JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Distribution of Nucleosides and Nucleobases in Edible Fungi

JIAN-PING YUAN,*,† SHU-YAN ZHAO,† JIANG-HAI WANG,† HUI-CONG KUANG,‡ AND XIN LIU†

State Key Laboratory of Biocontrol/Food Engineering Research Center of State Education Ministry, College of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, People's Republic of China, and Center for Disease Control and Prevention of Dongguan City, Dongguan 523016, People's Republic of China

A gradient reversed-phase high-performance liquid chromatography (HPLC) method was developed for the simultaneous determination of seven nucleosides, adenosine, cordycepin (3′-deoxyadenosine), cytidine, guanosine, thymidine, uridine, and inosine, and five nucleobases, adenine, cytosine, thymine, uracil, and hypoxanthine in *Cordyceps sinensis*, *Cordyceps militaris*, *Ganoderma lucidum*, *Agrocybe aegerita*, *Termitornyces albuminosus*, and *Lentinus edodes*. The results showed that total nucleoside and nucleobase contents ranged from 0.14 to 26.57 mg/g dry matter in these fungi. The higher total nucleoside and nucleobase levels (>10 mg/g dry matter) were found in the gills and the pilei of *A. aegerita* and *T. albuminosus*, and the gills of *L. edodes*. The lower levels (<1 mg/g dry matter) were detected in the stipe and the pileus of *G. lucidum*. The results indicated that *A. aegerita*, *T. albuminosus*, and *L. edodes* had much higher contents of nucleosides and nucleobases than *C. sinensis*, *C. militaris*, and *G. lucidum*. It is notable that the hymenophore tissues, which contained the spore-producing cells, such as the gills for *A. aegerita*, *T. albuminosus*, and *L. edodes*, the tubes for *G. lucidum*, and the perithecia for *C. sinensis*, were found to have considerably higher amounts of total nucleosides and nucleobases as compared to other parts of these fungi.

**KEYWORDS: Nucleosides; nucleobases; edible fungi; distribution; HPLC**

## INTRODUCTION

Edible fungi have long been appreciated for their flavor and texture and are recognized as a nutritious food as well as an important source of bioactive compounds of medicinal value (*1*). *Ganoderma lucidum* (Curt.: Fr.) Karst. and the caterpillar-shaped Chinese medicinal mushroom *Cordyceps sinensis* (Berk.) Sacc. have been widely used in traditional Chinese medicines as a herbal medicine for preventing and treating various diseases (*2, 3*). Because natural *C. sinensis* is rare and expensive, the cultivated *C. militaris* fruiting bodies, one of the substitutes for *C. sinensis* (*4*), are commonly sold as health food in Southeast Asia (*5*). *Lentinus edodes* is the most popular edible fungus used in Asia, particularly in China and Japan, and has a demonstrated range of beneficial actions (*6*). *Agrocybe aegerita* and *Termitomyces albuminosus* have been reported to show potent antioxidant activity (*7, 8*). These potential medicinal fungi contain many bioactive compounds, including terpenoids, steroids, phenols, nucleotides, polyketides, quinazoline, polysaccharides, chitin, lectin, and proteins (*1, 9–11*). The chemical analysis for these bioactive compounds not only provides information concerning nutritional value, but also differentiates the species and strains having morphological similarity (*12*).

Nucleosides are considered to be one of the major active components in *Cordyceps* (*5, 12–17*) and are involved in the regulation and modulation of various physiological processes in the central nervous system (*13*). More than 10 nucleosides and nucleobases have been isolated from *Cordyceps* and *Ganoderma* (*17, 18*). Adenosine and cordycepin isolated from *Cordyceps* have shown biological activity. Adenosine has merit as a cardioprotective and therapeutic agent against chronic heart failure, and cordycepin displays antileukemic activity against terminal deoxynucleotidyl transferase-positive leukemic cells (*5, 12, 19, 20*). Furthermore, nucleosides and nucleobases in *G. lucidum* were reported to be capable of inhibiting platelet aggregation and lowering the elevated serum aldolase level of experimental model mice (*18*). It had been suggested that the increased dietary nucleoside−nucleotide mixture might be associated with decreases in the age-induced deterioration of brain morphology and certain memory tasks (*21*).

The separation of nucleosides and nucleobases is very difficult because of their similar structure (*22*). A number of analytic methods have been reported for the determination of nucleosides and nucleobases in *Cordycedps* by using reversed-phase HPLC (*12–14, 16, 17, 20, 22, 23*), normal-phase HPLC (*4*),

* To whom correspondence should be addressed. Tel: +86-20-84112299. Fax: +86-20-84112005. E-mail: yuanjp@mail.sysu.edu.cn.
† Sun Yat-Sen University.
‡ Center for Disease Control and Prevention of Dongguan City.

10.1021/jf0719205 CCC: $40.75 
Published on Web 01/10/2008

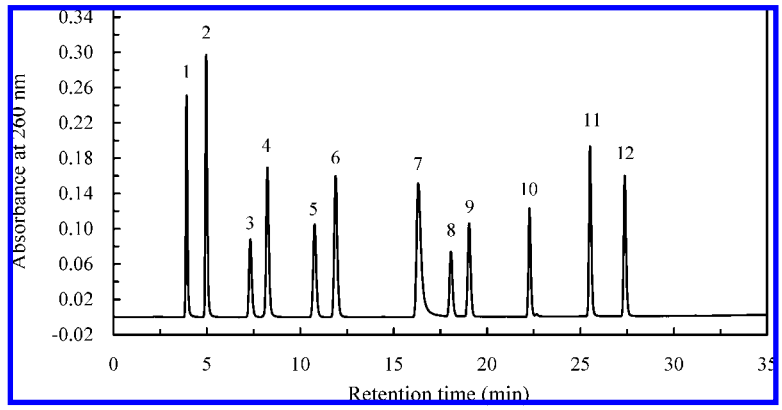


**Figure 1.** HPLC chromatogram of 12 nucleoside and nucleobase standards. Peaks: 1, cytosine; 2, uracil; 3, cytidine; 4, hypoxanthine; 5, uridine; 6, thymine; 7, adenine; 8, inosine; 9, guanosine; 10, thymidine; 11, adenosine; 12, cordycepin.

