Artificial cultivation and nutritional evaluation of proteins from a wild *Ganoderma gibbosum* strain

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ABSTRACT: *Ganoderma gibbosum* is a medicinal fungus widely distributed in China, but the scarcity of its wild resources has limited its application and potential economic value. In this research, we collected a macrofungus from the wild, isolated using tissue culture technique, and identified by morphological characteristic analysis combined with ITS sequencing and homology-based phylogenetic analysis. A DNA fragment of approximately 650 bp was obtained and the fungus was identified as *G. gibbosum*. Furthermore, the fungus was cultured in liquid medium and artificially cultivated for fruiting body development. The crude protein contents and amino acid compositions of the mycelia, artificially cultivated and wild fruiting bodies were systematically evaluated to assess the nutritional value. Protein quality was evaluated by calculating the amino acid score (AAS), chemical score (CS), score of ratio coefficient of amino acids (SRC) and essential amino acid index (EAAI), referring to the most recent version of the international amino acid reference patterns. The results revealed that 17 kinds of amino acids were detected, and the essential amino acid contents were superior to the WHO/FAO reference protein pattern value in the hydrolysates of samples. The most abundant essential amino acid in mycelia was Lys, whereas that in both artificially cultivated and wild fruiting bodies was Met. The SRC values were between 25.30 and 69.84, and the EAAI values were between 75.15 and 94.43, which basically conformed to the requirements of current international reference patterns. The study provides a scientific basis for further utilization of this fungus.

KEYWORDS: *Ganoderma gibbosum*, ITS identification, artificial cultivation, protein nutritional value, amino acid composition, medicinal fungus

INTRODUCTION

Ganoderma gibbosum (Blume & T. Nees) Pat. which belongs to the Ganodermataceae family [1] is mainly distributed in Jiangsu, Zhejiang, Hainan and other provinces of China [2]. There are abundant resources of Ganodermataceae fungi in China that are used as traditional Chinese medicines, and many species have medical and economic value [3]. The antibacterial, anticancer, antiviral, antioxidant, immune regulatory and sedative properties of *Ganoderma* make these fungi popular among the public. *G. gibbosum* is a medicinal fungus that not only presents a medical value similar to that of *Ganoderma applanatum* (Pers.) Pat. but also is considered useful for treating stomach diseases in Hainan province of China [4]. The wild resources of *G. gibbosum* are currently scarce, and there has been little research on this fungus. Chen et al [5]

studied the characteristics of mycelial cultures of *G. gibbosum* by measuring the mycelial growth rate and biomass. Zhang et al [6] explored the culture characteristics of wild *G. gibbosum* by cultivating its mycelia on solid plates and under artificial domestication cultivation, and they found that fruiting bodies could form on all three kinds of culture substrates. Many studies have focused on the biological characteristics of *G. gibbosum* mycelia, while research on the nutritional value of the proteins of *G. gibbosum* has rarely been reported.

The evaluation of protein quality is mainly based on the reference protein equivalent pattern [7], obtained by analyzing parameters such as the amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI), ratio coefficient of amino acids (RC) and score of ratio coefficient of amino acids (SRC). The systematic evaluation of protein quality using the above parameters has not

Fig. 1 Morphology of wild macrofungi.

yet been reported in *G. gibbosum* as a wild medicinal fungal resource. Chen et al [8] analyzed and compared the amino acid contents of the fruiting bodies of *Ganoderma* in Fujian province and found that the total amino acid content in *G. gibbosum* was lower than that in *G. applanatum*, but the proportion of essential amino acids was much higher; however, the amino acid characteristics and protein quality were not studied. In this study, mycelia, artificially cultivated fruiting bodies and wild fruiting bodies of *G. gibbosum* were used as the research materials, and their protein quality was analyzed by measuring the crude protein content and amino acid composition. The purpose of this work was to evaluate the nutritional value of the protein of *G. gibbosum*, thus providing a theoretical reference for further utilization of this fungus.

