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Original Article

AMINO ACID PROFILE OF A BASIDIOMYCETOUS EDIBLE MUSHROOM-LENTINUS SAJOR-CAJU

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ABSTRACT

Objective: In this paper amino acid profile of Lentinus sajor-caju (Fr.) Fr., a basidiomycetous mushroom has been investigated.

Methods: During the evaluation 15 amino acids (lysine, aspartic acid, serine, threonine, glutamic acid, cysteine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and histidine) were determined from the dried sample of *Lentinus sajor-caju* by following the standard technique of biochemistry using ion-exchange chromatography.

Results: The total amino acid content has been evaluated at 18.82 g/100g. Amongst the evaluated amino acids, exogenous amino acid lysine (6.66 g/100g) is preponderantly present in comparison to all other amino acids. The essential amino acid (EAA) index (44.64%) and biological value (BV=36.93%) has also been determined for the examined sample.

Conclusion: Lentinus sajor-caju (Fr.) Fr. is a potential source of quality protein with a substantial proportion of exogenous and endogenous amino acids.

Keywords: Lentinus sajor-caju, Amino acid, Nutritional value

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INTRODUCTION

Mushrooms have been recognized for their nutritional and nutraceutically properties because of their inherent composition. They are rich in essential nutrients including carbohydrates, proteins, vitamins, minerals, antioxidants and variety of bioactive components [1-4]. Because of the nutritional and medicinally important components, mushrooms are considered as functional foods worldwide [3] and can be compared favourably with the food value of milk, egg, meat and commonly consumed vegetables [5-9]. A number of Indian workers [6, 10-19] evaluated a variety of cultivated and wild edible mushrooms for their nutritional and nutraceutically profile including amino acids which are monomeric units of proteins and serve as antioxidant, energy metabolites and precursors of many biologically important nitrogen containing compounds [4, 20-21].

Lentinus sajor-caju (Fr.) Fr. is one such mushroom which is widely consumed in India [22, 23] and abroad [24, 25]. It is a basidiomycetous mushroom which grows in great abundance on dead and fallen branches, tree trunks and even on buried roots [26]. It is of common occurrence in the foot hills of North-West India and is being collected locally for consumption. So as to look into its culinary credentials amino acid profiling was done from the dried sporophores of the mushroom obtained in the laboratory through cultivation.

MATERIALS AND METHODS

The materials

The culture of *Lentinus sajor-caju* (Fr.) Fr. mushroom was raised through tissue culture from the sporophores collected from the wild. Subsequently, cultivation studies were undertaken under laboratory conditions so as to raise the sporophores. The mature sporophores were harvested and dried in a hot air drier at 40±1 °C [27] which was subsequently used for amino acid profiling.

Reagents and chemicals

All reagents and chemicals were of analytical grade. Amino acid standards, ninhydrin, hydrindantin, dimethyl sulfoxide were purchased from SIGMA-Aldrich; phenol, acetic acid, trisodium citrate dihydrate, sodium hydroxide, lithium hydroxide, lithium acetate dihydrate from FLUKA and HCl 30% from MERCK.

Solution preparation

The following solutions were prepared:

Eluent A

19.6 g trisodium citrate dihydrate was dissolved in 800 ml ultrapure water. 5 ml thiodiglycol, 1 g phenol crystals and 16 ml HCl were added to it. 2.2 pH was adjusted with HCl or NaOH solution. Raised the volume up to 1 L with water. This was used as elution buffer and extraction of amino acid standards.

Eluent B

1.25% lithium hydroxide and 0.1% phenol were dissolved in ultrapure water. It was used for column regeneration.

Ninhydrin reagent

4.0 g ninhydrin, 0.16 g hydrindantin were taken in bottle sparged with Argon/Nitrogen, 100 ml dimethylsulfoxide was poured and 100 ml 2 mol/l lithium acetate buffer (pH 5.2) was slowly dropped into the bottle. Ninhydrin reagent was stored at 4 °C temperature as it was a derivatization reagent.

Standard preparation

The standard methodology [28] was followed for analysis of 15 amino acids (lysine, aspartic acid, serine, threonine, glutamic acid, cysteine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and histidine). Each amino acid stock solution of concentration 2.5 mmol/l was prepared by dissolving each separately in 100 ml 1M HCl and diluted to the volume with triple distilled water. The working standard solution of each amino acid was prepared by diluting with eluent A using 1:20 ratio. Ninhydrin derivatized reagent was delivered and mixed at the flow rate 0.2 ml/min in the reaction column of the analyzer to let it react with amino acids at 120 °C temperature.

