# Composition of Cultivated Mushrooms (*Agaricus bisporus*) during the Growing Cycle as Affected by the Nitrogen Source Introduced in Composting

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Cultivated mushrooms (*Agaricus bisporus*) were grown on composts with different nitrogen integrators [urea or $(NH_4)_2SO_4$]. Total nitrogen, urea, ash composition, free and protein amino acids, lipids, and fatty acids were determined at the first and fourth breaks. Substitution of ammonium nitrogen by urea was followed by an increase in ash, potassium, urea, aspartic acid, alanine, valine, and sulfur amino acids, and a decrease in free and protein amino acids, proline, and arginine. Variations due to nitrogen integrators became less pronounced from the first to the fourth breaks. Following the succession of breaks in both $(NH_4)_2SO_4$ and urea integrated cultures, ash, total nitrogen, total amino acids, proline, and phenylalanine rose while aspartic and glutamic acids, lysine, ornithine, urea, and the sulfur amino acids decreased.

The effectiveness of various fertilizers introduced as integrators in composts used for growing cultivated mushrooms is generally evaluated on the basis of total production. Nevertheless, the determination of single components in the sporophores can provide further information on the suitability of the production for industrial preservation by canning.

Knowledge of the variations of such components during the growing cycle can also shed light upon the causes of the exhaustion of growing beds.

Hughes *et al.* (1958) demonstrated that the tyrosine content of mushrooms decreased through the first, third, and fifth crops; Hughes (1961) also observed some other variations in free amino acids throughout the growth period, and noted (1962) that the fatty acid composition was dependent upon the variety of the mushroom. Variations in the free amino acid pool from the first to the fourth breaks were also observed by Kissmeyer-Nielsen *et al.* (1966).

Ammonium sulfate, a commonly used nitrogen integrator, was used in the present study, either exclusively or by substituting for it large amounts of urea nitrogen, to compare the composition of sporophores. For both supplementation experiments, differences in the composition of the product at the beginning of the cycle (first break) and at an advanced stage (fourth break) were also evaluated.

## EXPERIMENTAL

Trials were carried out at a mushroom farm, using the cream (Somycel 87) variety of *Agaricus bisporus*. During the composting period, one portion of the horse manure being employed to prepare the growing beds was integrated with 1 kg. per cu. meter of nitrogen as $(NH_4)_2SO_4$, and another portion with the same quantity of nitrogen supplied as a mixture of urea and $(NH_4)_2SO_4$ with a nitrogen ratio of 2 to 1. Other practices concerning preparation and improvement of growing beds were carried out in an identical way.

Double samples (2 kg. each) from the first and fourth breaks were collected for each trial; after grinding and mixing, suitable aliquots of each sample were reserved for the various determinations which were run in duplicate. The results fell within the limits of precision of the analytical procedures.

**Dry Matter.** Fifteen grams of fresh material were dried to constant weight in a 105° C. oven.

**Ash.** Fifty grams of fresh product were ashed overnight at 550° C.; in the residue, which was dissolved in 2 ml. of concentrated HCl and diluted to 100 ml. with distilled water, K, Na, and Ca were determined by means of a flame photometer (Lange M6A), and Fe and P by colorimetry as a thiocyano complex (Charlot, 1966) and molybdenum blue (Rennie, 1956), respectively.

**Total Lipids and Fatty Acids.** One kilogram of fresh material, dried at 80° C., was ground in a mortar with calcinated quartz, and extracted in a Soxhlet extractor for 24 hours using ethyl ether; after evaporation of the solvent, the residue was placed in a vacuum desiccator until constant weight was attained. Ethyl esters of the fatty acids were prepared by refluxing in an absolute ethanol solution of sodium ethylate according to the "Metodi ufficiali di analisi" (1964). Refluxing was continued for 12 hours to produce complete esterification. The material obtained, viscous and dark brown due to oxidation, was purified on an alumina 50 × 10 mm. column ($Al_2O_3$ Merck) using ethyl ether as the eluent; then it was subjected to gas chromatography (Fractovap C Carlo Erba) for determination of fatty acids.

**Nitrogen Compounds.** Total nitrogen was determined by the Kjeldahl procedure according to the AOAC (1965) method.

**Free Amino Acids.** Five grams of the fresh product were homogenized in 50 ml. of water at 15,000 r.p.m. for 3 minutes, then poured into 200 ml. of boiling absolute ethanol. After centrifugation, the residue was extracted at 60° C. for 10 minutes, three times with 80% ethanol, then twice with 20% ethanol. The collected extracts

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were mixed, evaporated to dryness, dissolved in water, and poured onto an Amberlite IR 120 (20 × 500 mm.) column.

Amino acids and urea were eluted with a 2*N* ammonia solution, evaporated to dryness under vacuum, and dissolved in 10% 2-propanol.

Two-dimensional paper chromatography (Partridge's solvent in the first and phenol–$H_2O$, 100 to 5, v./v., in the second run) and ion exchange chromatography according to Moore *et al.* (1958) were carried out.