**Table 1.** Linear Regression Data, LOD, and LOQ of 12 Nucleosides and Nucleobases at 260 nm

| analyte | λ max (nm) | linear regression equation | $r^2$ | LOD (μg/mL) | LOQ (μg/mL) |
|---|---|---|---|---|---|
| cytosine | 266.2 | $y = 63353x - 37152$ | 0.9996 | 0.011 | 0.033 |
| uracil | 259.1 | $y = 80965x + 70544$ | 0.9997 | 0.009 | 0.027 |
| cytidine | 270.9 | $y = 38523x - 19341$ | 0.9995 | 0.018 | 0.054 |
| hypoxanthine | 249.7 | $y = 73208x - 18282$ | 0.9996 | 0.010 | 0.030 |
| uridine | 261.5 | $y = 50310x - 35731$ | 0.9995 | 0.014 | 0.042 |
| thymine | 265.0 | $y = 69707x - 640$ | 0.9995 | 0.010 | 0.030 |
| adenine | 260.3 | $y = 121931x - 52272$ | 0.9994 | 0.006 | 0.018 |
| inosine | 248.5 | $y = 34299x - 6724$ | 0.9995 | 0.020 | 0.060 |
| guanosine | 253.2 | $y = 47083x - 11106$ | 0.9997 | 0.015 | 0.045 |
| thymidine | 267.4 | $y = 45284x - 15488$ | 0.9996 | 0.015 | 0.045 |
| adenosine | 259.1 | $y = 70174x - 25792$ | 0.9995 | 0.010 | 0.030 |
| cordycepin | 260.3 | $y = 70490x + 58340$ | 0.9997 | 0.010 | 0.030 |

**Table 2.** Precision and Accuracy for the Determination of 12 Nucleosides and Nucleobases

| analyte | concentration (μg/mL) | found (μg/mL) | RSD (%) | accuracy (%) |
|---|---|---|---|---|
| cytosine | 25.0 | 24.825 | 1.042 | 99.3 |
| uracil | 25.0 | 24.675 | 1.265 | 98.7 |
| cytidine | 25.0 | 25.875 | 0.957 | 103.5 |
| hypoxanthine | 25.0 | 25.325 | 3.221 | 101.3 |
| uridine | 25.0 | 24.550 | 2.567 | 98.2 |
| thymine | 25.0 | 24.925 | 1.213 | 99.7 |
| adenine | 25.0 | 24.203 | 4.702 | 96.8 |
| inosine | 25.0 | 25.416 | 1.152 | 101.7 |
| guanosine | 25.0 | 24.625 | 3.025 | 98.5 |
| thymidine | 25.0 | 25.142 | 1.202 | 100.6 |
| adenosine | 25.0 | 24.951 | 2.359 | 99.8 |
| cordycepin | 25.0 | 25.725 | 2.534 | 102.9 |

**Table 3.** Recoveries for the Assay of 12 Nucleosides and Nucleobases in *C. militaris*

| analyte | original (mg/g) | spiked (mg/g) | found (mg/g) | recovery (mg/g) | RSD (%) |
|---|---|---|---|---|---|
| cytosine | 0.016 | 2.0 | 2.078 | 103.1 | 2.3 |
| uracil | 0.089 | 2.0 | 2.144 | 102.8 | 2.7 |
| cytidine | 0.396 | 2.0 | 2.428 | 101.6 | 4.5 |
| hypoxanthine | —[a] | 2.0 | 1.988 | 99.4 | 3.0 |
| uridine | 2.308 | 2.0 | 4.256 | 97.4 | 4.2 |
| thymine | — | 2.0 | 2.021 | 101.1 | 3.7 |
| adenine | 0.096 | 2.0 | 2.072 | 98.8 | 1.8 |
| inosine | 0.017 | 2.0 | 2.012 | 99.8 | 1.6 |
| guanosine | 0.640 | 2.0 | 2.595 | 97.8 | 4.5 |
| thymidine | 0.007 | 2.0 | 2.012 | 100.3 | 3.4 |
| adenosine | 1.583 | 2.0 | 3.595 | 100.6 | 2.3 |
| cordycepin | 4.380 | 2.0 | 6.345 | 98.3 | 3.9 |

[a] —, not detected.

and capillary electrophoresis (*5, 24–26*). Most studies to date have focused on the determination of nucleosides and nucleobases for the whole *C. sinensis* (*4, 13, 14, 16, 17, 20, 22–24, 26*). Although there has been some study to demonstrate that the two parts have different biological activities (*27, 28*), it is still not clear which part of *Cordyceps*, the stroma or the host caterpillar, is more important in terms of the main constituents and the biological activities (*25*). Li et al. (*25*) determined the contents of adenosine, guanosine, and uridine in the stroma and the host caterpillar of *C. sinensis* and suggested that the host caterpillar had chemical composition similar to that of the stroma. Hsu et al. (*12*) determined the adenosine and cordycepin contents in the stroma and the host caterpillar of *C. sinensis* and found that the adenosine concentration in stroma was approximately 6-fold higher than that in the host caterpillar. In addition, Gao et al. (*18*) determined six nucleosides and five nucleobases in the pilei and the stipes of *G. lucidum* and *G. sinense* and found that the contents of nine target analytes were different in the pileus and the stipes of the fruiting bodies and among the different species of *G.* spp. However, the said study did not separate the hymenophore tissue from the pileus. To our knowledge, there is no previous information concerning the determination of nucleosides and nucleobases in other edible fungi, such as *A. aegerita*, *T. albuminosus*, and *L. edodes*. The aim of the present study is to evaluate and compare the contents and distributions of nucleosides and nucleobases in *C. sinensis, C. militaris*, *G. lucidum*, *A. aegerita*, *T. albuminosus*, and *L. edodes*.

