

Content of Purine in Mushroom Fruiting Bodies and Mycelia

Shih-Jeng Huang

Department of Health Food, Chung Chou University of Science and Technology, Taiwan, ROC

Hsin-Wei Juan and Shu-Yao Tsai

Department of Health and Nutrition Biotechnology, Asia University, Taiwan, ROC

Email: sytsai@asia.edu.tw

Abstract—Levels of purine bases (adenine, guanine, hypoxanthine, and xanthine) were determined in 29 species of mushroom fruit body and mycelia from the Taiwan. Changes in purine-related compounds of mushroom during storage and cooking were investigated. Freeze-dried were hydrolyzed with water–trifluoroacetic acid–formic acid (1:5:5, v/v/v) at 120°C for 30 min for the quantitative liberation of bases from nucleic acids. Purine bases were then analyzed by reverse-phase liquid chromatography. The results indicated that total purine amounts in most mushroom fruit body were higher than mushroom mycelia. The principal purine bases were xanthine and adenine, and xanthine content was the highest in fruit body. The principal purine bases were hypoxanthine and adenine, and hypoxanthine content was the highest in mycelia. The purine content of mushroom differed depending on species, part, storage and cooking, which could be recommended for consumers as a healthy diet, especially for people with hyperuricemia and gout.

Index Terms—purine, mushroom, HPLC, storage, cooking

I. INTRODUCTION

Dietary purines, including adenine, guanine, hypoxanthine and xanthine, occur widely in food as constituents of nucleic acids, nucleotides and free bases [1]–[3]. The end product of purine metabolism is uric acid, and the increase of serum uric acid level causes gout and hyperuricemia [4]–[7]. Monosodium urate crystals accumulate in the joints and other soft tissues, causing painful inflammation of arthritis. Apart from arthritis, gout may also trigger complications such as diabetes and cardiovascular disease, which has been prevalent throughout the world in recent years [8]–[10].

A high-protein diet typically contains large quantities of purines. Although it has been postulated that diet plays an important role in the development of hyperuricemia and gout, data directly linking food intake with hyperuricemia is limited [11], [12]. In nutritional therapy, it is recommended that the amount of dietary purines should be less than 400 mg per day [13], [14].

Mushrooms are considered valuable health foods, since they have a significant amount of dietary fiber and are

poor in calories and fat. Mushrooms have been used as food flavoring materials in soups and sauces for centuries, due to their unique and subtle flavor [15].

However, flavorful food generally contains high levels of purine, such as umami components. [2] The objectives of this study were to investigate the purine compounds of mushroom fruit body and mycelia, and changes in purine compounds of mushroom storage and cooking.

II. MATERIALS AND METHODS

A. Standards and Chemicals

Standards were obtained from Sigma Chemical Company (St. Louis, MO, USA). The purines used in this study were adenine, guanine, hypoxanthine, and xanthine, which were chromatography-grade and all assayed at more than 98% purity. Potassium acetate, trimethylamine, trifluoroacetic acid, and formic acid were purchased from Merck (Darmstadt, Germany); they were all guaranteed reagents (GR).

B. Mushroom Collection

Totally, 29 species of mushrooms were used and categorized into edible and medicinal mushrooms. Nineteen species of mushroom fruiting bodies, including *Agaricus bisporus*, *Auricularia mesenterica*, *Boletus edulis*, *Clitocybe maxima*, *Flammulina velutipes*, *Ganoderma lucidum*, *Ganoderma tsugae*, *Grifola frondosa*, *Hypsizigus marmoreus*, *Inonotus obliquus*, *Lentinula edodes*, *Pholiota nameko*, *Pleurotus citrinopileatus*, *Pleurotus cystidiosus*, *Pleurotus eryngii*, *Pleurotus ferulae*, *Pleurotus eryngii* var. *ferulae*, *Pleurotus ostreatus* and *Pleurotus salmoneostramineus* were obtained from Q-Yo Bio-Technology Farm, Pusin, Chunghua, Taiwan. Fruiting bodies of *C. maxima* and *L. edodes* were divided into caps and stipes as described in Chen *et al.* [15] Three strains of *P. ostreatus*, including Japan, Korea and Taiwan strains, were commercially available. Thirteen species of mushroom mycelia, including *Agaricus blazei*, *Antrodia camphorata*, *Antrodia salmonea*, *Armillariella mellea*, *Cordyceps cicadae*, *Cordyceps militaris*, *Cordyceps sinensis*, *Coriolus versicolor*, *Ganoderma lucidum*, *G. frondosa*, *Hericius erinaceus*, *H. marmoreus*, and *Phellinus linteus*