Sample preparation

The mushroom sample was extracted with 0.1M HCl by stirring with a magnetic stirrer for 30 min. 1 ml aliquot was taken in 50 ml volumetric flask and diluted up to the mark with eluent A. and kept in the refrigerator until when required for analysis. The extracted mushroom sample was filtered through 0.22 μm cellulose acetate membrane filter units before injection.

Instrumentation

The amino acid composition of the mushroom sample was determined with ion-chromatograph equipped with metrohm 881 compact IC pro anion, metrohm 882 compact IC plus cation, metrohm 886 professional thermostat reactor, metrohm 887 professional auto sampler and metrohm 887 professional uv-vis detector. The amino acids in the standards and in the mushroom sample were separated in the column containing the elution buffers at a temperature of 60 °C with 20-50 μ l injection volume at a flow rate of 0.4 ml/min. Finally, products were sent to the detector and detection was achieved with a uv-vis detector at 570 nm.

Expression of data

Each amino acid was expressed as g/100g dry weight basis and essential amino acid (EAA-geometric mean of the ratios of the essential amino acids in a protein relative to their respective

amounts in reference protein/whole egg) index and biological value (BV) were calculated by using the following formulas [29].

EAA Index

_	7	$\frac{Lys_{p} \times (Phe + Tyr)_{p} \times Leu_{p} \times His_{p} \times Ile_{p} \times Val_{p} \times (Met + Cys)_{p}}{Lys_{s} \times (Phe + Tyr)_{s} \times Leu_{s} \times His_{s} \times Ile_{s} \times Val_{s} \times (Met + Cys)_{s}}$
-	1	$Lys_s \times (Phe + Tyr)_s \times Leu_s \times His_s \times Ile_s \times Val_s \times (Met + Cys)_s$

Where subscript p refers to the food protein; s the standard protein and n the number of amino acids (counting pairs such as methionine and cysteine as one).

Biological Value = 1.09 (EAA Index)-11.73

RESULTS

From the dried sample of *Lentinus sajor-caju* in all 15 amino acids were quantified following standard biochemical procedure [28]. The estimated amino acid amounts (g/100g) are summarized in table 1, 2 and 3 while fig. 1 and 2 depicts the chromatogram of amino acid standards and amino acid content in mushroom sample respectively.

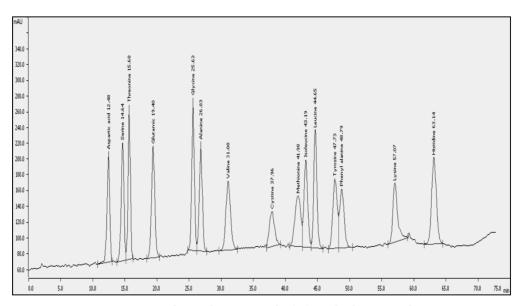


Fig. 1: Ion-exchange chromatograph of 15 standard amino acids

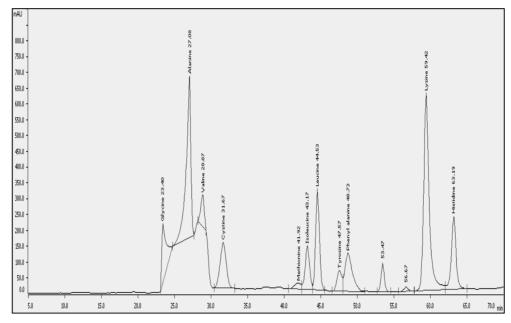


Fig. 2: Ion-exchange chromatograph amino acid profile of Lentinus sajor-caju

The composition of analyzed amino acids revealed the presence of essential and non-essential free amino acids in varying quantity ranging from 6.66 g/100g to 0.16g/100g in the cultivated mushroom. The total amino acid content has been evaluated at 18.82 g/100g. Lysine, alanine, cysteine, phenylalanine, leucine and histidine were present in large amounts while isoleucine, tyrosine, glycine, valine and methionine in reduced amount. Glutamic acid, aspartic acid, threonine and serine were not detected during estimation.

