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Full Length Research Paper

Compositional analysis and nutritional studies of *Tricholoma matsutake* collected from Southwest China

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The chemical composition and nutritional value of *Tricholoma matsutake* was determined in various laboratory assays. The contents of crude protein, crude fibre, crude fat, carbohydrate, soluble sugars, ash, mineral elements (K, Na, P, Ca, Zn, Fe, Cu, Mn) and the profile of essential amino acids, fatty acids were analyzed. The analysis indicated that the wild edible mushroom contained rich sources of crude protein (20.3%), crude fibre (29.10%) and carbohydrate (36.67%). The total essential amino acid accounted for 34.65% of the total amino acid and the first limited amino acid was methionine. The score of ratio coefficient of amino acid of its protein was 80.16. The mushroom studied had good amounts of minerals. Content of fat was low (5.04%) with oleic and linoleic acids accounted for more than 75% of total fatty acids. The research proves that *T. matsutake* is a nutritional plant and has great value to health.

Key words: *Tricholoma matsutake*, nutritional value, chemical composition, amino acids, fatty acids.

INTRODUCTION

Fungi have influenced human affairs for thousands of years, either as a direct food source and in a food process, or as a medicine. The nutritive and medicinal values of mushrooms have long been recognized all over the world (Cochran, 1978). Many fungi are of considerable medical significance. Research suggested they help in the treatment of certain types of cancer, boost the immune system and reduce the risk of coronary heart disease, because some of the edible mushroom species possess pharmacological properties (Kalač, 2009). Mushrooms were also among the best sources of other essential nutrients. Previous studies have indicated that edible mushroom species were highly nutritious. Their nutritional value can be compared favourably with that of meat, eggs and milk (Breene, 1990; Gruen and Wong, 1982).

Tricholoma matsutake (TM) can be found in many parts of Africa, America, Europe and Asia (Redhead, 1997). It is a valuable species throughout the world, exhibiting a characteristic delicate flavour as well as several biological activities, such as lower cholesterol, anti- oxidant and immuno-modulating effects in humans (Hoshi et al., 2005; Mau et al., 2002). A mycelial preparation of this mushroom prepared in bulk culture was shown to have antitumor activity against the in vivo growth of mouse syngeneic fibrosarcoma (Ebina et al., 2002) as well as preventive activity against the formation of azoxymethane-induced precancerous lesions in the colon (Matsunaga et al., 2003). A recent study examined the free radical-scavenging ability and inhibition of nitric oxide (NO) production by various solvent extracts obtained from TM (Lim et al., 2007). Besides pharmacological properties and bioactive compounds already available in the literature, little work has been carried out on the nutritional value and chemical composition of the mushroom.

In this investigation we have determined the proximate chemical composition, including crude protein, crude fibre, crude fat, carbohydrate, soluble sugars, ash and mineral elements of TM. Moreover, we have examined amino acid and fatty acid profiles of proteins and lipids in order to establish a preliminary guide for assessing the relative nutritive qualities and provide a basis for taping the nutritive potential of the natural resource. The results showed that TM was a good nutritional plant for their unique contributions to health.

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MATERIALS AND METHODS

Sample preparation

TM samples were collected from Yunnan province in southwest China. The collected samples were cleaned, cut and freeze-dried. Dried samples were crushed by using a mortar with pestle and stored in pre-cleaned polyethylene bottles until the analysis started.

Chemical composition

The proximate analysis (carbohydrates, fats, proteins and ash) of all plant samples were determined by using AOAC (1990) methods. Crude protein (N × 6.25) was determined by micro Kjeldahl method described by Pearson (1976), in which the sample was digested with a known quantity of acid.

The digested material was distilled after the addition of alkali. The released ammonia was collected in 4% boric acid. The resultant boric acid, which now contained the ammonia, was then titrated against 0.1 N HCl, manually.

The nitrogen content thus determined was multiplied by a factor of 6.25 to arrive at the amount of crude protein. Crude fat content of the samples was determined by extracting a known weight of powdered plant sample with petroleum ether, using by Soxhlet. The ash content was determined by combusting the plant material in silica crucibles in a muffle furnace at 535 ± 15^{0}C for 3 h. Fibre content was estimated by the method of Van (1967). The carbohydrate content was calculated by subtracting the contents of crude ash, fat, fibre and protein from dry matter. Soluble sugars were determined as follows: The powdered samples were extracted with boiling water for 30 min, absolute alcohol were added to precipitate the soluble sugars.

