

Comparison of Protein and Amino Acids in the Extracts of Two Edible Mushroom, *Pleurotus sajor-caju* and *Schizophyllum commune*

Sujjat Al Azad*, Vivian Chong Ai Ping

¹Borneo Marine Research Institute, University Malaysia Sabah, Kota Kinabalu, Malaysia ²Triniti Farm Enterprise, Kota Belud, Malaysia Email: *sujjat@ums.edu.my, vivian.1103@ymail.com

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Abstract

This study was undertaken to determine total protein (%) and profiles of amino acid and made comparison between the aqueous and organic solvent extracted mushroom. Extraction was made from two edible, Pleurotus sajor-caju (commercial) and Schizophyllum commune (wild) types of mushrooms. Four types of solvents were used for the extraction include 100% aqueous, 50% ethanol, 50% methanol and 50% acetone. True protein of mushroom extract was analyzed with colorimetric Lowry method and amino acids were determined by using high-performance liquid chromatograph (HPLC). The range of 1.06% to 3.43% and 1.30% to 2.17% total protein value were obtained in the extracts of *P. sajor-caju* and *S. commune* respectively, while the highest total protein of 3.43% was determined in aqueous extracted *P. sajor-caju* mushroom. The amount of total amino acids of S. commune and P. sajor-caju were in the range of 308.65 mg/g to 443.84 mg/g and 172.52 mg/g to 400.76 mg/g, respectively. The highest content of 443.84 mg/g total amino acids and 77.08 mg/g of essential amino acids were obtained in the aqueous extracted Schizophyllum commune. On the other hand the total content of essential amino acids (EAA). Essential amino acid of both mushrooms was dominated by leucine along with threonine and alanine, but the highest contents were determined from the extract of Schizophyllum commune. Aqueous extraction was effective in both types of mushroom for the protein components as well essential amino acids compared to other organic solvents that were used in extraction process in this study.

Keywords

Protein, Amino Acids, Extracts, Cultivated, Wild Mushroom

1. Introduction

Nutritional values of mushrooms are closely related to their high protein content. Mushrooms are among the best sources of protein comparable to soybean and chicken meat making them major choice for vegetarians [1]. Fresh mushrooms are known to contain most of the essential amino acids [2]. Bioactive compounds of mushrooms protein compose of lectins, functional immune-modulatory protein, ribosome-inactivating protein, ribonucleases and laccases [3].

Pleurotus sajor-caju and Schizophyllum commune are the two most edible and marketable mushrooms in Sabah (East Malaysia) and are favorable as local dish. In local language, P. sajor-caju is known as "cendawan tiramkelabu" which means gray oyster mushroom and S. commune is named as "cendawan kodop". Both the types are edible, but only *P. sajor-caju* is cultivated commercially by agriculturist inside indoor environment with treated media, while S. commune normally not cultivated commercially rather it grew naturally in rubber wood, but growth depends on the suitable environment conditions like temperature, moist and wood substrates. This wild-grown mushroom generally harvest by people for local consumptions. Schizophyllum commune is abundantly available during the rainy season. Other than that, locals are educated with the benefits of mushrooms as they are reported to exhibit pharmacological properties such as antitumor, cardiovascular regulator, immunity booster, and antioxidant activities (personal communication with local peoples). Wild edible mushrooms are becoming more important because of their medicinal values [4]. In general, fresh mushroom contained high carbohydrates followed by proteins, but the least amounts of lipids. Carbohydrate in *P. sajor-caju* constituted of 65.14% (DW) [5], while in *S. commune* mushroom the carbohydrate content is 81.59% (DW) [6]. Crude protein content of *P. sajor-caju* meal contained higher protein (21.3%) than S. commune [5]. On the other hand higher protein content of 22.51% - 26.34% was observed in the cultivated P. sajor-caju than of 14.55% - 20.67% that obtained from wild varieties. The comprehensive and proportional of amino acids in mushroom is important to know for the quality and quantity of total protein in particular species. Certain amino acid-like threonine, valine and phenylalanine are present in mushroom in sufficient quality like meat protein, while presence of methionine and cysteine are lower in mushroom protein. In addition amounts of amino acids such as lysine and tryptophan in protein of mushroom are quite comparable to that of protein from vegetables [7]. So it is essential to understand the nutritional values of wild and cultures mushroom in terms of amino acid and total protein in locally available mushrooms.

