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Chemical Ingredients of *Cordyceps militaris*

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Medicinal mushrooms, including *Cordyceps militaris*, have received attention in Korea because of their biological activities. In the fruiting body and in corpus of *C. militaris*, the total free amino acid content was 69.32 mg/g and 14.03 mg/g, respectively. In the fruiting body, the most abundant amino acids were lysine, glutamic acid, proline and threonine. The fruiting body was rich in unsaturated fatty acids, which comprised about 70% of the total fatty acids. The most abundant unsaturated acid was linoleic acid. There were differences in adenosine and cordycepin contents between the fruiting body and the corpus. The adenosine concentration was 0.18% in the fruiting body and 0.06% in the corpus, and the cordycepin concentration was 0.97% in the fruiting body and 0.36% in the corpus.

KEYWORDS : Adenosine, Amino acid, Cordycepin, *Cordyceps militaris*, Fatty acid

"Winter Worm Summer Grass" (*Cordyceps*), known as "Dong Chung Ha Cao" in Korea and "Dong Chong Xia-Cao" in China, has been used as a traditional folk medicine in Asia for hundreds of years. *Cordyceps* is in the family Clavicipitaceae in the class Pyrenomycetes of the order Hypocreales of the ascomycetous fungi. It parasitizes insects (Kobayashi, 1982; Spatafora and Blackwell, 1993) and colonizes dead or living *Hepialus* (Lepidoptera) caterpillars. Spores germinate inside the caterpillars, hyphae fill the caterpillar body, and a stalked fruiting body is produced (Li *et al*., 1998). The fruiting body of *Cordyceps* is collected from infected pupae or larvae.

Various bioactive compounds are found in *Cordyceps* spp. Compounds from *C. sinensis* can modulate immune responses (Kuo *et al*., 1996), inhibit the growth of tumor cells (Bok *et al*., 1999), enhance hepatic energy (Manabe *et al*., 1996) and promote the secretion of adrenal hormones (Wang *et al*., 1998), and it posseses hypotensive and vasorelaxant activities (Chiou *et al*., 2000). Cordycepin from *C. militaris* has several biological activities, including inhibition of RNA and DNA synthesis and suppression of viral replication (Kuo *et al*., 1994). Galactomannan from *C. cicadae* prevents the growth of sarcoma in mice (Huang *et al*., 1997). Polysaccharides purified from *C. ophioglossoides* have antitumor properties (Wu *et al*., 2001). N^6-(2-hydroxyethyl) adenosine (HEA), a nucleoside derivative isolated from *C. pruinosa*, has a Ca^{2+} antagonistic effect and negative inotropic response (Furuya *et al*., 1983). Compounds from *C. pruinosa* suppress inflammation through the suppression of NF-κB-dependent inflammatory gene expression (Kim *et al*., 2003).

Naturally grown fruiting bodies of *Cordyceps* are expensive and scarce but the demand for *Cordyceps* has increased. The *Cordyceps* species, *C.militaris* and *C. pruinosa* are cultivated mainly in Korea. The aim of this study was to find and to compare the chemical ingredients of the fruiting body and corpus of *Cordyceps militaris*

Materials and Methods

Samples. *Cordyceps militaris* was purchased at the Kyong-dong market in Seoul. Specimens were divided into fruiting body and corpus, then milled.

Amino acid assay. The amino acid composition was determined by hydrolyzing *Cordyceps militaris* samples with 6 N HCl for 24 h at 105°C, then deriving the amino acids in a Waters Pico-Tag work station (Pico-Tag System, Waters Co.). The derivative amino acids were analyzed by liquid chromatograph consisting of Waters 515 pumps, Waters 486 UV detector, and Reodyne injector (Waters Co.), equipped with Waters Pico-Tag column (3.9 × 150 mm, Waters Co.). Amino acids were identified by comparing retention times and areas to an authentic standard mixture.

Fatty acid assay. Fatty acids were extracted from dried samples using the method of Hamilton & Hamilton (1992). Fatty acids were determined as fatty acid methyl esters (FAMEs) by gas chromatography using Hewlett-Packard, Model 5890 Series II gas chromatograph (Agilent Co.) equipped with a fused silica capillary column (SP-2560, with a 0.25 mm diameters, 100 m length, and 0.20 μm film thickness; Supelco Ltd.). The sample was injected into the GC using a Hewlett-Packard 7673 autoinjector (Agilent Co.). The temperature program was:

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140°C for 5 min; increase to 240°C at 4°C/min; maintain at 240°C for 15 min. Helium was used as the carrier gas and was maintained at a flow rate of 20 cm/s. The injection port and the flame ionization detector oven temperatures were 260°C. FAMEs were identified by comparing retention times to an authentic standard mixture (Supelco 37 Component FAME Mix, Supelco Co.).