The present work revealed that lysine (6.66 g/100g) was the preponderant essential amino acid in this mushroom. Non-essential amino acid alanine (3.40 g/100g) was found to be the second and cysteine (1.99 g/100g) third most abundant amino acid. Amongst the essential and non-essential amino acids, methionine (0.16 g/100g) was observed as the least abundant amino acid. Amongst

the 7 essential amino acids detected in the presently evaluated mushroom sample, lysine (6.66 g/100g), phenylalanine (1.63 g/100g), leucine (1.39 g/100g) and histidine (1.28 g/100g) were detected in substantial amount in comparison to isoleucine (0.76 g/100g), valine (0.43 g/100g) and methionine (0.16 g/100g). Amino acid threonine was not detected at all.

In the presently evaluated sample lysine (6.66 g/100 g) was found to be in higher concentration in comparison to its required value (5.20 g/100g) in the human diet as prescribed pattern by [30] of the amino acid requirement for 3-10 y old individual. Also in the presently evaluated mushroom sample the concentration (2.25 g/100g) of aromatic amino acids (phenylalanine+tyrosine), histidine (1.28 g/100g) and concentration (2.15 g/100g) of Sulfur amino acids (methionine+cysteine) were found to be very close to the value of reference protein recommended for human nutrition [30].

S. No.	Amino acid	g/100g	
Essential Amino A	cids		
1	Lysine (K)	6.66	
2	Phenylalanine (F)	1.63	
3	Leucine (L)	1.39	
4	Histidine (H)	1.28	
5	Isoleucine (I)	0.76	
6	Valine (V)	0.43	
7	Methionine (M)	0.16	
8	Threonine (T)	ND	
Non-Essential Am	ino Acids		
9	Alanine (A)	3.40	
10	Cysteine (C)	1.99	
11	Tyrosine (Y)	0.62	
12	Glycine (G)	0.50	
13	Glutamic acid (E)	ND	
14	Aspartic acid (D)	ND	
15	Serine (S)	ND	
Total Amino Acid Content		18.82	

ND = not detected

Table 2: Amino acid composition in L. sajor-caju compared to pattern by [30] of amino acid requirements for 3-10 y old individual

S.	Amino	g/100g dry weight	FAO 2013
No.	acid		
1	Lysine	6.66	4.8
2	Phenylalanine+Tyrosine	1.63 + 0.62 = 2.25	4.1
3	Leucine	1.39	6.1
4	Histidine	1.28	1.6
5	Isoleucine	0.76	3.0
6	Valine	0.43	4.0
7	Methionine+Cysteine	0.16 + 1.99 = 2.15	2.3

In the presently evaluated mushroom sample so as to look into its nutritional quality, essential amino acid index (44.63%), biological value (36.93%), ratio of total essential amino acid and total amino acid (65.41%), ratio of total non-essential amino acid and total amino acid (34.59%), ratio of total essential amino acid and total non-essential

amino acid (1.89%), total Sulphur amino acid (2.15 g/100g) and total aromatic amino acid (2.25 g/100g) were also calculated. From the evaluated data as above, it becomes apparent that EAA Index and biological value of this mushroom are reasonably good which are the important indicators of any nutritionally rich food substrate.

Table 3: Nutritional quality evaluation

S. No.	Nutritional quality	Results
1	Total Amino Acid (TAA)	18.82 g/100g
2	Total Essential Amino Acid/Total Non-Essential Amino Acid	1.89 g/100g
3	Total Sulphur Amino Acid (TSAA=Meth+Cys)	2.15 g/100g
4	Total Aromatic Essential Amino Acid (TArEAA=Phe+Tyr)	2.25 g/100g
5	Total Essential Amino Acids (%)	65.41%
6	Essential Amino Acid (EAA) Index (%)	44.64 %
7	Total Non-Essential Amino Acid (TNEAA)/Total Amino Acid (TAA) %	34.59%
8	Biological Value (BV %)	36.93 %

DISCUSSION

Mushrooms are rich sources of proteins as well as amino acids. Because of the variety of substrates used in the cultivation of edible mushrooms and their genetic profile, there is substantial variation in their amino acid content [31]. Amongst the cultivated species, the total amino acid content reported in *Pleurotus ostreatus* grown on different substrates have been reported to range from 35.97 g/100g-42.30 g/100g [32]. As compared only 18.82 g/100g total amino acid content was evaluated presently from the dried sporophores of *Lentinus sajor-caju* raised on the mixture of wheat straw, paddy straw, saw dust and wooden flakes substrate, which is much less in comparison to the range of total amino acids documented in oyster mushroom [32].