The extraction is one of the common techniques used for the evaluation of bioactive compounds and antioxidant activity in mushroom research. Among the extraction techniques, hot water extraction, and extraction with organic solvents and alkali extraction, etc. are very common in the extraction of those compounds. Researchers are not only looking for bioactive compounds, but also interested to evaluate performances of the yield of product [8]. In *P. sajor-caju* to-

tal polysaccharide of 56% (crude extract) was obtained using boil water (80°C -90°C) extraction process followed by 95% ethanol precipitation [9]. S. commune extracted in autoclaved water at 121°C contained 29% (crude extract) of total polysaccharide [10]. Boiling water and ethanol for precipitation are the common solvents used to extract polysaccharide from mushrooms reported by researchers mentioned above. In *P. sajor-caju* phosphate buffer extract obtained 0.64 mg/mL (crude extract) concentration of protein [11]. In P. sajor-caju aqueous extract contained five EAAs [12], while in Dichloromethane extract contained eight EAAs [13]. On the other hand, seven EAAs were identified in *S. commune* meal [14] and eight of EAAs were identified in *P. sajor-caju* dried meal [15]. So far, there is limited information on protein content and presence of amino acids in S. commune extract. Amino acids profile of P. sajor-caju and S. commune mushrooms have been documented nevertheless information of amino acids contained in mushroom aqueous extracts has yet to be reported. This study was undertaken to evaluate true protein and amino acids of commercially cultured P. sajor-caju and wild variety of S. commune mushrooms extracted with aqueous and three types of organic solvents.

2. Materials and Methods

2.1. Mushroom Preparation

Commercially cultivated mushroom *Pleurotus sajor-caju* and wild mushroom *Schizophyllum commune* were used in this study and collection locally. Freshly plucked *P. sajor-caju* was collected from the local commercial oyster mushroom farm located (General Mushrooms Sdn. Bhd., Kundasan Sabah) While and *S. commune* was purchased from farmer of village weekend market (Tambunan, Sabah), who collected this wild variety mushroom from the rubber woods. Fresh mushrooms were brought to laboratory for cleaning and rinsing to remove debris and insects. Mushrooms were chopped into 2 cm² and placed in the oven at a temperature of 40°C until complete dryness. Dried mushrooms were then blended into fine powder using food processor and stored in zipped plastic bag for extraction.

2.2. Extraction

Aqueous and three types of organic solvents such as, 50% ethanol, 50% methanol and 50% acetone were used in extraction. Mushroom powder and solvent with ratio of 1:10 were mixed well in conical flasks and homogenized by stirring with glass rod. Flasks was kept in incubator shaker to shake at 100 rpm for three days. The mixture was then centrifuged at 5000 rpm for 20 minutes to separate the liquid from mushroom debris. Liquid, extracted portion was collected and filtered with Whatman G/C filter paper. Filtrate was concentrated with rotary evaporator at 40°C temperature. The concentrated extracts were freeze at -80° C prior to freeze drying process. Freeze dried extracts were kept in zipped plastic bag and freeze at -20° C while not in use.

2.3. Determination of True Protein

True protein of mushroom extract was analyzed using modified Lowry method [16]. In brief, protein precipitation was done from 0.5 g of mushroom extract dissolved in 10 mL of 0.1N sodium hydroxide solution and placed in shaker for eight hours. Mixture was centrifuged at 1000 rpm for 10 minutes and five mL of supernatant was collected. One mL of 20% trichloroacetic acid was added to supernatant and stored at 4°C in the fridge overnight for complete precipitation of mushroom extract protein. Supernatant with precipitation was centrifuged at 1000 rpm for 20 minutes. Liquid was discarded and 5 mL 1N NaOH was added into the precipitate. Color development proceeded by obtaining 0.5 mL of sample added with three mL of protein reagent and mixed well. Mixture was left for 10 minutes. Absorbance of the developed color was read in spectrophotometer at absorbance of 660 nm, while Bovine Serum Albumin (BSA) in the range of 100 - 500 µg was used as standard

