

Nutritional value evaluation of wild edible mushroom (*Helvella leucopus*) from western China

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Abstract

Helvella leucopus, a wild mushroom species, is considered a valuable food by local residents. In the present work, the nutritional value of *H. leucopus* was evaluated against some commonly cultivated edible and medicinal fungi as controls. *H. leucopus* showed significantly higher crude protein content. The amino acid composition of *H. leucopus* met the FAO/WHO ideal protein standard, with the highest ratio of essential amino acid to total amino acid (EAA/TAA) reaching 58.27%. *H. leucopus* showed the highest correlation with *Ganoderma lucidum*. The contents of flavour amino acid (FAA) and medicinal amino acid (MAA) in *H. leucopus* were significantly higher than those in the controls, and a high ratio of branched-chain amino acid to aromatic amino acid (BCAA/AAA) was observed in *H. leucopus*. The EAA composition of *H. leucopus* was 54.74%, 1.56- and 1.10-fold higher than that of the FAO/WHO model and the egg model, respectively. On the basis of the amino acid score, nutrition index, and other indices, *H. leucopus* could be a good protein source rich in amino acids, particularly in EAA and MAA. Therefore, this mushroom species is worthy of further investigation and utilisation, with huge market potential.

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Introduction

In many countries, mushrooms have become a popular dietary nutrient, not only due to their attractive culinary features or sensory characteristics, but also their nutritional properties. They are rich in proteins, carbohydrates, fibres, vitamins, and minerals, but low in fats (Jacinto-Azevedo *et al.*, 2021). To meet the increasing demands of consumers, the edible mushroom market has grown significantly, and the variety of mushroom products available in the market has considerably increased over the last decade (Roncero-Ramos and Delgado-Andrade, 2017). In addition to cultivated varieties, several wild mushrooms are considered delicacies worldwide due to their aroma and taste (Falandysz and Borovicka, 2013).

The wild edible mushroom *Helvella leucopus*, also locally known as Bachu mushroom, is distributed in the northern Tarim Basin, Xinjiang Province, China. Due to its unique flavour and delicious taste, *H. leucopus* is considered a valuable food by the

locals. Extracts of *H. leucopus* show various beneficial properties, including immunoenhancement effects (Zhang *et al.*, 2020; 2022), and antioxidant and anticancer effects (Zeng and Zhu, 2018). Abdureyim *et al.* (2022) found that polysaccharides of *H. leucopus* can alleviate colonic injury by modulating the gut microbiota. Presently, the research on *H. leucopus* is mainly focused on species validation (Zhao *et al.*, 2016); investigation of ecological factors (Li *et al.*, 2016); and extraction, purification, and identification of bioactive ingredients such as polysaccharides, mannan, and phenolic compounds (Hou and Wei, 2008; Hou *et al.*, 2008; Acar *et al.*, 2021; Zhang *et al.*, 2022). However, to date, there has been no systematic evaluation on the nutritional components of *H. leucopus*, particularly assessment of its nutritional value, based on the analysis of crude protein content, amino acid content, and amino acid correlation analysis.

The high protein content of edible mushrooms and the variety of amino acids present in them provide both nutritional and health benefits (Kalac, 2013).

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Several methods are available to evaluate the nutritional value of edible fungi, including amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI), biological value (BV), and nutrition index (NI) (Bano *et al.*, 1988; Su *et al.*, 2022). The present work comprehensively evaluated and analysed the nutritional value of *H. leucopus* to enhance the utilisation and protection of this wild mushroom species, and provide a theoretical basis for using this species as a functional food.

Materials and methods

Sample preparation

A fresh, wild-grown *H. leucopus* strain was collected from Seven Village, Tiremu Town, Yuepu County, Kashgar Prefecture, Xinjiang Province, China. As controls, five common edible and medicinal fungi available in the market, namely *Lentinula edodes*, *Ganoderma lucidum*, *Auricularia auricula-judae*, *Flammulina velutipes*, and *Pleurotus ostreatus* were cultivated mainly on wood chips, and collected from Changji City, Changji Hui Autonomous Prefecture, Xinjiang Province, China. After collection, all samples were washed with deionised water, dried at low temperature (40°C for 1.5 h, 55°C for 2 h, and 65°C for 3 h or more), crushed, and kept in a dryer at room temperature prior to analysis.

Sample analysis

The crude protein contents of *H. leucopus* and other mushrooms were determined following the Chinese standard GB 5009.5-2016 (Standards China, 2016) by the Kjeldahl method using an automatic Kjeltex nitrogen analyser (Kjeltex™ 8400, FOSS, Denmark). Their amino acid compositions were determined by ion exchange chromatography with post-column derivatisation (with ninhydrin) using a high-speed amino acid analyser (LA8080, Hitachi, Japan) (Rahgo *et al.*, 2019).