Pleurotus citrinopileatus worked by [33] and documented eight essential amino acids, namely leucine, valine, threonine, lysine, phenylalanine, isoleucine, methionine and tryptophan in their decreasing order of abundance. All the essential amino acids reported [34] in adequate quantity in Lentinus polychrous. However, in the case of Lentinus sajor-caju the order of their abundance starts with lysine followed by phenylalanine, leucine, histidine, isoleucine, valine and methionine. Threonine which has been reported [33] to be present in an abundant amount in P. citrinopileatus was found to be altogether absent in *L. sajor-caju* during the present investigation. The total essential amino acid percentage (65.41%) documented in the cultivated sample of L. sajor-caju is substantially low in comparison to the average range of essential amino acid percentage reported [35] for cultivated (42.91%-43.67%) and wild mushrooms (42.90%-43.73%). It is even less than the total essential amino acid percentage (34.65%) documented for Tricholoma matsutake [36].

However amongst the essential amino acids documented in the presently investigated species, lysine (6.66 g/100g) which is vital for hormone production and bone formation [37] is present in substantial amount in comparison to its amount in Lentinus connatus (2.15 g/100g) as documented 38], Lentinus polychrous (1.74 g/100g) as documented [38], Agaricus bisporus (3.57 g/100g) and Pleurotus florida (3.20 g/100g) as documented by [39]. Even it is much higher than reported for *Pleurotus* species (5 g/100g) by [40] and Agaricus bohusii (1.5%) by [41]. However higher percentage of lysine [42] in Agaricus species (6.26%) and Pleurotus sajor-caju (6.33 %) which is comparable to the net amount of lysine (6.66 g/100g) evaluated in *L. sajor-caju* during the present investigations. The deficiency of this amino acid has been reported to result in anemia and chronic fatigue syndrome [37]. L. sajor-caju being a good source of lysine can be of immense utility to take care of the lysine deficiency problems by making it an obligatory part of human diet.

Phenylalanine (1.63 g/100g) was found to be the second most abundant exogenous amino acid in L. sajor-caju. This amount is slightly less than the amount of this amino acid documented in Pleurotus ostreatus (2 g/100g) by [40], in Lentinus connatus (1.90 g/100g) by [38], in Agaricus abruptibulbus (1.92 g/100g) by [43] and on the higher side in comparison to its range (1.27 g/100g-1.44 g/100) documented by [32] in Pleurotus ostreatus. While working with Agaricus species, much higher amount of this amino acid (6.67%) was documented by [42] while much less percentage (0.65%) of this amino acid was also documented by [33] in Pleurotus citrinopileatus. Higher amount of phenylalanine (3.28 g/100g) was reported by [34] in Lentinus polychrous while [41] documented its much less percentage (0.60%) in Agaricus bohusii. This amino acid has been reported to serve as a precursor of tyrosine, which in turn is the precursor of neurotransmitters and is a powerful antidepressant [37].

Different workers have been reported the variable amount of leucine in different edible mushrooms which is a potent stimulator of insulin [37]. The amount of this amino acid evaluated during the present investigations in *L. sajor-caju* (1.39 g/100g) is almost comparable to its percentage documented in *Pleurotus citrinopileatus* (1.07%) by [33]. However, comparatively much lower amount of leucine has been reported in *P. sajor-caju* (0.46%) by [42]. There are number of reports including that for *Lentinus connatus* (2.29 g/100g) [38], for *Pleurotus* species (4.4 g/100g) [40], for *P. ostreatus* (1.76 g/100g-2.31 g/100g) [32], for *Agaricus* species

(14.2%) [42], for *Agaricus bohusii* (2.7%) [41] and for *Lentinus polychrous* (7.64 g/100g) [34] where the presence of much higher percentage of leucine has been reported in comparison to its amount (1.39 g/100g) in the presently evaluated sample of *L. sajor-caju*. Deficiency of leucine has been reported to result in chronic fatigue syndrome [37].

