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Non-volatile flavour components of *Ganoderma tsugae*

Yu-Hsiu Tseng, Yu-Ling Lee, Ruei-Chian Li, Jeng-Leun Mau *

Department of Food Science, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC

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Abstract

Ganoderma tsugae Murrill are currently popular and used in the formulation of nutraceuticals and as functional foods. The non-volatile components in the form of mature and baby fruit bodies (Ling chih), mycelia and fermentation filtrate from submerged culture were studied. Mycelia and filtrate contained significantly higher moisture contents (10.3% and 19.8%) and higher contents of carbohydrates, reducing sugars, crude ash and crude protein. Four forms of *G. tsugae* contained from 7.65% to 10.1% dry weight of total soluble sugars and polyols. Total free amino acid contents ranged from 2.50 to 149 mg g^{-1} dry weight and in the descending order of filtrate, mycelia, baby Ling chih and Ling chih. Contents of monosodium glutamate-like components ranged from 0.16 to 26.0 mg g^{-1} whereas, contents of sweet components ranged from 0.50 to 24.6 mg g^{-1}. The bitter components were predominant. Contents of total and flavour 5′-nucleotides were high in filtrate (5.48 and 3.10 mg g^{-1}, respectively). The umami intensities were expected to be in the descending order of filtrate, mycelia, baby Ling chih and Ling chih.

Keywords: *Ganoderma tsugae*; Soluble sugars; Free amino acids; 5′-Nucleotides

1. Introduction

Medicinal mushroom *Ganoderma* are traditionally used in Chinese medicine and are currently popular in regions of China, Japan, Korea and Taiwan. *Ganoderma* is highly valued as a folk medicine and functional food for its antitumor and other physiological benefits. Recently, *Ganoderma* was found to be medically active with several therapeutic effects including antiinflammatory, antitumor, antiviral (e.g., anti-HIV), antibacterial and antiparasitic, blood pressure regulation, cardiovascular disorders, immunomodulating, kidney tonic, hepatoprotective, nerve tonic, sexual potentiator and chronic bronchitis (Wasser & Weis, 1999).

Ganoderma tsugae Murrill (Ling chih, Sung-shan-ling-chih or reishi) is the most widely cultivated species in Taiwan. Normally, mature Ling chih, the fruit body of *G. tsugae*, is harvested from polypropylene bags at 1–2 months after fruiting whereas, baby Ling chih is harvested at 2–3 weeks after fruiting. The total yield of baby Ling chih is higher than that of mature Ling chih for the entire crop (Tseng, Tsai, Lee, & Mau, 2003). In addition, baby Ling chih will not cause the ecological damage to trees since no spores are discharged. Recently, people in Mid-Taiwan have presented anecdotal evidence suggesting that baby Ling chih is more effective in therapeutic effects than mature Ling chih. It was thought that baby Ling chih is soft in texture and contains more soluble substances than mature Ling chih that has become woody after one month of growth.

In polypropylene bag cultivation, *G. tsugae* not only requires a long time to produce fruit bodies, but also needs a considerable volume of sawdust for fruit body development. However, sawdust preparation will shrink the area of forest and thereby cause some ecological damage. On the other hand, the submerged culture that require a short time to yield mycelia and fermented filtrate do not need sawdust for growth. Both fruit bodies and mycelia of *G. tsugae* are mainly prepared for use in the formulation of nutraceuticals and functional foods. As a food ingredient, the chemical composition and non-volatile flavour components of *G. tsugae* may correlate with product acceptability. Mau, Lin, and Chen (2001a) examined the non-volatile flavour components

* Corresponding author. Tel.: +886-4-2285-4313; fax: +886-4-2287-6211/2483-3508.
E-mail address: jlmau@dragon.nchu.edu.tw (J.-L. Mau).

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in fruit bodies of *G. tsugae*. However, the profile of non-volatile flavour components of *G. tsugae* in the form of baby Ling chih, mycelia and fermentation filtrate is not available.

Therefore, our objective was to examine the non-volatile flavour components in mature and baby fruit bodies (Ling chih), mycelia and filtrate from the submerged culture, including their proximate compositions, soluble sugars or polyols, free amino acids and 5′-nucleotides. The differences in the non-volatile components in four forms of *G. tsugae* were also compared.

