

A Study on the Amino Acids in Shii-take (*Cortinellus Berkeleyana* ITO et IMAI) and Masu-take (*Polyporus sulphureus* FRIES)

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Contents

Introduction	215	Discussion	218
Experimental		Summary	218
Materials	215	References	218
Extraction of materials	216	和文摘要	219
Chromatographic method and results	216	Plates V	

INTRODUCTION

The use of fungi as foodstuffs from olden days and especially that of the mushroom doubtless came among first.

The nutritive value of fungi is discussed chiefly from the standpoints of protein content and value, vitamin, fat and mineral. The value of the protein of fungi is determined by its amino acids composition and ROSE (1938)¹⁾ after long series of careful experiments, determined that some nine or ten of amino acids were essential for mankind. The studies of the amino acid composition of yeasts and other proteins have been carried out by many investigators.^{2),3),4),5),6),7),8),9)}

Less complete reports are available as to the amino acid composition of fleshy fungus.^{10),11),12)} The writer tried to find out the amino acids in Shii-take (*Cortinellus Berkeleyana*) and Masu-take (*Polyporus sulphureus*) qualitatively by the paper partition chromatographic method.

EXPERIMENTAL

Materials

Shii-take was collected in Ashikaga, Tochigi prefecture, in Oct., 1951, from the cultured wood, inoculated with the mycerium of the Government Forestry Experiment

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Station. Masu-take was collected from the dead stump, in Oze, Gumma prefecture, in Oct., 1951.

All the experiments were carried out in Feb.-Aug., 1952.

Extractions of Materials

The mushrooms were dried and crushed in a mortar and rinsed with petroleum ether in a Soxhlet's extractor about six hrs. Five grams of each sample was confused in 50 ml. of 80% ethanol, the extractive was added with water and evaporated to 5 ml. under reduced pressure at 60°C.....(Sa) (Shii-take extract), (Sula) (Masu-take extract).

The residue of Shii-take, extracted with ethanol, was dried and confused in 100 ml. of 0.2% KOH about 24 hrs., the upper suspension was transferred into another vessel, the procedure was repeated 3 times and all the suspension was neutralized with dilute solution of acetic acid. Then the crude protein was precipitated.

It was gathered on a filter paper, rinsed with water, and over again the above process was repeated. The precipitate was rinsed with water, then ethanol and ethyl-ether in this order. 5 mg. of crude protein was mixed with 10 volumes of 30% HCl and hydrolyzed by keeping it in an oil bath at 110°-115°C for 10 hrs. The solution was filtered, added with water and then evaporated to 3 ml. (Hs).

The color reactions of these samples were tested and the results were shown in Table 1.

Table 1. Color Reactions of the Samples

	(Sa)	(Hs)	(Sula)	Amino acid whose presence is expected (Polypeptide)
Xanthoprotein Reaction	—	—	—	Tyrosine or Tryptophan
Millon R.	—	—	—	Tyrosine
Adamkiewitz R.	—	—	—	Tryptophan
Liebermann R.	—	—	—	Tryptophan
Pauly R.	+	+	+	Histidine or Tyrosine
Sulphur R.	—	—	—	Cystine or Cysteine
Sakaguchi R.	+	+	+	Arginine

Chromatographic Method and Results

The method of CONSDEN et al. (1944)¹³⁾ as applied to 22 pure amino acids by OHASHI and HATANO (1953)¹⁴⁾ was used. The sample to be analyzed (0.03-0.09 ml.) was placed near the 5 cm. from either edge of the paper (No. 50, Toyo Filter Paper Co. (Tokyo)). The first solvent phenol (containing 10% of 0.1% aqua ammonia)

was allowed to run for 24 hrs. (27-28 cm in length) at 25°C. Then the paper was removed and dried, and the same process was repeated over again in a perpendicular direction. The second solvent butanol-acetic acid-water (4-1-1) was allowed to run for 12 hrs. (27-28 cm in length) at 25°C. After dried, the paper was sprayed with a solution of ninhydrin (0.25% in n-butanol saturated with water) and heated at 95°C for 3 min.

The identification of ninhydrin sensitive substances on the chromatogram was attempted by comparing the positions of various spots (R_f values) and colors with the previous results. Moreover, the presumed pure amino acids were added to the sample in order to see their superposition with unknown on the developed chromatogram could be recognized or not.

The H_2O_2 (perhydrol) pre-treatment was performed to get methionine and cystine. But in this case methionine sulphone and cysteic acid were not obtained. The chromatograms and the amino acids in these samples are shown on Plate V (Fig. 1~3).

Table 2. Amino acids detected by Paper Chromatography

	(Sa)	(Hs)	(Sula)	Color by Ninhydrin Reaction on the Chromatogram
1. Glycine	+	+	+	Reddish purple
2. α -Alanine	+	+	+	R. p.
3. Valine	+	+	+	"
4. Leucines	+	+	+	"
5. Serine		+	+	"
6. Threonine		+	+	"
7. Phenylalanine		+	+	Purplish blue
8. Tyrosine		+	+	Dull purple
9. Lysine		+		Reddish purple
10. Arginine	+	+	+	Purple
11. Proline		+		Yellow
12. Aspartic acid	+	+	+	Purple
13. Gultamic acid	+	+	+	Reddish purple
14. Histidine	+	\pm	+	Blue
15. Unknown (a)	+	+		Brownish purple
16. Unknown (b)	+	+		Reddish purple
17. Unknown (c)	+		+	Purple

Eight amino acids and unknown (a), (b) and (c) were obtained in (Sa). Twelve amino acids and unknown (a) were obtained in (Sula). Fourteen amino acids, unknown (a) and (b) were obtained in (Hs). Unknown (a) was brownish yellow in

color above leucine. Unknown (b) was red purple between α -alanine and valine. Unknown (c) was purplish red under valine.

