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Amino Acid Composition of the Morel Mushroom

Richard L. McKellar and Robert E. Kohrman*

Freeze-dried samples of the morel mushroom (*Morchella spp.*) as well as other genera were hydrolyzed with hydrochloric acid and the free amino acids isolated by ion exchange chromatography. The amino acids were derivatized with *n*-propyl alcohol and heptafluorobutyric anhydride. The volatile *N*-heptafluorobutyl-*n*-propyl derivatives were separated and quantitatively determined by gas chromatography. Seventeen amino acids were identified as major components in most species. The method of analysis did not permit the identification of tryptophan, although a few unidentified minor components were detected. Variation of composition as a function of species, genus, and sample source is discussed as well as the efficacy of the gas chromatographic technique.

The morel mushroom (*Morchella spp.*) is avidly sought by connoisseurs throughout the world. Because of its delicate flavor and nutritional potential as a protein source (Litchfield et al., 1963a; Litchfield, 1967a) morel mycelia have been grown in submerged culture on a commercial scale while fruiting bodies of the morel have so far resisted efforts toward cultivation.

Previous investigators have reported marked variances in chemical composition among samples of cultured mushroom mycelium as a function of growth conditions and nutrients (Litchfield et al., 1963a; Litchfield, 1967b).

In the case of the cultivated mushroom, *Agaricus bisporus*, alanine, ammonia, and arginine contents of the tissue decreased, and the aspartic acid content increased when gelatin was added to the compost (Kissmeyer-Nielsen et al., 1966). However, when hydrolyzed casein was added to the compost, the arginine content decreased. There is a lack of information in the literature on the chemical composition of mushrooms as affected by environmental conditions in natural habitats.

While morel mushrooms are easy to recognize in the field (Boudier, 1897), distinguishing between various species and varieties is difficult and is a matter of some uncertainty (Gilbert, 1960; Imbach, 1968). The amino acid composition of morels might provide an improved means for identifying these organisms as well as provide an index of protein quality and flavor characteristics.

Previous studies have determined qualitatively (Litchfield et al., 1963b; Litchfield, 1967b; Janardhanan et al., 1970) and quantitatively (Litchfield et al., 1963b) the amino acid pattern in cultured morel mycelium, while Hatanaka and Terakawa (1968) have reported the qualitative examination of morel fruiting bodies for nonprotein amino acids and recently Hatanaka (1969) has isolated a new amino acid, *cis*-3-aminoproline, from three morel species. The literature contains no quantitative data that compare the amino acid composition of naturally occurring morels with that of the cultured mycelium.

Interest in gas chromatography as a reasonably inexpensive and rapid means of determining amino acid composition has increased with particular emphasis upon single column analysis. Gehrke and others have developed the derivatizing techniques enabling efficient separation (Gehrke et al., 1971). A recent study suggests that complete single column separation of 20 amino acids may be effected via the *N*-heptafluorobutyl-*n*-propyl derivatives (Moss et al., 1971).

The rapidity with which gas chromatographic techniques may be applied suggested the applicability of the single column technique to amino acid analysis of fungal tissue. This paper reports the results of such a study.

EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard Model 5750 gas chromatograph, equipped with a flame ionization detector, was used in all of the analyses. The recorder has an input of 0–5 mV and a chart speed of 0.25 in./min with a working sensitivity of 16×10^{-11} A. The column was a 3.65 m × 3 mm i.d. coiled glass column packed with 3% OV-1 on Chromosorb W (HP), 80–100 mesh. A detector temperature of 290° and an injector temperature of 270° were maintained throughout the investigation. The column

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Table I. Results of Analyses of Morel Mushrooms for Amino Acid Content (g/16 g of Protein N)