## MATERIALS AND METHODS

**Chemicals and Reagents.** HPLC-grade methanol was obtained from Merck KGaA (Darmstadt, Germany). Adenosine, cordycepin (3′-deoxyadenosine), cytidine, guanosine, thymidine, uridine, inosine, adenine, cytosine, thymine, uracil, and hypoxanthine were purchased from Sigma (St. Louis, MO). Water was purified using a Millipore Simplicity system (Billerica, MA). Sodium dihydrogen phosphate and disodium hydrogen phosphate were of analytical grade.

**Fungal Samples.** Dried edible fungi *A. aegerita*, *T. albuminosus*, *L. edodes*, and *G. lucidum*, and cultured stroma of *C. militaris* through solid-state fermentation were purchased from the local supermarkets. Four dried *C. sinensis* samples (250–500 g) were obtained from Tibet, Qinghai, Sichuan, and Yunnan, China, respectively. The identities of these fungal samples were confirmed by one of us (Professor X. Liu).

*G. lucidum*, *A. aegerita*, *T. albuminosus*, and *L. edodes* were divided into three parts, the pileus, the hymenophore (tubes or gills), and the stipe. *C. sinensis* (∼10 g) was divided into two parts, the stroma and the host caterpillar. Moreover, in order to look into the distribution of nucleosides and nucleobases in the stroma and the host caterpillar, each of the 9 individual *C. sinensis* specimens (4 from the Tibet sample, 2

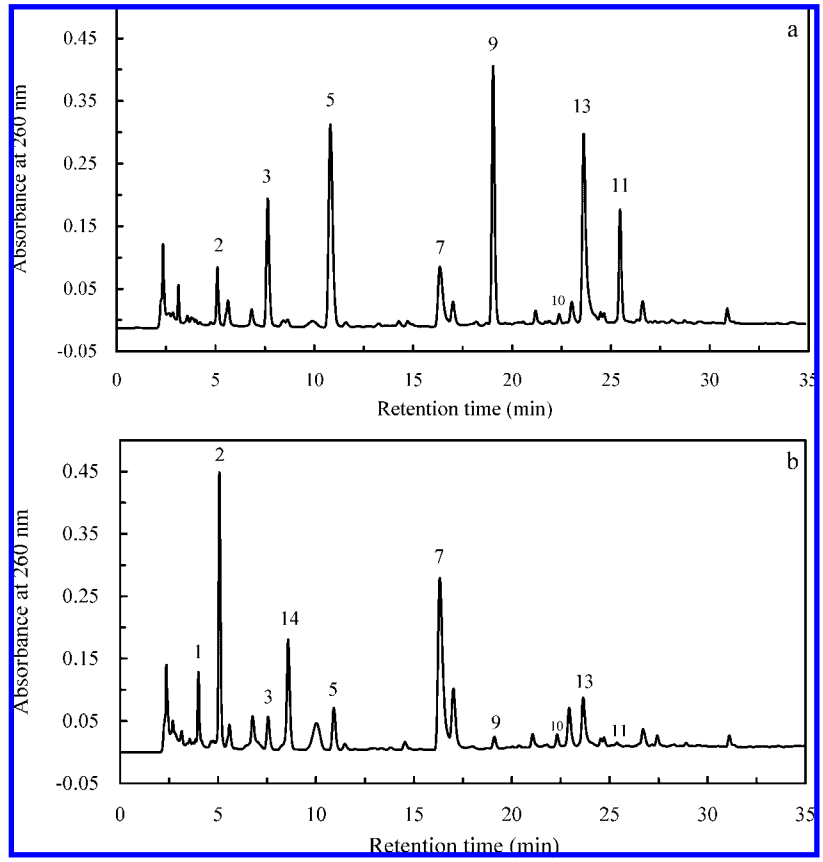


**Figure 2.** HPLC chromatograms of the water extract from the pileus of *A. aegerita* after the extract solution was stored for 0 days (**a**) and 5 days (**b**) at room temperature. Peaks: 1, cytosine; 2, uracil; 3, cytidine; 5, uridine; 7, adenine; 9, guanosine; 10, thymidine; 11, adenosine; 13, adenosine analogue; 14, guanine.

from the Yunnan sample, 2 from the Qinghai sample, and 1 from the Sichuan sample) was divided into eight segments. (The stroma was divided into three equal parts, and the host caterpillar was divided into five equal parts.) These samples were ground into powder with the help of a mortar and a pestle. A 0.1–0.3 g amount of a sample powder was accurately weighed and mixed with 5 mL of water followed by sonication for 10 min and centrifugation at 10,000*g* for 5 min. The extraction procedure was repeated three times, and the total extracts were sampled for HPLC analysis. The extract of *A. aegerita* was determined again after storage at room temperature for 5 days to investigate the stability of nucleosides in the extract solution.

**HPLC Analysis of Nucleosides and Nucleobases.** HPLC was conducted on a Waters liquid chromatograph equipped with a 1525 binary pump and a 2996 photodiode array detector from Waters Corporation (Milford, MA). Twelve nucleosides and nucleobases were separated and analyzed by using a ZORBAX Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 $\mu$m) and a Guard column (4.6 mm × 12.5 mm, 5 $\mu$m), from Agilent Technologies, Inc. (Santa Clara, CA), at 30 °C. The mobile phase consisted of solvent A (aqueous sodium phosphate solution containing 20 mmol/L sodium dihydrogen phosphate and 20 mmol/L disodium hydrogen phosphate prepared with purified water) and solvent B (methanol). The following linear gradient procedure was used: 0–5 min, 0% B; 5–15 min, 0–5% B; 15–40 min, 5–40% B; 40–50 min, 40% B. The flow rate was 1.0 mL/min. The detecting wavelength was set between 200 and 400 nm, and the chromatographic peaks were measured at a wavelength of 260 nm to facilitate the detection of nucleosides and nucleobases. Aliquots of 20 $\mu$L were directly injected into the HPLC column for the determination. All injections were repeated three times. The identifications of nucleosides and nucleobases were achieved by comparing their retention time and spectrum against known standards. The external standard method was used for the determinations of nucleosides and nucleobases.

