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Chemical Components of *Paecilomyces tenuipes* (Peck) Samson

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The caterpillar-shaped Chinese medicinal mushroom (DongChongXiaCao) looks like a worm in the winter and like a grass in the summer. The fruiting body has been regarded as popular folk or effective medicines used to treat human diseases such as asthma, bronchial and lung inflammation, and kidney disease. The fruiting bodies of *Paecilomyces tenuipes* that formed on the living silkworm (*Bombyx mori*) host were used in this examination. This study was carried out to investigate the proximate composition, soluble sugar, amino acid and fatty acid profiles, and contents of the bioactive ingredient including adenosine and D-mannitol in the fruiting-bodies. The moisture content was 57.56%. Soluble sugars found were glycerol, glucose, mannitol and sucrose, and the contents exceeded 24 mgg^{-1}dry weight. Total free amino acid content was 17.09 mg g^{-1}dry weight. Arginine, glycine, proline and tyrosine were main amino acids. The content of oleic acid in fatty acids was high. Adenosine was more abundant in fruiting bodies than corpus.

KEYWORDS: Amino acid, *Paecilomyces tenuipes*, Proximate composition, Soluble sugars

Cordyceps, "Winter-Worm-Summer-Grass" called "Dong-ChungHaCao" in Korea and "DongChongXiaCao" in Chinese has been used as a traditional folk medicine for hundreds of years in Asia countries.

Cordyceps is placed in the family Clavicipitaceae of the order Hypocreales in the class Pyrenomycetes of ascomycetous fungi, and known to parasitize on insects (Kobayashi, 1982; Spatafora and Blackwell, 1993). The fruiting body of *Cordyceps* is derived from the pupa or larva of insects infected by the entomopathogenic fungus *Cordyceps*. These fungi endophytically parasitize on dead or living caterpillars of the moth *Hepialus* spp. Spores of them germinate inside the caterpillars, filling the caterpillars with hyphae, and produce a stalked fruiting body (Li *et al.*, 1998).

Various bioactive components were found in the genus *Cordyceps*. *C. sinensis* is one of the best known fungi possessed many important pharmacological activities. It 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), promote the secretion of adrenal hormones (Wang *et al.*, 1998) and possess hypotensive and vasorelaxant activities (Chiou *et al.*, 2000). Cordycepin identified from *C. militaris* has several biological activities such as inhibition of RNA and DNA synthesis and suppression of viral replication (Kuo *et al.*, 1994). Galactomannan isolated from *C. cicadae* is shown to prevent the growth of sarcoma 180 mice (Huang *et al.*, 1997). Polysaccharides purified from *C. ophioglossoides* have been reported as antitumor agents (Wu *et al.*, 2001). *Paecilomyces tenuipes* possesses anti-cancer activity *in vivo* (Cho *et al.*, 1999) and significant cytotoxicity against cancer cell lines (Shim *et al.*, 2000). The nucleoside derivative N^6-(2-hydroxyethyl) adenosine (HEA) isolated from *C.* pruinosa showed a Ca^{2+} antagonistic effect and negative inotropic response (Furuya *et al.*, 1983). *C.* pruinosa suppresses inflammation through suppression of NF-κB-dependent inflammatory gene expression (Kim *et al.*, 2003) Nevertheless the natural fruiting bodies are expensive and scarce, the demand for *Cordyceps* has been increased. Recently the artificial cultivation method of *P. tenuipes* using living silkworm as the growth substrate was developed in Korea. The aim of this study was to find the chemical compositions of the fruiting body of *P. tenuipes* grown on living silkworm host.

Materials and Methods

Samples. Conidia were obtained by cultivating *P. tenuipes* on rice medium for 20 days.

The harvested conidia were adjusted to 10^8 concentrations and sprayed on the fifrth instar one day silkworm. The silkworm inoculated with *P. tenuipes* was reared for 45 days by traditional standard method. After cutting the cocoon, the inoculated pupae were cultivated under temperature 25°C and humidity 90% for fruiting body forma-

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tion. The fruiting bodies formed on silkworm pupae were dried, milled and used as sample for analysis.