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were obtained in freeze-dried form from the Biotechnology Centre, Grape King Inc., Chungli City, Taiwan. Two strains of *C. militaris*, including strains cm1 and cm5, were commercially available. Normal and white

strains of *H. marmoreus* were also used. Three species of mushrooms, including *G. lucidum*, *G. frondosa* and *H. marmoreus*, were analyzed for both fruiting bodies and mycelia.

TABLE I. THE CONTENT OF PURINE IN MUSHROOM FRUIT BODIES

Fruit bodies	mg/g of sample (dry weight)				
	Adenine	Guanine	Hypoxanthine	Xanthine	Total purine
<i>A. mesenterica</i>	0.13±0.01N	0.06±0.01F	0.77±0.05G	0.10±0.01K	0.94±0.02M
<i>B. edulis</i>	1.49±0.03K	0.06±0.00F	3.63±0.10D	0.52±0.01JK	5.69±0.12I
<i>C. maxima</i> (cap)	2.05±0.07H	nd	0.18±0.00J	1.12±0.02I	3.35±0.09J
<i>C. maxima</i> (stem)	0.98±0.02L	0.08±0.00E	0.30±0.04I	0.54±0.01JK	1.90±0.06L
<i>G. lucidum</i>	0.96±0.08L	0.06±0.00F	0.94±0.02F	4.61±0.02F	6.57±0.08H
<i>G. tsugae</i>	0.70±0.07M	0.06±0.00F	0.51±0.01H	2.36±0.10G	3.63±0.18J
<i>G. frondosa</i>	1.83±0.08I	0.09±0.00D	0.32±0.01I	0.81±0.01IJ	3.05±0.10JK
<i>H. marmoreus</i>	1.95±0.03H	0.09±0.00D	0.78±0.01G	0.63±0.01J	3.44±0.03J
<i>I. obliquus</i>	nd	0.06±0.00F	0.19±0.01J	nd	0.25±0.04M
<i>L. edodes</i>	2.04±0.01H	0.08±0.01E	nd	0.67±0.02IJ	2.79±0.31JK
<i>L. edodes</i> (cap)	2.31±0.10G	0.08±0.00E	nd	0.89±0.02IJ	3.28±0.12J
<i>L. edodes</i> (stem)	1.50±0.00K	0.06±0.00F	nd	0.45±0.00JK	2.01±0.01L
<i>P. nameko</i>	6.89±0.14A	0.06±0.00F	nd	0.45±0.00JK	7.40±0.10G
<i>P. citrinopileatus</i>	4.16±0.08B	0.12±0.00A	0.22±0.00IJ	33.63±0.54A	38.13±0.62A
<i>P. cystidiosus</i>	1.57±0.04K	0.08±0.00E	nd	0.72±0.00IJ	2.35±0.04KL
<i>P. eryngi</i> var. <i>ferulae</i>	3.17±0.03E	0.11±0.00B	0.18±0.04J	32.14±0.10B	35.60±0.09B
<i>P. ferulae</i>	1.69±0.05J	0.09±0.00D	3.19±0.03E	4.80±0.35E	9.77±0.28F
<i>P. ostreatus</i>	3.01±0.04F	0.10±0.00C	4.74±0.04C	6.87±0.81D	14.72±0.84E
<i>P. ostreatus</i> (Japan)	3.12±0.04E	0.11±0.00B	nd	1.82±0.03H	5.05±0.06I
<i>P. ostreatus</i> (Korea)	3.99±0.10C	nd	9.42±0.21A	8.57±0.59C	21.97±1.78C
<i>P. salmoneostramineus</i>	3.30±0.05D	nd	7.43±0.13B	8.56±0.08C	19.30±0.18D

Each value is expressed as mean ±SD (n = 3). Means with different letters within a column are significantly different (p < 0.05). Total= adenine + guanine + hypoxanthine + xanthine; nd, not detected.

