The Content Analysis of Amino Acids in Auricularia auricula from Heilongjiang and Jilin

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Received 28 June 2020; Accepted 16 November 2021; Published 27 November 2021

Academic Editor: Fatma M. El-Demerdash

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The content of amino acids in Auricularia auricula was analyzed by the method of acid hydrolysis and automatic online analysis. The content of total amino acids in A. auricula produced in Heilongjiang was between 68.287 and 110.949 mg/g, and the average was 90.848 mg/g. The content of essential amino acids in A. auricula from Heilongjiang was between 28.847 and 45.757 mg/g, and the average was 37.987 mg/g. The proportion of essential amino acids (EAA) to total amino acids (TAA) in A. auricula from Heilongjiang was between 41.24% and 42.26%. However, the content of total amino acids in A. auricula from Jilin was between 71.716 and 124.143 mg/g, and the average was 94.318 mg/g. The content of essential amino acids (EAA) to total amino acids (TAA) in A. auricula from Jilin was between 39.75% and 41.94%. The content of total amino acids and essential amino acids in A. auricula from Jilin was higher than that from Heilongjiang. However, EAA/TAA in A. auricula from Heilongjiang was higher than that of Jilin. The content of total amino acids in different batches of A. auricula in the same production area was quite different, but the ratio of essential amino acids content to total amino acids content was basically the same.

1. Introduction

Auricularia auricula is a precious edible and medicinal glial fungus [1] and mostly distributed in temperate regions of the northern hemisphere, mainly in Asia, China, Japan, and other countries, and production of China is higher. In China, Heilongjiang, Jilin, Hubei, Yunnan, Sichuan, Guizhou, Hunan, and Guangxi provinces, A. auricula is cultivated and naturally grown [2]. A. auricula has a thousand years of dual-use history of food and drug in China, first recorded in the “Shennong Herbal Medicine.” In the Ming Dynasty, Li Shizhen recorded in the “Compendium of Materia Medica.” A. auricula can treat “Duan-gu-zhi-zhi, Yi-qi-bu-ji, and Qing-shen-qiang-zhi” [3]. A. auricula contains a variety of nutrients such as crude polysaccharide, protein, dietary fiber, minerals, and melanin [4], with antioxidant [5], bacteriostasis [6], anticoagulant [7], hypoglycemic [8], and prevention of atherosclerosis [9] and other pharmacological effects. Studies have shown that the protein content in A. auricula was as high as 10.0–16.2 g/100 g [10]. With the improvement of living standards, people’s awareness of healthcare gradually increased, the healthcare function of A. auricula gradually give rise to attention, but the quality of A. auricula from different production area is uneven. Therefore, the differences of amino acids content in A. auricula produced in two main producing areas (Heilongjiang and Jilin) were analyzed in this study to provide some foundation for the evaluation of nutritional components in A. auricula.
2. Materials and Methods

2.1. Instruments. The content of amino acids in *A. auricula* was detected on an LC-20A amino acid automatic analyzer (Shimadzu, Japan), equipped with a DUG-20A5R online degassing machine, LC-20AB pump, SIL-20A automatic sampler, CTO-20A column temperature box, and SPD-20A UV detector. The data were analyzed on LabSolutions chromatographic workstation (Shimadzu, Japan). Amino acids specific analysis column (C18, 3 μm, 4.6 × 150 mm) was purchased from Shimadzu, Japan. A/10000 electronic balance (ME104/02) was purchased from Swiss Mettler-Toledo Instruments Limited. An Electric blast dryer (DHG-9053A) was purchased from Shanghai Yiheng Scientific Instrument Co., Ltd. Rotary evaporator (EYELA) was purchased from Hangzhou Wahaha Group Co., Ltd. Hydrochloric acid was purchased from Xilong Science Limited (analytical grade). Acetonitrile was purchased from Shanghai Yiheng Scientific Instrument Co., Ltd. Pure water was obtained from Hangzhou Wahaha Group Co., Ltd. 2.5 μM of mixed amino acid standard solution (lot no. 081A1803), internal standard solution (lot no. C3242V01), OPA-derived reagent A liquid (lot no. G4344V02), OPA-derived reagent B liquid (lot no. 129018C02), and FMOC-derived reagents (lot no: F8404V02) were purchased from Shimadzu. All other basic reagents were analytical grade.

2.2. Reagent. Sodium hydrogen phosphate dodecahydrate and sodium tetraborate decahydrate were obtained from the Chinese Medicine Group Chemical Reagent Co., Ltd. (analytical grade). Hydrochloric acid was purchased from Beijing Chemical Factory (superior grade). Methanol and acetonitrile were purchased from Xilong Science Limited (chromatographic grade). Pure water was obtained from Hangzhou Wahaha Group Co., Ltd. 2.5 μM of mixed amino acid standard solution (lot no. 081A1803), internal standard solution (lot no. C3242V01), OPA-derived reagent A liquid (lot no. G4344V02), OPA-derived reagent B liquid (lot no. 129018C02), and FMOC-derived reagents (lot no: F8404V02) were purchased from Shimadzu. All other basic reagents were analytical grade.