2.4. Amino Acids Assay

Mushrooms extract amino acids were analyzed using high-performance liquid chromatograph (HPLC) amino acid analyzer (SHIMADZU, model Lab10X) with specific procedure [5]. Hydrolysis was carried out by using 0.2 g of mushrooms powdered extract in 8 mL hydrochloric acid (6N) for 24 hours at 110°C. Hydrolyzed samples were diluted with 25 mL of double distilled water. An aliquot 0.1 mL of sample was added with 0.8 mL of sodium diluent and 0.1 mL of DL-2-aminobutyric acid (AABA). Samples were then filtered with membrane disc filter polytetrafluoroethylene (PTFE), for analysis. The Amino acid separation was derivatized using post column derivatizer (Pickering, model lab PCX5200) with two reagent, sodium hydrochloride and O-pthaladehyde (OPA). The mobile phases consisted of three eluents, eluent A filled with sodium (1700-0112), eluent B filled with sodium (Na₂OH) and eluent C filled with column regeneration (RG011). The amino acids quantification was using a ultra-violet detector at wavelength excitation of 330 nm and emission at 465 nm. Calibration chromatogram was established from 17 known amino acids standards (aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, tryptophan, arginine and valine). The value of amino acids was presented as mg per g of extract.

2.5. Statistical Analysis

The data of true protein and amino acids were collected in triplicates and calculate mean value and standard deviation (SD). The results of true protein were subjected to one way analysis of variance (ANOVA) using SPSS for windows (version 21.0). The significance of difference of true protein was determined according to Duncan's multiple range test. P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Mushroom Dried Extract Yield (%)

Aqueous extraction method of both mushrooms obtained the highest dry extract yield with the percentage comprised of 39.65 and 14.48 in *P. sajor-caju* and *S. commune* respectively. The dry extract yielded in *P. sajor-caju* (25.62% to 39.65%) showed two times higher compared to the amounts of extract obtained in *S. commune* (9.20% to 14.48%). Aqueous extraction observed more efficient in dry extract yield in both types of mushrooms (**Table 1**).

3.2. True Protein in Extracted Mushroom

The true protein value of *P. sajor-caju* and *S. commune* extracts were in the range of 1.38% to 3.43% and 1.30% to 2.17%, respectively (**Table 2**). The highest values (%) of 3.43 ± 0.32 and 2.17 ± 0.28 were determined in aqueous extracts of *P. sajor-caju* and *S. commune* respectively. Although the true protein (%) was obtained higher in all extracts of *P. sajor-caju*, but observed no significance (P > 0.05) differences except in the aqueous extracted sample.

3.3. Amino Acids Profile

A total of 17 types of amino acids were identified in *Pleurotus sajor-caju* and *Schizophyllum commune* mushroom extracts, but variations were observed within species and also among various extracts (**Table 3**). All the nine essential amino acids (EAA) were identified from *S. commune* extract, while only seven types of EAA were found in *P. sajor-caju* mushroom extract. In addition to that the highest amounts of 20.29% to 31.62% amino acid were observed in the extracts of *S. commune* compared to 19.04% to 20.34% of amino acids in *P. sajor-caju* extracts. Aqueous extract of both mushrooms had the highest amount of total EAA (8.95% \pm 0.02%) particularly threonine and leucine contributed the highest

Γa	Ы	e 1.	Mus	hroom	dry	extract	yield	(%)) from	each	extraction	solvents.
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SolventsPleurotus sajor-cajuSchizophyllum commune	
Aqueous 39.65 ± 3.2 14.48 ± 1.2	
Ethanol 27.13 ± 2.0 10.11 ± 0.9	
Methanol 25.62 ± 0.5 9.65 ± 0.2	
Acetone 26.50 ± 2.1 9.20 ± 0.9	

Table 2. True protein (%) contents in the *Pleurotus sajor-caju* and *Schizophyllum commune* after aqueous and different types of organic solvents extraction (mean \pm SD; n = 3).

	Aqueous	Ethanol	Methanol	Acetone
Pleurotus sajor-caju	3.43 ± 0.32^{a}	$1.76 \pm 0.19^{\rm b}$	$1.38\pm0.44^{\rm b}$	$2.06\pm0.24^{\rm b}$
Schizophyllum commune	$2.17\pm0.28^{\rm b}$	$1.69\pm0.24^{\rm b}$	$1.30\pm0.35^{\text{b}}$	$2.04 \pm 1.03^{\rm b}$

In each row different letters imply significant differences (P < 0.05).

