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Chemical Composition of Main Cordyceps species in Korea

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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 *Cordyceps militaris*, *C. pruinosa* and *Paecilomyces tenuipes* that formed on the living silkworm (*Bombyx mori*) host were used in this examination. This study was carried out to investigate the soluble sugar, amino acid and fatty acid profiles in the fruiting-bodies. Soluble sugars such as glycerol, glucose, mannitol and sucrose were mainly found in the fruiting bodies of *C. militaris*, *C. pruinosa* and *P. tenuipes*. Total soluble sugar content was 29.23 mg/g in *C. militaris*, 8.61 mg/g in *C. pruinosa* and 24.00 mg/g [1]in *P. tenuipes* on dry weight basis. Total free amino acid content was 14.09 mg/g [1]in *C. militaris*, 34.60 mg/g in *C. pruinosa* and 17.09 mg/g in *P. tenuipes*. The content of oleic acid in fatty acids was above high more than 30% regardless of species.

Key words: Amino acid, *Cordyceps* species, Fatty acid, Soluble sugar

Introduction

Cordyceps, “Winter-Worm-Summer-Grass” called “DongChungHaCo” 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 a 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 fungi *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). *P. tenuipes* possess anti-cancer activity *in vivo* and significant cytotoxicity against cancer cell lines (Cho *et al*., 1998; 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). *C. militaris* has similar pharmacological activities to the well known Chinese traditional medicine *C. sinensis* (Cai *et al*,

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2004). Because the demand for *Cordyceps* has been increased and natural fruit bodies are rare, many scientists have researched to develop the techniques for artificial cultivation. Cordyceps species such as *C. militaris*, *C. pruinosa* and *P. tenuipes* mainly cultivated using living silkworm as the growth substrate in Korea. The aim of this study was to investigate the chemical compositions of the fruiting body of Cordyceps grown on living silkworm host.

Materials and Methods

Samples

Conidia were obtained after cultivating of Cordyceps species on rice medium for 20 days.

The harvested conidia were adjusted to 10^8 concentrations and sprayed on first day of the firth instar silkworm. The silkworm inoculated with Cordyceps 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 formation (Fig. 1). The fruiting bodies formed on silkworm pupae were dried, milled and used as sample for analysis.

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 the 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 & 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 15min. 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.).

Results

Soluble sugar

The total content of soluble sugar contained glycerol, glucose, mannitol and sucrose was 29.23 mg/g in *C. militaris*, 8.61 mg/g in *C. pruinosa* and 24.00 mg/g in *P. tenuipes* on dry weight basis (Table 1). The total content of soluble sugar of *C. militaris* was approximately two-fold higher than in the silkworm (14.00 mg/g), however that of *C. pruinosa* was decreased to 8.61 mg/g. Also, our results showed that the content of glucose was increased, sucrose content was reduced, and glycerol was no big change according to growing of fungi on the silkworm host. Namely, the glucose concentration in the fruiting body of

Cordyceps militaris *Cordyceps pruinosa* *Paecilomyces tenuipes*

Fig. 1. Fruiting bodies of Cordyceps mushroom used in this experiment.

Table 1. Content of soluble sugars of *Cordyceps* species

Material	Content (mg g^{-1} dry wt.)				
	Glycerol	Glucose	Mannitol	Sucrose	Total
C. militaris	3.56±0.52	9.07±0.63	13.03±3.48	3.57±0.31	29.23±1.24
C. pruinosa	Tr	7.90±0.53	Tr	0.71±0.07	8.61±0.300
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

Table 2. Content of free amino acids in *Cordyceps* species

Amino acid	Content (mg g^{-1} dry wt)			
	C. militaris	*C. pruinosa*	*P. tenuipes*	Silkworm
Aspartic acid	0.67	1.55	0.76	0.82
Serine	1.09	2.00	1.19	1.42
Glutamic acid	0.76	2.16	0.85	1.14
Glycine	1.96	1.39	1.77	3.43
Histidine	0.54	1.93	0.61	0.68
Arginine	1.05	5.40	2.21	0.98
Threonine	1.04	2.66	1.07	0.96
Alanine	0.90	1.18	0.97	1.38
Proline	0.88	2.00	1.68	0.90
Tyrosine	0.75	3.30	1.51	0.97
Valine	0.83	1.45	0.87	0.93
Methionine	0.36	1.25	0.36	0.28
Lysine	0.75	1.16	0.53	0.55
Isoleucine	0.57	1.40	0.64	0.74
Leucine	0.97	1.95	1.08	1.16
Phenylalanine	0.97	3.82	0.99	1.07
:	:	:	:	:
Total			17.09	17.41

C. militaris, *C. pruinosa* and *P. tenuipe* (9.07, 7.90 and 9.21 mg/g) was much higher than in the silkworm (1.12 mg/g), whereas the sucrose in the fruiting body of them (3.57, 0.71 and 2.67 mg/g) was lower than in the silkworm (6.33 mg/g). Glucose was abundantly present in the *Cordyceps* species.