MATERIALS AND METHODS

Sample collection

In July 2018, a large fungus was found on a beech tree (*Zelkova serrata* (Thunb.) Makinoz) at the Shanghai Academy of Agricultural Sciences (SAAS), Shanghai, China (Fig. 1). This place used to be wild areas before the SAAS moved in. Furthermore, cultivation of *G. gibbosum* isolated from this area has never been reported. After isolation, fungus has been preserved on PDA medium at the Edible Fungi Institute of the SAAS by tissue culture and designated as SS.

Culture medium

Solid medium: One litre of potato dextrose agar (PDA) medium was composed of 200 g peeled potato extract, 20 g dextrose, and 20 g agar. The PDA medium was sterilized for 20 min at 121 °C and aseptically poured into a petri-dish.

Liquid medium: One litre of potato dextrose (PD) medium was composed of 200 g peeled potato extract, and 20 g dextrose, and the PD medium was sterilized for 20 min at 121 °C.

Morphological identification

The morphological characteristics of the wild fungi, including the shape, size, color, stipe, cap, and ring pleats of the fruiting bodies and the size and color of the spores, were observed.

Molecular identification

Total DNA extraction: For preservation, the fungal mycelia were subcultured on PDA plates. After incubation for 10 days at 25 °C, mycelia were collected and thoroughly ground in liquid nitrogen using a mortar, after which DNA was extracted with the cetyltrimethylammonium bromide (CTAB) method [9].

PCR amplification was performed using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The reaction system included 12.5 µl of 2 × Easy Taq Mix, 1 µl of each primer, 1 µl of template DNA, and 9.5 µl of ddH$_2$O. PCR amplification was performed as follows: predegeneration at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. After the PCR product was detected by 1.0% agarose gel electrophoresis, the target bands were sequenced by Sangon Biotech (Shanghai) Co., Ltd. The obtained internal transcribed spacer (ITS) sequence was analyzed with DNAMAN software and compared with the DNA database with the BLAST tool on the NCBI website, and the most homologous sequences were downloaded. A phylogenetic tree was constructed using the neighbor-joining method with MEGA 6.0 software, and the bootstrap parameter was set to 1000.

Mycelia collection

Fungal mycelia were subcultured on a PDA plate. After incubation for 7 days at 25 °C, the culture was transferred to 250 ml flasks containing 100 ml of PD medium, and the flasks were incubated at 25 °C with shaking at 150 rpm for 12 days. Mycelia were collected, washed with ddH$_2$O, blotted with filter paper to remove excess moisture, dried at 60 °C overnight, ground immediately thereafter using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany), and reserved after sieving through a 40 mesh [10].

Fungal cultivation and preparation

The main substrate culture formulation for the SS fruiting bodies was composed of 78% sawdust, 20% wheat bran, 1% gypsum, 0.5% corn meal, and 0.5% KH_2PO_4 by weight/weight. The moisture content of the culture substrate was maintained at 60–65% (w/w). The homogenized substrate (900 g) was placed in polyethylene bags (17 cm × 33 cm × 0.03 cm). The culture substrate was added to a total of eighty polyethylene bags as replicates. Then, the culture substrate was sterilized under atmospheric pressure at 121 °C for 2 h. The sterilized substrate was inoculated with 20 g of fully colonized spawn, which was then cultured in a darkened ventilated spawn running room at 25 °C with 60–62% relative humidity until mushroom mycelium running was complete (approximately 30 days). Thereafter, the fungal mycelia entered the ripe stage, and culture continued for 10 days; when the mycelia became physiologically mature, the culture bags were removed. Fungal fruiting bodies were grown at 25 °C with 85–95% relative humidity in the mushroom growing room. Samples of the fruiting bodies were harvested at the fruiting developmental stages, dried at 60 °C, and then ground into a fine powder (40 mesh) using a mill. The wild fruiting bodies were similarly treated as described for the artificially cultivated ones.