The precipitated polysaccharides were collected by centrifugation at 5000 rpm for 10 min in a bench centrifuge and subsequently dried at 60^{0}C to remove residual ethanol. Soluble sugars were determined by the phenol-sulfuric reaction described by Whistler and Wolfrom (1962). Minerals were determined by atomic absorption spectrophotometer (AAS-6800, Shimadzu, Japan) after dry-ashing the samples (AOAC, 1990). Phosphorus was determined by using the molybdovanadate method (AOAC, 1990).

Nutritional analysis

The amino acid composition of proteins of the mushroom was determined by the Wiedmeier et al. (1982) method after hydrolysis of freeze-dried samples with 6 N HCl for 24 h at 110^{0}C. A separate portion of the samples was oxidized with performic acid according to the procedure of Moore (1963) and then hydrolyzed with 6 N HCl in order to obtain reliable cystine and methionine values. For tryptophan analysis, samples were hydrolyzed with 5 N NaOH, according to the Hugli and Moore (1972) method. Amino acid analysis was carried out by ion-exchange chromatography in an automatic amino acid analyzer (Hitachi 835-50, Japan). Each essential amino acid was expressed as a percentage of the corresponding amino acid in the reference protein. Score of ratio coefficient of amino acid (SRC) was calculated by using the following formula (Zhu and Wu, 1988).

$$\mathrm{RAA} = \frac{\text{amino acid in test protein}}{\text{amino acid of WHO/FAO standard}}$$

$$\mathrm{RC} = \frac{\text{ratio of amino acid}}{\text{average ratio of amino acid}}$$

SRC = 100-CV × 100 (CV was the coefficient of variation of RC)Fatty acid methyl esters were analyzed by gas chromatography (GC) on a Shimadzu GC 14A equipped with a 30 × 0.32 mm silica capillary column packed with 10% Silar 10CP on Chromosorb W-AW DMCS (80/100) and a flame ionization detector (FID).

Statistical analysis

Each experiment was carried out three times for each parameter and we got three replications (n = 3) from which we derived the mean values and standard error (SE) (Muhammad et al., 2010).

RESULTS

Chemical composition

The results of the proximate and mineral composition are shown in Table 1. It shows that the dominant compounds are protein, fibre and carbohydrates which contents are 20.3, 29.1 and 36.67%, respectively. Crude fat and ash contents here are 5.04 and 8.89%, respectively. Of the mineral analyzed, the potassium, sodium, phosphorus, calcium content were 2352, 31, 504, 41 mg·100 g^{-1} and the iron, copper, manganese, zinc content were 36.9, 8.72, 8.31, 14 mg·100 g^{-1}, respectively.

Componential analysis of protein and amino acid

As shown in Table 2, there are 17 amino acids in TM including 7 essential amino acids.

The total amino acid content is 24.73 g·kg^{-1}. The essential amino acid is 8.57 g·kg^{-1} and the ratio of essential amino acid to total amino acid is 34.65% which close to FAO/WHO (1989) model (35.0%). The ratio of essential and non-essential amino acids is 0.53. These values are similar to the ideal protein requirements suggested by the FAO/ WHO (1989).

Glutamic is the major amino acid and the first limited amino acid is methionine. The SRC of the mushroom is 80.16. The typical chromatographic profile of standard solution of 17 amino acids is shown in Figure 1. It shows that the 17 amino acids were well separated in chromatogram.

Fatty acid composition

Table 3 shows the examined results of fatty acid composition of TM. The fatty acid profile of other species of *Tricholoma* (Senatore et al., 1987) is also listed for comparison. In this study, oleic, linoleic and palmitic acid were the main fatty acid constituents, as occurs in many other species (Longvah and Deosthale, 1998; Senatore, 1990).

Other fatty acids, for example C12:0, C14:0, C16:1 and C18:0 were only found in very small amounts.

Table 1. Proximate and mineral compositions of *T. matsutake* (per 100 g sample, n = 3).