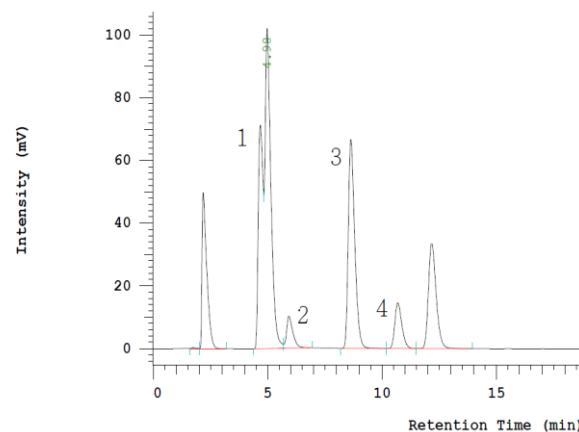


Figure 1. HPLC chromatograms of purine standard. 1. adenine; 2. guanine; 3. hypoxanthine; 4. xanthine.

C. Storage and Cooking Conditions

Mushrooms from *A. bisporus*, *L. edodes*, *F. velutipes*, and *P. ostreatus* were randomly selected into 12 samples and packaged by placing about 100g in 300 mL polystyrene (PS) trays and overwrapping them with MK-

PVC film using a B-105 diawrapper. After packaging, three trays of mushrooms were randomly selected for day 0 analyses, and the remaining nine trays were stored in a 4 °C incubator. At days 3, 6, and 9, (*A. bisporus*, *L. edodes* and *F. velutipes*), and at days 7, 14, and 21, (*P. ostreatus*) three trays were randomly selected for the

study. *P. ostreatus* (10 g) was heated with 100 mL hot water for 5, 10, 15 and 20 min and through filter., then divided into fruiting bodies and soup. All samples were freeze dried and ground using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany) to obtain a coarse powder (60 mesh).

D. Purine Analysis [16]

The powdered samples (0.05 g) were digested in a triangular glass flask containing 2.75 mL water–trifluoroacetic acid–formic acid (1:5:5, v/v/v) at 120°C for 30min. After chilling in cold water, the resultant hydrolysates were transferred to a 50 mL flask and dried using a rotary vacuum evaporator at 50°C to dryness, redissolved in potassium phosphate monobasic (0.02 M, pH 4.0), and filtered prior to injection into a high-performance liquid chromatograph (HPLC). The HPLC system consisted of a Hitachi L-2130 pump, Hitachi L-2400 UV detector, and LiChrospher 100 RP-18e column (4.6 × 250 mm, 5μm, Merck). The mobile phase was 0.02M potassium acetate (pH 4.0, contain 0.1% triethylamine) at a flow rate of 1.0 mL/min and UV detection was at 254 nm. Content of adenine, guanine, hypoxanthine, and xanthine was calculated based on the calibration curve of authentic standard. A chromatogram for standard mixture is depicted in Fig. 1.

E. Statistical Analysis

All the measurements were in triplicate. The experimental data were subjected to an analysis of variance for a completely random design to determine the Fisher least significant difference among means at $P < 0.05$.

III. RESULTS AND DISCUSSION

A. Purine Content in Fruiting Bodies

Results are expressed in mg/g of sample dry weight. Total purine content was the sum of adenine, guanine, hypoxanthine and xanthine content. Purine concentrations in mushroom fruit body are shown in Table I. The amount of total purines was classified into three groups: low group: <2.5 mg/g dry weight, moderate group: 2.5-10 mg/g dry weight, and high group: >10 mg/g dry weight.