2. Materials and methods

2.1. Medicinal mushrooms

The pure culture of *G. tsugae* GT01 was originally obtained from the Department of Plant Pathology, Taiwan Agricultural Research Institute, Wufeng, Taichung County, Taiwan. Fresh mature (6 weeks old) and baby (2 weeks old) Ling chih was harvested from the mushroom room of the Department of Food Science, National Chung-Hsing University, Taichung, Taiwan, and air-dried in an oven at 40 °C for 2–3 days before sample preparation. Mycelia and fermentation filtrate, both in a freeze-dried form, were obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan. For each of mature, baby Ling chih, mycelia and filtrate, three dried samples (~50 g each) were randomly selected and ground using a mill (Retsch ultra centrifugal mill and sieving machine, Haan, Germany) to obtain coarse powder (20 mesh).

2.2. Proximate analysis

The proximate compositions of four forms of *G. tsugae*, including moisture, ash, crude fat, crude fibre and crude protein, were determined according to AOAC methods (1990). The nitrogen factor used for crude protein calculation was 4.38 (Crisan & Sands, 1978). The carbohydrate content was calculated by subtracting the contents of crude ash, fat, fibre and protein from 100% of dry matter and expressed as the percentage of dry weight. Total reducing sugars were determination using the 3,5-dinitrosalicylic acid (DNS) method as described by James (1995). The absorbance of each sample solution was measured at 540 nm on a Hitachi 2001 spectrophotometer. Total reducing sugars were calculated based on a calibration curve of glucose.

2.3. Soluble sugar or polyol assay

Soluble sugars or polyols were extracted and analysed as described by Ajlouni, Beelman, Thompson, and Mau (1995). Dried powder (600 mg) was extracted with 50 ml of 80% aqueous ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei). This suspension was shaken for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The residue was washed five times with additional 25 ml portions of 80% ethanol. The combined filtrate was then rotary-evaporated at 40 °C and redissolved in deionised water to a final volume of 10 ml. The aqueous extract was passed through a filter unit (13 mm, Lida, Corp., Kenosha, WI), and filtered using a 0.45 μm CA non-sterile filter (Lida) prior to HPLC analysis.

The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20 μl sample loop, a Hitachi D-2500 chromato-integrator, a Shimadzu RID-10A detector, and a Phase Sep-NH_2 column (4.6×250 mm, 5 μm, Phase Separation Inc., Norwalk, CT). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH)/deionised water, 85:15 (v/v) at a flow rate of 1 ml min^{-1}. Each sugar or polyol was identified using the authentic sugar or polyol (Sigma Chemical Co., St. Louis, MO) and quantified by the calibration curve of the authentic compound.

2.4. Free amino acid assay

Dried powder (500 mg) was shaken with 50 ml of 0.1 N HCl (Union Chemical Co., Hsinchu, Taiwan) for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was then passed through a filter unit (13 mm, Lida), and filtered using a 0.45 μm CA non-sterile filter (Lida). This filtrate was mixed with *o*-phthalaldehyde reagent (Sigma) in an Eppendorf tube, shaken to facilitate derivatisation and then immediately injected onto the HPLC.

The HPLC system was the same as for sugar analysis but included a Hitachi L-7485 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a LiChrospher 100 RP-18 column (4.6×250 mm, 5 μm, Phenomenex Inc., Torrance, CA). The mobile phases were A, 50 mM sodium acetate (pH 5.7) containing 0.5% tetrahydrofuran; B, deionised water; and C, methanol. The gradient was A:B:C 80:0:20–33:0:67 for 0–38 min, 0:33:67 for 38–40 min, and 0:100:0 for 40–43 min. The flow rate was 1.2 ml min^{-1}. Each amino acid was identified using the authentic amino acid (Sigma) and quantified by the calibration curve of the authentic compound.

2.5. 5′-Nucleotide assay

5′-Nucleotides were extracted and analysed as described by Taylor, Hershey, Levine, Coy, and Olivelle (1981). Dried powder (500 mg) was extracted with 25 ml of deionised water. This suspension was heated to boiling for one min, cooled, and then centrifuged at 22,200*g* for 15 min. The extraction was repeated once

with 20 ml of deionised water. The combined filtrate was then evaporated, and filtered prior to HPLC injection in the same manner as in the soluble sugar or polyol assays.