Amino acid	*M. esculenta*		*M. deliciosa*	*M. crassipes*	*M. angusticeps*	*M. esculenta*		*M. crassipes* cultured	*H. esculenta*	*V. bohemica*	*A. campestris*
	Stalks	Caps				Cultured	Cultured (Litchfield)				
Ala	4.4	4.6	2.2	4.2	3.3	1.9	4.8	8.7	3.5	3.4	4.7
Gly	3.7	4.0	2.3	2.8	3.2	1.2	2.9	4.7	3.0	3.1	2.8
Val	4.0	4.1	2.5	2.1	2.8	1.4	3.4	5.2	3.2	3.3	3.4
Thr	3.1	3.0	3.0	3.6	2.6	0.8	3.0	3.6	2.4	2.4	2.8
Ser	2.4	2.8	2.9	3.3	2.1	0.6	3.0	3.4	2.2	1.8	2.7
Leu	4.8	4.3	3.6	4.8	3.6	2.0	5.1	6.7	4.0	4.0	3.9
Ile	4.7	2.9	2.7	2.9	2.5	1.2	2.7	3.7	2.8	3.0	2.4
Pro	3.0	3.8	2.2	2.5	2.6	0.9	4.2	4.0	2.6	2.1	2.8
Met	0.8	0.7	0.9	0.6	0.8	0.4	0.9	1.1	0.8	0.6	0.8
Asp	4.8	5.5	5.2	4.9	3.2	1.8	5.0	5.1	3.7	5.1	4.2
Phe	3.0	2.6	3.2	3.2	2.6	1.2	2.5	4.1	3.0	2.7	3.1
Glu	8.6	8.8	10.0	8.3	7.5	2.6	15.0	10.0	9.6	8.4	9.1
Lys	4.2	3.8	3.6	3.4	3.1	1.5	3.8	5.9	3.2	3.8	4.0
Tyr	2.1	1.8	2.5	2.5	1.3	0.7	1.7	2.7	1.4	2.0	1.0
Arg	1.5	3.2	3.2	3.0	2.9	1.4	8.0	4.7	3.7	3.4	3.0
His	2.3	0	0	0	0	1.5	2.1	0.6	0	0	0
Trp							0.9				
Cys	0	0.3	0.6	0.6	0.2	0.	0.3	0.9	0.5	0.8	0.9

temperature was 75° at the time of injection. After injection the column temperature was held at 75° for 3 min and then programmed at 4°/min to 255° and held until the last amino acid, cystine, was eluted. Gas flows were optimized at 40 ml/min for the carrier gas nitrogen and at 40 and 300 ml/min for the burner gases, hydrogen and oxygen, respectively.

Samples. All samples of morels were collected in the environs of Mount Pleasant, Mich. Samples of *Agaricus campestris* were obtained at a local grocery, while cultures of *M. esculenta* and *M. crassipes* were obtained from the American Type Culture Collection, Rockville, Md. The cultures were grown on synthetic media as generally described in previous work (Block et al., 1953; Litchfield et al., 1963a). Shortly after collection the samples were cleaned, homogenized with a Waring Blendor, frozen, and dried under reduced pressure.

Hydrolysis. A 250-mg portion of a freeze-dried mushroom was weighed into a 1.5 × 15 cm screw-cap culture tube. Fifteen milliliters of 6 *N* hydrochloric acid was added to the tube which was capped tightly and shaken for 3 min on a vortex shaker. The sample was heated for 4 hr at 145°. After cooling, the sample was filtered on a 0.5-cm pad of filter-aid placed in a 15-ml Buchner funnel fitted with a fritted disk. The solution was evaporated under vacuum to about 1 ml on a rotary evaporator with the flask immersed in an 80° water bath. This solution was chromatographed on a 2 × 5 cm cation exchange column (50W-X8, hydrogen form). The amino acids were eluted from the column with 25 ml of 3 *N* ammonium hydroxide, followed by 25 ml of deionized water. The eluate was evaporated to dryness under vacuum on a rotary evaporator at 80°. The residue was redissolved in 5 ml of 0.1 *N* hydrochloric acid. A 2-ml aliquot was then taken for derivatization. The optimum hydrolysis conditions were determined utilizing the common field mushroom (*A. campestris*).

Derivatization. The *n*-propyl esters of the amino acids were prepared by pipetting a 2-ml aliquot of a standard solution containing a mixture of 0.4 mg/ml in 0.1 *N* hydrochloric acid into a 1.5 × 7 cm culture tube and evaporating to near dryness in an 80° water bath under a stream of nitrogen. The remaining water was then taken off by successive azeotropic distillations with two 1-ml portions of methylene chloride. A 1-ml portion of *n*-propyl alcohol, made 3 *N* in hydrochloric acid, was then allowed to react at 100° (steam bath), for approximately 30 min. The tube was cooled, and the excess *n*-propyl alcohol and/or hydrochloric acid was evaporated to near dryness. The sample was again azeotropically distilled with one 1-ml portion of methylene chloride. Optimal esterification conditions were determined for each amino acid. The acyl derivatives were prepared by taking the esters and adding 0.5 ml of ethyl acetate and 0.5 ml of heptafluorobutyric anhydride. This mixture was heated at 150° for 30 min and cooled immediately. Optimal acylation conditions were determined for each amino acid.