The AAS, EAAI, BV, and NI were calculated according to Bano *et al.* (1988) using Eqs. 1 – 4:

$$\text{AAS} = A_x/A_s \quad (\text{Eq. 1})$$

where, A_x = content (mg) of an essential amino acid in 1 g of test protein, and A_s = same amino acid content (mg) in 1 g of reference protein (the FAO/WHO scoring standard model).

$$\text{EAAI} = \sqrt[n]{a_1/b_1 \times a_2/b_2 \times \dots \times a_n/b_n} \quad (\text{Eq. 2})$$

where, n = number of essential amino acids for comparison, a_n = essential amino acid content (mg) of 1 g test protein, and b_n = content (mg) of the corresponding essential amino acids of 1 g reference protein in the FAO/WHO scoring standard model.

$$\text{BV} = 1.09 \times \text{EAAI} - 11.7 \quad (\text{Eq. 3})$$

$$\text{NI} = (\text{EAAI} \times \text{PP}) / 100 \quad (\text{Eq. 4})$$

where, PP = percentage of protein in the sample (%).

CS was estimated following the FAO methods (FAO, 1970) using Eq. 5:

$$\text{CS} = A_x/A_e \quad (\text{Eq. 5})$$

where, A_x = content (mg) of an essential amino acid in 1 g of test protein, and A_e = same amino acid content (mg) in 1 g of standard egg albumen.

Statistical analysis

All measurements were obtained from triplicate experiments. Random univariate analysis of variance (ANOVA) was performed using SPSS software (IBM SPSS Statistics for Windows, Version 23.0., Armonk, NY: IBM Corp.). Tukey's test was used to analyse significant differences ($p < 0.05$).

Results

Analysis of protein nutrient value of *H. leucopus*

The crude protein composition of the samples, with values based on dry weight (dw), was determined. Compared to other common edible and medicinal fungi, *H. leucopus* showed significantly higher protein content of 17.10 g/100 g dw (close to *F. velutipes* and *P. ostreatus*, 18.43 and 18.86 g/100 g dw, respectively), which was 1.25-, 1.51-, and 2.18-fold that of *L. edodes*, *A. auricula-judae*, and *G. lucidum*, respectively.

Analysis of amino acid composition of *H. leucopus*

Amino acids are the basic units of proteins, and they participate in many physiological activities in the bodies of living organisms, and play an important role in routine functioning. As shown in Table 1, 17 amino acids, namely threonine, serine, aspartic acid, glycine, valine, methionine, leucine, phenylalanine, arginine,

proline, tyrosine, glutamic acid, cysteine, isoleucine, histidine, alanine, and lysine were detected in *H. leucopus*. The total amino acid (TAA) content was 15.42 g/100 g dw in *H. leucopus* (Table 1). Seven types of essential amino acid (EAA) were detected (tryptophan was not detected because of the detection method used). The EAA content of *H. leucopus* was 8.98 g/100 g dw, and the ratio of EAA/TAA was 58.27%. The content of non-essential amino acid (NEAA) was 6.43 g/100 g dw.

A comparison of the TAA content in the fruiting bodies of *H. leucopus* and the other common test mushrooms showed the following result in

descending order: *H. leucopus* > *L. edodes* > *P. ostreatus* > *F. velutipes* > *A. auricula-judae* > *G. lucidum* (Table 1). The Met content was the highest in *H. leucopus* and *G. lucidum*; the Glu content was the highest in *L. edodes*, *P. ostreatus*, and *A. auricula-judae*; and the His content was the highest in *F. velutipes*. The EAA content showed a consistent trend with the result of TAA. The EAA/TAA ratio in the descending order was as follows: *H. leucopus* > *G. lucidum* > *L. edodes* > *P. ostreatus* > *A. auricula-judae* > *F. velutipes*, and this trend was the same as that observed for the EAA/NEAA ratio.

Table 1. Comparison of amino acid content of *H. leucopus* and other test fungi.