Mushroom	Mushroom Pleurotus sajor-caju					Schizophllum commune				
Extract	Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol	Acetone		
Aspartic acid	11.23 ± 0.28	10.31 ± 0.7	10.59 ± 1.10	12.86 ± 1.42	6.74 ± 0.16	28.7 ± 4.2	40.91 ± 0.71	22.84 ± 0.46		
Threonine*	18.29 ± 0.89	7.88 ± 0.61	8.13 ± 0.98	10.37 ± 1.66	24.4 ± 0.40	13.13 ± 1.91	20.14 ± 0.34	15.63 ± 0.28		
Serine	9.00 ± 0.07	11.41 ± 0.7	11.78 ± 1.32	11.52 ± 1.16	9.25 ± 0.12	15.8 ± 2.14	22.61 ± 0.39	16.58 ± 0.31		
Glutamic Acid	14.17 ± 0.37	77.67 ± 4.52	81.6 ± 11.1	81.11 ± 8.72	111.49 ± 1.36	49.51 ± 7.36	64.93 ± 1.16	150.65 ± 2.55		
Glycine	19.43 ± 0.04	7.66 ± 0.45	7.97 ± 0.96	9.83 ± 1.12	24.14 ± 0.19	14.38 ± 1.95	21.70 ± 0.51	15.62 ± 0.26		
Valine*	N.d.	N.d.	N.d.	N.d.	1.16 ± 0.42	8.53 ± 1.42	16.62 ± 0.89	8.44 ± 0.19		
Alanine	39.10 ± 0.43	18.17 ± 1.08	18.93 ± 2.28	23.46 ± 2.48	52.05 ± 0.80	23.31 ± 3.36	32.99 ± 0.55	22.17 ± 0.38		
Cystine	9.00 ± 0.08	4.39 ± 0.46	4.47 ± 0.57	6.70 ± 2.04	23.47 ± 1.70	11.02 ± 1.35	20.03 ± 0.47	13.35 ± 0.33		
Methionine*	3.38 ± 0.34	0.80 ± 0.13	N.d.	1.80 ± 0.34	1.14 ± 0.22	0.32 ± 0.0	0.65 ± 0.23	0.81 ± 0.03		
Isoleucine*	14.37 ± 0.19	3.93 ± 0.17	4.11 ± 0.48	7.04 ± 0.75	1.14 ± 0.22	9.54 ± 1.12	17.67 ± 0.23	9.45 ± 0.16		
Leucine*	24.2 ± 0.02	8.1 ± 0.48	8.38 ± 0.91	13.12 ± 1.45	17.57 ± 1.13	16.91 ± 1.71	22.13 ± 7.88	14.81 ± 0.36		
Tyrosine	N.d.	3.19 ± 0.21	4.04 ± 0.41	5.73 ± 1.38	1.02 ± 0.17	3.10 ± 0.0	16.15 ± 28.73	1.97 ± 0.07		
Phenyalanine*	8.11 ± 0.04	4.08 ± 0.44	4.20 ± 0.65	6.92 ± 13.53	1.27 ± 1.01	4.60 ± 6.36	1.34 ± 0.27	2.70 ± 0.1		
Lysine*	8.36 ± 0.74	11.20 ± 1.16	11.30 ± 1.31	10.7 ± 1.15	7.14 ± 0.11	14.00 ± 1.72	21.71 ± 0.79	13.72 ± 0.3		
Histidine*	9.65 ± 0.13	5.04 ± 0.65	4.86 ± 0.54	5.82 ± 0.66	11.06 ± 0.81	4.60 ± 0.54	6.67 ± 0.45	6.03 ± .57		
Tryptophan*	N.d.	N.d.	N.d.	N.d.	1.48 ± 0.66	14.97 ± 9.27	0.75 ± 0.33	1.40 ± 0.37		
Arginine	2.20 ± 0.06	0.43 ± 0.08	0.43 ± 0.08	0.76 ± 0.69	1.92 ± 0.32	16.22 ± 1.74	20.06 ± 2.3	16.47 ± 0.46		
Total EAA	76.71	35.99	36.12	50.02	77.08	51.44	88.56	58.75		
Total NEAA	113.78	138.27	144.67	157.79	227.00	151.48	232.97	265.32		
Total amino acids	172.52	182.68	430.53	400.76	443.84	308.65	417.82	408.14		

Table 3. Content of amino acids (mg/g) in different solvent extraction of *Pleurotus sajor-caju* and *Schizophyllum commune* (mean \pm SD; n = 4).