The HPLC system was the same as for sugar or polyol assay except for a Shimadzu UV detector and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Phenomenex). The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.3, Wako Pure Chemical Co., Osaka, Japan) at a flow rate of 1 ml min^{-1} and UV detection at 254 nm. Each 5′-nucleotide was identified using the authentic 5′-nucleotide (Sigma) and quantified by the calibration curve of the authentic compound.

2.6. Statistical analysis

For each of mature, baby Ling chih, mycelia and filtrate, three samples were used for the determination of every quality attribute. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel, Torrie, and Dickey (1997), to determine the least significant difference among means at the level of 0.05.

3. Results and discussion

Mycelia and fermentation filtrate from the submerged culture of *G. tsugae* contained significantly higher moisture contents (10.3% and 19.8%) than the fruit bodies (mature and baby Ling chih, 8.84% and 7.27%, respectively) (Table 1). The filtrate contained the highest amount of crude ash (14.1%), and the mycelia contained the second highest amount (5.18%). The mycelia contained the highest amount of crude fat (21.9%). However, high contents of crude ash in the mycelia and the filtrate and high content of crude fat in the mycelia resulted from the supplementation of soybean meal and mineral solution to submerged culture.

Contents of carbohydrate and total reducing sugar ranged from 10.4% to 39.6% and from 5.35% to 24.9%, respectively, and both were in the descending order of filtrate, mycelia, baby Ling chih and Ling chih. However, the difference when the reducing sugar content was subtracted from the carbohydrate content was the content of soluble polysaccharides. Soluble polysaccharides were thought to be the biologically active component in mushrooms (Wasser & Weis, 1999), and their contents were 5.06%, 11.3%, 11.6% and 14.7% for Ling chih, baby Ling chih, mycelia and filtrate, respectively. Generally, carbohydrate contents of fruit bodies were in the range 44.0–74.3% (Crisan & Sands, 1978). Furthermore, Mau et al. (2001a) found that contents of carbohydrate in fruit bodies of *Ganoderma* spp. ranged from 21.8% to 27.8%. However, the carbohydrate contents of mature and baby Ling chih were low as compared to those reported by Mau et al. (2001a).

The crude fibre contents were high in Ling chih and baby Ling chih (73.4% and 59.9%, respectively). Crisan and Sands (1978) reported that most fruit bodies contained 3–32% crude fibre. Obviously, the fruit bodies of *G. tsugae* are a good source of fibre. However, the high amount of fibre that was acid-, alkali- and alcohol-insoluble was ineffective in taste. Since the fibre accumulated as fruit bodies grew, contents of other components were consistently reduced. Therefore, the contents of carbohydrates, ash, fat and protein in baby Ling chih and Ling chih were low as compared to those in the mycelia. However, the major component of crude fibre in mushrooms is chitin, which is an important structural polysaccharide found in the cell wall (Michalenko, Hohl, & Rast, 1976). Evidently, fruit bodies contained plenty of chitin whereas, the fibre content of mature Ling chih even reached 73.4%. Ling chih were usually cooked in boiling water for several hours and the concentrate thus obtained was used as folk medicine. Apparently, the fibre (mainly the chitin) was a waste after folk medicine preparation; however, it was also a wound healing enhancer (Su, Juan, Sun, & Tung, 1996).

The protein contents ranged from 8.81% to 39.6% and were in the descending order of filtrate, mycelia, baby Ling chih and Ling chih. Mushrooms are though

Table 1
Proximate composition of *G. tsugae* fruit bodies, mycelia and fermentation filtrate

Component[a]	Content[b] (%)			
	Ling chih	Baby Ling chih	Mycelia	Filtrate
Moisture	8.84 ± 0.19c	7.27 ± 0.12d	10.3 ± 0.04b	19.8 ± 0.07a
Dry matter	91.2 ± 0.19b	92.7 ± 0.12a	89.7 ± 0.04c	80.2 ± 0.07d
Carbohydrate	10.4 ± 0.08d	17.2 ± 0.20c	27.0 ± 0.34b	39.6 ± 0.23a
Reducing sugar	5.35 ± 0.40c	5.86 ± 0.22c	15.4 ± 0.31b	24.9 ± 0.43a
Crude ash	1.69 ± 0.05d	2.62 ± 0.11c	5.18 ± 0.22b	14.1 ± 0.10a
Crude fat	5.72 ± 0.63b	6.50 ± 0.53b	21.9 ± 0.31a	5.12 ± 0.21b
Crude fibre	73.4 ± 0.53a	59.9 ± 0.24b	19.4 ± 0.34c	1.58 ± 0.07d
Crude protein	8.81 ± 0.08d	13.8 ± 0.66c	26.6 ± 1.20b	39.6 ± 0.27a

