young leaves were the main site transforming selenium into organoselenium (Pickering et al., 2000). In the cultivation of *C. militaris*, relatively short and thick fruit bodies rather than long fruit bodies should be considered the best products. However, in the traditional production, daylight or white light are always used for fruit body development, which make the fruit bodies grow very long, and furthermore longer fruit bodies are traditionally considered excellent. In our previous research, light of short wavelengths could significantly increase the contents of cordycepin (Dong, Liu, Lei et al., 2012), so further research should be focused on special cultivation conditions such as short light wavelength or lower temperature to induce short and thick fruit bodies.