Amino acid	Amino acid content (g/100 g)					
	<i>H. leucopus</i>	<i>L. edodes</i>	<i>G. lucidum</i>	<i>A. auricula-judae</i>	<i>F. velutipes</i>	<i>P. ostreatus</i>
Asp	1.32 ± 0.04 ^a	0.91 ± 0.04 ^c	0.64 ± 0.05 ^e	1.05 ± 0.03 ^b	0.83 ± 0.03 ^d	1.27 ± 0.02 ^a
Thr*	0.84 ± 0.01 ^a	0.50 ± 0.02 ^d	0.21 ± 0.01 ^e	0.62 ± 0.01 ^b	0.51 ± 0.02 ^d	0.55 ± 0.02 ^c
Ser	0.64 ± 0.01 ^a	0.56 ± 0.01 ^b	0.27 ± 0.01 ^d	0.56 ± 0.01 ^b	0.49 ± 0.02 ^c	0.56 ± 0.02 ^b
Glu	1.38 ± 0.01 ^c	2.78 ± 0.06 ^a	0.68 ± 0.01 ^e	1.24 ± 0.02 ^d	1.38 ± 0.01 ^c	1.54 ± 0.01 ^b
Gly	0.66 ± 0.01 ^a	0.49 ± 0.02 ^c	0.37 ± 0.02 ^e	0.47 ± 0.01 ^c	0.44 ± 0.01 ^d	0.58 ± 0.02 ^b
Ala	0.61 ± 0.01 ^d	0.94 ± 0.01 ^a	0.37 ± 0.01 ^e	0.86 ± 0.02 ^b	0.83 ± 0.02 ^c	0.96 ± 0.03 ^a
Cys	0.10 ± 0.02 ^d	0.39 ± 0.03 ^a	0.09 ± 0.02 ^d	0.18 ± 0.02 ^b	0.14 ± 0.02 ^c	0.17 ± 0.01 ^b
Val*	1.57 ± 0.04 ^a	0.66 ± 0.03 ^b	0.34 ± 0.02 ^d	0.53 ± 0.02 ^c	0.54 ± 0.02 ^c	0.68 ± 0.03 ^b
Met*	3.37 ± 0.08 ^a	0.84 ± 0.05 ^c	1.38 ± 0.03 ^b	0.14 ± 0.01 ^d	0.11 ± 0.01 ^d	0.18 ± 0.02 ^d
Ile*	0.53 ± 0.01 ^b	0.83 ± 0.02 ^a	0.23 ± 0.02 ^e	0.34 ± 0.02 ^d	0.38 ± 0.01 ^c	0.52 ± 0.03 ^b
Leu*	1.78 ± 0.11 ^a	0.81 ± 0.08 ^b	0.42 ± 0.02 ^d	0.73 ± 0.04 ^{bc}	0.68 ± 0.06 ^c	0.85 ± 0.08 ^b
Tyr	0.28 ± 0.01 ^b	0.26 ± 0.02 ^b	0.13 ± 0.02 ^c	0.28 ± 0.02 ^b	0.35 ± 0.03 ^a	0.25 ± 0.02 ^b
Phe*	0.45 ± 0.02 ^b	0.54 ± 0.02 ^a	0.22 ± 0.03 ^c	0.42 ± 0.03 ^b	0.52 ± 0.05 ^a	0.51 ± 0.02 ^a
Lys*	0.45 ± 0.04 ^d	0.5 ± 0.01 ^{cd}	0.21 ± 0.02 ^e	0.51 ± 0.02 ^c	0.58 ± 0.04 ^b	0.71 ± 0.05 ^a
His	0.27 ± 0.02 ^d	0.21 ± 0.02 ^d	0.11 ± 0.01 ^e	0.88 ± 0.05 ^c	1.93 ± 0.07 ^a	0.97 ± 0.05 ^b
Arg	0.49 ± 0.01 ^b	0.64 ± 0.03 ^a	0.23 ± 0.02 ^d	0.48 ± 0.05 ^b	0.36 ± 0.04 ^c	0.59 ± 0.06 ^a
Pro	0.69 ± 0.04 ^a	0.26 ± 0.02 ^d	0.25 ± 0.02 ^d	0.42 ± 0.03 ^b	0.34 ± 0.03 ^c	0.46 ± 0.02 ^b
TAA	15.42 ± 0.25 ^a	12.14 ± 0.35 ^b	6.16 ± 0.24 ^f	9.7 ± 0.24 ^e	10.43 ± 0.19 ^d	11.35 ± 0.30 ^c
EAA	8.98 ± 0.16 ^a	4.69 ± 0.17 ^b	3.01 ± 0.12 ^e	3.30 ± 0.09 ^d	3.33 ± 0.09 ^d	3.99 ± 0.19 ^c
NEAA	6.43 ± 0.11 ^c	7.45 ± 0.19 ^a	3.15 ± 0.12 ^d	6.41 ± 0.16 ^c	7.10 ± 0.11 ^b	7.35 ± 0.14 ^a
EAA/TAA(%)	58.27 ± 0.33 ^a	38.64 ± 0.28 ^c	48.92 ± 0.17 ^b	33.97 ± 0.24 ^e	31.94 ± 0.34 ^f	35.18 ± 0.87 ^d
EAA/NEAA(%)	139.64 ± 1.88 ^a	62.97 ± 0.74 ^c	95.76 ± 0.66 ^b	51.46 ± 0.55 ^e	46.92 ± 0.73 ^f	54.30 ± 2.06 ^d

TAA: total amino acid; EAA: essential amino acid (marked with asterisk (*)); and NEAA: non-essential amino acid; different lowercase superscripts indicate statistical difference ($p < 0.05$).

Correlation analysis of *H. leucopus* based on amino acids

As shown in Figure 1A, based on the amino acid content, *H. leucopus* showed a positive correlation with *G. lucidum* ($p = 0.921$), *L. edodes* ($p = 0.351$), and *P. ostreatus* ($p = 0.055$). *G. lucidum* showed the closest correlation with *H. leucopus*,

while *H. leucopus* shared a distant relationship with *A. auricula-judae* ($p = 0.015$). A negative correlation ($p = -0.130$) was observed between *H. leucopus* and *F. velutipes*.