[a] Moisture and dry matter were presented based on air-dried weight, others were presented based on dry weight.
[b] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

to be a good source of protein, and their protein contents generally range from 19% to 35% of dry weight (Crisan & Sands, 1978). Evidently, the fruit bodies of *G. tsugae* were not a good source of protein. However, with regards to medicinal properties, the protein contents of baby Ling chih and Ling were not the major concern.

As compared to Ling chih, the fruit bodies of *G. tsugae* in Mau et al. (2001a) contained lower ash (0.72%), fat (4.62%), fibre (65.3%) and protein (7.54%) but higher carbohydrate (21.8%). The discrepancy in the profiles of proximate compositions might be due to the difference in the strains used. From the results shown in Table 1, four forms of *G. tsugae* were evidently different in the proximate composition profile. The mycelia and filtrate contained higher contents of carbohydrates, reducing sugars, crude ash and crude protein than Ling chih and baby Ling chih. These results were consistent with the existence of soluble polysaccharides and extracellular enzymes that were produced and excreted during the growth of mycelia. Although some differences were observed in proximate compositions, mature and baby Ling chih were related as compared to mycelia and filtrate.

Four forms of *G. tsugae* contained from 7.65% to 10.1% dry weight of total soluble sugars and polyols (Table 2). Glucose, myo-inositol and trehalose were detected in *G. tsugae*. However, fructose was not found in Ling chih and baby Ling chih whereas, mannitol was not present in the filtrate. Mannitol was the major polyol found in fruit bodies, glucose was the major sugar in mycelia, and fructose and glucose were the two major sugars in filtrate.

On the dry weight basis, with regards to fruit bodies, contents of total soluble sugars were found to be 349–458 mg g^{-1} in paddy straw mushrooms (*Volvariella volvacea*) (Mau, Chyau, Li, & Tseng, 1997), 205–319 mg g^{-1} in common mushrooms (*Agaricus bisporus*) (Tseng & Mau, 1999), 98.7–316 mg g^{-1} in ear mushrooms (*Auricularia* spp. and *Tremella fuciformis*) (Mau, Wu, Wu, & Lin, 1998a), 56–86.1 mg g^{-1} in black poplar mushrooms (*Agrocybe cylindracea*) (Mau & Tseng, 1998), and 6.96–20.8 mg g^{-1} in king oyster mushrooms (*Pleurotus eryngii*) (Mau, Lin, Chen, Wu, & Peng, 1998b). However, Mau et al. (2001a) found that the fruit bodies of *Ganoderma* spp. contained low amounts of total soluble sugars, ranged from 1.68% to 8.37%. In addition, Chang, Chao, Chen, and Mau (2001) found that contents of total soluble sugars in three medicinal mushroom mycelia, including Brazilian mushroom (*Agaricus blazei*), chang-chih (*Antrodia camphorata*) and northern *Cordyceps* (*Cordyceps militaris*), ranged from 101 to 115 mg g^{-1}. Soluble sugars contained in the mushrooms contributed a sweet taste (Litchfield, 1967). Therefore, the results revealed that four forms of *G. tsugae* would not give a palatably sweet perception.

The total free amino acid contents in four forms of *G. tsugae* ranged from 2.50 to 149 mg g^{-1} dry weight and in the descending order of filtrate, mycelia, baby Ling chih and Ling chih (Table 3). Table 4 tabulates the free amino acids into several classes on the basis of their flavour characteristics as described by Komata (1969). Aspartic and glutamic acids were monosodium glutamate-like (MSG-like) components, which gave the most typical mushroom taste, the umami taste or palatable taste that was the characteristic flavour of MSG and 5′-nucleotides (Yamaguchi, 1979). Contents of MSG-like components were relatively lower and insignificant in Ling chih (0.16 mg g^{-1}) and baby Ling chih (0.46 mg g^{-1}), but significant higher in filtrate (26.0 mg g^{-1}). Contents of sweet components ranged from 0.50 to 24.6 mg g^{-1} dry weight and in the descending order of filtrate, mycelia, baby Ling chih and Ling chih.