Calculations. Analyses which had been previously done (Kohrman and Allen, 1971) provided the basis for the calculation of protein nitrogen. Kjehldahl analyses gave percent nitrogen values consistent with previously reported percentages (Litchfield et al., 1963b). Multiplication of percent nitrogen by 6.25 gave a representative value for crude protein content.

Procedures and supplementary data assessing the reproducibility of the gas chromatographic technique utilized are available on microfilm (see paragraph at end of paper regarding supplementary material).

RESULTS AND DISCUSSION

The data obtained for the morels as well as data for several other mushrooms are shown in Table I. As has been previously observed (Litchfield, 1967a), all samples analyzed proved to be deficient in the sulfur-containing amino acids. Presumably, if morel tissue were to be used as a protein source, it would be used as a food supplement. Moreover, it is clear that these values do not necessarily relate to actual protein nitrogen and are used here primarily as a means of comparison with previous work.

The freeze-dried samples include both stalks and caps as contained in the natural samples. However, one species, *Morchella esculenta,* was analyzed separately with respect to cap tissue and stalk tissue. The data suggest that while the amino acid pattern of these tissue types is similar, there are significant differences. The arginine concentration in the stalks (1.5 g/16 g of protein N) is somewhat lower than in the caps (3.2 g/16 g of protein N). The stalks contain histidine (2.3 g/16 g) but no de-