Amino acid

Amino acid compositions of fungi were presented in Table 2. The total free amino acid contents ranged from 0.36 to 1. mg/g in *C. militaris*, 1.16 to 5.40 mg/g in *C. pruinosa* and 0.36 to 2.21 mg/g in *P. tenuipes* on dry weight basis. The total content of amino acids of *C. pruinosa* (34.60 mg/g) was two-times increased, *C. militaris* (14.09 mg/g) was reduced, and *P. tenuipes* (17.09 mg/g) was no big change comparing to silkworm (17.41 mg/g). The contents of the main amino acids were 1.96 mg/g glycine (13.9%), 1.09 mg/g serine (7.7%), 1.05 mg/g arginine (7.7%) in *C. militaris*, 5.40 mg/g arginine (15.6%), 3.82 mg/g phenylalanine (11.0%), 3.30 mg/g tyrosine (9.5%) in *C. pruinosa* and 2.21 mg/g arginine (12.9%), 1.77 mg/g glycine (10.4%), 1.68 mg/g proline (9.8%) in *P. tenuipes*. Also in silkworm, glycine (19.7%), serine (8.2%) and alanine (7.9%) were abundantly presented.

Table 3. Content of fatty acids of *Cordyceps* species

Fatty acid	Content (% of total FA)			
	C. militaris	*C. pruinosa*	*P. tenuipes*	Silkworm
Palmitic acid (C16:0)	15.17	16.90	17.08	17.86
Palmitoeic acid (C16:1)	0.75	1.19	0.76	0.42
Stearic acid (C18:0)	2.47	3.95	3.16	10.83
Oleic acid (C18:1)	39.33	34.29	38.35	32.45
Linoleic acid (C18:2)	11.87	19.20	12.35	6.9
Linolenic acid (C18:3)	32.57	23.78	28.3	38.77

Fatty acid

Fatty acid compositions of fungi were presented in Table 3. The fruiting body of fungi was rich in unsaturated fatty acids with about 79~83% of the total fatty acids comparing to silkworm (73%). While the main saturated acid of fungi was palmitic acid, the most abundant unsaturated acids were oleic acid and linolenic acid, and their levels were 39.3% and 32.6% in *C. militaris*, 34.3% and 23.8% in *C. pruinosa*, and 38.4% and 28.3% in *P. tenuipes*, irrespectively.

Discussion

The use of DongChongXiaCao as health or functional foods has been appreciated for thousands of years in Asia. *Cordyceps* species such as *C. militaris*, *C. pruinosa* and *Paecilomyces tenuipes* have been mainly cultivated using living silkworm as the growth substrate in Korea. There are also edible mushroom, typically cooked with chicken, as a medicinal repast, for restoring health.

The basic information of Cordyceps species which have been cultivated in Korea was obtained by soluble sugar, amino acid and fatty acid composition.

The total content of soluble sugar contained glycerol, glucose, mannitol and sucrose was 29.23 mg/g in *C. militaris*, 8.61 mg/g in *C. pruinosa* and 24.00 mg/g in *P. tenuipes* on dry weight basis. The total content of soluble sugar of *C. militaris* was approximately two-fold higher than in the silkworm (14.00 mg/g), however that of *C. pruinosa* was decreased to 8.61 mg/g. Glucose was abundantly present in the *Cordyceps* species. 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-diabetic and hypocholesterolemic activities (Ooi and Liu, 1999). Soluble sugars, especially mannitol contained in the mushroom, contribute a sweet taste (Litchfield, 1967).

The total contents of amino acid in the fruiting body were 14.09 mg/g in *C. militaris*, 34.60 mg/g in *C. pruinosa* and 17.09 mg/g in *P. tenuipes* on dry weight basis. The main amino acid contents were glycine (13.9%) in *C. militaris*, arginine (15.6%) and, phenylalanine (11.0%) in *C. pruinosa*, and arginine (12.9%) and glycine (10.4%) in *P. tenuipes*. Chang *et al.* (2001) reported that the contents of the main amino acids in *C. militaris* mycelia were aspartic acid (2.66 mg/g), valine (2.21 mg/g) and tyrosine (1.57 mg/g). Chen (1986) found that alanine, glycine and threonine were sweet, and aspartic and glutamic acids (MSG-like) were taste-active amino acids in common mushrooms.

The fruiting body of fungi was rich in unsaturated fatty acids with about 79~83% of the total fatty acids. While the main saturated acid of fungi was palmitic acid, the most abundant unsaturated acids were oleic acid and linolenic acid. Therefore, the results showed that the fruiting body of *Cordyceps* species is a source of essential fatty acids such as oleic (C18:1), linoleic acid (C18:2) and linolenic acidacid (C18:3), and can be used as health food.

Our studies have clarified the difference with regard to the soluble sugar, amino and fatty acid profiles in the fruiting body of *Cordyceps* species which are cultivated in Korea

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