Contents of bitter components were significantly high in total free amino acid contents of filtrate. Interestingly, contents of bitter components were higher than contents of MSG-like and sweet components in four forms of *G. tsugae*. Furthermore, in baby Ling chih, mycelia and filtrate, contents of bitter components were almost three fold higher than contents of sweet components. Obviously, the bitter components were predominantly present and may contribute most to the taste.

Contents of MSG-like components were found to be 22.7–47.1 mg g^{-1} dry weight in common mushrooms (Tseng & Mau, 1999), 11.2–26.2 mg g^{-1} in paddy straw

Table 2
Content of soluble sugars and polyols of *G. tsugae* fruit bodies, mycelia and fermentation filtrate

Sugar or polyol	Content[a] (mg g^{-1} dry weight)			
	Ling chih	Baby Ling chih	Mycelia	Filtrate
Fructose	nd[b]	nd	1.53 ± 0.23b	4.59 ± 0.29a
Glucose	1.75 ± 0.32b	1.26 ± 0.07c	3.32 ± 0.15a	3.53 ± 0.14a
Mannitol	4.72 ± 0.29a	3.33 ± 0.15b	1.39 ± 0.11c	nd
myo-Inositol	1.43 ± 0.12b	1.79 ± 0.03a	1.17 ± 0.01c	1.29 ± 0.02bc
Trehalose	0.72 ± 0.01bc	1.27 ± 0.02a	0.81 ± 0.09b	0.68 ± 0.03c
Total	8.62 ± 1.21b	7.65 ± 0.96b	8.22 ± 0.34b	10.1 ± 1.28a

[a] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).
[b] nd: not detected.

Table 3
Content of free amino acids of *G. tsugae* fruit bodies, mycelia and fermentation filtrate

Amino acid	Content[b] (mg g^{-1} dry weight)			
	Ling chih	Baby Ling chih	Mycelia	Filtrate
L-Alanine	0.23 ± 0.01c	0.26 ± 0.01c	3.93 ± 0.02b	5.84 ± 0.08a
L-Arginine	0.18 ± <0.01d	0.68 ± <0.01c	3.67 ± 0.01b	5.29 ± 0.06a
L-Aspartic acid	0.07 ± <0.01c	0.15 ± <0.01c	0.98 ± 0.01b	10.4 ± 0.24a
L-Cysteine	0.75 ± <0.01d	2.47 ± 0.02a	1.99 ± 0.01c	2.23 ± 0.07b
L-Glutamic acid	0.09 ± <0.01d	0.31 ± 0.01c	1.66 ± 0.02b	15.6 ± 0.11a
Glycine	0.06 ± <0.01c	0.16 ± <0.01c	0.49 ± 0.01b	5.34 ± 0.09a
L-Histidine[a]	0.14 ± <0.01d	0.91 ± 0.04c	3.22 ± 0.02b	13.3 ± 0.68a
L-Isoleucine[a]	0.07 ± <0.01c	0.17 ± <0.01c	1.61 ± 0.01b	6.56 ± 0.09a
L-Leucine[a]	0.10 ± 0.01d	0.24 ± 0.01c	3.52 ± 0.01b	10.8 ± 0.14a
L-Lysine[a]	0.16 ± <0.01d	0.64 ± 0.01c	2.77 ± 0.01b	13.8 ± 0.27a
L-Methionine[a]	0.05 ± <0.01d	0.13 ± <0.01c	1.20 ± 0.02b	2.18 ± <0.01a
L-Phenylalanine[a]	0.12 ± <0.01d	0.37 ± 0.02c	3.10 ± 0.02b	10.5 ± 0.02a
L-Serine	0.16 ± <0.01c	0.23 ± <0.01c	2.44 ± 0.02b	9.25 ± 0.52a
L-Threonine[a]	0.05 ± <0.01c	0.12 ± <0.01c	0.37 ± 0.01b	4.18 ± 0.11a
L-Tryptophan[a]	0.08 ± <0.01c	0.35 ± 0.01c	3.29 ± 0.05b	14.9 ± 0.27a
L-Tyrosine	0.10 ± <0.01c	0.08 ± <0.01c	1.54 ± 0.02b	11.1 ± 0.14a
L-Valine[a]	0.09 ± <0.01c	0.19 ± 0.01c	1.55 ± 0.01b	7.28 ± 0.07a
Total	2.50 ± 0.03d	7.46 ± 1.10c	37.3 ± 2.19b	149 ± 3.08a