**Method Validation.** The stock standards of nucleosides and nucleobases were prepared at 300 $\mu$g/mL, and additional calibration levels were prepared by a serial dilution with ethanol. The standard calibration curves were constructed using these standard solutions. The linear regression analysis was carried out by plotting the peak areas (*A*) against the concentrations (*C*). The linearity was demonstrated by a correlation coefficient ($r^2$) greater than 0.999. The limit of detection (LOD) and the limit of quantification (LOQ) were determined on the basis of a signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. Four replicate determinations of analytes were performed to evaluate precision, which was calculated as a relative standard deviation (%RSD) for the repeated measurements. For recovery studies, known volumes of standard solutions were added to *C. militaris* sample at three levels. The spiked samples were extracted with water following the described procedure. Background levels were subtracted in all recovery determinations.

## RESULTS AND DISCUSSION

In the present work, *C. sinensis*, *C. militaris*, *G. lucidum, A. aegerita, T. albuminosus*, and *L. edodes* were chosen as subjects of investigation because all of these edible fungi are popular in China. This study describes an HPLC method for the separation of nucleosides and nucleobases in fungi. Because of little or no retention on a reversed-phase column, cytosine and uracil could serve as the column-void volume markers (*29*). Therefore, the initial mobile phase strength was kept low to promote the retention of the polar compounds. Nucleosides are both weak acids and bases; therefore, the retention on a reversed-phase column can be altered by the changes in pH of a mobile phase (*30*). An aqueous sodium phosphate solution was run as the initial mobile phase so that cytosine and uracil could be retained and separated on the chosen reversed-phase column. Methanol was then systematically increased over time to elute each sample component. In the present work, six reversed-phase columns, Symmetry C18, SymmetryShield RP18, Kromasil 100-5-C18,

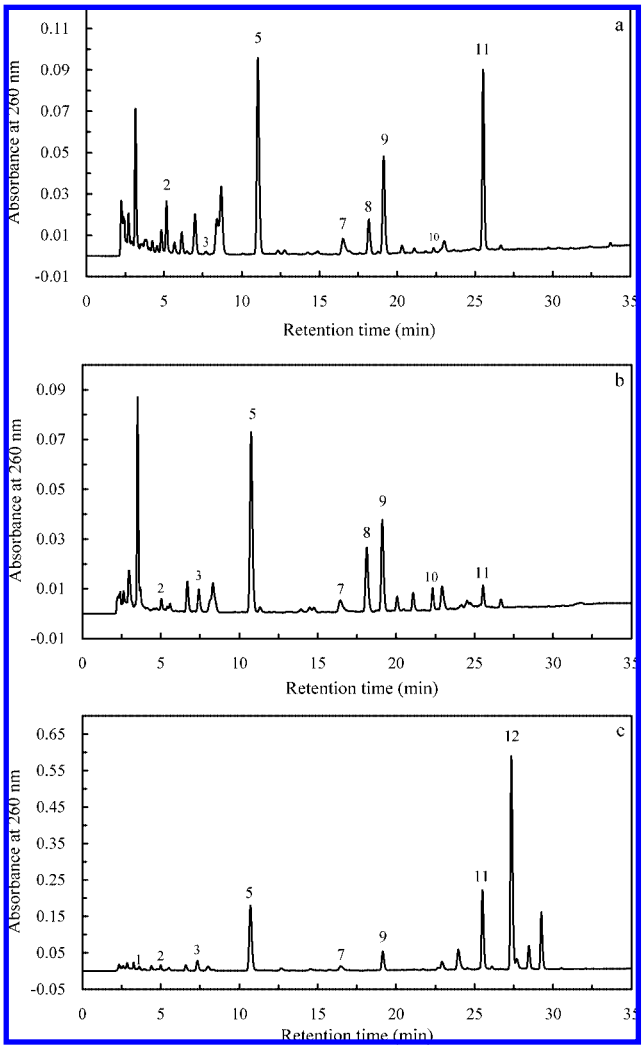


**Figure 3.** HPLC chromatograms of the water extracts from the stroma (**a**) and the host caterpillar (**b**) of *C. sinensis*, and the solid cultured stroma of *C. militaris* (**c**). Peaks: 1, cytosine; 2, uracil; 3, cytidine; 5, uridine; 7, adenine; 8, inosine; 9, guanosine; 10, thymidine; 11, adenosine; 12, cordycepin.

ZORBAX Eclipse XDB-C18, XTerra RP18, and Nova-Pak C18, obtained from different manufacturers or with packing materials of different surface areas, carbon load, and silanol activities, were tested for the separation of 12 nucleosides and nucleobases. The results showed that the ZORBAX Eclipse XDB-C18 column could retain and separate cytosine and uracil better than the other columns tested. **Figure 1** shows the typical HPLC chromatogram of seven nucleoside (adenosine, cordycepin, cytidine, guanosine, thymidine, uridine, and inosine) and five nucleobase (adenine, cytosine, thymine, uracil, and hypoxanthine) standards using this column. As shown in **Figure 1**, the better separation and peak shapes were obtained by using an aqueous sodium phosphate solution as the initial mobile phase in comparison with using an ammonium acetate solution, by which adenine shows severe peak tailing (*13*).

The calibration curves of the peak-area (*A*) against the concentration (*C*) for 12 nucleosides and nucleobases at 260 nm gave good linear responses over a wide range of concentrations (5–300 $\mu$g/mL) (**Table 1**). Precision was determined, and the results are shown in **Table 2**. In order to determine the recovery, a known amount of analytes was spiked into an accurately weighed portion of *C. militaris*. The spiked samples were extracted and analyzed following the described procedure. The recoveries were found to be between 97.4 and 103.1% (**Table 3**). The data showed that this HPLC method was sensitive for the qualitative and quantitative determination of these nucleosides and nucleobases.