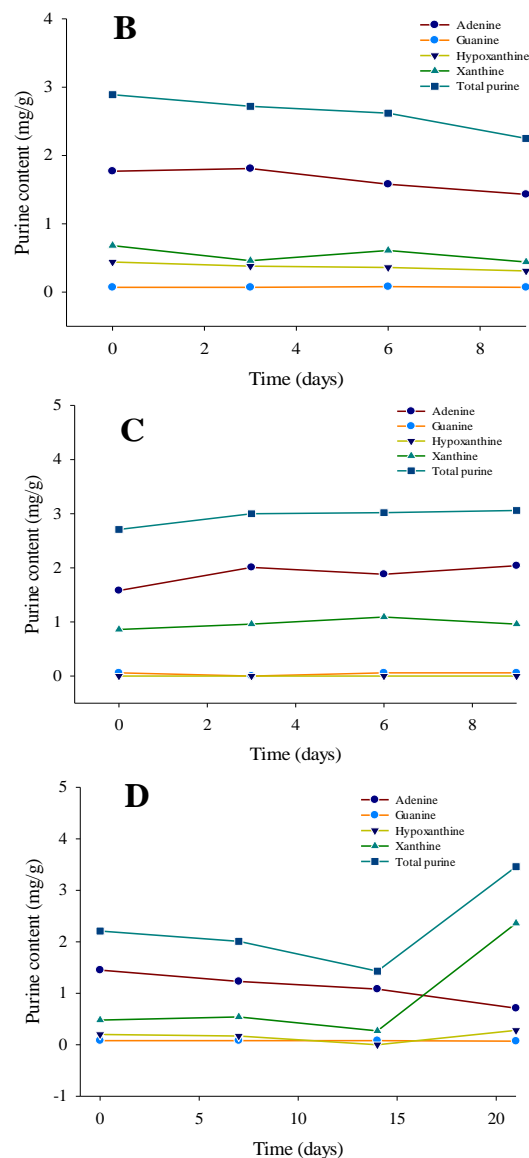
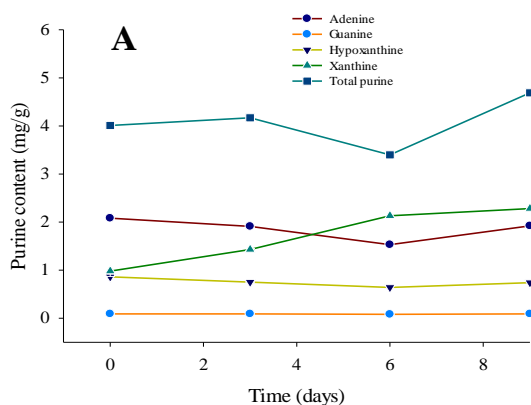


Figure 2. Content change of purine in mushroom during storage: (A) *A. bisporus*; (B) *F. velutipes*; (C) *L. edodes*; (D) *P. eryngii*.

Purine content in *P. citrinopileatus*, *P. eryngii* var. *ferulae*, *P. ostreatus* (Korea), *P. salmoneostramineus*, *P. ostreatus* contained 14.72-38.13 mg/g dry weight purines, so they were classified in the high group (Table I). Most *Pleurotus* sp. contained a high ratio of xanthine (*P. citrinopileatus* and *P. eryngii* var. *ferulae* as 88-90% of their total purines). Eleven mushroom fruit body (11/21 items) contained 2.79-9.77 mg/g purines; they were clarified in the moderated group. Five mushroom fruit body (5/21 items) contained 0.25-2.35 mg/g purines; they were clarified in the low group.

Each purine will transform to uric acid equivalently. Furthermore, adenine and hypoxanthine had a greater hyperuricemic effect [17]. Differences in the metabolic effect of individual purine base would suggest that the restriction of some foods should be based on the content of uricogenic purines rather than on the total purine content [1]-[3]. Overall, the mushroom fruit body

contains mainly adenine and xanthine, and xanthine does not tend to elevate serum concentration of uric acid.

B. Purine Content in Mycelia

Twelve mushroom mycelia (12/15 items) contained 2.84-5.12 mg/g purines; they were clarified in the moderated group (Table II). Three mushroom mycelia (3/15 items) contained 1.62-2.25 mg/g purines; they were clarified in the low group. Most mushroom mycelia contained 47-84% ratio of hypoxanthine. The content of hypoxanthine and adenine in mushroom mycelia was higher than those in other parts. Furthermore, the purine content was related to mushroom species and mycelium purine content is low.