[a] Essential amino acid.
[b] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

Table 4
Content of taste characteristics of free amino acids in *G. tsugae* fruit bodies, mycelia and fermentation filtrate

Taste characteristic[a]	Content[b] (mg g^{-1} dry weight)			
	Ling chih	Baby Ling chih	Mycelia	Filtrate
MSG-like	0.16 ± 0.01c	0.46 ± 0.01c	2.64 ± 0.03b	26.0 ± 0.33a
Sweet	0.50 ± 0.02c	0.77 ± 0.03c	7.22 ± 0.02b	24.6 ± 0.29a
Bitter	0.84 ± 0.01d	3.05 ± 0.17c	21.2 ± 0.25b	70.8 ± 1.55a
Tasteless	1.00 ± <0.01d	3.18 ± 0.01c	6.30 ± 0.02b	27.1 ± 0.76a
Total	2.50 ± 0.03d	7.46 ± 1.10c	37.3 ± 2.19b	149 ± 3.08a

[a] MSG-like: monosodium glutamate-like, Asp + Glu; sweet: Ala + Gly + Ser + Thr; bitter: Arg + His + Ile + Leu + Met + Phe + Try + Val; tasteless: Cys + Lys + Tyr.
[b] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

mushrooms (Mau et al., 1997), 10.9–11.9 mg g^{-1} in black poplar mushrooms (Mau & Tseng, 1998), 3.75–9.06 mg g^{-1} in shiitake (*Lentinula edodes*) (Lin, 1999), 1.01–1.77 mg g^{-1} in king oyster mushrooms (Mau et al., 1998b), and 0.05–0.34 mg g^{-1} in ear mushrooms (Mau et al., 1998a). Furthermore, Mau, Lin, Ma, and Song (2001b) found that contents of MSG-like components in four specialty mushrooms, including basket stinkhorn (*Dictyophora indusiata*), maitake, lion's mane (*Hericium erinaceus*) and white matsutake (*Tricholoma giganteum*), ranged from 0.68 to 1.09 mg g^{-1}. In addition, Yang, Lin, and Mau (2001) found that contents of MSG-like components in several commercial mushrooms, including shiitake, winter (*Flammulina velutipes* strain white), abalone (*P. cystidiosus*) and tree oyster mushrooms (*P. ostreatus*), ranged from 0.84 to 1.93 mg g^{-1}. Yang et al. (2001) also found that that in the strain yellow of winter mushrooms was 7.06 mg g^{-1}. However, Mau et al. (2001a) found that contents of MSG-like components in medicinal mushrooms, including the fruit bodies of *Ganoderma lucidum*, *G. tsugae* and *Coriolus versicolor* were in the range 0.17–0.50 mg g^{-1}, similar to those of Baby Ling chih and Ling chih.

In addition, Weng (2003) found that contents of MSG-like components in three mushroom mycelia, including maitake (*Grifola frondosa*), morel (*Morchella esculenta*) and termite mushrooms (*Termitomyces albuminosus*), ranged from 3.11 to 6.51 mg g^{-1}. Chang et al. (2001) found that contents of MSG-like components in three medicinal mushroom mycelia, including Brazilian mushroom, chang-chih and northern *Cordyceps*, ranged from 0.47 to 2.97 mg g^{-1}. Furthermore, Yang et al. (2001) reported that contents of MSG-like components could be divided into three ranges: low (<5 mg g^{-1}), middle (5–20 mg g^{-1}) and high ranges (>20 mg g^{-1}). Based on the previous results, the contents of MSG-like

Table 5
Content of 5′-nucleotides of *G. tsugae* fruit bodies, mycelia and fermentation filtrate