N.d.: Not detected; EAA: Essential amino acids (*). NEAA: Non-essential amino acids.

EAA of total EAA composition. On the other hand, total AA of 32.41% was obtained in *S. commune* acetone extract.

4. Discussion

The different extraction solvents used in this study affect the amount of yield extracted as selectivity is characterized by different strength of polarity [17]. Even the variation observed by the same extracts or fresh mushroom being used are consequence of different cultivation process drying temperature, and genetic variation [18]. In this aqueous extracts of *P. sajor-caju* and *S. commune* consistently produced higher yield compared to organic solvents, which might be due to the high amount of polar contents such as protein [12] and antioxidant properties [19] in the mushroom. Antioxidant and antimicrobial properties of these two mushrooms extract reported elsewhere, although was not considered in this study. Extract with organic solvents (ethanol, methanol, acetone) were yielded

lower amounts in both mushrooms than the yield obtained in aqueous extract, but the extractability for some essential compound is better than aqueous extract. The yield, purity, chemical composition, molecular weight distribution, microstructure, and bioactivities of polysaccharides are the resultants of extraction methods and solvents used in the process of extraction [20]. The extraction methods are very specific and depended on the goals of particular study. Methanol extraction which vielded 16.7% to 19.5% observed more efficient on high antioxidant activity in lipid peroxidation [21]. However, present study also able to obtain average of extract yield of 25.62% higher than the extraction obtained in the commercial species of *P. sajor-caju* than the wild mushroom *S. commune*. On the other hand hot aqueous extraction was observed less efficient than the cold aqueous extraction, as bioactive compounds might lose the potency with high temperature [20]. In addition, extraction duration, amount of solvents used and higher temperatures were identified as it block wide commercial application. So optimization of process play very important roles to obtained better yield for specific purposes components [22]. Methanol extract of this mushroom was not observed below the concentration of 20 mg/mL extract [23]. Although the aqueous extraction in this study found to be more effectives, but lower yield was determined in wild S. commune compared to the yield obtained in commercial P. sajor-caju, which may not be effective against other species of edible mushroom [20].

Crude protein contained of *P. sajor-caju* meal was reported higher than that of crude protein determined in *Schizophyllum commune*. In general, the meal of P. sajor-caju contained 21.3% of protein on dry weight basis [5], while 9.63% (dry weight) of crude protein was reported in S. commune [6]. On the other hand, variation was determined in commercially cultivated and wild collected P. sajor-caju. The higher of 22.51% - 26.34% crude protein was observed in the cultivated P. sajor-caju mushroom meal than that of 14.55% - 20.67% crude protein obtained from wild varieties of same species [15]. Variation in Protein contents of different species of mushrooms were depended on mushroom strain/type, composition of growth media, and time of harvest, management techniques, handling conditions, and the preparation of the substrates [24]. True protein is the actual amount of protein contained in the mushrooms whereas crude protein consisted of nitrogen content, taking account of crude protein value as true protein can over estimate the amount of protein for including non-protein nitrogen. Obviously, true protein value of are the resultant of crude protein that contained mushrooms species. Phosphate buffer extract of P. sajor-caju contained 0.64 mg/mL concentration of true protein [11], while methanol extract of Tremellla fuciformis obtained 3.02% of protein. In this study, aqueous extraction was observed the best among the extraction. True protein of 17.14% and 10.7% was determined in *P. sajor-caju* and S. commune respectively. Aqueous extract is the common liquid extraction method used to extract protein from mushroom, Agaricus bisporus [25]. However, the published data on the true protein in S. *commune* extract are limited. This study also revealed that commercially grown *P. sajor-caju* extracts had higher protein compared to protein determined in wild collected mushroom, *S. commune* suggested that the highest protein content in the cultivated mushroom was due to formulated substrate and supplemented media that used in the cultivated mushroom compared to wild mushroom where the derived nutrition depended on natural conditions available in that particular wild [15].