The biological activity of proteins is closely associated not only with the type and quantity of amino acids, but also with the correlation between the

different amino acids. The correlation between various amino acid components of *H. leucopus* showed significant difference (Figure 1B). The contents of some amino acids were negatively correlated with each other. For instance, Asp was negatively correlated with Glu, Ala, Val, Met, Leu, Arg, and Pro at 99% probability level, while Ser was negatively correlated with Thr, Glu, Ala, Lys, Arg, and Pro at 95% probability level. The correlation of

the contents of some amino acids showed opposite results. For example, Arg was positively correlated with Glu, Ala, Val, Met, and Leu at 99% probability level, while Asp was positively correlated with Ser and Glu at 95% probability level. The absolute value of correlation coefficients between most amino acid components ranged between 0.85 and 1.00, indicating a very strong correlation between the content of different amino acids of *H. leucopus*.

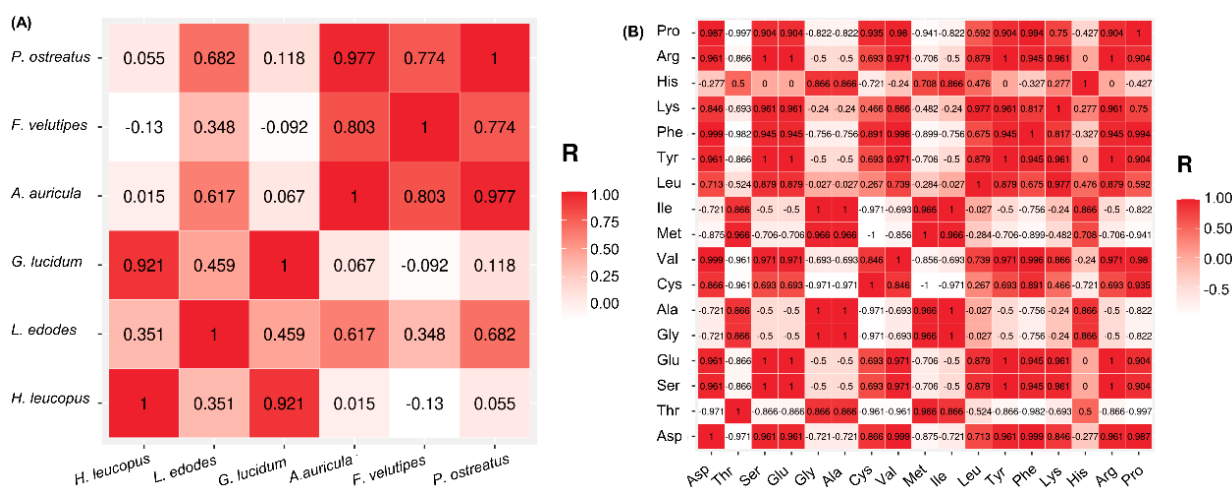


Figure 1. Correlation heat map of *H. leucopus*. (A) Correlation analysis between *H. leucopus* and other test fungi; and (B) Correlation analyses of different amino acids of *H. leucopus*.

Content and composition of flavour amino acid of *H. leucopus*

Edible fungi are a popular component in cooking due to their unique flavour. After cooking or processing, the protein present in edible fungi is degraded into small peptides and amino acids, thereby gradually imparting the unique flavour of edible fungi to the food. Amino acids play an important role in the unique flavour of edible fungi. Figure 2A shows the contents and compositions of flavour amino acids analysed in *H. leucopus*.

The umami amino acid (UAA) content of *H. leucopus* was 2.70 g/100 g dw. The UAA content of *H. leucopus* was higher than that of *G. lucidum*, *A. auricula-judae*, and *F. velutipes*. The contents of sweet amino acid (SAA) and bitter amino acid (BAA) of *H. leucopus* were the highest among all the analysed mushrooms. The SAA content of *H. leucopus* was calculated to be 2.60 g/100 g dw, which was significantly different from that of *L. edodes*, *G. lucidum*, *A. auricula-judae*, and *F. velutipes*. The content of flavouring and colouring amino acids (FCAA: Glu, Cys, Val, Leu, Lys, His, and Arg) of *H. leucopus* was 6.02 g/100 g dw, which was significantly higher than that of all the other test

mushrooms, except *L. edodes*. The content of aromatic amino acids (AAA: Phe, Tyr, and Trp) of *H. leucopus* was calculated to be 0.73 g/100 g dw.

Content and composition of special functional amino acids of *H. leucopus*

Amino acids play an important role in food flavour, and are also critical material sources for physiological activities in humans. Figure 2B shows the contents and compositions of children's amino acids (CE: His and Arg), medicinal amino acids (MAA: Asp, Glu, Gly, Met, Leu, Phe, Tyr, Lys, and Arg), antibacterial amino acids (ABAA: Phe, Trp, and Gly), primary amino acids (PAAA: Asp, Glu, Pro, Gly, Ala, Lys, and Arg), branched-chain amino acids (BCAA: Ile, Leu, and Val), and other special functional amino acids of *H. leucopus*.