Histidine which has a role in the formation of RBCs and WBCs and treatment of rheumatoid arthritis [37] is present in fairly comparable amount (1.28 g/100g) in *L. sajor-caju* in comparison to its percentage documented in *P. ostreatus* (1.03 g/100g-1.10 g/100g) by [32], but it is on the lower side than documented by [40] in *Pleurotus* species (2.1 g/100g), *Lentinus polychrous* (1.82 g/100g) [34], *Lentinus connatus* (1.45 g/100g) [38] and almost more than double in comparison to its percentage documented in *P. citrinoplieatus* (0.51%) [33]. Much less amount of histidine in *Agaricus bohusii* (0.3%) was reported by [41]. Much higher quantity of histidine (8.26 g/100g) in *Agaricus abruptibulbus* was documented by [43]. The concentration of histidine (1.28 g/100g) in the presently evaluated sample was found to be very close to the suggested pattern of amino acid [30].

Amongst the essential amino acids documented in L. sajor-caju in comparison to lysine (6.66 g/100g), phenylalanine (1.63 g/100g), leucine (1.39 g/100g) and histidine (1.28 g/100g) much less percentage of isoleucine (0.76 g/100g), valine (0.43 g/100g) and methionine (0.16 g/100g) has been evaluated. Almost comparable percentage of isoleucine (0.61%) has been reported in P. citrinopileatus by [33], while in P. ostreatus (1.10 g/100g-1.26 g/100g), in Lentinus connatus (1.35 g/100g) and in Agaricus bohusii (1.8%) much higher percentage of this amino acid has been documented [32,38,41] respectively. In comparison, presence of 5.8 g/100g of isoleucine was reported by [40] in unidentified species of Pleurotus, 9.46 g/100g of isoleucine in Agaricus abruptibulbus was documented by [43] and 3.61 g/100g of this amino acid was reported in Lentinus polychrous [34] which is still on the higher side in comparison to the percentage of its occurrence in all other mushrooms discussed above. Like other amino acids, isoleucine is also important for human health. It is reported to have a role in the formation of hemoglobin and regulation of blood sugar and energy level and its deficiency has been reported to result in chronic fatigue syndrome [37].

Substantially higher percentage of valine (4.65 g/100g), which has a role in tissue repair [37], has been documented in *Pleurotus* species [40], in *P. ostreatus* (1.42 g/100g-1.63 g/100) [32], in *P. sajor-caju* (7.81%) [42], in *P. citrinopileatus* (0.85%) [33], in *Lentinus polychrous* (5.42 g/100g) [34], in *L. connatus* (1.62 g/100g) [38], in *Agaricus abruptibulbus* (4.18 g/100g) 43] and in *Agaricus bohusii* (3%) [41] as compared to the percentage of its occurrence (0.43 g/100g) in *L. sajor-caju*.

Amongst the essential amino acids evaluated in *L. sajor-caju*, lowest amount was documented for methionine (0.16 g/100g), which is substantially on the lower side than documented in *P. citrinopileatus* (0.30%) by [33], *P. ostreatus* (0.42 g/100g-0.53 g/100g) by [32], *P. florida* (1.84 g/100g) by [39], *Agaricus bisporus* (0.98 g/100g) by [39], *Pleurotus* species (1.26 g/100g) by [40], *Agaricus abruptibulbus* (1.62g/100g) by [43], *Lentinus polychrous* (2.25 g/100g) by [33] and *L. connatus* (0.55 g/100g) by [38]. Almost comparable amount of methionine (0.1%) has been documented in *Agaricus bohusii* [41]. Methionine is reported to assist in the breakdown of fats and also serves as an antioxidant [37].

Out of the non-essential amino acids, alanine (18%-45%) which is a major part of connective tissues and help to boost immune system [37] has been reported to be the main component in almost all the mushrooms [44]. Presently also in *L. sajor-caju*, alanine (3.40 g/100g) which has a role in the sugar metabolism and boosting the immune system [44], was found in maximum proportion in comparison to cysteine (1.99 g/100g), tyrosine (0.62 g/100g) and glycine (0.50 g/100g). The documented amount of alanine in the presently investigated species is almost comparable to its amount in *Lentinus connatus* (3.74 g/100g) by [38] and *Pleurotus ostreatus* (3.55 g/100g) as reported by [32]. However, in comparison much less percentage of alanine (1.60%) has been documented in *Agaricus*