Proximate analysis. The proximate compositions including moisture, carbohydrate, crude fat, crude fiber and crude protein were analyzed according to the AOAC (1995) procedures.

Soluble sugar assay. The free sugars were extracted with 10 *ml* of 85% ethanol in 1.0-g samples on the basis of dry weight for 24 h. The free sugars were analyzed by HPLC at the following conditions: column, high-performance carbohydrate column (4.6 × 250 mm, Waters Co.); column temperature, 35°C; detection, refractive index (Waters Model 410); mobile phase, 75% acetonitrile; flow rate, 1.2 *ml*/min. The free sugars were measured by comparison of standards using the Millennium Program (Waters Co.).

Amino acid assay. The amino acid composition of the samples was determined by hydrolyzing them with 6 N HCl for 24 h at 105°C and them 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 composed 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 with those of an authentic standard mixture.

Fatty acid assay. The fatty acid composition of the total lipids, extracted from dried samples according to Hamilton and Hamilton (1992), was 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.). Temperature of the oven was programmed at 140°C for 5 min, followed by ramping to 240°C at 4°C/min and kept there for 15 min. Helium at a flow rate of 20 cm/s was used as the carrier gas, The injection port and the flame ionization detector oven temperatures were set at 260°C. FAMEs were identified by comparing retention times with those of an authentic standard mixture (Supelco 37 Component FAME Mix, Supelco Co.).

Adenosine. Accurate amounts of adenosine were dissolved in a mobile phase solution, as described later, to give various concentrations for calibration. Samples were extracted with 100°C hot water for 2 h and filtered through a 0.45 μm filter membrane prior to analysis. 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) of Nacalai Tesque (Kyoto, Japan) was used. The mobile phase was a mixture of methanol/0.02 M potassium dihydrogenphosphate (15 : 85). Elution was performed at a solvent flow rate of 1 *ml*/min starting with 30% methanol, which remained isocratic for 15 min; then a gradient was installed to obtain 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, and 80% methanol at 52 min, subsequently becoming isocratic for 60 min. Detection was performed with a variable-wavelength UV detector (L-4250) at 260 nm.

Results

Proximate. Proximate compositions of *P. tenuipes* were presented in Table 1. The moisture content was 57.56%. The conspicuously high fat content (21.76%) comprised the main part of *P. tenuipes* compared with 6.83% in the protein and 3.49% in the carbohydrate, respectively.

Soluble sugar. The total content of soluble sugar contained glycerol, glucose, mannitol and sucrose was 24 mgg^{-1} in the fruiting body of *P. tenuipes*, but 14 mgg^{-1} in the

Table 1. Proximate composition of fruiting bodies of *P. tenuipes*

Chemical composition (%)				
Moisture	Carbohydrate	Crude fat	Crude fiber	Crude protein
57.56 ± 0.07	3.49 ± 0.00	21.76 ± 0.00	6.20 ± 0.26	6.83 ± 0.02

Table 2. Content of soluble sugars of *P. tenuipes*

Material	Content (mgg^{-1} dry wt)				
	Glycerol	Glucose	Mannitol	Sucrose	Total
P. tenuipes	3.71 ± 0.44	9.21 ± 1.31	8.40 ± 0.58	2.67 ± 0.81	24.00 ± 3.14
Silkworm powder	3.56 ± 0.19	1.12 ± 0.00	2.96 ± 0.09	6.33 ± 0.38	14.00 ± 0.66

silkworm (Table 2). The results showed that the contents of glucose and mannitol were increased, sucrose content was reduced, and glycerol was no big change according as *P. tenuipe* grew on the silkworm host. Namely, the glucose and mannitol contents in the fruiting body of *P. tenuipe* (9.21 and 8.40 mgg^{-1}) was much higher than in the silkworm (1.12 and 2.96 mgg^{-1}), whereas the sucrose in the fruiting body of *P. tenuipes* (2.67 mgg^{-1}) was lower than in the silkworm (6.33 mgg^{-1}). Glucose and mannitol were abundantly present in the *P. tenuipes*.