Component	Concentration (Mean ± SE)
Protein (g)	20.30 ± 0.02
Fat (g)	5.04 ± 0.01
Ash (g)	8.89 ± 0.05
Fibre (g)	29.10 ± 0.09
Carbohydrate (g)	36.67 ± 0.04
Soluble sugars (g)	7.07 ± 0.03
Potassium (mg)	2352 ± 0.44
Natrium (mg)	31 ± 0.06
Phosphorus (mg)	504 ± 0.11
Calcium (mg)	41 ± 0.04
Zinc (mg)	14 ± 0.07
Iron (mg)	36.9 ± 0.03
Copper (mg)	8.72 ± 0.06
Manganese (mg)	8.31 ± 0.02

Table 2. Amino acid compositions in the mushroom compared to FAO whole egg protein (n=3).

Amino acids	*T. matsutake*[b] (Mean ± SE)	% of total amino acid	FAO whole egg
Threonine[a]	0.98 ± 0.002	4.0	5.1
Valine[a]	1.59 ± 0.004	6.5	6.9
Methionine[a]	0.45 ± 0.001	1.8	3.3
Isoleucine [a]	1.16 ± 0.007	4.6	6.3
Leucine[a]	1.87 ± 0.002	7.6	8.8
Phenylalanine[a]	1.05 ± 0.004	4.2	5.7
Lysine[a]	1.47 ± 0.002	6.0	7.0
Tyrosine	0.64 ± 0.001	2.6	4.2
Serine	0.66 ± 0.001	2.7	7.6
Glutamic	5.49 ± 0.007	22.2	12.7
Glycine	1.42 ± 0.003	5.8	3.3
Alanine	2.36 ± 0.005	9.6	5.9
Cysteine	0.10 ± 0.001	0.4	5.9
Aspartate	2.31 ± 0.002	9.4	9.6
Histidine	0.56 ± 0.001	2.2	2.4
Arginine	1.11 ± 0.002	4.4	6.1
Proline	1.51 ± 0.002	6.2	4.2
Total amino acid	24.73		
Total essential amino acid	8.57	34.65	
SRC		80.16	

[a] Means essential amino acid, [b]Values are expressed as $g \cdot kg^{-1}$ of dry matter.

DISCUSSION

Proximate compositions

In the samples, the dominant compounds were protein, fibre and carbohydrates. Some edible fungi were highly valued as a good source of protein and their protein contents usually range from 19 to 35% of dry weight (Crisan and Sands, 1978), from 15.4 to 26.7% (Yang et al., 2001), from 14.6 to 22.3% (Mau et al., 2001) or from 16.5 to 59.4% (Kalač, 2009). Here, protein content in TM (20.3%) seemed to have a normal protein content compared with other edible mushrooms. Crude fibre content in TM (29.1%) was quite high in comparison with many other edible mushrooms (3 - 32%) (Crisan and Sands, 1978; Mau et al., 2001; Yang et al., 2001). The

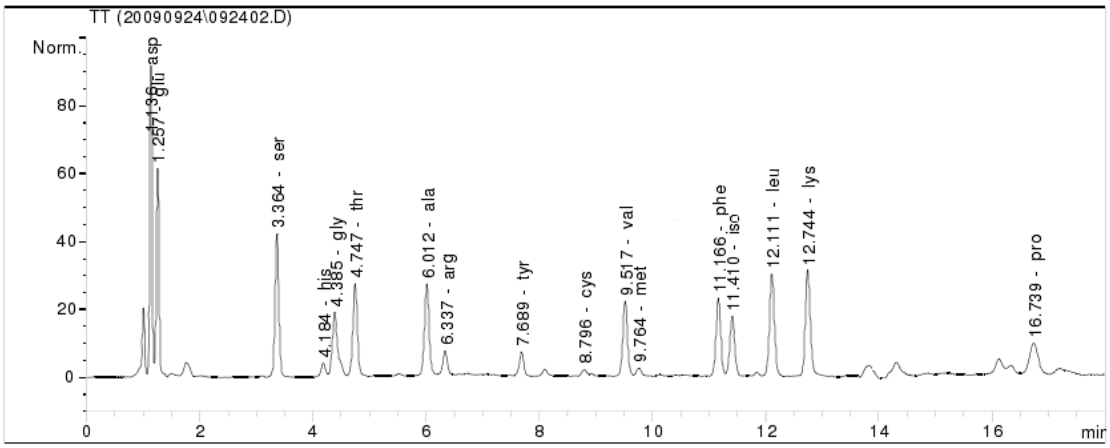

Figure 1. The typical chromatogram of standard solution of 17 amino acids.