Determination of the crude protein content

The crude protein content was measured according to China National Standard GB5009.5-2016, "Determination method of protein in food", by Kjeldahl determination.

Assay of amino acids

Powdered samples (60 mg) were suspended in 5 ml of 6 N HCl (36%) and 20 µl of phenol, after vacuum treatment applied, the ampere tube was filled with pure nitrogen and then sealed. Hydrolysis was performed in a drying oven at a constant temperature of 110 °C for 22 h. After the hydrolysate was cooled at ambient temperature and transferred to a 25 ml volumetric flask, a 1 ml aliquot was transferred to a 5 ml volumetric flask and dried at 40–50 °C two times until the residues were completely evaporated. Then, the extracted solids were redissolved in buffer (pH 2.2) to a final volume of 1 ml, the solution was centrifuged at $12\,000 \times g$ for 25 min, and the supernatant was filtered with a filter membrane (0.22 µm cellulose filter, Millipore, Shanghai, China) prior to analysis [11, 12]. The amino acid
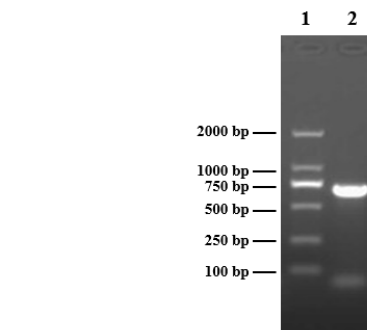
Fig. 2 PCR amplification results from wild fungi. Lane 1: DNA marker; lane 2: PCR amplification of ITS from mycelia of SS.

content using an L-8900 automatic amino acid analyzer (Hitachi Ltd., Japan) was determined.

Nutritional evaluation of proteins

The AAS was calculated according to the WHO/-FAO/UNU method [13]. The CS was calculated using the reference method recommended by FAO and Seligson and Mackey [14]. The EAAI was evaluated according to Oser [15, 16], and the RC and SRC were calculated according to Zhu and Wu [17].
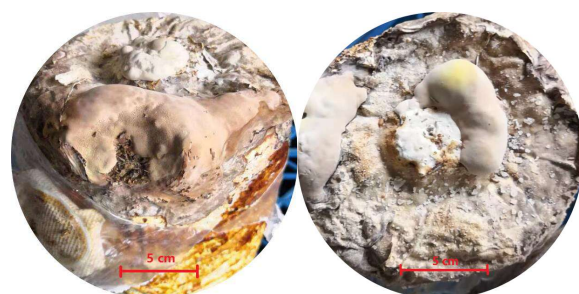
RESULTS

Morphological characteristics

The fruiting body of the collected wild fungi was fan-shaped, extended laterally and had a flat cap, with a diameter of 15.2 cm, a short diameter of 6.8 cm, and a thickness of 2.9 cm. The surface was reddish brown, with a concentric annulus and tumor-like protrusions. The orifice on the underside was white or yellow-white, and the tube was clearly visible, similar to the recorded characteristics of *G. gibbosum*. The spores of this fungus were brown, and the size was approximately 9 µm × 5 µm under the light microscope examination.

ITS analysis and construction of the phylogenetic tree

Using the DNA of SS mycelia as the template for ITS region amplification, a DNA fragment of approximately 650 bp was obtained (Fig. 2), and the successfully amplified product bands were sequenced. Thereafter, using BLAST tools for comparison, the sequences with the highest homology were selected for analysis and to construct the phylogenetic tree (Fig. 3). The results showed that SS presented similarity to the *Ganoderma* genus and belonged
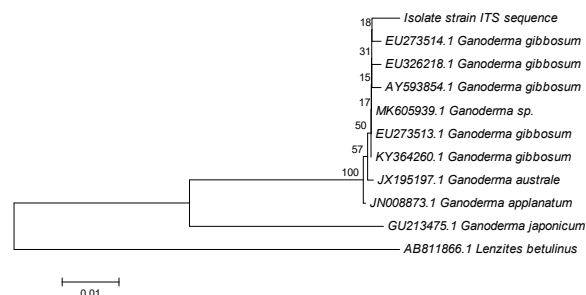
Fig. 3 Neighbor-joining (NJ) phylogenetic tree based on the rDNA ITS region sequence. Bar indicates 0.01 sequence divergence substitutions per nucleotide position.