Amino acid. Amino acid compositions of *P. tenuipes* were presented in Table 3. The total contents of amino acid in the fruiting body of *P. tenuipes* (17.09 mgg^{-1}) were similar to those in the silkworm (17.41 mgg^{-1}). The total free amino acid contents in the fruiting body of *P. tenuipes* ranged from 0.36 to 2.21 mgg^{-1} dry weight The amino acids with contents of more than 1.50 mgg^{-1} and their levels were 2.21 mgg^{-1} arginine, 1.77 mgg^{-1} glycine, 1.68 mgg^{-1} proline and 1.51 mgg^{-1} tyrosine in the fruiting body of *P. tenuipe*, respectively and 3.43 mgg^{-1} glycine in the silkworm.

Table 3. Content of free amino acids in *P. tenuipes*

Amino acid	Content (mgg^{-1} dry wt)	
	P. tenuipes	Silkworm powder
Aspartic acid	0.76	0.82
Serine	1.19	1.42
Glutamic acid	0.85	1.14
Glycine	1.77	3.43
Histidine	0.61	0.68
Arginine	2.21	0.98
Threonine	1.07	0.96
Alanine	0.97	1.38
Proline	1.68	0.90
Tyrosine	1.51	0.97
Valine	0.87	0.93
Methionine	0.36	0.28
Lysine	0.53	0.55
Isoleucine	0.64	0.74
Leucine	1.08	1.16
Phenylalanine	0.99	1.07
:	:	:
Total	17.09	17.41

Table 4. Content of fatty acids of *P. tenuipes*

Fatty acid	Content (% of total FA)	
	P. tenuipes	Silkworm powder
Palmitic acid (C16:0)	17.08	17.86
Palmitoeic acid (C16:1)	0.76	0.42
Stearic acid (C18:0)	3.16	10.83
Oleic acid (C18:1)	38.35	32.45
Linoleic acid (C18:2)	12.35	6.9
Linolenic acid (C18:3)	28.3	38.77

Table 5. Content of adenosine and D-mannitol of *P. tenuipes*

Bioactive ingredient	Content (%)	
	Fruiting body	Corpus
Adenosine	0.033 ± 0.003	0.013 ± 0.001
D-mannitol	4.676 ± 0.095	7.436 ± 0.617

Fatty acid. Fatty acid compositions of *P. tenuipes* were presented in Table 4. The fruiting body of *P. tenuipes* was rich in unsaturated fatty acids with about 79% of the total fatty acids. While the main saturated acid and its level of *P. tenuipes* was 17.08% palmitic acid, the most abundant unsaturated acids and their levels were 38.35% oleic acid, 12.35% linoleic acid and 28.30% linolenic acid. Therefore, the results showed that the fruiting body of *P. tenuipes* is a source of essential fatty acids such as linoleic acid (C18:2) and can be used as health food.

Adenosine. Adenosine and D-mannitol concentrations in the *P. tenuipes* are presented in Table 5. There were differences in adenosine and D-mannitol contents between the fruiting body and the corpus of *P. tenuipes*. The D-mannitol concentration, consisting of 7.4% in the corpus, was approximately higher 1.5 times than in the fruiting-body. However, the adenosine concentration of 0.013% in copus and 0.033% in fruiting body were relatively low for the *P. tenuipes*.

Discussion

The use of DongChongXiaCao as health or functional foods has been appreciated for thousands of years in Asia. Recently, the artificial cultivation method of *P. tenuipes* which uses living silkworm as the growth substrate was developed in Korea. It is also edible mushroom, typically cooked with chicken, as a medicinal repast, for restoring health. The basic information of *P. tenuipes* was obtained by proximate, soluble sugar, amino acid and fatty acid composition. It contains about 22.8% fat, 6.8% protein, 6.2% fiber and 3.5% carbohydrate. Our study agrees with a report by Crisan and Sands (1978) that the carbohydrate content was low and the fat content high in the most mushrooms.