H. leucopus showed a high content of MAA (10.16 g/100 g dw), with the MAA/TAA ratio of 65.92%, significantly higher than that of the other test common mushrooms. The BCAA content of *H. leucopus* was 3.88 g/100 g dw, with the highest ratio of BCAA/AAA. The contents of ABAA and PAAA of *H. leucopus* were 1.10 and 5.59 g/100 g dw. The ABAA content of *H. leucopus* was significantly

higher than that of all the other test mushrooms, and the PAAA content of *H. leucopus* was significantly

higher than that of *G. lucidum*, *A. auricula-judae*, and *F. velutipes*.

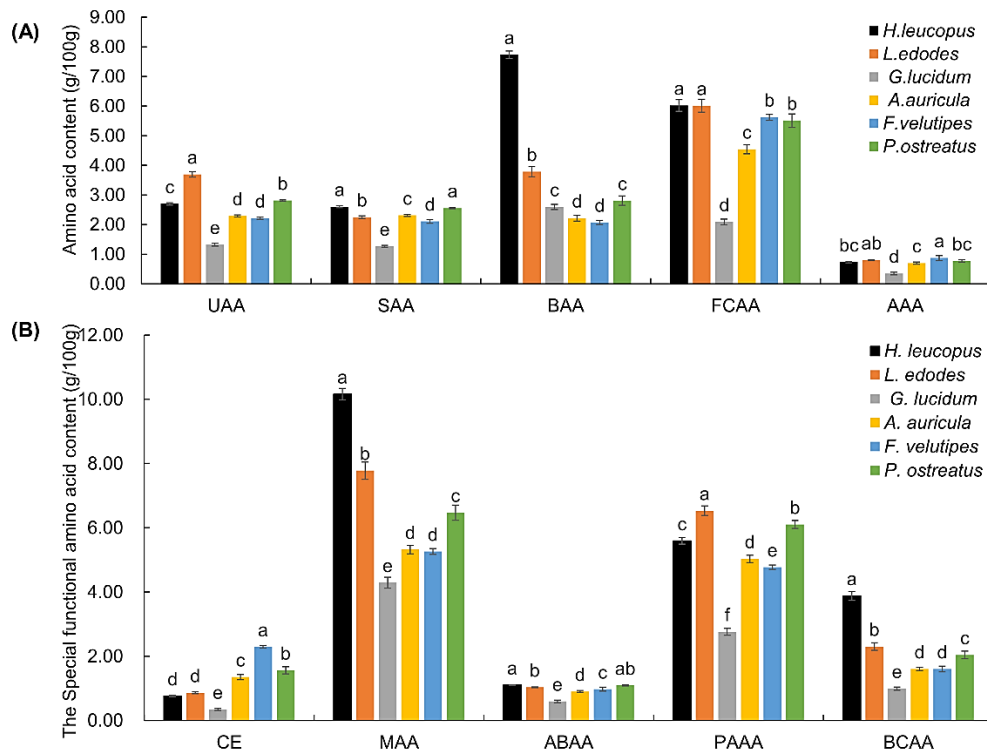


Figure 2. Content and composition of flavour amino acids (A) and special functional amino acids (B) of *H. leucopus*. UAA: umami amino acid; SAA: sweet amino acid; BAA: bitter amino acid; FCAA: flavouring and colouring amino acid; AAA: aromatic amino acid; CE: children's amino acid; MAA: medicinal amino acid; ABAA: antibacterial amino acid; PAAA: primary amino acid; and BCAA: branched-chain amino acid. Different lowercase letters indicate statistical difference ($p < 0.05$).

Evaluation of *H. leucopus* nutritional value

The nutritional value of food is associated with the type and quantity of proteins contained in the food, and with the composition of EAA (Luo *et al.*, 2015). As shown in Table 2, the composition of the seven types of EAA of *H. leucopus* was close to that of the egg model and the FAO/WHO model (FAO, 1973). The total EAA composition of *H. leucopus* was 54.74%, which was 1.56- and 1.10-fold that of the FAO/WHO and egg model, respectively. This finding indicated that the consumption of *H. leucopus* could meet the nutritional requirements of infants, children (10 - 12 years old), and adults for EAA. The EAA composition of *H. leucopus* was also higher than or close to that of some conventional protein crops such as peanut kernel, soybean, and wheat, thus suggesting that it could be used as an alternative protein source for some food crops.

AAS is used to measure the percentage of an EAA to the corresponding amino acid in the

FAO/WHO model, and it mainly reflects the deficiency level of the limiting amino acids in food (Wang *et al.*, 2021). The AAS of *H. leucopus* was higher than that of *F. velutipes*. The first limiting amino acid of *H. leucopus* was Lys, which was identical to that of *L. edodes* and *G. lucidum*. The second limiting amino acid of *H. leucopus* was Phe+Tyr (Figure 3A).