Fig. 4 Primordium formation in *Ganoderma gibbosum* under artificial cultivation.

Table 1 Amino acid composition of the proteins of *G. gibbosum*.

Amino acid composition		Content (mg/g DW)		
		CM	AF	WF
EAA	Thr	12.99 ± 0.14	5.94 ± 0.09	4.82 ± 0.10
	Lys	18.11 ± 0.27	5.41 ± 0.03	3.56 ± 0.13
	Leu	15.64 ± 0.18	8.90 ± 0.05	5.43 ± 0.14
	Ile	8.08 ± 0.10	4.86 ± 0.16	1.83 ± 0.04
	Met	2.67 ± 0.07	30.15 ± 0.36	27.71 ± 0.33
	Phe	10.06 ± 0.11	4.96 ± 0.08	3.66 ± 0.03
	Val	13.87 ± 0.18	6.07 ± 0.06	4.32 ± 0.09
	Total	81.43 ± 0.97	66.28 ± 0.26	51.32 ± 0.31
NEAA	Asp	21.87 ± 0.27	9.96 ± 0.04	7.29 ± 0.16
	Ser	12.99 ± 0.14	5.94 ± 0.09	4.82 ± 0.10
	Glu	32.68 ± 0.37	11.75 ± 0.39	6.96 ± 0.21
	Gly	12.75 ± 0.19	5.97 ± 0.31	4.20 ± 0.08
	Ala	17.64 ± 0.26	6.38 ± 0.46	4.64 ± 0.12
	Cys	1.40 ± 0.11	1.16 ± 0.02	0.54 ± 0.02
	Tyr	5.83 ± 0.04	2.25 ± 0.14	2.37 ± 0.24
	His	6.65 ± 0.07	2.07 ± 0.06	1.43 ± 0.05
	Arg	17.40 ± 0.18	4.09 ± 0.15	3.16 ± 0.07
	Pro	10.79 ± 0.15	4.72 ± 0.17	3.60 ± 0.06
	Total	139.80 ± 1.72	54.55 ± 1.77	38.73 ± 0.66
TAA		221.23 ± 2.69	120.83 ± 1.67	90.05 ± 0.79
E/T		0.37 ± 0.00	0.55 ± 0.01	0.57 ± 0.00
E/N		0.58 ± 0.00	1.22 ± 0.04	1.33 ± 0.02

CM, cultured mycelia; AF, artificially cultivated fruiting bodies; WF, wild fruiting bodies; EAA, essential amino acids; NEAA, nonessential amino acids; TAA, total amino acids; E/T, essential/total amino acids; E/N, essential/nonessential amino acids. Data were calculated from triplicated samples.

to *G. gibbosum*, with 99% homology. Combined with the observed morphological characteristics, the isolated SS was identified as *G. gibbosum*.

Artificial cultivation

G. gibbosum could grow normally in the cultivation medium, and the time required for the mycelia to completely fill the bag was approximately 30 days. After 10 days of further culture, the bags were removed for fruiting body formation, and the primordia formed for approximately one month (Fig. 4). Compared with the wild fruiting bodies, the artificially cultivated fruiting bodies exhibited a lighter color, softer texture and decreased thickness, which may be related to the age of the fruiting bodies, light conditions or cultivation substrate.

Crude protein content

The crude protein contents of the mycelia, artificially cultivated fruiting bodies and wild fruiting bodies were 26.00 ± 0.40, 14.13 ± 0.10 and 14.02 ± 0.21 g/100 g DW, respectively. The results showed that the mycelia exhibited the highest crude protein content and that the crude protein contents of the artificially cultivated and wild fruiting bodies

were similar.