The total content of soluble sugar contained glycerol, glucose, mannitol and sucrose was 24 mgg^{-1} in the fruiting body of *P. tenuipes*. The contents of glucose and mannitol in the fruiting body of *P. tenuipe* were 9.21 and 8.40 mgg^{-1}, respectively. Glucose and mannitol was abundantly present in the *P. tenuipes*. Recently, much interest has arisen in characterizing relationships between the structure and function of water-soluble and water-unsoluble polysaccharides obtained from mushrooms because of their antioxidant, free radical scavenging, antiviral, hepatoprotective, antifibrotic, anti-inflammatory, anti-dia-

betic and hypocholesterolemic activities (Ooi and Liu, 1999). Soluble sugars, especially mannitol contained in the mushroom, contribute a sweet taste (Litchfield, 1967).

The contents of amino acid in the fruiting body of *P. tenuipes* ranged from 0.36 to 2.21 mgg^{-1} dry weight. The main amino acid contents were arginine (2.21 mgg^{-1}), glycine (1.77 mgg^{-1}), and praline (1.68 mgg^{-1}).

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 *P. tenuipes* was rich in unsaturated fatty acids with about 79% of the total fatty acids. The main fatty acids and their levels of *P. tenuipes* were 38.35% oleic acid, 28.30% linolenic acid and 12.35% linoleic acid in unsaturated acids, and 17.08% palmitic acid in saturated acids.

There were differences in adenosine and D-mannitol contents between the fruiting body and the corpus of *P. tenuipes*. The adenosine concentration was 0.033% in the fruiting body and 0.013% in the corpus of *P. tenuipes*. It was believed that the fruiting body and the corpus of *P. tenuipes* had different functions, due to the former growing above ground and the latter existing underground.

Our studies have clarified the difference with regard to the proximate composition, soluble sugar, amino and fatty acid profiles, and adenosine in the fruiting body of *P. tenuipes*.

References

AOAC. 1995. *Official methods of analysis* (16th ed). Washington, DC. Association of Official Analytical Chemists.

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**(7): 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 Chemistry* **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**(14): 1369-1376.

Cho, S. Y., Shin, K. H., Song, S. K. and Sung, J. M. 1999. Mass production and useful material development of the entomopathogenic fungi growing on the silkworm, *Rural Development Administration Report*, 1-234.

Crisan, E. V. and Sands, A. 1978. Nutritional value. In S. T. Chang and W. A. Hayes, *The biology and cultivation of edible mushrooms* (pp. 137-165). New York: Academic Press.

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.

Huang, B. M., Hsu, C. C., Tsai, S. J., Sheu, C. C. and Leu, S. F. 2001. Effect of *Cordyceps sinensis* on testosterone production in normal mouse Leydig cells. *Life Sci.* **69**: 2593-2602.

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**(5): 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 nivo inflammatory mediators by suppressing NF-κB activation. *Toxicology and Applied Pharmacology* **190**: 1-8.

Kobayashi, Y. 1982. Key to the taxa of the genera *Cordyceps* and *Torrubiella*. *Trans. Mycol. Soc. Jpn.* **23**(3): 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 Investi.* **12**(6): 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**(2): 111-125.

Li, Q. S., Zeng, W., Yi, D. H. and Huang, T. F. 1998. Studies on the alternation of generations in *Cordyceps sinensis*. *ChungKuoYaoTsaChil.* **23**(4): 210-212.

Litchfield, J. H. 1967. Morel mushroom mycelium as a food flavoring material. *Biotechnology Bioengineering* **9**: 289-304.

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**(1): 85-88.

Ooi, V. E. C. and Liu, F. 1999. A review of pharmacological activity of mushroom polysaccharides. *International Journal of Medicinal Mushrooms* **1**(3), 195-206.

Shim, J. S., Min, E. G., Chang, H. R., Lee, C. Y., Kim, S. S. and Han, Y. H. 2000. Cytoctxicity against human cancer cell lines by *Paecilomyces tenuipes* DGUM 32001. *Kor. J. Microbial.* **36**: 312-315

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**(4): 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**(5): 245-254.

Zhu, J. S., Halpern, G. M. and Jones, K. 1998a. The Scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: part I. *J. Altern. Complement. Med.* **4**(3): 289-303.

Zhu, J. S., Halpern, G. M. and Jones, K. 1998b. The Scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: part II. *J. Altern. Complement. Med.* **4**(4): 429-457.