Table 3. Fatty acid compositions of mushroom [a] (values are percent of total fat, n=3).

Fatty acids	*Tricholoma matsutake*	*Tricholoma acerbum* [b]	*Tricholoma batschii* [b]	*Tricholoma nudun* [b]	*Tricholoma pardinum* [b]
C12:0 Lauric	0.2 ± 0.02	0.8	T[c]	0.3	0.4
C14:0 Myristic	1.3 ± 0.05	0.5	0.2	0.3	T[c]
C16:0 Palmitic	9.1 ± 0.07	16.2	23.0	21.2	18.2
C16:1 Palmitolsic	1.0 ± 0.03	1.1	0.5	1.3	0.7
C18:0 Stearic	1.8 ± 0.03	4.9	5.3	1.9	10.1
C18:1 Oleic	57.6 ± 0.22	5.8	7.1	4.8	4.0
C18:2 Linoleic	26.7 ± 0.14	65.6	58.5	63.9	61.4
Total Saturated	12.4	22.4	28.5	23.8	28.7
Total Unsaturated	85.3	72.5	66.1	70.0	66.1
L/O[d]	0.46	11.3	8.23	13.3	15.35

[a] values are percent of total fatty acids, [b] Data reported by Senatore et al. (1987), [c]T, trace amounts, [d]L/O, linoleic-oleic acid ratio.

fairly high level of fibre in the mushroom was a desirable characteristic since fibre plays an important role in human diet. Obviously, the fruit bodies of TM were a good source of fibre.

Carbohydrate content of TM (36.67%) was similar to those reported by Crisan and Sands (1978) and Kalač (2009) in the range 44.0 - 74.3% and 16.4 - 75%, respectively. Soluble sugars in edible mushrooms were highly regarded as biologically or medically active compounds and were used as functional food ingredients or nutraceuticals (Wasser and Weis, 1999). The content was 7.07% for TM.

Crude fat and ash contents here were 5.04 and 8.89% respectively. These results were similar to those obtained by Crisan and Sands (1978), Yang et al. (2001) and Kalač (2009) in several edible mushrooms. From the results shown in Table 1, the macronutrient profile, in general, revealed that TM had rich sources of protein and fibre and had low amounts of fat. This high protein and low fat characteristic of the edible wild mushrooms has been previously reported by many workers (Aletor, 1995; Diez and Alvarez, 2001; Longvah and Deosthale, 1998).

Mineral elements

The metal content in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil (Gast et al., 1988). The uptake of metal ions in mushrooms is in many respects different from other plants. For this reason the concentration variations of metals depend on mushroom species and their ecosystems (Seeger, 1982).

In general, most of the mushrooms studied had good amount of minerals including trace minerals. A similar range for calcium, phosphorus, iron, manganese, copper and zinc have been reported for popular wild edible mushrooms from northern Thailand (Sanmee et al., 2003)

where the climatic conditions are similar to Yunnan province, China. The concentration of sodium is relatively, low and is of very great nutritional benefit to the consumer, a finding that has been corroborated by Vetter (2003). It is known that adequate iron in a diet is very important for decreasing the incidence of anemia. The iron values of TM are in agreement with those reported in the literature. (Sesli and Tuzen, 1999; Kalač and Svoboda, 2000; Kalač, 2010). Copper concentrations in the accumulating mushroom species are usually 100 - 300 mg/kg of dry matter, which is not considered as a health risk (Kalač and Svoboda, 2000; Kalač, 2010). Copper contents in mushrooms higher than those in vegetables should be considered as a nutritional source of the element. Nevertheless, for people, bioavailability from mushrooms was reported to be low, due to limited absorption from the small intestine. Copper contents found in this study are in agreement with results reported in the literature (Sesli and Tuzen, 1999 v 9-35). Mushrooms were known as zinc accumulator and sporophore, substrate ratio for Zn ranges from 1 to 10 mg·kg-1(Isiloglu et al., 2001). Zinc concentrations of mushroom samples in the literature have been reported in the range of 29.3 - 158 $\mu g \cdot g^{-1}$ (Isiloglu et al., 2001), 45 -188 $\mu g \cdot g^{-1}$ (Tuzen, 2003). The zinc values of TM are in agreement with the reported in the literature.