Conversion coefficient of nitrogen in protein

It was determined that the total amino acid content of the mycelia accounted for 85.09% of the crude protein content, and the corresponding proportions in the artificially cultivated fruiting bodies and wild fruiting bodies were 85.51% and 64.23%, respectively. The calculated conversion coefficients of nitrogen in protein in the mycelia, artificially cultivated fruiting bodies and wild fruiting bodies were 5.32, 5.35 and 4.02, respectively, which were lower than the traditional conversion factor of 6.25, suggesting that this coefficient shows some degree of specificity in different research fields [18].

Assay of the amino acid composition

The composition and content of amino acids were analyzed in the mycelia, artificially cultivated fruiting bodies and wild fruiting bodies. The three types of samples contained 17 common amino acids (tryptophan was not detected) (Table 1). The contents of total amino acids and essential amino acids were highest in the mycelia, and their contents in

artificially cultivated fruiting bodies were higher than those in wild fruiting bodies. Glutamic acid was found to be the major amino acid in the mycelia, accounting for 14.77% of the total amino acids, whereas high levels of methionine were observed in artificially cultivated fruiting bodies and wild fruiting bodies, accounting for 24.95% and 30.77% of the total amino acids, respectively.

According to the ideal protein model factors proposed by the FAO/WHO, the ratio of essential amino acids to total amino acids (E/T) was approximately 0.40, and the ratio of essential to nonessential amino acids (E/N) was greater than 0.60. The E/T and E/N in mycelia were 0.37 and 0.58, respectively, which showed that the protein composition of the mycelia was close to the ideal protein model proposed by the FAO/WHO, belonging to the better-quality protein category. The E/T (0.55 and 0.57, >0.40) and E/N (1.22 and 1.33, >0.60) of both the artificially cultivated fruiting bodies and wild fruiting bodies were superior to the reference values, and the protein composition of the fruiting bodies of *G. gibbosum* suggested that they are an excellent source of protein.

Composition of essential amino acids

The nutritional value of proteins depends on the composition and proportion of the amino acids within them, especially the composition and proportion of essential amino acids. All three types of samples exhibited abundant essential amino acids (400.78–603.14 mg/g protein) (Table 2). The highest content of essential amino acids was found in wild fruiting bodies, followed by that in artificially cultivated fruiting bodies, while the content of essential amino acids in mycelia was lowest; all three materials showed markedly superior essential amino acid contents to the WHO/FAO reference protein pattern value (350 mg/g protein). The content of essential amino acids in mycelia was inferior to the ovalbumin model value (497 mg/g protein), while the contents in artificially cultivated fruiting bodies and wild fruiting bodies were superior to that value.

Methionine and cysteine were found to be the major essential amino acids in artificially cultivated fruiting bodies and wild fruiting bodies. Their contents accounted for approximately half of the total essential amino acids and were more than 7 times and 4 times the WHO/FAO reference value and the ovalbumin reference value, respectively. Lysine was the major essential amino acid in mycelia. The wild fruiting bodies exhibited higher levels of methionine and cysteine than the artificially cultivated fruiting bodies.

Nutritional profile of amino acids in proteins

The AAS, representing the percentage of a single essential amino acid in a tested protein sample in relation to the corresponding amino acid in the FAO/WHO reference protein pattern, was the main index used to evaluate the nutritional value of the amino acid compositions in the current study. The closer the AAS to 100% and the closer the composition of amino acids to the standard model, the higher the nutritional value of protein. The lowest AAS is taken as the total AAS of the sampled protein, and the corresponding amino acid is also known as the first-limiting amino acid. Met+Cys were the first-limiting amino acids in mycelia, and Lys and Ile were the first-limiting amino acids in artificially cultivated fruiting bodies and wild fruiting bodies (Table 3). In addition, the AASs of other amino acids in the mycelia and artificially cultivated fruiting bodies were higher or closer to the FAO/WHO reference protein pattern. However, the AASs of Lys and Leu in wild fruiting bodies were much lower than those from the FAO/WHO reference protein pattern values. According to the AAS value, the composition and proportion of essential amino acids in artificially cultivated fruiting bodies were closer to the amino acid composition required by humans than those in wild fruiting bodies. It is worth noting that the AASs of Met+Cys in artificially cultivated fruiting bodies and wild fruiting bodies were much higher than the model values (741.24 and 892.47, respectively).