In this study a total of 15 amino acids were detected in *Pleurotus sajor-caju*, whereas 17 types of amino acids were forum in Schizophyllum commune crude extract. But total of 17 amino acids were identified from fresh mushroom of P. sajor-caju [5] and dried mushroom of S. commune [14]. On the other hand, 11 types of amino acids were reported in the aqueous extract of wild *P. sajor-caju* mushroom [12] less than the amino acids that was determined in this study. Irrespective of extraction all the nine types of essential amino acids was detected in S. commune, but in P. sajor-caju extract only seven EAA was identified. Ffluctuations and abundances of EAA in the species of mushroom are the influence of genetic factors (*i.e.* species and strains), the stage of development, the nature of pre- and post-harvest treatments and the type of growth substrate [24]. Five types of EAA obtained in *P. sajor-caju* aqueous extract [12], eight types of EAA was determined in dichloromethane extract [13] and seven types of EAA in S. commune meal [14]. Variation of EAA obtained by same extracts or fresh mushroom being used are consequence of different cultivation process and also because of drying temperature, and genetic variation [26].

Aqueous extracts of this study are proven the highest distribution of threonine in total essential amino acid composition. In P. sajor-caju extract threonine contributed 24% of essential amino acid and S. commune contributed 32%. Threonine of 37% obtained from P. sajor-caju mushroom meal extracted in distilled water [12]. Aqueous extraction demonstrated better extraction of threonine as in mushroom meal. In dry mushroom of P. sajor-caju, threonine and leucine were the main EAA [27]. In this study, leucine, threonine and lysine were the most predominant EAA in both mushrooms extracts. While leucine and lysine were the main EAA in S. commune. Tryptophan was observed limited in P. sajor-caju extract. This might be due that the amount of tryptophan in *P. sajor-caju* is too scarce as reported in mushroom meal, tryptophan was the least amino acid reported of 0.41% [12]. In general, glutamic acid is a dominant amino acid in mushrooms accounted of 13% in S. commune meal [14], 22% in P. sajor-caju meal [5] and 22% in *P. sajor-caju* single cell protein [28]. High glutamic content in mushrooms explained the unique monosodium glutatamate (MSG)-like taste produced from mushrooms. The main EAA of S. commune is leucine [14]. Present study revealed that glutamic acid accumulated 7% to 46% of total amino acid composition in mushrooms extracts which the highest obtained in aqueous extract, but threonine and leucine contributed the highest EAA of total EAA composition. On the other hand, in P. sajor-caju ethanol extract lysine was determined the major component, but also detected in fresh mushroom of same species [28]. Most *P. sajor-caju* is rich with leucine and threonine as essential amino acid when used fresh mushroom in analysis [5]. Proportion of amino acids in a mushroom related with the amount of protein contained in that particular species. The total amino acids in present study were determined in the range of 308.65 mg/g to 443.84 mg/g and 172.52 mg/g to 400.76 mg/g in *S. commune* and *P. sajor-caju* respectively, irrespective of aqueous and solvents extraction. On the other hand, total EAA obtained comparatively higher (51.44 - 88.56 mg/g) in *S. commune* than the total EAA that was found in *P. sajor-caju* (35.44 to 76.71 mg/g). The values of total EAA in *S. commune* in this study was observed higher than that of 34% obtained in edible wild mushroom of same species [14]. The total essential amino acids values of dried mushroom varieties *Pleurotus ostreatus* and *Agaricus bisporus* were determined 39.25 and 44.95 gm/16gm N, respectively, but 41.4% of total essential amino acids was identified in other species of *P. pistillaris* [14].

5. Conclusion

A large amount of protein was found in aqueous extracts of both mushrooms. The amount of protein varied from extract to extract in the same mushroom species of *Pleurotus sajor-caju*. A total of 17 amino acids were identified. All the nine essential amino acids were identified from the extracts of *Schizophyllum commune*, while only seven EAA obtained in was *Pleurotus sajor-caju*. Essential amino acid of both mushrooms was dominated by leucine along with threonine and alanine. These amino acids play an important role in human growth and metabolism. It is apparent that aqueous extraction is effective, obtained total highest of 8.95% in which threonine and leucine contributed the highest EAA of total EAA composition compare to organic solvents.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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