CS is calculated based on the ratio of EAA in food to the corresponding amino acid in standard egg albumen (Luo *et al.*, 2015). Figure 3B shows that the CS of *H. leucopus* was higher than those of *F. velutipes* and *P. ostreatus*. The first and second limiting amino acids of *H. leucopus* were consistent with the results of AAS.

The EAAI, BV, and NI were calculated based on the FAO model, and the results are shown in Figure 3C. The EAAI is used to evaluate the percentage of EAA in food to that in standard egg albumen; this parameter was first proposed by Oser

Table 2. Comparison of essential amino acid composition of *H. leucopus* and other test fungi (%).

	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Val	Total
<i>H. leucopus</i>	3.14 ± 0.19 ^b	10.38 ± 0.59 ^a	2.63 ± 0.16 ^d	20.26 ± 1.33 ^a	4.26 ± 0.09 ^b	4.91 ± 0.30 ^b	9.15 ± 0.24 ^a	54.74 ± 2.48 ^a
<i>L. edodes</i>	6.08 ± 0.36 ^a	5.98 ± 0.73 ^b	3.70 ± 0.27 ^b	9.02 ± 0.74 ^b	5.91 ± 0.45 ^a	3.71 ± 0.45 ^c	4.88 ± 0.41 ^b	39.29 ± 3.18 ^b
<i>G. lucidum</i>	2.92 ± 0.49 ^b	5.44 ± 0.66 ^{bc}	2.74 ± 0.41 ^{cd}	18.89 ± 1.65 ^a	4.53 ± 0.63 ^b	2.74 ± 0.28 ^d	4.32 ± 0.42 ^c	41.58 ± 4.31 ^b
<i>A. auricula-judae</i>	3.02 ± 0.09 ^b	6.41 ± 0.43 ^b	4.52 ± 0.22 ^a	2.76 ± 0.16 ^c	6.16 ± 0.01 ^a	5.50 ± 0.27 ^a	4.67 ± 0.07 ^{bc}	33.05 ± 0.79 ^c
<i>F. velutipes</i>	2.05 ± 0.13 ^c	3.68 ± 0.46 ^d	3.16 ± 0.13 ^c	1.36 ± 0.14 ^c	4.73 ± 0.58 ^b	2.79 ± 0.06 ^d	2.95 ± 0.18 ^e	20.72 ± 1.41 ^d
<i>P. ostreatus</i>	2.74 ± 0.21 ^b	4.50 ± 0.54 ^{cd}	3.75 ± 0.34 ^b	1.86 ± 0.19 ^c	4.07 ± 0.34 ^b	2.93 ± 0.06 ^d	3.61 ± 0.25 ^d	23.47 ± 1.82 ^d
FAO/WHO	4.00	7.00	5.50	3.50	6.00	4.00	5.00	35.00
Infant*	3.50	8.00	5.20	2.90	6.30	4.40	4.70	35.00
Children (10 -12 years old)*	3.70	5.60	7.50	3.40	3.40	4.40	4.10	32.10
adult*	1.80	2.50	2.20	2.40	2.50	1.30	1.80	14.50
Egg**	6.60	8.80	6.40	5.50	10.00	5.10	7.30	49.70
milk**	4.70	9.50	7.80	3.30	10.20	4.40	6.40	46.30
peanut***	3.30	6.60	3.90	2.00	9.30	2.50	3.90	31.50
soybean***	4.50	7.80	4.90	1.80	9.10	3.80	4.80	36.70
wheat***	3.40	6.90	2.60	2.40	6.90	3.40	4.30	29.90

*Reference value of EAA content for different age groups with FAO (1973) recommendation. **Reference value of EAA in milk and egg protein proposed by FAO (1973). ***Reference value of EAA in common crops reported by Feng *et al.* (2016). Different lowercase superscripts indicate statistical difference ($p < 0.05$).