The CS is used to evaluate the proximity of the relative content of an essential amino acid in a tested protein to the relative content of the corresponding essential amino acid in the ovalbumin reference protein pattern. The closer the CS to 100% and the closer the composition of amino acids to the reference protein pattern, the higher the nutritional value of protein. A CS below 100% indicates a limiting amino acid. Meanwhile, the amino acid that exhibits the lowest CS will be the first-limiting amino acid. Met+Cys, Phe+Tyr and Ile were the first-limiting amino acids in mycelia, artificially cultivated fruiting bodies and wild fruiting bodies, respectively (Table 3). Ile was the second-limiting amino acid in mycelia and artificially cultivated fruiting bodies, whereas Lys was the second-limiting amino acid in wild fruiting bodies. The CS of Ile in artificially cultivated fruiting bodies was 96.61% higher than that in wild fruiting bodies.

Table 2 Essential amino acid composition of proteins in *G. gibbosum*.

Amino acid	Content (mg/g protein)				
	CM	AF	WF	WHO/FAO[a]	Ovalbumin[a]
Thr	58.71 ± 0.15	49.22 ± 0.80	53.94 ± 0.18	40	51
Lys	81.86 ± 0.26	44.80 ± 0.74	39.84 ± 0.61	55	64
Leu	70.70 ± 0.07	73.73 ± 0.15	60.76 ± 0.89	70	88
Ile	36.53 ± 0.07	40.22 ± 0.11	20.46 ± 0.32	40	66
Met+Cys	18.43 ± 0.26	259.44 ± 3.27	312.37 ± 3.59	35	55
Phe+Tyr	71.86 ± 0.18	59.78 ± 0.75	67.47 ± 2.07	60	100
Val	62.69 ± 0.04	50.28 ± 0.24	48.29 ± 0.15	50	73
Total	400.78 ± 0.56	577.47 ± 3.98	603.14 ± 5.36	350	497

[a] WHO/FAO and ovalbumin reference protein pattern values. CM, cultured mycelia; AF, artificially cultivated fruiting bodies; WF, wild fruiting bodies. Data were calculated from triplicated samples.

Table 3 Nutritional profile of amino acids in the proteins of *G. gibbosum*.

Amino acid	AAS (%)			CS (%)			RC		
	CM	AF	WF	CM	AF	WF	CM	AF	WF
Thr	146.77 ± 0.39	123.05 ± 2.01	134.86 ± 0.47	115.12 ± 0.30	96.51 ± 1.58	105.77 ± 0.37	1.31 ± 0.01	0.64 ± 0.01	0.65 ± 0.01
Lys	148.84 ± 0.47	81.45 ± 1.36	72.44 ± 1.12	127.91 ± 0.40	69.99 ± 1.17	62.25 ± 0.97	1.33 ± 0.00	0.42 ± 0.00	0.35 ± 0.01
Leu	101.00 ± 0.11	105.33 ± 1.64	86.80 ± 0.41	80.34 ± 0.09	83.79 ± 1.30	69.05 ± 0.38	0.90 ± 0.00	0.55 ± 0.01	0.42 ± 0.02
Ile	91.33 ± 0.17	100.56 ± 2.78	51.15 ± 0.80	55.35 ± 0.10	60.95 ± 1.68	31.00 ± 0.49	0.81 ± 0.00	0.52 ± 0.02	0.25 ± 0.00
Met+Cys	52.66 ± 1.59	741.24 ± 17.92	892.47 ± 10.28	33.51 ± 1.14	471.70 ± 11.40	567.93 ± 6.54	0.47 ± 0.02	3.84 ± 0.04	4.32 ± 0.04
Phe+Tyr	119.77 ± 0.30	99.64 ± 1.25	112.46 ± 0.79	71.86 ± 0.18	59.78 ± 0.75	67.47 ± 0.47	1.07 ± 0.00	0.52 ± 0.01	0.54 ± 0.03
Val	125.38 ± 0.09	100.55 ± 0.02	96.59 ± 0.31	85.88 ± 0.06	68.87 ± 0.02	66.16 ± 0.21	1.12 ± 0.00	0.53 ± 0.01	0.47 ± 0.01