DISCUSSION

The amino acids in Shii-take and Masu-take, as a whole, are similar in composition compared with other microbial cells.^{3),4),5),6),7),8),9)}

Unknown (b) was found in the ethanol extract of Shii-take, considered to be β -alanine which has been shown to be the derivative of the decarboxylation of aspartic acid.^{16),17)} Unknown (c) found in ethanol extract of Masu-take, considered to be tryptophan.

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SUMMARY

1. The amino acids in Shii-take and Masu-take have been studied qualitatively by the paper chromatographic method.
2. Eight amino acids and unknown (a), (b), (c) were obtained in (Sa). Twelve amino acids and unknown (c) were obtained in (Sula). Fourteen amino acids and unknown (a) and (b) were obtained in (Hs).
3. Proline were not found in ethanol extracts.
4. Leucine and isoleucine were not analysed.
5. Lysine, histidine and arginine were not completely analysed.

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シイタケ・マスタケ中のアミノ酸について (摘要)

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1. ペーパークロマトグラフィー (フェノール-ブタノール・醋酸二次元展開) により, シイタケ・マスタケ中の遊離アミノ酸及びシイタケ粗蛋白構成アミノ酸の検出を行つた。
2. 検出されたアミノ酸は Table 2 に示す如くである。
3. 遊離アミノ酸中にはプロリンが検出出来なかつた。
4. ロイシンとイソロイシンとは分離出来なかつた。
5. リジン, ヒスチジン, アルギニン等は展開しにくかつた。

Fig. 1 The spots show the positions of amino acids taken up on the chromatogram by (Sa).

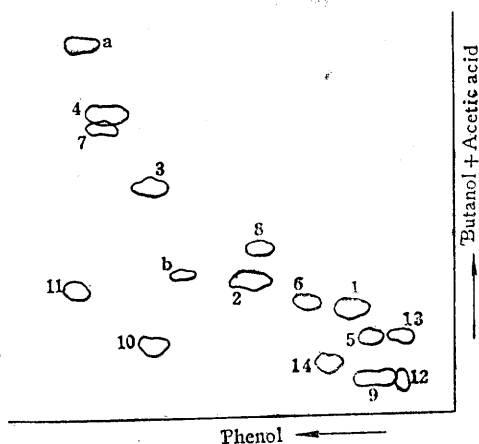


Fig. 2 The spots show the positions of amino acids taken up on the chromatogram by (Sula).

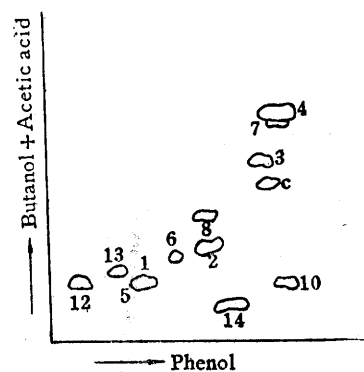
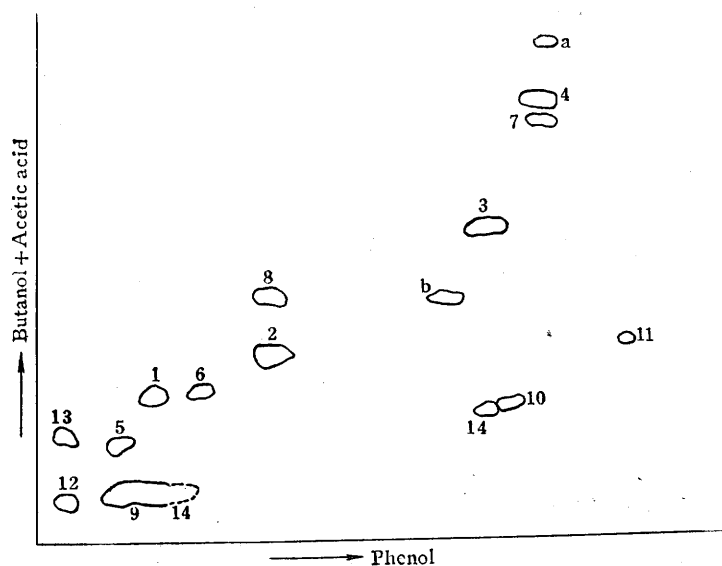


Fig. 3 The spots show the positions of amino acids taken up on the chromatogram by (Hs) of 14 amino acids.



Figs. 1~3 The maps of the spots of the two dimensional Paper Chromatograms of samples from Shii-take and Masu-take.

The marks on the maps;

1. glycine 2. α -alanine 3. valine 4. leucines 5. serine 6. threonine 7. phenyl-alanine 8. tyrosine 9. lysine 10. arginine 11. proline 12. aspartic acid 13. glutamic acid 14. histidine a. unknown (a) b. unknown (b) c. unknown (c)