Adenosine and cordycepin. Known amounts of adenosine and cordycepin were dissolved in mobile phase solution to give various concentrations for calibration. Samples were extracted in hot water at 100°C for 2 h, and then filtered through a 0.45 μm filter membrane. HPLC analysis was performed using a HITACHI L-6200 pump with a RHEODYNE M-4250 detector and D-2500 integrator. A pre-packed RP column Cosmosil 5C18 (4.6 × 250 mm, 5 μm particle size) from Nacalai Tesque (Kyoto, Japan) was used. The mobile phase was a mixture of methanol and 0.02 M potassium dihydrogenphosphate (15 : 85). Elution was performed at a solvent flow rate of 1 *ml*/min starting with 30% methanol for 15 min. A gradient was then used to obtain 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, and 80% methanol at 52 min. Elution then remained isocratic at 80% methanol for another 60 min. Detection was performed with a variable-wavelength UV detector (L-4250) at 260 nm.

Results

Amino acids. Amino acid compositions of *C. militaris* are presented in Table 1. The total amino acid content (dry weight) was higher in the fruiting body (69.32 mg/g) than in the corpus (14.03 mg/g). The content of individual amino acids in the fruiting body and corpus of *C. militaris* ranged from 1.15 to 15.06 mg/g and from 0.36 to 2.99 mg/g, respectively. Amino acids present at concentrations of more than 5.00 mg/g were lysine (15.06 mg/g), glutamic acid (8.79 mg/g), prolin (6.68 mg/g), threonine (5.99 mg/g), arginine (5.29 mg/g), and alanine (5.18 mg/g) in the fruiting body. Chang *et al.* (2001) reported that the most abundant amino acids in *C. militaris* mycelia were aspartic acid (2.66 mg/g), valine (2.21 mg/g) and tyrosine (1.57 mg/g).

Table 1. Contents of free amino acids in *Cordyceps militaris*

Amino acid	Content (mg/g dry wt)	
	Fruiting body	Corpus
Aspartic acid	4.75	0.36
Serine	3.13	0.39
Glutamic acid	8.79	1.40
Glycine	1.84	0.52
Histidine	1.84	0.46
Arginine	5.29	0.65
Threonine	5.99	0.86
Alanine	5.18	0.98
Proline	6.68	2.99
Tyrosine	3.39	1.27
Valine	3.46	0.65
Methionine	0.18	0.07
Lysine	15.06	2.20
Isoleucine	1.16	0.35
Leucine	1.43	0.46
Phenylalanine	1.15	0.42
Total	69.32	14.03

Table 2. Contents of fatty acids of *Cordyceps militaris*

Fatty acid	Content (% of total FA)	
	Fruiting body	Corpus
Palmitic acid (C16:0)	24.5	21.5
Palmitoeic acid (C16:1)	2.3	2.1
Stearic acid (C18:0)	5.8	5.0
Oleic acid (C18:1)	6.0	17.7
Linoleic acid (C18:2)	61.3	33.0
Linolenic acid (C18:3)	–	20.6

Table 3. Contents of adenosine and cordycepin in *Cordyceps militaris*

Bioactive ingredient	Content (%)	
	Fruiting body	Corpus
Adenosine	0.18	0.06
Cordycepin	0.97	0.36

Fatty acids. Fatty acid compositions of *C. militaris* are presented in Table 2. The fruiting body of *C. militaris* was rich in unsaturated fatty acids (about 70% of the total fatty acids). The most abundant saturated acid was palmitic acid. Its levels were 24.5% in fruiting body and 21.5% in the corpus. The most abundant unsaturated acid was linoleic acid. Its levels were 61.3% in fruiting body and 33.0% in the corpus.

Adenosine and cordycepin. Adenosine and cordycepin concentrations in the *Cordyceps militaris* are presented in Table 3. The adenosine concentration was 0.18% in the fruiting body and 0.06% in the corpus. The cordycepin concentration was 0.97% in the fruiting body and 0.36% in the corpus There were differences in adenosine and cordycepin contents between the fruiting body and the corpus of *C. militaris*. The adenosine and cordycepin concentration in the fruiting body was approximately 3 fold higher than in the corpus. The adenosine concentration was lower than the concentration of cordycepin.

Discussion

The use of Dong Chong Xia Cao as a health or func-

tional food has been appreciated for hundreds of years in Asia. Recently, an artificial cultivation method was developed in Korea. It uses living silkworm larvae and pupae as growth substrates for *Cordyceps militaris*.The amino acid and fatty acid composition of *Cordyceps militaris* was obtained. The total content of amino acids in the fruiting body was much higher than in the corpus. The most abundant amino acids of *C. militaris* were lysine, glutamic acid, proline and threonine in the fruiting body, and proline and lysine in the corpus. Chang *et al.* (2001) reported that the most abundant amino acids in *C. militaris* mycelia were aspartic acid (2.66 mg/g), valine (2.21 mg/g) and tyrosine (1.57 mg/g). These results show that the most abundant amino acids of *C. militaris* are not similar to the fermented mycelia and the fruiting body. Chen (1986) found that alanine, glycine and threonine (sweet), and aspartic and glutamic acids (MSG-like) were taste-active amino acids in common mushrooms.