bisporus [42] and in Agaricus bohusii (0.8%) [41]. In five wild edible species of Lentinus, namely L. sajor-caju, L. connatus, L. torulosus, L. cladopus and L. squarrosulus, the alanine content has been reported to range from 0.09%-0.15% [45]. Also amongst the five wild Pleurotus species evaluated by [46], maximum (0.19%) percentage of alanine was documented in P. pulmonarius, which is much less in comparison to the proportion of alanine present in L. sajor-caju (3.40 g/100g). The documented amount of alanine in the presently investigated species is much less in comparison to its amount in Agaricus abruptibulbus (7.15 g/100g) reported by [43] and in Lentinus polychrous (11.29 g/100g) reported by [34]. This amino acid is reported to be responsible for giving sweet taste to mushroom [4].

The second most abundant non-essential amino acid evaluated in the presently investigated mushroom is cysteine (1.99 g/100g)which is a natural detoxifier and its deficiency in the body results in food allergy [37]. In comparison, much less cysteine percentage has been documented in *Agaricus bisporus* (0.86%) and *Pleurotus florida* (0.55%) [39]. Even in *P. ostreatus* (0.46%-0.53%) cysteine has been documented in much less proportion [32]. Same is true for *P. citrinopileatus* in which substantially low percentage of cysteine (0.20%) has been evaluated [33], for *L. connatus* (0.43 g/100g) [38] and for *Agaricus bohusii* (0.1%) by [41]. However, in *Lentinus polychrous* almost comparable (1.92 g/100g) amount of cysteine has been documented [34]. In comparison, high cysteine quantity has been documented in *Agaricus abruptibulbus* (2.75 g/100g) by [43].

Tyrosine, which is a precursor of neurotransmitters-dopamine, norepinephrine, epinephrine and melanin [37], has been documented in very less (0.62 g/100g) proportion in L. sajor-caju during the present investigation in comparison to 0.81%-1.13% tyrosine reported in Pleurotus ostreatus[32], Agaricus bohusii (1.4%) by [41], Lentinus polychrous (5.52 g/100g) by [34], L. connatus (1.43 g/100g) by [38] and P. sajor-caju (3.36%) by [42]. However, in P. citrinopileatus almost comparable (0.58%) amount of tyrosine has been documented [33]. While working with five edible Lentinus species from North-West India, tyrosine content ranged from 0.16%-0.24% [45] and in five wild Pleurotus species ranged from 0.14%-0.20% [46], with maximum in P. sapidus (0.20%). As in all other edible mushrooms, the presence of tyrosine in the presently investigated mushroom adds to the nutraceutically credentials of L. sajor-caju, as the deficiency of this amino acid has been reported to result in hypothyroidism.

Glycine, although the present in substantially low (0.50 g/100g) amount in *L. sajor-caju*, is an important structural constituent of hemoglobin and its deficiency results in hypoglycemia and anemia [37]. It is an integral part of commonly cultivated mushrooms including *Agaricus bisporus* (3.02%) and *Pleurotus sajor-caju* (11.1%) as documented by [42]. In the case of *Pleurotus ostreatus*, glycine content ranged from 0.43 g/100g to 1.65 g/100g [32], which is almost comparable to the amount of glycine documented in *L. sajor-caju* (0.50 g/100g). Another oyster mushroom, *Pleurotus citrinopileatus*, also possesses glycine (0.84%) in the proportionate amount [33]. However, in *Agaricus abruptibulbus* (3.21 g/100g) much higher amount of glycine has been documented [43], in *Agaricus bohusii* (1.9%) [41], in *Lentinus polychrous* (5.55 g/100g) by [34] and in *L. connatus* (1.55 g/100g) by [38].

CONCLUSION

Lentinus sajor-caju (Fr.) Fr. is a potential source of quality protein with a substantial proportion of exogenous and endogenous amino acids. In view of its nutritional quality parameters, amino acids composition and the presence of some such amino acids as lysine, phenylalanine, leucine, histidine amongst the exogenous amino acids and alanine, cysteine, tyrosine and glycine amongst the non-essential amino acids, this mushroom can be a good food supplement.

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CONFLICT OF INTERESTS

The authors do not have any conflict of interests

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