To test the stability of nucleosides in extract solution, the water extracts of *A. aegerita* were determined after storage for 5 days at room temperature. **Figure 2** shows the HPLC chromatograms of the extract solutions from the pileus of *A. aegerita* stored for 0 days and 5 days, respectively. As can be seen from **Figure 2**, peaks 3, 5, 9, 11, and 13 considerably decreased, and peaks 1, 2, 7, and 14 noticeably increased after the extract solution was stored for 5 days. Although the retention time of peak 14 is similar to that of hypoxanthine, their UV spectra are different. It was found that peak 14 was the hydrolysate of guanosine (peak 9). Therefore, peak 14 was tentatively identified as guanine. The results showed that nucleosides such as cytidine (peak 3), uridine (peak 5), guanosine (peak 9), adenosine (peak 11), and an adenosine analogue (peak 13) in the extract solution were labile and could be spontaneously hydrolyzed to produce their respective nucleobases, such as cytosine (peak 1), uracil (peak 2), guanine (peak 14), and adenine (peak 7). This indicated that a longer storage time (e.g., 5 days) not only favored the hydrolysis of nucleosides but also markedly promoted the degradation of nucleosides and nucleobases. Therefore, storage for the water extract of fungal samples should be avoided before HPLC analysis, and all of the extracts were analyzed immediately after they were obtained.

The contents of nucleosides and nucleobases in the stroma and the host caterpillar of natural *C. sinensis*, the solid cultured stroma of *C. militaris* (**Figure 3**), and the hymenophore (gills or tubes), pileus, and stipe of *G. lucidum*, *A. aegerita*, *T. albuminosus*, and *L. edodes* were analyzed, and the results are shown in **Table 4**. The results showed that the contents of nucleosides and nucleobases varied in different parts of the fungal tissue or different fungal samples. Total nucleoside and nucleobase contents ranged from 0.14 to 26.57 mg/g dry matter. The higher total nucleoside and nucleobase levels (>10 mg/g dry matter) were found in the gills and pilei of *A. aegerita* and *T. albuminosus*, and the gills of *L. edodes*. The lower levels (<1 mg/g dry matter) were detected in the stipe and the pileus of *G. lucidum*. The results indicate that *A. aegerita*, *T. albuminosus*, and *L. edodes* have much higher contents of nucleosides and nucleobases than *C. sinensis*, *C. militaris*, and *G. lucidum* (**Table 4**).

As can be seen from **Table 4**, these fungi contained remarkably high amounts of nucleosides. In contrast, only a very small quantity of nucleobases (2.1–8.9% of total nucleosides and nucleobases) was found in these fungal samples with the exception of the stipes of *A. aegerita* (14.9%) and *G. lucidum* (19.8%), and the pileus of *L. edodes* (14.0%). Among the individual nucleosides, guanosine, uridine, and cytidine were the main nucleosides and constituted more than 81.6% of the total nucleosides and nucleobases in *A. aegerita*. Uridine, guanosine, and adenosine were the main nucleosides in *T. albuminosus* (>77.6%), *L. edodes* (>72.9%), and *C. sinensis* (>62.0%). For *C. militaris*, cordycepin, uridine, and adenosine (>86.9%) were the main nucleosides. For *G. lucidum*, uridine and inosine (>60.1%) were the main nucleosides. The results showed that uridine was the most abundant nucleoside in *G. lucidum*, which was in accordance with the previous study (*18*), especially in its hymenophore tissue. Gao et al. (*18*) divided the fruiting body of *G. lucidum* into the pileus (including hymenophore) and the stipes and also found that the contents of nine nucleosides and nucleobases were different in the two parts of the fruiting bodies and that the pileus contained a relatively higher content of target compounds than the stipe.

**Table 4.** Average Contents (mg/g Dry Matter) of Nucleosides and Nucleobases in Different Parts of Edible Fungi ($n = 3$)[a]