Urea was determined colorimetrically using the method of Ceriotti and Spandrio (1963) as modified by Kissmeyer-Nielsen *et al.* (1966).

**Protein Amino Acids.** Hydrolysis in boiling 6*N* HCl was performed on the residue from the extractions, and the amino acids were analyzed using the above methods.

Cystine was determined directly from 2 grams of fresh material by performic acid oxidation to cysteic acid according to Moore (1963) and column chromatography by the method of Lewis (1966).

## RESULTS AND DISCUSSION

Total production of fresh material [8.70 kg. per sq. meter within six breaks on urea integrated compost, and 8.25 kg. per sq. meter when $(NH_4)_2SO_4$ was used] and total nitrogen in the sporophores were not affected by the kind of nitrogen integrator. Ash was greater when urea was used (Table I), and the difference consisted of potassium, which represented over 50% of the ash, and iron. Other elements did not vary remarkably.

The most striking changes concerned the free amino acids (Table II), whose total content was lower when urea was used.

At the beginning of the growing cycle, urea limited the proline content greatly and to a lesser degree the arginine content, while methionine, aspartic acid, valine, and α-alanine increased.

At the fourth break, the differences were attenuated, and only the proline and α-alanine variations persisted.

The increase in amino acid content with $(NH_4)_2SO_4$ was also evident in the acid hydrolyzates (Table III), although the single variations with respect to urea were not so striking as in the free amino acid pool.

Urea was present in larger amounts in mushrooms grown on urea integrated beds, at both the first and fourth breaks; however, its content was rather low compared with the data reported by Ivanoff (1923).

Synthesis of sulfur amino acids is stimulated by urea; therefore, gypsum introduced in composting appears to provide the required amount of available sulfate.

The variations due to the progress of the growing cycle and the consequent exhaustion of composts concerned the ash and nitrogen compounds. An increase in total nitrogen, ash content, without noticeable differences in single elements, and in both free and protein amino acids was observed.

**Table I. Composition (Per Cent of Dry Matter) of *Agaricus bisporus* at First and Fourth Breaks, Obtained on Urea and $(NH_4)_2SO_4$ Integrated Composts**

| | Break | | | |
|---|---|---|---|---|
| | 1 | 4 | 1 | 4 |
| | Urea | | $(NH_4)_2SO_4$ | |
| Moisture | 90.50 | 88.36 | 89.00 | 88.00 |
| Dry matter | 9.50 | 11.64 | 11.00 | 12.00 |
| Nitrogen | 6.29 | 7.95 | 6.78 | 7.73 |
| Fat | 1.86 | 2.15 | 2.20 | 1.66 |
| Ash | 10.75 | 11.74 | 9.13 | 10.29 |
| Ca | 0.18 | 0.20 | 0.18 | 0.17 |
| K | 4.68 | 4.37 | 3.77 | 3.80 |
| Na | 0.50 | 0.85 | 0.45 | 0.75 |
| P | 1.15 | 1.19 | 1.25 | 1.26 |
| Fe (mg. per 100 grams) | 23.0 | 32.0 | 19.0 | 27.0 |

**Table II. Free Amino Acids and Urea of *Agaricus bisporus* Grown on Urea and $(NH_4)_2SO_4$ Integrated Composts at First and Fourth Breaks**

| | Break | | | |
|---|---|---|---|---|
| | 1 | 4 | 1 | 4 |
| | Urea | | $(NH_4)_2SO_4$ | |
| Amino acid nitrogen (mg. per gram) | 11.01 | 14.78 | 12.17 | 13.96 |
| Glutamic acid | 25.04 | 21.90 | 24.74 | 22.27 |
| Proline | 6.49 | 21.61 | 18.39 | 26.17 |
| α-Alanine | 15.72 | 15.99 | 13.44 | 13.05 |
| Aspartic acid | 13.40 | 6.50 | 11.86 | 6.17 |
| Lysine + ornithine | 10.12 | 6.76 | 11.86 | 6.45 |
| Serine | 7.56 | 8.28 | 6.97 | 8.60 |
| Arginine | 2.02 | 4.08 | 3.66 | 3.24 |
| Glycine | 3.48 | 2.56 | 2.53 | 3.83 |
| Leucine | 3.01 | 1.70 | 2.02 | 1.35 |
| Valine | 2.12 | 2.65 | 0.69 | 2.00 |
| Threonine | 2.46 | 1.13 | 1.77 | 1.14 |
| Isoleucine | 1.83 | 1.35 | 1.45 | 1.18 |
| Phenylalanine | 0.43 | 1.86 | 0.41 | 2.54 |
| Tyrosine | 0.76 | 1.70 | 0.72 | 0.65 |
| Methionine | 3.48 | 0.56 | 0.43 | 0.51 |
| Histidine | 0.80 | 0.77 | 0.71 | 0.59 |
| Urea | 1.21 | 0.51 | 0.87 | 0.20 |