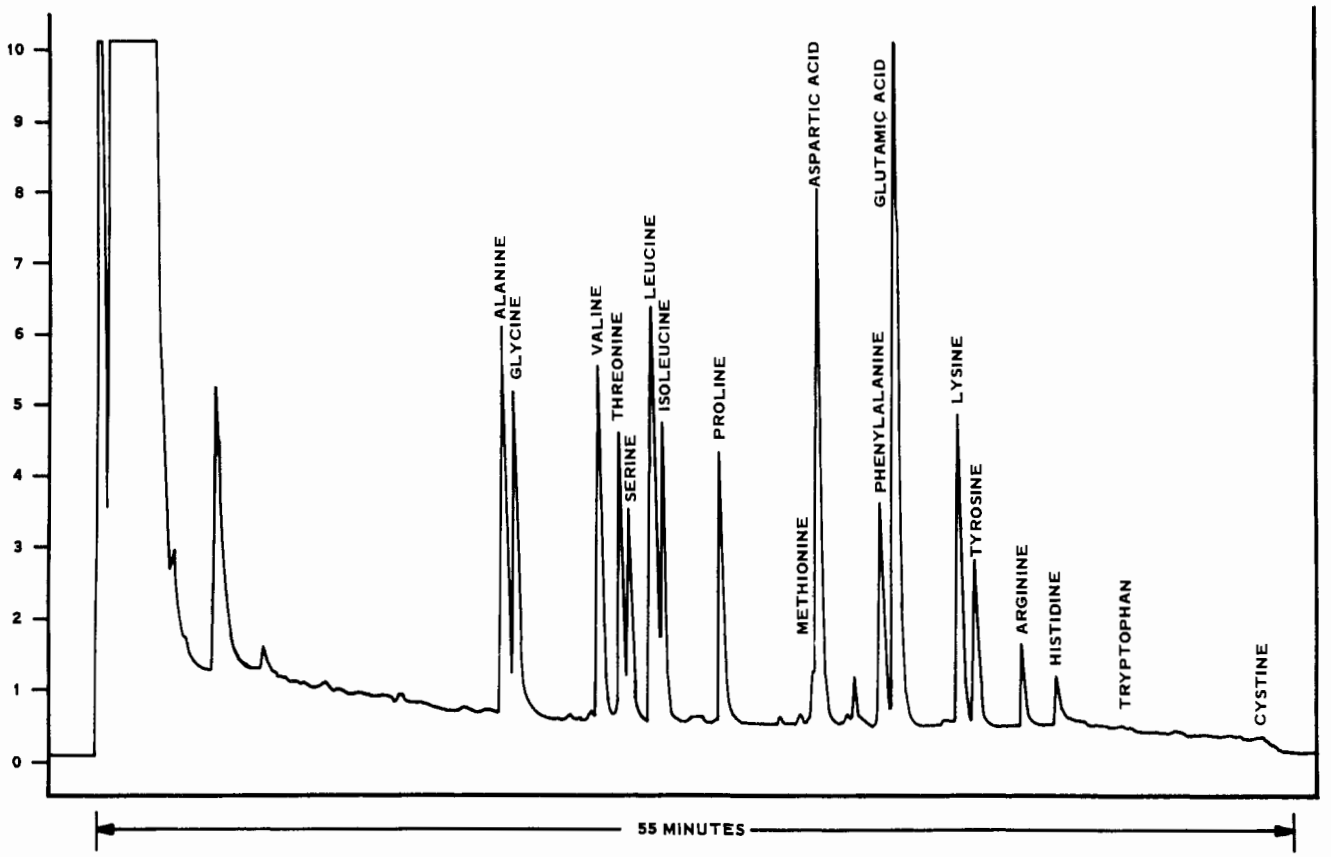

Figure 1. Chromatogram of the *N*-heptafluorobutyl-*n*-propyl derivatives of the amino acids of the mushroom *Morchella esculenta*.