| samples | | C | U | Cy | H | Ur | T | A | In | Gu | Th | Ad | Co | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. sinensis* from Tibet | stroma | 0.144 | 0.087 | 0.055 | 0.017 | 1.423 | 0.016 | 0.064 | 0.026 | 1.150 | 0.008 | 1.770 | 0.003 | 4.763 |
| | HC | 0.064 | 0.041 | 0.039 | 0.027 | 0.868 | 0.010 | 0.069 | 0.381 | 0.659 | 0.081 | 0.522 | 0.048 | 2.809 |
| *C. sinensis* from Qinghai | stroma | 0.009 | 0.062 | − | 0.013 | 1.553 | 0.006 | 0.056 | 0.084 | 0.570 | − | 1.029 | − | 3.382 |
| | HC | 0.052 | 0.027 | − | 0.022 | 1.294 | − | 0.059 | 0.464 | 0.680 | 0.081 | 0.379 | 0.049 | 3.107 |
| *C. sinensis* from Yunnan | stroma | 0.035 | 0.074 | 0.157 | 0.006 | 1.895 | 0.003 | 0.036 | 0.033 | 1.320 | − | 1.919 | − | 5.478 |
| | HC | 0.017 | 0.033 | 0.061 | 0.015 | 1.068 | 0.003 | 0.057 | 0.441 | 0.750 | 0.052 | 0.622 | 0.032 | 3.151 |
| *C. sinensis* from Sichuan | stroma | 0.069 | 0.047 | − | 0.014 | 1.703 | − | 0.042 | 0.102 | 0.862 | − | 1.087 | − | 3.926 |
| | HC | 0.039 | 0.030 | − | 0.078 | 1.376 | − | 0.075 | 0.463 | 0.837 | 0.046 | 0.416 | − | 3.360 |
| *C. militaris* | stroma | 0.016 | 0.089 | 0.396 | − | 2.308 | − | 0.096 | 0.017 | 0.640 | 0.007 | 1.583 | 4.380 | 9.532 |
| | tubes | − | 0.095 | 0.120 | − | 1.264 | − | 0.004 | 0.568 | 0.254 | 0.018 | 0.202 | − | 2.525 |
| *G. lucidum* | pileus | − | 0.013 | 0.012 | − | 0.121 | − | 0.001 | 0.028 | 0.016 | − | 0.027 | − | 0.218 |
| | stipe | − | 0.026 | 0.004 | − | 0.045 | − | 0.001 | 0.038 | 0.006 | − | 0.018 | − | 0.138 |
| | gills | − | 0.515 | 3.769 | − | 5.638 | − | 1.140 | 0.108 | 6.614 | 0.129 | 1.644 | − | 19.557 |
| *A. aegerita* | pileus | − | 0.325 | 2.230 | − | 3.719 | − | 0.572 | 0.079 | 3.573 | 0.106 | 0.902 | − | 11.506 |
| | stipe | − | 0.142 | 0.496 | − | 1.167 | − | 0.312 | 0.031 | 0.812 | 0.021 | 0.053 | − | 3.034 |
| | gills | − | 0.430 | 1.502 | − | 6.143 | − | 0.151 | 1.552 | 6.186 | 0.146 | 4.808 | − | 20.918 |
| *T. albuminosus* | pileus | − | 0.377 | 0.855 | + | 5.302 | − | 0.223 | 1.311 | 4.319 | 0.092 | 3.431 | − | 15.910 |
| | stipe | − | 0.077 | 0.410 | + | 2.375 | − | 0.380 | 0.469 | 1.516 | 0.048 | 0.895 | − | 6.170 |
| | gills | − | 0.881 | 4.315 | + | 6.463 | − | 0.148 | 0.125 | 6.977 | − | 7.662 | − | 26.571 |
| *L. edodes* | pileus | − | 0.161 | 0.169 | 0.087 | 1.160 | − | 0.231 | 0.134 | 0.312 | − | 0.635 | − | 2.889 |
| | stipe | − | 0.158 | 0.225 | 0.061 | 1.324 | − | 0.074 | 0.301 | 0.543 | − | 1.124 | − | 3.810 |

[a] C, cytosine; U, uracil; Cy, cytidine; H, hypoxanthine; Ur, uridine; T, thymine; A, adenine; In, inosine; Gu, guanosine; Th, thymidine; Ad, adenosine; Co, cordycepin; HC, Host caterpillar; −, not detected; +, below the limit of quantitation. The mean values (R.S.D.s <5%) of three determinations are presented.

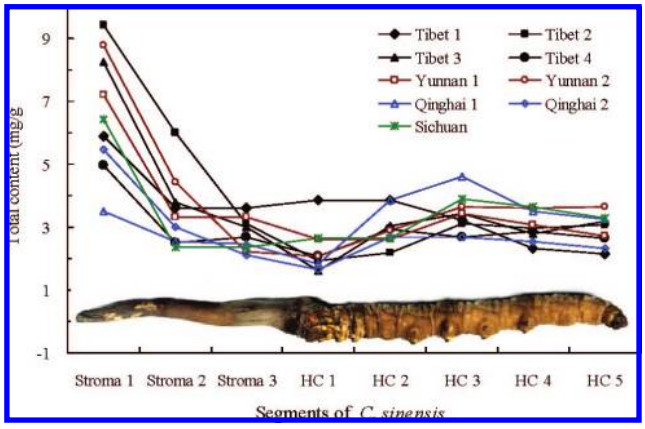


**Figure 4.** Distribution of total nucleosides and nucleobases in the stroma (stromata 1, 2, and 3) and the host caterpillar (HCs 1–5) of individual *C. sinensis* specimens.

In the present study, the hymenophore tissue, which had been separated from the pileus, was found to contain a much higher content of total nucleosides and nucleobases than the pileus and the stipe. In addition, no cytosine was found in *G. lucidum*, *A. aegerita*, *T. albuminosus*, and *L. edodes* in opposition to *C. sinensis* and *C. militaris*, and no thymidine was found in *L. edodes* and in the pileus and the stipe of *G. lucidum*.

As shown in **Table 4**, the hymenophore tissue that bore the hymenium containing the spore-bearing cells contained considerably higher amounts of total nucleosides and nucleobases as compared to other fungal parts, the pileus and the stipe, for *A. aegerita*, *T. albuminosus*, *L. edodes*, and *G. lucidum*. These results indicate that pileus, hymenophore, and stipe may be in different growth phases or have different physiological functions for the growth and multiplication of fungi. The younger tissues, such as gills or tubes, tend to have a higher concentration of total nucleosides and nucleobase than the older tissues, such as the stipe and the pileus.

For *C. sinensis*, the contents of total nucleosides and nucleobases in the stroma were higher than that in the host caterpillar (**Table 4**). In order to look into the distribution of total nucleosides and nucleobases in *C. sinensis*, 9 individual *C. sinensis* specimens were divided into eight segments: stromata 1, 2, and 3, host caterpillars (HCs) 1, 2, 3, 4, and 5 (**Figure 4**), which were analyzed. **Figure 4** shows the distribution of total nucleosides and nucleobases in 9 individual *C. sinensis* specimens. The results showed that stroma 1, the top segment of the stroma in which its spore-producing cells known as asci were developed, contained considerably higher amounts of total nucleosides and nucleobases, especially uridine, guanosine, and adenosine, than other segments of the stroma and the whole host caterpillar. It is speculated that the stroma of *C. sinensis* with well developed asci has higher contents of total nucleosides and nucleobases than that without well developed asci. The growth of natural *C. sinensis* has a very restricted habitat, and the yield is decreasing every year (*15*). The intensive collection made the natural *C. sinensis* become rare and more expensive. At the onset of the collection season, the fungus had been dug up before it had enough time to develop its spore-producing cells, and thus, some or none of the fungi offered for sale showed signs of maturity of the asci (*31*).