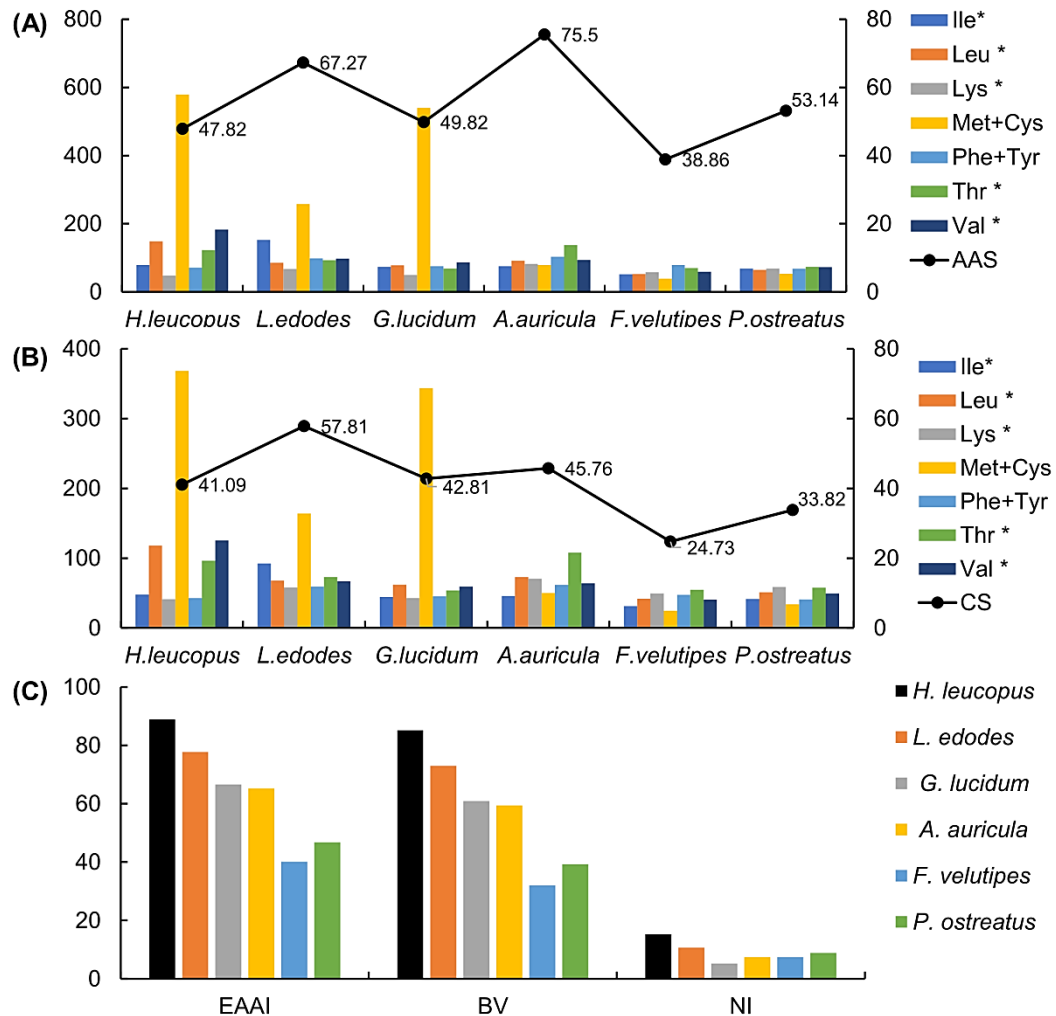


Figure 3. Amino acid score (A); chemical score (B); and essential amino acid indexes, biological values, and nutritional indexes (C) of essential amino acids of *H. leucopus*. AAS: amino acid score; CS: chemical score; EAAI: essential amino acid indexes; BV: biological values; and NI: nutritional indexes.

(1951; 1959). The closer the EAAI is to the value of 100, the closer the EAA composition of the food analysed is to the standard protein, which indicates that it has a high nutritional value. To differentiate protein quality and guide production practices, EAAI was classified into a high-quality protein source (EAAI > 0.95), a good protein source (0.86 < EAAI < 0.95), and an available protein source (0.75 < EAAI < 0.86) (Chen *et al.*, 2014). As shown in Figure 3C, the EAAI of *H. leucopus* reached the standard of a good protein source, with the highest EAAI of 88.84%. The EAAI of the other common edible and medicinal fungi reached the standard of an available protein source.

BV is a biological method to evaluate the nutritional value of food proteins, calculated based on the utilisation efficiency of proteins after digestion and absorption by the human body, and proportional to utilisation efficiency (Su *et al.*, 2019). *H. leucopus* showed the highest BV (85.13%), followed by *L.*

edodes. *F. velutipes* showed the lowest BV of 31.98% (Figure 3C). The results of EAAI and BV indicated that *H. leucopus* is not only a good protein source, but it can also be easily absorbed and utilised by the human body.

The NI is a comprehensive evaluation parameter based on the amino acid composition and protein content of food (Su *et al.*, 2021). *H. leucopus* showed the highest NI (15.19%, stipe to pileus). Compared to the other common edible and medicinal fungi, the NI of *H. leucopus* was 1.43-, 2.92-, 2.05-, 2.06-, and 1.72-fold that of *L. edodes*, *G. lucidum*, *A. auricula-judae*, *F. velutipes*, and *P. ostreatus*, respectively, showing a higher nutritional value of *H. leucopus*.

Discussion

Edible mushrooms belong to the rare category of high-protein alkaline foods, which can balance the

influence of acidic foods such as fish and meat in the diet on the human body, thus making them consistent with the modern healthy diet (Chen *et al.*, 2014). Moreover, as a wild edible mushroom, *H. leucopus*, has become a local delicacy due to its unique flavour and delicious taste.