CM, cultured mycelia; AF, artificially cultivated fruiting bodies; WF, wild fruiting bodies. Data were calculated from triplicated samples.

Furthermore, the CSs of Met+Cys in artificially cultivated fruiting bodies and wild fruiting bodies were also much higher than the model values (55 mg/g protein).

The RC is used to evaluate the equilibrium of the amino acid. The more scattered the RC is, the greater negative contribution of the amino acids to their equilibrium and the worse nutritional value of the protein will be acquired. The closer the SRC to 100, the higher the nutritional value of protein. The RCs of the amino acids in mycelia, artificially cultivated fruiting bodies and wild fruiting bodies were 0.47–1.33, 0.42–3.84 and 0.25–4.32, respectively (Table 3). Lys and Met+Cys, Met+Cys and Lys, and Met+Cys and Ile deviated positively and negatively from the amino acid equilibrium spectra of the three types of tested samples, respectively, which was consistent with the AAS results. The second-limiting amino acids in the mycelia, artificially cultivated fruiting bodies, and wild fruiting bodies were Ile, Ile and Lys, respectively, which was consistent with the CS results. The SRC values for the tested samples were 69.84, 25.30 and 46.02, respectively, which were also calculated by the method of Zhu and Wu [17] and showed significant differences. According to the SRC values, the nutritional value of the proteins in the mycelia was superior to that of the proteins in the artificially cultivated fruiting

bodies and wild fruiting bodies. This scattered data may be caused by the high proportion of Met in the artificially cultivated fruiting bodies and wild fruiting bodies, leading to the deviation proportion of essential amino acids in these materials from the FAO/WHO reference protein pattern.

EAAI values

The EAAI is used to estimate the overall effects of all essential amino acids on the tested protein and calculated as the geometric mean of the essential amino acids in the tested protein compared with the reference amino acids in the standard protein patterns (ovalbumin used as standard). The EAAI values of the proteins in mycelia, artificially cultivated fruiting bodies and wild fruiting bodies were 75.15 ± 0.36, 94.43 ± 1.00 and 86.31 ± 1.23, respectively. The data obtained from this research revealed that the proteins of *G. gibbosum* exhibited a relatively balanced amino acid content, meaning that this species could serve as a favorable source of proteins with suitable proportions.

DISCUSSION

As a traditional Chinese medicine, *Ganoderma* possesses important medical and economic value. In this study, the ITS1/ITS4 primers were used to amplify the ITS sequence of a wild macrofungus,

which was identified by homologous and phylogenetic analysis, combined with its wild morphological characteristics. It was found that the isolated wild strain was *G. gibbosum* based on classical morphology supplemented by systematic molecular identification [19]. The isolated strain was further cultured in liquid and artificially cultivated, and the amino acid profile and protein quality of the mycelia, artificially cultivated fruiting bodies and wild fruiting bodies were then systematically determined and evaluated according to current international amino acid reference patterns [20, 21]. The results showed that the crude protein content of the tested *G. gibbosum* samples was higher than 14%, which is an intermediate level and is higher than those of common cereals such as *Oryza sativa* L. (7.1–7.7%) [22], *Zea mays* L. (10.8%) [7] and *Hordeum vulgare* L. (11.2–13.8%) [23] but lower than those of some common edible fungi (*Lentinula edodes* (Berk.) Sing 21.4–32.5%, *Pleurotuseryngii* (DC.) Quél. 18.6–27.8%, *Agaricusbisporus* (J. E. Lange) Imbach 26.5–27.5%) [18, 24, 25]. The contents of essential amino acids in the proteins of the tested materials were sufficient, and the proportions were basically reasonable, conforming to the requirements of current international reference amino acid patterns.