C. Purine Content in Mushroom Storage

The change in purine content of mushroom during storage at different days was almost constant (Fig. 2). Mushroom fruit body during storage, except *F. velutipes*, at 9 or 21 days, contained higher total purine content. Furthermore, *A. bisporus* and *P. eryngii* during storage, contained higher ratio of xanthine. Therefore, mushrooms in the storage conditions for a long time, will increase the purine content. May be due to mushroom self-dissolving phenomenon, it is recommended that fresh mushrooms should be finished as soon as possible to avoid frozen storage for too long.

TABLE II. THE CONTENT OF PURINE IN MUSHROOM MYCELIA

Mycelia	mg/g of sample (dry weight)				
	Adenine	Guanine	Hypoxanthine	Xanthine	Total purine
<i>A. blazei</i>	1.13±0.02F	0.09±0.01B	2.65±0.02D	nd	3.87±0.03BCD
<i>A. camphorata</i>	0.50±0.03J	0.06±0.01B	1.00±0.04H	0.07±0.01H	1.62±0.06F
<i>A. salmonea</i>	0.82±0.09H	0.07±0.01B	1.80±0.11E	0.15±0.01G	2.84±0.20DE
<i>A. mellea</i>	0.70±0.06I	0.07±0.01B	1.48±0.16F	nd	2.25±2.22EF
<i>C. cicadae</i>	1.56±0.01C	0.07±0.01B	3.24±0.03C	nd	4.86±0.04ABC
<i>C. militaris</i> (Cm 1)	1.31±0.01E	0.07±0.01B	3.14±0.03C	0.18±0.01G	4.70±0.02ABC
<i>C. militaris</i> (Cm 5)	1.51±0.02C	0.18±0.07A	nd	0.38±0.03E	2.07±0.05EF
<i>C. sinensis</i>	0.67±0.01I	0.06±0.01B	1.41±0.02FG	1.61±0.10B	3.75±0.11CD
<i>C. versicolor</i>	1.39±0.07D	0.07±0.01B	3.37±0.17B	nd	4.83±0.27AB
<i>G. lucidum</i>	0.57±0.01J	0.10±0.01B	3.66±0.02A	nd	4.34±0.02ABC
<i>G. frondosa</i>	1.65±0.01B	0.08±0.01B	1.77±0.01E	0.26±0.01F	3.76±0.02CD
<i>H. erinaceus</i>	0.99±0.05G	0.06±0.01B	1.32±0.02G	2.75±0.07A	5.12±0.13A
<i>H. marmoreus</i>	2.23±0.04A	0.09±0.01B	0.70±0.01I	0.95±0.01D	3.97±0.06BC
<i>H. marmoreus</i> (white)	2.23±0.05A	0.09±0.01B	1.03±0.02H	1.11±0.01C	4.46±0.07ABC
<i>P. linteus</i>	1.55±0.06C	0.07±0.01B	3.21±0.11C	nd	4.83±0.18ABC

Each value is expressed as mean ±SD (n = 3). Means with different letters within a column are significantly different (p < 0.05). Total= adenine + guanine + hypoxanthine + xanthine; nd, not detected.

TABLE III. THE CONTENT OF PURINE IN P. FERULAE MUSHROOM COOKING

mg/g of sample (dry weight)						
	Time (min)	Adenine	Guanine	Hypoxanthine	Xanthine	Total purine
Fruit body	5	1.35±0.03B	0.08±0.00A	0.23±0.01A	3.48±0.19BC	5.14±0.27AB
	10	0.46±0.02D	0.08±0.00A	0.23±0.03A	3.96±0.01A	4.73±0.19B
	15	1.20±0.05C	0.08±0.00A	0.24±0.00A	3.16±0.36C	4.68±0.51B
	20	1.43±0.02A	0.08±0.00A	0.24±0.00A	3.63±0.04AB	5.38±0.08A
Soup	5	1.20±0.04B	0.08±0.00B	1.24±0.01B	0.01±0.00B	2.53±0.17B
	10	1.18±0.01B	0.07±0.00A	1.05±0.01D	0.01±0.00B	2.31±0.01C
	15	1.16±0.02B	0.07±0.00A	1.16±0.02C	0.08±0.00A	2.47±0.03BC
	20	1.32±0.01A	0.08±0.00B	1.38±0.03A	nd	2.78±0.03A
mg/g of sample (dry weight)						
	Time (min)	Adenine	Guanine	Hypoxanthine	Xanthine	Total purine
Fruit body	5	1.35±0.03B	0.08±0.00A	0.23±0.01A	3.48±0.19BC	5.14±0.27AB
	10	0.46±0.02D	0.08±0.00A	0.23±0.03A	3.96±0.01A	4.73±0.19B
	15	1.20±0.05C	0.08±0.00A	0.24±0.00A	3.16±0.36C	4.68±0.51B
	20	1.43±0.02A	0.08±0.00A	0.24±0.00A	3.63±0.04AB	5.38±0.08A
Soup	5	1.20±0.04B	0.08±0.00B	1.24±0.01B	0.01±0.00B	2.53±0.17B
	10	1.18±0.01B	0.07±0.00A	1.05±0.01D	0.01±0.00B	2.31±0.01C
	15	1.16±0.02B	0.07±0.00A	1.16±0.02C	0.08±0.00A	2.47±0.03BC
	20	1.32±0.01A	0.08±0.00B	1.38±0.03A	nd	2.78±0.03A
mg/g of sample (dry weight)						