The present work showed that the crude protein content of *H. leucopus* was significantly higher than that of common edible and medicinal fungi such as *L. edodes*, *A. auricula-judae*, and *G. lucidum*. The protein quality of foods mainly depends on the type, content, and composition ratio of EAA (Luo *et al.*, 2015). Seven types of EAA were detected in *H. leucopus*. In comparison with the other common edible and medicinal fungi, *H. leucopus* showed a high content of several amino acids, and was particularly rich in Met and Leu. As EAA, Met exerts certain effects on liver cirrhosis, fatty liver, and cardiovascular diseases. It also promotes antioxidant defence, and modulates cell and protein activities (Savino *et al.*, 2022). Moreover, a diet deficient in Met can affect DNA methylation (Aissa *et al.*, 2022). Leu plays a critical role in reducing fatigue and ethanol concentration in blood (Mannheim and Cheryan, 1992). It also regulates blood glucose levels, contributes to metabolise visceral fat (Wei *et al.*, 2011), and effectively improves skeletal muscle (Camajani *et al.*, 2022). According to the ideal protein model proposed by FAO, protein quality is considered to be better at the EAA/TAA ratio of 40%, and the EAA/NEAA ratio of > 60% (Luo *et al.*, 2021). The results of the present work showed that, among all the analysed mushrooms, the amino acid composition of only *H. leucopus* and *G. lucidum* could meet the FAO ideal protein standard. The EAA/NEAA and EAA/TAA ratios of *H. leucopus* were 139.64 and 58.27%, which were significantly higher than those of *G. lucidum*.

On the basis of the amino acid content, the correlation between *H. leucopus* and other fungi in the descending order was as follows: *G. lucidum* > *L. edodes* > *P. ostreatus* > *A. auricula-judae* > *F. velutipes*. *H. leucopus* showed the strongest correlation with the medicinal fungus *G. lucidum*; this finding was consistent with the percentage of MAA in the TAA. Nine types of MAA (Asp, Gly, Met, etc.) are necessary to maintain the nitrogen balance of the human body; however, they are found in small amounts in common plant-based foods (Jiang, 1996). Moreover, some of them belong to EAA, which cannot be naturally synthesised by the human body.

This shows that *H. leucopus* has a high medicinal value, and broad development prospects for preparing healthy foods and modern medicinal diets. The specific correlation of the content of different amino acids in *H. leucopus* contributes to its special edible and medicinal values.

Amino acids, particularly FAA, play a key role in forming food flavour substances (Hidalgo and Zamora, 2008; Tian *et al.*, 2022). The amino group of FCAA can react with the carbonyl group of reducing sugar to produce a brown substance with a pleasant flavour, thereby promoting the flavour and colour of foods. The unique flavour of *H. leucopus* is closely related to the type, content, and composition of amino acids. The higher FAA content of *H. leucopus* accounted for its unique flavour, thus offering it market potential. The abundant biological function and activity of *H. leucopus* are reflected by CE, MAA, ABAA, PAAA, BCAA, AAA, and other special functional amino acids. ABAA at certain concentrations can inhibit the growth of microorganisms that cause food spoilage. PAAA can inhibit the formation of dimethyl amino nitrosamine by replacing the secondary amine and nitrite to produce nitrogen and organic acids (Jiang, 1996). BCAA are important energy sources in the absence of carbohydrates and fats, and can prevent metabolic disorders. They have functions such as regulating protein synthesis and decomposition, regulating the entry of aromatic amino acids into the blood-brain barrier, and preventing liver and kidney failure (Jiang, 1996). The present work showed a high ratio of BCAA/AAA in *H. leucopus*. The high ratio of BCAA/AAA in foods has a significant effect on treating liver insufficiency (Guo *et al.*, 1990), and this may be promising area of research for *H. leucopus*.

Regarding the EAA pattern, various EAA compositions of *H. leucopus* met the standard egg and the FAO/WHO model pattern; in particular, the content of both Leu and Met+Cys far exceeded the standard values. Presently, *H. leucopus* remains a wild mushroom resource. If it is protected properly and exploited rationally, this mushroom species could be a good substitute for some protein foods, with a wide market potential. Based on AAS and CS, the first and second limiting amino acids of *H. leucopus* were Lys and Phe+Tyr. The balance of EAA of *H. leucopus* can be improved when consumed with foods rich in Leu and Met+Cys.

To have a more comprehensive understanding of the nutritional value of wild resources, the EAAI,

BV, and NI were summarised and analysed by the FAO model. The results showed that *H. leucopus* had higher values for these three indices than the other common edible and medicinal fungi analysed in the present work.

Conclusion

The nutritional value of *H. leucopus*, a wild mushroom, was evaluated. Some common edible and medicinal fungi such as *L. edodes*, *G. lucidum*, *A. auricula-judae*, *F. velutipes*, and *P. ostreatus* were used as controls for comparison. Results showed that the NI in the descending order was as follows: *H. leucopus* > *L. edodes* > *P. ostreatus* > *A. auricula-judae* > *F. velutipes* > *G. lucidum*. The present work revealed that the amino acids of *H. leucopus* protein are diverse, can meet the nutritional requirements of people of all ages for EAA, and can also meet the ideal protein model proposed by FAO. Therefore, the wild mushroom *H. leucopus* is worthy of further investigation and utilisation in the field of modern healthy diets.

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