The fruiting body of *C. militaris* was rich in unsaturated fatty acids.The fruiting body of *C. militaris* is a better source of essential fatty acids, such as linoleic acid (C18:2).

There were differences in adenosine and cordycepin contents between the fruiting body and the corpus of *C. militaris.* This cordycepin concentration in the fruiting body was relatively high compared with previous reports of 0.46% (Yun *et al*., 2003).

It was believed that the fruiting body and the corpus of *C. militaris* had different functions, due to the former growing above ground and the latter existing underground (Hong *et al*., 2007). This study has clarified the differences with regard to amino and fatty acid profiles, and the cordycepin and adenosine concentrations of *C. militaris*.

References

Bok, J. W., Lermer, L., Chilton, J., Klingeman, H. G. and Towers, G. H. 1999. Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry* 51:891-898.

Chang, H. L., Chao, G. R., Chen, C. C. and Mau, J. L. 2001. Non-volatile taste components of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mtcelia. *Food Chem.* 74:203-207.

Chen, H. K. 1986. Studies on the characteristics of taste-active components in mushroom concentrate and its powderization. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan.

Chiou, W. F., Chang, P. C., Chou, C. J. and Chen, C. F. 2000. Protein constituent contributes to the hypotensive and vasorelaxant activities of *Cordyceps sinensis*. *Life Sci.* 66:1369-1376.

Furuya, T., Hirotani, M. and Matsuzawa, M. 1983. N^6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. *Phytochemistry* 22:2509-2512.

Hong, I. P., Nam, S. H., Sung, G. B., Chung, I. M., Hur, H., Lee, M. W., Kim, M. K. and Guo, S. H. 2007. Chemical components of *Paecilomyces tenupes* (Peck) Samson. *Mycobiology* 35:215-218.

Huang, B. M., Stocco, D. M. and Norman, R. L. 1997. The cellular mechanism of corticotropin-releasing hormone (CRH) stimulated steroidogenesis in mouse Leydig cells are similar to those for LH. *J. Androl.* 18:528-534.

Kim, K. M., Kwon, Y. G., Chung, H. T., Yun, Y. G., Pae, H. O., Han, J. A., Ha, K. S., Kim, T. W. and Kim, Y. M. 2003. Methanol extract of *Cordyceps pruinosa* inhibits *in vitro* and *in vivo* inflammatory mediators by suppressing NF-κB activation. *Toxicol. Appl. Pharm.* 190:1-8.

Kobayashi, Y. 1982. Key to the taxa of the genera *Cordyceps* and *Torrubiella*. *Trans. Mycol. Soc. Jpn*. 23:29-364.

Kuo, Y. C., Lin, C. Y., Tsai, W. J., Wu, C. L., Chen, C. F. and Shiao, M. S. 1994. Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides. *Cancer Invest.* 12:611-615.

Kuo, Y. C., Tsai, W. J., Shiao, M. S., Chen, C. F. and Lin, C. Y. 1996. *Cordyceps sinensis* as an immunomodulatory agent. *Am. J. Chin. Med.* 24:111-125.

Li, Q. S., Zeng, W., Yi, D. H. and Huang, T. F. 1998. Studies on the alternation of generations in *Cordyceps sinensis*. *Chung Kuo Yao Tsa Chil.* 23:210-212.

Manabe, N., Sugimoto, M., Azuma, Y., Taketomo, N., Yamashita, A., Tsuboi, H., Tsunoo, A., Kinjo, N., Nian-Lai, H. and Miyamoto, H. 1996. Effect of the mycelial extract of cultured *Cordyceps sinensis* on *in vivo* hepatic energy metabolism in the mouse. *Jpn. J. Pharmacol.* 70:23-29.

Spatafora, J. W. and Blackwell, M. 1993 .Molecular systematics of unitunicate perithecia ascomycetes; the Clavicipitales-Hypocreales connection. *Mycologia* 85:912-922.

Wang, S. M., Lee, L. J., Lin, W. W. and Chang, C. M. 1998. Effect of a water-soluble extract of *Cordyceps sinensis* on steroidogenesis and capsular morphology of lipid droplet in cultured rat adrenocortical cell. *J. Cell Biochem*. 69:483-489.

Wu, C. S., Leu, S. F., Yang, H. Y. and Huang, B. M. 2001. Melatonin inhibits the expression of steroidogenic acute regulatory protein and steroidogenesis in MA-10 cells. *J. Androl.* 22:245-254.

Yun, Y. H., Han, S. H., Lee, S. J., Ko, S. K., Lee, C. K., Ha, N. J. and Kim, K. J. 2003. Anti-diabetic effects of CCCA, CMESS and cordycepin from *C. militaris* and the immune responses in streptozotocin-induced diabetic mice. *Kor. J. Nat. Product Sci.* 9:291-298.