5′-Nucleotide[a]	Content[c] (mg g^{-1} dry weight)			
	Ling chih	Baby Ling chih	Mycelia	Filtrate
5′-AMP	0.07 ± <0.01c	0.05 ± <0.01c	0.47 ± 0.23a	1.72 ± 0.01b
5′-CMP	0.71 ± 0.02a	0.46 ± 0.01b	0.33 ± 0.01c	0.47 ± 0.03b
5′-GMP	0.15 ± 0.01b	0.19 ± 0.01b	0.19 ± <0.01b	1.40 ± 0.11a
5′-IMP	0.11 ± 0.01b	0.17 ± 0.01b	0.06 ± <0.01b	1.04 ± 0.09a
5′-UMP	0.20 ± 0.01a	0.13 ± <0.01b	0.09 ± 0.01c	0.19 ± 0.02a
5′-XMP	0.16 ± 0.02c	1.25 ± 0.06a	0.54 ± 0.01b	0.66 ± 0.10b
Flavour 5′-nucleotides[b]	0.42 ± 0.01d	1.61 ± 0.04b	0.79 ± 0.01c	3.10 ± 0.08a
Total	1.40 ± 0.02d	2.25 ± 0.05b	1.68 ± 0.12c	5.48 ± 0.14a

[a] 5′-AMP: 5′-adenosine monophosphate; 5′-CMP: 5′-cytosine monophosphate; 5′-GMP: 5′-guanosine monophosphate; 5′-IMP: 5′-inosine monophosphate; 5′-UMP: 5′-uridine monophosphate; 5′-XMP: 5′-xanthosine monophosphate.
[b] Flavour 5′-nucleotide, 5′-GMP + 5′-IMP + 5′-XMP.
[c] Each value is expressed as mean ± SE ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

components in Ling chih, baby Ling chih and mycelia were below the low range (<5 mg g^{-1}).

Contents of total 5′-nucleotides were high in filtrate (5.48 mg g^{-1}), moderate in baby Ling chih (2.25 mg g^{-1}), and low in mycelia and Ling chih (1.68 and 1.40 mg g^{-1}, respectively) (Table 5). Flavour 5′-nucleotides were found to be 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP) and 5′-xanthosine monophosphate (5′-XMP) (Chen, 1986). Contents of flavour 5′nucleotides were high in filtrate (3.10 mg g^{-1}), moderate in baby Ling chih (1.61 mg g^{-1}), and low in mycelia and Ling chih (0.79 and 0.42 mg g^{-1}, respectively). Yang et al. (2001) reported that contents of flavour 5′-nucleotides could be divided into three ranges: low (<1 mg g^{-1}), middle (1–5 mg g^{-1}) and high ranges (>5 mg g^{-1}). In addition, contents of flavour 5′-nucleotides in medicinal mushrooms, including the fruit bodies of *G. tsugae* and *C. versicolor*, were in the range 1.18–5.65 mg g^{-1} (Mau et al., 2001a). Therefore, contents of flavour 5′-nucleotides in baby Ling chih and filtrate were also in the middle range but the contents of flavour 5′-nucleotides in Ling chih and mycelia were in the low range.

Chang et al. (2001) found that the contents of flavour 5′-nucleotides in three medicinal mushroom mycelia, including Brazilian mushroom, chang-chih and northern *Cordyceps*, ranged from 7.00 to 38.0 mg g^{-1}. In addition, Weng (2003) found that the contents of flavour 5′-nucleotides in three mushroom mycelia, including maitake, morel and termite mushrooms, ranged from 7.33 to 10.5 mg g^{-1}. As compared to the previous mycelia, the contents of flavour 5′-nucleotides in mycelia of *G. tsugae* were considerably low.

Based on contents of the total soluble sugar and sweet components, it was anticipated that the sweetness was consistent with their sugar content and in the descending order of filtrate, mycelia, Ling chih and baby Ling chih. 5′-GMP gave the meaty flavour, and is a much stronger flavour enhancer than MSG (Litchfield, 1967). The synergistic effect of flavour 5′-nucleotides with MSG-like components might greatly increase the umami taste of mushrooms (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). Based on the contents of MSG-like components and flavour 5′-nucleotides, the umami intensities in four forms of *G. tsugae* were expected to be in the descending order of filtrate, mycelia, baby Ling chih and Ling chih. Overall, filtrate contained relatively higher amounts of sweet components, MSG-like components and flavour 5′-nucleotides, as well as higher amounts of bitter components.

In addition, bitter triterpenoids, which showed profound medicinal effects, also were present and dominated the taste of medicinal mushrooms (Shiao, Lee, Lin, & Wang, 1994). Besides the sweet and umami taste, the bitter taste from bitter components and bitter triterpenoids might affect consumers' acceptability of *G. tsugae* as a functional food. Further study is needed of the interaction of taste components to eliminate or suppress the bitter taste and to enhance the sweet and umami taste in *G. tsugae*.

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