As shown in **Table 4**, the contents of both total nucleosides and nucleobases and the individual nucleosides, such as cytidine, uridine, and cordycepin, in natural *C. sinensis* are much lower than that in cultured *C. militaris*. In contrast, natural *C. sinensis*, especially the host caterpillar, contains a much higher amount of inosine (0.381–0.464 mg/g dry matter) than the cultured *C. militaris* (0.017 mg/g dry matter), which is in accordance with the result of Yu et al. (*16*). In fact, the content of inosine in most cultured *Cordyceps* mycelia was too low to be determined (*26*). Cordycepin, formed by the enzymatic reduction of adenosine (*32*), was first detected from *C. militaris* but rarely detected in *C. sinensis* (*13*). In the present study, only very small amounts of cordycepin were found in several natural *C. sinensis*. In general, cultured *C. militaris* showed a higher concentration of cordycepin when compared to that in natural products (*5*). Although trace amounts of cordycepin have also been detected in the fermented mycelium of *C. sinensis*, cordycepin was not detectable in several *Cordyceps* species (*12*). As shown in **Table 4**, adenosine was abundant in stromata (1.087 –1.919 mg/g dry matter), amounting to considerably more than that in the host caterpillars (0.379–0.622 mg/g dry matter) of the natural *C.*

*sinensis*, which was in accordance with the findings of Hsu et al. (*12*) and Weng et al. (*27*).

The profile of nucleosides, especially adenosine, inosine, and cordycepin, could be used for the discrimination of natural and cultured *C. sinensis* and *C. militaris* (*15, 16, 22*). As can be seen from **Figure 3**, the chromatograms with the profiles of each analyzed species would be helpful to control the quality of *Cordyceps*, indicating the importance of nucleoside profiles in the discrimination of the species. To date, nucleosides are believed to be the active components in *Cordyceps* (*5, 12–17*), but the synergistic effect of ergosterol (*33*), the glycosylated form of ergosterol peroxide (*2*), polysaccharides, a glycoprotein and peptides containing α-aminoisobutyric acid (*34*), and nucleosides could not be neglected.

In conclusion, from the results of distribution investigation, the hymenophore tissues containing the spore-producing cells, such as the gills for *A. aegerita*, *T. albuminosus*, and *L. edodes*, the tubes for *G. lucidum*, and the perithecia for *C. sinensis*, were found to have considerably higher amounts of total nucleosides and nucleobases as compared to that in other parts of these fungi. This indicates that the contents of nucleosides and nucleobases increase during sporulation. The amount of nucleosides and nucleobases in fungi is not constant and depends on the fungal species and developmental stage (growth phase, hyphal formation, and sporulation). Although little is known about the synergistic pharmacological activities of nucleosides and nucleobases, the study provided valuable insight on distribution in these fungi, which could also be developed as a reliable means to differentiate various fungi.

## LITERATURE CITED


(1) Cheung, L. M.; Cheung, P. C. K.; Ooi, V. E. C. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* **2003**, *81*, 249–255.

(2) Bok, J. W.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. N. Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry* **1999**, *51*, 891–898.

(3) Liu, X.; Wang, J. H.; Yuan, J. P. Pharmacological and anti-tumor activities of Ganoderma spores processed by top-down approaches. *J. Nanosci. Nanotechnol.* **2005**, *5*, 2001–2013.

(4) Li, S. P.; Li, P.; Lai, C. M.; Gong, Y. X.; Kan, K. K. W.; Dong, T. T. X.; Tsim, K. W. K.; Wang, Y. T. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured *Cordyceps* by pressurised liquid extraction and high-performance liquid chromatography. *J. Chromatogr., A.* **2004**, *1036*, 239–243.

(5) Rao, Y. K.; Chou, C. H.; Tzeng, Y. M. A simple and rapid method for identification and determination of cordycepin in *Cordyceps militaris* by capillary electrophoresis. *Anal. Chim. Acta* **2006**, *566*, 253–258.

(6) Wojtas, M.; Bieñkowski, T.; Tateyama, S.; Sagami, H.; Chojnacki, T.; Danikiewicz, W.; Swiezewska, E. Polyisoprenoid alcohols from the mushroom *Lentinus edodes*. *Chem. Phys. Lipids* **2004**, *130*, 109–115.

(7) Mau, J. L.; Chang, C. N.; Huang, S. J.; Chen, C. C. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem.* **2004**, *87*, 111–118.

(8) Lo, K. M.; Cheung, P. C. K. Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. *Food Chem.* **2005**, *89*, 533–539.

(9) Yang, N.; Tong, X.; Xiang, Y.; Zhang, Y.; Sun, H.; Wang, D. C. Crystallization and preliminary crystallographic studies of the recombinant antitumour lectin from the edible mushroom *Agrocybe aegerita*. *BBA-Proteins Proteome* **2005**, *1751*, 209–212.

(10) Manzi, P.; Marconi, S.; Aguzzi, A.; Pizzoferrato, L. Commercial mushrooms: nutritional quality and effect of cooking. *Food Chem.* **2004**, *84*, 201–206.

(11) Wang, Y. T.; Huang, Z. J.; Chang, H. M. Proteomic analysis of human leukemic U937 cells incubated with conditioned medium of mononuclear cells stimulated by proteins from dietary mushroom of *Agrocybe aegerita*. *J. Proteome Res.* **2004**, *3*, 890–896.

(12) Hsu, T. H.; Shiao, L. H.; Hsieh, C.; Chang, D. M. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chem.* **2002**, *78*, 463–469.

(13) Fan, H.; Li, S. P.; Xiang, J. J.; Lai, C. M.; Yang, F. Q.; Gao, J. L.; Wang, Y. T. Qualitative and quantitative determination of nucleosides, bases and their analogues in natural and cultured *Cordyceps* by pressurized liquid extraction and high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). *Anal. Chim. Acta* **2006**, *567*, 218–228.