2.3. Samples. 10 batches of *A. auricula* were purchased from market, there into, 5 batches were from Heilongjiang and 5 batches were from Jilin.

2.4. Preparation of Samples. 10 batches of *A. auricula* were crushed over 20 mesh. 0.5 g of *A. auricula* was accurately weighed, placed in the hydrolytic tube, and 20 mL of hydrochloric acid (6 mol/L) was added. The hydrolysate tube was put into ice and frozen for 3–5 min and then filled with nitrogen and sealed. The hydrolysate tube was then placed in an electrothermal blast dryer, hydrolyzed at 110°C for 22 h, and then removed and cooled to room temperature. Then, the hydrolysate was filtered into 50 mL capacity. The hydrolysate tube was washed with a small amount of water many times, and the water lotion was transferred to the volumetric flask together, and the distilled water was added to the volume to the scale line and shook well.

Accurately take 1.0 mL of the above constant volume solution in glass bottle with a micropipette, decompress, and concentrate, and 1 mL of water was added to dissolve the residue after drying, then decompress, concentrate, and steam dry. Then, 1 mL of sodium citrate buffer solution (pH 2.2) was added, dissolved, oscillated, and mixed, filter with 0.22 μm, that is, to get the sample solution. 2 parallel was set for each batch sample.

2.5. Chromatographic Conditions. Chromatographic conditions refer to the AJS-01 amino acid analysis method (Shimadzu). The chromatographic column was special column for amino acid analysis (C18, 3 μm, 4.6 × 150 mm), the column temperature was 50°C, and the detection wavelengths were 338 nm and 262 nm. Mobile phase A was buffer (pH 8.2) with disodium hydrogen phosphate dodecylsulfate and sodium tetraborate decahydrate, and mobile phase B was methanol: acetonitrile: water (4.5: 4.5: 1). The elution gradient and flow rate are given in Table 1.

2.6. Determination of Samples. The mobile phase was arranged according to the method of amino acid analysis of Shimadzu (AJS-01), and the amino acid analysis of *A. auricula* was carried out by an automatic analyzer of amino acids (LC-20A).

3. Results

The content of amino acids for 5 batches of *A. auricula* from Heilongjiang and Jilin was determined, respectively, 16 amino acids were detected, including 7 essential amino acids: threonine, valine, methionine, isoleucine, phenylalanine, lysine, and leucine. The results are given in Tables 2 and 3. In general, the higher amino acids in *A. auricula* were aspartic acid and glutamic acid. The highest content of essential amino acids was leucine.

In Table 2, the content of total amino acids in *A. auricula* from Heilongjiang was between 68.287 and 110.949 mg/g, and the average was 90.848 mg/g. The content of essential amino acids in *A. auricula* from Heilongjiang was between 28.847 and 45.757 mg/g, and the average was 37.987 mg/g. The proportion of essential amino acids (EAA) to total amino acids (TAA) in *A. auricula* from Heilongjiang was between 41.24% and 42.26%.

In Table 3, the content of total amino acids in *A. auricula* from Jilin was between 71.716 and 124.143 mg/g, and the average was 94.318 mg/g. The content of essential amino acids from Heilongjiang was between 68.287 and 110.949 mg/g, and the average was 38.498 mg/g. The content of essential amino acids for 5 batches of *A. auricula* from Heilongjiang and Jilin was determined, respectively, 16 amino acids were detected, including 7 essential amino acids: threonine, valine, methionine, isoleucine, phenylalanine, lysine, and leucine. The results are given in Tables 2 and 3.

4. Discussion

Liu et al. [11] determined the content of 17 hydrolysate amino acids in *A. auricula* from 9 different producing areas in Jilin, Heilongjiang, Liaoning, Hebei, Hubei, Xinjiang, Zhejiang, Jiangxi, and Tibet. The content of 7 essential amino acids in *A. auricula* produced in Jilin and Heilongjiang was 26.50 mg/g and 37.07 mg/g, respectively. In this study, the contents of essential amino acids in 5 batches of *A. auricula* produced in Jilin and Heilongjiang were 28.847 and 45.757 mg/g; the results are basically the same. However, different batches of *A. auricula* from the same origin have a large difference in amino acids content, so if we want to evaluate the quality of *A. auricula* from Heilongjiang and Jilin, there into, 5 batches were from Heilongjiang and 5 batches were from Jilin.
from one origin, more than one batch should be selected for experiment.