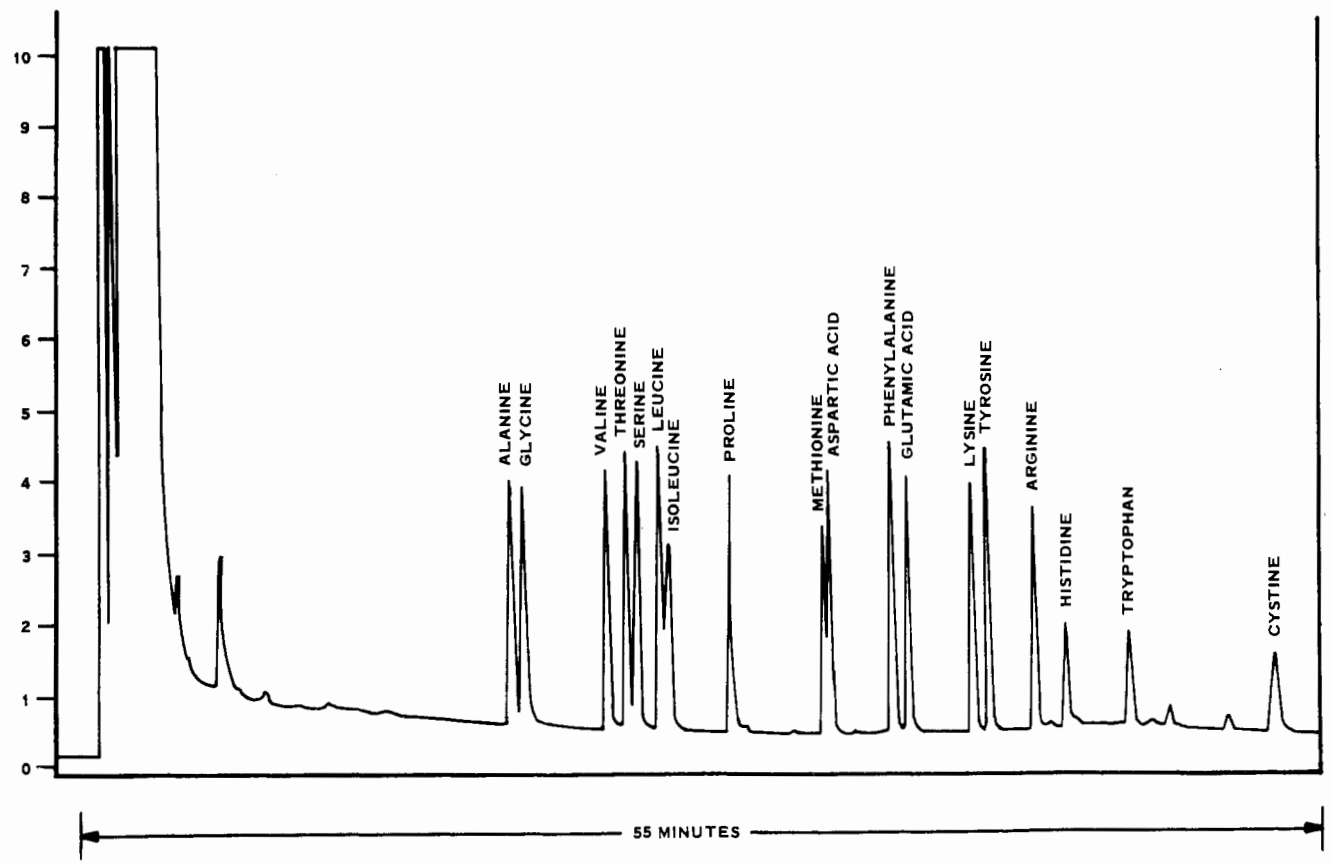

Figure 2. Chromatogram of the *N*-heptafluorobutyl-*n*-propyl derivatives of a standard mixture of 18 amino acids.

tectable cystine, and the caps contain cystine (0.3 g/16 g) and no histidine. The vegetative stipe tissue thus parallels cultured mycelial tissue because it contains significant amounts of histidine, while the reproductive cap tissue does not contain detectable amounts.

Other similarities between natural and cultured tissue

exist in the qualitative distribution of amino acids although differences exist in the relative order of magnitude. With respect to *M. esculenta,* there is reasonably good agreement between values previously obtained (Litchfield, 1967a; see also Table I) and the naturally occurring samples. There is poor agreement with those values obtained from *M. esculenta* cultured in the present investigation. This discrepancy may be related to the differences in culture medium which may significantly alter growth patterns (Block et al., 1953; Litchfield, 1967a,b; Brock, 1951; Reusser et al., 1958). Several of the previously mentioned authors have indicated a lack of reproducibility with respect to flavor of different cultures of morel tissue. Should the amino acid fraction be significantly involved in the characteristic morel flavor, this would be understandable in light of the above results.

Because of the ease of commercial availability of morel mycelial cultures and for purposes of comparison to previous studies, cultured samples were not obtained from specimens collected in the wild. A more precise comparison of amino acid composition of fruiting bodies to that of the mycelium would be obtained if the collected sporacarp tissue were cultured and analyzed.

Although collection of fruiting bodies from natural habitats somewhat precludes rigorous control over variables affecting growth, it is clear that a similarity exists between different species of morels. *M. esculenta, M. deliciosa, M. crassipes,* and *M. angusticeps* have similar distributions of amino acids as well as similar relative orders of magnitude. *Hevella esculenta* and *Verpa bohemica* occasionally are mistaken as morels and consequently have earned the label "false morels". These mushrooms have amino acid patterns similar to that of the true morels as does *Agaricus campestris,* the common field mushroom. Indeed, it would appear that amino acid analysis of fungi would not be useful as a chemotaxonomic tool but rather reflects a distribution of primary metabolites. Recent investigations suggest that unique amino acids may act as marker compounds because they would be indicative of either a particular species or genus. One such compound has been discovered for the morel mushroom (Hatanaka, 1969). The present study did not identify this compound although it may have been present as one of the unidentified minor constituents (see Figure 1).

Single column gas chromatography does appear to be a viable technique for amino acid analysis of fungal tissue. It is fast, inexpensive, and reproducible. Typical chromatograms are shown for both a typical mushroom sample, *M. esculenta* (Figure 1), and a standard amino acid mixture (Figure 2). A slight overlap of peaks is seen where leucine and isoleucine as well as methionine and aspartic acid are not completely resolved. The total chromatographic time is 55 min.

The method as designed will detect a minimum of 0.1 μg of each amino acid except tryptophan, which is completely destroyed during the hydrolysis. Converting 0.1 μg, the minimum detectable amount, into grams per 16 g of protein nitrogen gives a minimum detectable limit for each amino acid of 0.05 g/16 g of protein nitrogen. It would appear from the data that the error in measurement is very small with respect to the variance in results due to sampling, growth nutrients, and maturity. Although the technique is quite effective, amino acid profiles of morels do not appear to be a reliable chemotaxonomic aid. Present evidence indicates that morel tissue, cultured or natural, may be a valuable protein supplement while further studies are in progress to determine major components of the distinctive morel flavor.

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Supplementary Material Available. Quantitation procedures and three tables of data utilized for determining gas chromatographic reproducibility will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-464.

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