(14) Guo, F. Q.; Li, A.; Huang, L. F.; Liang, Y. Z.; Chen, B. M. Identification and determination of nucleosides in *Cordyceps sinensis* and its substitutes by high performance liquid chromatography with mass spectrometric detection. *J. Pharm. Biomed.* **2006**, *40*, 623–630.

(15) Li, S. P.; Yang, F. Q.; Tsim, K. W. K. Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. *J. Pharm. Biomed.* **2006**, *41*, 1571–1584.

(16) Yu, L.; Zhao, J.; Li, S. P.; Fan, H.; Hong, M.; Wang, Y. T.; Zhu, Q. Quality evaluation of *Cordyceps* through simultaneous determination of eleven nucleosides and bases by RP-HPLC. *J. Sep. Sci.* **2006**, *29*, 953–958.

(17) Fan, H.; Yang, F. Q.; Li, S. P. Determination of purine and pyrimidine bases in natural and cultured *Cordyceps* using optimum acid hydrolysis followed by high performance liquid chromatography. *J. Pharm. Biomed.* **2007**, *45*, 141–144.

(18) Gao, J. L.; Leung, K. S. Y.; Wang, Y. T.; Lai, C. M.; Li, S. P.; Hu, L. F.; Lu, G. H.; Jiang, Z. H.; Yu, Z. L. Qualitative and quantitative analyses of nucleosides and nucleobases in *Ganoderma* spp. by HPLC-DAD-MS. *J. Pharm. Biomed.* **2007**, *44*, 807–811.

(19) Kodama, E. N.; McCaffrey, R. P.; Yusa, K.; Mitsuya, H. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT+) leukemic cells. *Biochem. Pharmacol.* **2000**, *59*, 273–281.

(20) Yu, H. M.; Wang, B. S.; Huang, S. C.; Duh, P. D. Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. *J. Agric. Food Chem.* **2006**, *54*, 3132–3138.

(21) Chen, T. H.; Wang, M. F.; Liang, Y. F.; Komatsu, T.; Chan, Y. C.; Chung, S. Y.; Yamamoto, S. A nucleoside-nucleotide mixture may reduce memory deterioration in old senescence- accelerated mice. *J. Nutr.* **2000**, *130*, 3085–3089.

(22) Huang, L. F.; Liang, Y. Z.; Guo, F. Q.; Zhou, Z. F.; Cheng, B. M. Simultaneous separation and determination of active components in *Cordyceps sinensis* and Cordyceps militarris by LC/ESI-MS. *J. Pharm. Biomed.* **2003**, *33*, 1155–1162.

(23) Yu, R. M.; Ye, B.; Yan, C. Y.; Song, L. Y.; Zhang, Z.; Yang, W.; Zhao, Y. Fingerprint analysis of fruiting bodies of cultured *Cordyceps militaris* by high-performance liquid chromatography-photodiode array detection. *J. Pharm. Biomed.* **2007**, *44*, 818–823.

(24) Li, S. P.; Li, P.; Dong, T. T. X.; Tsim, K. W. K. Determination of nucleosides in natural *Cordyceps sinsensis* and cultured *Cordyceps mycelia* by capillary electrophoresis. *Electrophoresis* **2001**, *22*, 144–150.

(25) Li, S. P.; Su, Z. R.; Dong, T. T. X.; Tsim, K. W. K. The fruiting body and its caterpillar host of *Cordyceps sinensis* show close resemblance in main constituents and anti-oxidation activity. *Phytomedicine* **2002**, *9*, 319–324.

(26) Gong, Y. X.; Li, S. P.; Li, P.; Liu, J. J.; Wang, Y. T. Simultaneous determination of six main nucleosides and bases in natural and cultured *Cordyceps* by capillary electrophoresis. *J. Chromatogr., A* **2004**, *1055*, 215–221.

(27) Weng, S. C.; Chou, C. J.; Lin, L. C.; Tsai, W. J.; Kuo, Y. C. Immunomodulatory functions of extracts from the Chinese medicinal fungus *Cordyceps cicadae*. *J. Ethnopharmacol.* **2002**, *83*, 79–85.

(28) Lo, H. C.; Tu, S. T.; Lin, K. C.; Lin, S. C. The anti-hyperglycemic activity of the fruiting body of *Cordyceps* in diabetic rats induced by nicotinamide and streptozotocin. *Life Sci.* **2004**, *74*, 2897–2908.

(29) Luo, H. B.; Cheng, Y. K. A comparative study of void volume markers in immobilized-artificial-membrane and reversed-phase liquid chromatography. *J. Chromatogr., A* **2006**, *1103*, 356–361.

(30) Kowalska, S.; Krupczyńska, K.; Buszewski, B. The influence of the mobile phase pH and the stationary phase type on the selectivity tuning in high performance liquid chromatography nucleosides separation. *J. Sep. Sci.* **2005**, *28*, 1502–1511.

(31) Winkler, D. *Cordyceps sinensis* (Berk.) Sacc: economy, ecology, and ethno-mycology of yartsa gunbu, a medicinal fungus endemic for the Tibetan Plateau. *Int. J. Med. Mushrooms* **2005**, *7*, 481–482.

(32) Masuda, M.; Urabe, E.; Sakurai, A.; Sakakibara, M. Production of cordycepin by surface culture using the medicinal mushroom Cordyceps militaris. *Enzyme Microb. Technol.* **2006**, *39*, 641–646.

(33) Slominski, A.; Semak, I.; Zjawiony, J.; Wortsman, J.; Gandy, M. N.; Li, J. H.; Zbytek, B.; Li, W.; Tuckey, R. C. Enzymatic metabolism of ergosterol by cytochrome p450scc to biologically active 17α, 24-dihydroxyergosterol. *Chem. Biol.* **2005**, *12*, 931–939.

(34) Ng, T. B.; Wang, H. X. Pharmacological actions of *Cordyceps*, a prized folk medicine. *J. Pharm. Pharmacol.* **2005**, *57*, 1–12.

**Received for review June 28, 2007. Revised manuscript received December 15, 2007. Accepted December 16, 2007.**

JF0719205