Wang et al. [12] determined the amino acid content of 8 varieties of \textit{A. auricula}. 16 hydrolyzed amino acids were detected, including 7 essential amino acids. The EAA/TAA of 8 varieties was between 35.61% and 45.91%, with an average of 39.56%. In this study, the EAA/TAA mean of 10 batches of \textit{A. auricula} was 41.30%, and the data were basically consistent.

Lí et al. [13] determined the content of 17 amino acids of \textit{A. auricula} with different cultivation base materials. The results showed that the total content of 17 amino acids in \textit{A. auricula} cultivated with mulberry branches was 10.51% ± 0.05%, the total content of 17 amino acids in \textit{A. auricula} cultivated with wild tusshah trees was 8.49% ± 0.08%, and the total content of 17 amino acids in common \textit{A. auricula} was 9.91% ± 0.05%. It revealed that the content of amino acids in \textit{A. auricula} with different cultivation bases was different. Yuan et al. [14] analyzed the content of amino acids in \textit{A. auricula} with various substrates. The results showed that the content of total amino acids in \textit{A. auricula} cultivated with mulberry, pear, and miscellaneous sawdust was 13.58%, 14.08%, and 9.91%, respectively. It was further proved that different cultivation substrates have great influence on the content of amino acids in \textit{A. auricula}. In this study, the cultivation matrix of \textit{A. auricula} was not clear, but the difference of total amino acids content in different batches of \textit{A. auricula} was obvious from the test results, and the reasons may be different specific areas of origin, different cultivation substrates, and so on.

In addition to the content of amino acids, Liu et al. [11] also analyzed the conventional nutrients of \textit{A. auricula} from different regions. The results showed that the content of ash,
dietary fiber, and total sugar in *A. auricula* from Jilin was higher than that from Heilongjiang; however, the content of crude fat, crude protein, and crude polysaccharide in *A. auricula* from Jilin was lower than that from Heilongjiang. This indicated that the nutrient contents except amino acids of *A. auricula* in different regions were also different.

5. Conclusion

The content of total amino acids and essential amino acids in *A. auricula* from Jilin was higher than that of Heilongjiang. However, EAA/TAA in *A. auricula* from Heilongjiang was higher than that of Jilin. The content of total amino acids in different batches of *A. auricula* in the same production area was quite different, but the ratio of essential amino acids content to total amino acids content was basically the same.

### Abbreviations

| Amino acids | J1 | J2 | J3 | J4 | J5 |
|-------------|----|----|----|----|----|
| Asp | 9.415 | 0.853 | 11.975 | 1.245 | 11.987 |
| Glu | 8.634 | 0.793 | 12.346 | 1.120 | 11.222 |
| Ser | 5.037 | 0.527 | 6.787 | 0.853 | 6.556 |
| His | 1.716 | 0.314 | 2.609 | 0.385 | 2.385 |
| Gly | 3.733 | 0.332 | 5.169 | 0.359 | 4.521 |
| Thr* | 5.229 | 0.736 | 7.389 | 0.032 | 6.153 |
| Arg | 3.828 | 0.432 | 6.136 | 0.524 | 5.417 |
| Ala | 6.022 | 0.492 | 8.146 | 0.882 | 7.828 |
| Tyr | 2.412 | 0.361 | 3.269 | 0.414 | 3.378 |
| Cys-Cys | 1.146 | 0.070 | 1.496 | 0.318 | 1.321 |
| Val* | 4.504 | 0.261 | 5.327 | 0.058 | 5.271 |
| Met* | 2.978 | 0.420 | 3.160 | 0.463 | 3.033 |
| Ile* | 2.910 | 0.176 | 3.859 | 0.066 | 3.287 |
| Phe* | 3.564 | 0.335 | 5.146 | 0.617 | 4.374 |
| Lys* | 4.168 | 0.346 | 6.495 | 0.720 | 5.668 |
| Leu* | 6.423 | 0.555 | 8.601 | 0.705 | 8.251 |
| TAA | 71.716 | 97.907 | 90.648 | 87.176 | 124.143 |
| EAA | 29.775 | 39.976 | 36.035 | 34.644 | 52.063 |
| EAA/TAA (%) | 41.52 | 40.83 | 39.75 | 39.74 | 41.94 |
| TAA mean | 94.318 | 40.83 | 39.75 | 39.74 | 41.94 |
| EAA mean | 38.498 |

* TAA, the content of total amino acids; EAA, the content of essential amino acids; TAA mean, the average of total amino acids content for 5 batches of *A. auricula* from Jilin; EAA mean, the average of essential amino acids content for 5 batches of *A. auricula* from Jilin.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Acknowledgments**

This work was funded by the National Key Research and Development Program of China (2018YFD0400200) and Kaifeng Science and Technology Bureau Project (1908007).

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