The total content of essential amino acids in *G. gibbosum* even exceeded the requirements of the WHO/FAO reference protein pattern value, especially in the artificially cultivated and wild fruiting bodies, which showed much higher contents than the ovalbumin reference protein pattern values. The contents of crude proteins and amino acids are related to the levels of elements such as nitrogen and phosphorus in the environment, and the nutritional value of proteins mainly depends on the species and the quantity and composition ratio of essential amino acids. The AAS and CS of essential amino acids in the artificially cultivated fruiting bodies of *G. gibbosum* were higher than those in wild fruiting bodies, but the balance of essential amino acids was slightly inferior to that in the wild fruiting bodies. Compared with the research on the total amino acid contents and the total proportion of essential amino acids reported by Chen et al [8], the amino acid contents of *G. gibbosum* determined in this study were higher, which may be related to the effects on the examined variety and growth conditions.

This study showed that the relative content of lysine was the highest among the essential amino acids in the mycelia, while the relative content of methionine was the highest in the artificially cultivated fruiting bodies and wild fruiting bodies. Lysine plays an important role in promoting human growth and development, enhancing immunity, promoting fat oxidation, and relieving anxiety [26, 27]. For example, it promotes calcium and iron absorption [28], wound healing and antibody secretion [29]. Methionine is a sulfur-containing amino acid and is closely related to the metabolism of various sulfur-containing compounds in living bodies. Long-term methionine deficiency may cause liver and heart muscle damage [30]. It can also be converted into cysteine or cystine in the body, and the reaction is irreversible [31]. Sulfur-containing amino acids are not only the main donors of sulfur in foods (e.g., providing sulfur atoms for sulfur compounds such as coenzyme A and taurine) but can also inhibit food browning and the Maillard reaction, thereby preventing decreases in food flavor and nutritional value [32]. In addition, sulfur-containing amino acids such as methionine can produce glutathione (GSH) through the sulfur transfer pathway, thereby achieving oxidation protection in the body [33, 34]. It is concluded that *G. gibbosum* presents great potential for the development of functional foods that may promote human growth and development, delay the aging of the body and protect the cardiovascular system.

CONCLUSION

In the present study, a wild *G. gibbosum* strain was identified through morphological and molecular analyses and then cultivated artificially. The artificially cultivated fruiting bodies could grow at 25 °C under 85–95% relative humidity. The amino acid profile and protein quality of *G. gibbosum* were systematically investigated according to the most recent version of international reference patterns. The results indicated that the crude protein content of the mycelia of cultivated *G. gibbosum* was higher than that of artificially cultivated and wild fruiting bodies. The contents of essential amino acids in the proteins of *G. gibbosum* were sufficient, and the proportions were basically reasonable, conforming to the requirements of current international reference amino acid patterns. In addition, Lys in the mycelia and Met+Cys in the artificially cultivated fruiting bodies and wild fruiting bodies of *G. gibbosum* were much more plentiful than the model values. In the comprehensive analysis of all indicators, the protein nutritional value of the artificially cultivated fruiting bodies was slightly higher than that of the wild fruiting bodies. In terms of the conservation and rational utilization of medicinal fungal biodiver-

sity, artificially cultivated *G. gibbosum* could supply amino acids and protein to humans on a large scale based on the results of this research. In summary, this study provides a theoretical foundation for the exploration and utilization of the nutritional value of *G. gibbosum* in the near future.

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