Amino acid

As shown in Table 2, there are 17 amino acids in TM, including 7 essential amino acids. The total amino acid content is 24.73 $g \cdot kg^{-1}$. Glutamic acid, alanine, aspartic and leucine are present in large amounts. The mushroom is especially rich in glutamic acid (22.2% of total) as occurs in other wild and cultivated fungi (Beuchat et al., 1993; Ogawa et al., 1987; Sato et al., 1985; Senatore., 1990) including species of the genus *Tricholoma* (Fujita et al., 1991; Senatore et al., 1987). Previous studies have reported that glutamic acid was an active participant in metabolism and the synthesis of nucleotide and some amino acids. It also plays a role in the function of brain tissue and nerve system and participates in the disintoxication of ammonia in lever, muscle and brain. And it is well known that amino acids, especially highly basic amino acid and glutamic acid contribute to the flavour properties of the mushrooms (Maga, 1981; Sugahara et al., 1975), thus the high levels of glutamic acid in TM probably contribute most to their characteristic flavour. Essential amino acids accounted for 34.65% of total amino acid contents of TM, which was close to FAO/WHO (1989) model (35.0%). The ratio of essential and non-essential amino acids was 0.53. These values are similar to the ideal protein requirements suggested by the FAO/ WHO (1989). However, other amino acids such as lysine, which was usually limited in many vegetable foods, and threonine, leucine, isoleucine, phenylalanine and valine, were presented in amounts exceeding the FAO/WHO (1973) reference protein requirements. Only methionine was slightly deficient in the mushroom. The results of this study clearly indicate the potential of the mushroom species for their use as sources of essential amino acids.

From the results, we could see that the SRC was accounted for 80.16. Recent work in modern nutriology showed that not only insufficient amino acid reduces the nutrition value of protein, but also over-sufficient amino acid limits the nutrition value of protein. Therefore, the theory of balanced amino acid was proposed. The method of ratio coefficient of amino acid, based on the theory of amino acid balance theory, was a design to evaluate nutritional value of food protein. The meaning of SRC was that if the essential amino acids components ratio in the food protein matches the standard essential amino acids pattern, then CV = 0, SRC = 100. The more dispersed the RC is (meant the EAA contribute more negatively in the amino acid balancing physiological action, thus CV increases and SRC decreases), the lower nutrition value the protein gets. As a result, the protein gets higher nutrition value when SRC reaches closer to 100. The results of SRC of amino acid showed that TM had a better nutritional protein quality. Fatty acids Fatty acid analysis of the mushroom in the present study showed that the unsaturated fatty acids were higher than the saturated ones (Table 3). This is consistent with the observations that, in mushrooms, unsaturated fatty acids predominate over the saturated (Senatore et al., 1988). Oleic, linoleic and palmitic acids counted for almost the whole of the fatty acids determined. Similar observations have been made in other mushrooms (Senatore et al., 1988; Senatore, 1990; Bárbara et al., 2009). Other fatty acids detected were found only in minor amounts.

It is also important to point out that, in contrast to other fungi (Stancher et al., 1992), no other fatty acids with an odd number of carbon atoms have been identified. All species cited in Table 3 are characterized by a high concentration of unsaturated fatty acids and more than 65% of total fatty acid content. Nevertheless, the oleic acid content in TM is much higher (57.6%) than in other *Tricholoma* sp. reported, while the percentage of linoleic acid is practically half; consequently, the linoleic-oleic acid ratio is practically the reverse. Realistically, this fact is not of importance from the nutritional point of view, since the amounts of fat in TM are quite small. However, the linoleic-oleic acid ratio could constitute an important parameter from a chemotaxonomic viewpoint and could be useful for the taxonomical differentiation between species of the same genus. On the whole, the mushrooms studied were found to be a good source of protein, fibre and minerals. Furthermore, detailed analysis of the mushroom species for other nutrients, anti-nutrients and secondary metabolites with medicinal potential should be undertaken. The results from the present analysis allow a direct comparison of the

chemical composition and amino acid content of TM with those of other mushrooms species.

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