	Time (min)	Adenine	Guanine	Hypoxanthine	Xanthine	Total purine
Fruit body	5	1.35±0.03B	0.08±0.00A	0.23±0.01A	3.48±0.19BC	5.14±0.27AB
	10	0.46±0.02D	0.08±0.00A	0.23±0.03A	3.96±0.01A	4.73±0.19B
	15	1.20±0.05C	0.08±0.00A	0.24±0.00A	3.16±0.36C	4.68±0.51B
	20	1.43±0.02A	0.08±0.00A	0.24±0.00A	3.63±0.04AB	5.38±0.08A
Soup	5	1.20±0.04B	0.08±0.00B	1.24±0.01B	0.01±0.00B	2.53±0.17B
	10	1.18±0.01B	0.07±0.00A	1.05±0.01D	0.01±0.00B	2.31±0.01C
	15	1.16±0.02B	0.07±0.00A	1.16±0.02C	0.08±0.00A	2.47±0.03BC
	20	1.32±0.01A	0.08±0.00B	1.38±0.03A	nd	2.78±0.03A

Each value is expressed as mean ± SD (n = 3). Means with different letters within a column are significantly different (p < 0.05). Total= adenine + guanine + hypoxanthine + xanthine; nd, not detected.

D. Purine Content in Mushroom Cooking

Change in purine content of *P. ferulae* because of cooking time was evaluated (Table III). The content of total purine from fruit body decreased after 10 and 15 min cooking, and then increased slightly up to 20 min. The content of total purine from soup was increased with increased cooking time. The content of hypoxanthine in soup was higher than those in other parts. Overall, longer cooking time could result in higher total purine content of the soups.

IV. CONCLUSIONS

The total purine content of most mushroom fruiting bodies was higher than that of mushroom mycelia. The cold storage of mushroom fruit body had little effect on total purine content. Longer cooking time could result in higher total purine content of the soup. Thus, this show time cooking procedure could reduce the purine content of mushroom. Also, the changing purine content of mushrooms could provide consumers with a healthy dietary guideline. Mushrooms are not high-purine content; it is recommended they be more intake foodstuffs.

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owns the membership of IFT.



Shih-Jeng Huang was born in Changhua, Taiwan. He received his Ph.D. degree in Department of Food Science from National Chung-Hsing University in 2005. He is currently an associate professor in the Department of Health Food at Chung-Chou Institute of Technology in Taiwan. His main research interests include aroma chemical, fermentation, γ -irradiation technology and edible and medicinal mushroom. Dr. Huang

Hsin-Wei Juan was born in Taipei, Taiwan. His major is mushroom analysis. He is now studying in Asia university for his master's degree. He will graduate in 2018. His main research interests include fermentation and edible and medicinal mushroom.



Shu-Yao Tsai was born in Chiayi, Taiwan. She is currently a professor in the Department of Health and Nutrition Biotechnology at Asia University in Taiwan. She received her Ph. D. degree in Department of Food Science and Biotechnology from National Chung-Hsing university in 2006. Her main research interests include aroma chemical and edible and medicinal mushroom.