**Table III. Protein Amino Acids of *Agaricus bisporus* Grown on Urea and $(NH_4)_2SO_4$ Integrated Composts at First and Fourth Breaks**

| | Break | | | |
|---|---|---|---|---|
| | 1 | 4 | 1 | 4 |
| | Urea | | $(NH_4)_2SO_4$ | |
| Amino acid nitrogen (mg. per gram) | 15.79 | 17.54 | 17.76 | 21.86 |
| Tyrosine | 14.53 | 16.24 | 14.28 | 14.14 |
| Glutamic acid | 9.28 | 9.57 | 8.58 | 9.68 |
| Aspartic acid | 8.14 | 8.73 | 8.71 | 8.55 |
| α-Alanine | 8.40 | 8.20 | 7.78 | 8.36 |
| Glycine | 8.00 | 8.24 | 8.40 | 8.03 |
| Leucine | 7.12 | 6.83 | 6.77 | 7.41 |
| Lysine + ornithine | 6.45 | 5.80 | 7.12 | 6.09 |
| Valine | 5.78 | 5.83 | 5.75 | 5.61 |
| Serine | 5.65 | 6.10 | 5.53 | 5.76 |
| Threonine | 4.97 | 4.96 | 5.26 | 5.61 |
| Isoleucine | 4.83 | 4.57 | 4.38 | 4.55 |
| Arginine | 4.63 | 3.85 | 4.62 | 4.43 |
| Proline | 4.03 | 4.23 | 4.73 | 4.14 |
| Phenylalanine | 4.04 | 3.05 | 4.03 | 3.71 |
| Histidine | 2.38 | 2.47 | 2.60 | 2.75 |
| Methionine | 1.68 | 1.26 | 1.37 | 1.12 |
| Cysteic acid[a] | 1.33 | 1.07 | 0.72 | 0.65 |

[a] Data shown as nitrogen per cent of total amino acid nitrogen.

The composition of the free amino acid pool substantially agrees with the data of Hughes (1958, 1961); seven amino acids—namely, glutamic acid, proline, α-alanine, aspartic acid, lysine plus ornithine, and serine—comprised about 80% of the total free amino acid nitrogen.

Among the free amino acids, a remarkable decrease of lysine plus ornithine and aspartic and glutamic acids was evident at the fourth break; the glutamic acid decrement was largely balanced by the increase in proline. Phenylalanine also increased.

The variations observed agree with those of Hughes (1961) with regard to proline and ornithine. However, they differ from Hughes' observations in that a decrease in tyrosine and phenylalanine and an increase in histidine were not noted, but rather an increase in phenylalanine and a decrease in aspartic and glutamic acids.

The composition of the protein amino acids was regular; only the tyrosine percentage (14 to 16%) was strikingly high.

Total protein amino acids increased from the first to the fourth breaks, with negligible differences in the individual percentages. Nevertheless, considering the analogous variations in the free amino acids, the decrease in lysine plus ornithine appears significant. Both urea and cysteic acid decreased during the growing cycle.

The per cent composition of fatty acids in the lipid extract was very similar to that reported by Hughes (1962) for the same variety of *Agaricus bisporus*.

Among the fatty acids determined (Table IV), linoleic was prevailing and, with palmitic acid, comprised about 90% of the total fatty acids, with a slight increase found in the $(NH_4)_2SO_4$ integrated compost.

In the chromatogram (Figure 1), two peaks were unidentified. The relative retentions ($C_{16:0} = 1$) are reported in Table IV; they account for a $C_{22:0}$ and a $C_{22:1}$ compound (Burchfield and Storrs, 1962).

**Table IV. Fatty Acid Composition of *Agaricus bisporus* at First and Fourth Breaks, Obtained on Urea and $(NH_4)_2SO_4$ Integrated Composts**

| | Break | | | |
|---|---|---|---|---|
| | 1 | 4 | 1 | 4 |
| | Urea | | $(NH_4)_2SO_4$ | |
| Capric | Trace | Trace | Trace | Trace |
| Lauric | Trace | 0.18 | Trace | 0.22 |
| Myristic | 0.71 | 0.59 | 0.40 | 0.57 |
| Pentadecanoic | 0.79 | 1.22 | 0.60 | 0.87 |
| Palmitic | 15.79 | 17.98 | 13.24 | 16.18 |
| Palmitoleic | 0.28 | Trace | 0.07 | Trace |
| Eptadecanoic | 0.89 | 0.35 | 0.82 | 0.38 |
| Stearic | 3.30 | 3.18 | 2.94 | 3.29 |
| Oleic | 2.31 | 2.63 | 1.38 | 1.85 |
| Linoleic | 71.03 | 70.51 | 78.32 | 75.29 |
| Linolenic | 0.50 | 0.73 | 0.50 | 0.73 |
| $X_1$ (relative retention = 5.00) | 0.46 | Trace | 0.54 | Trace |
| $X_2$ (relative retention = 6.03) | 3.90 | 2.45 | 1.17 | 0.57 |

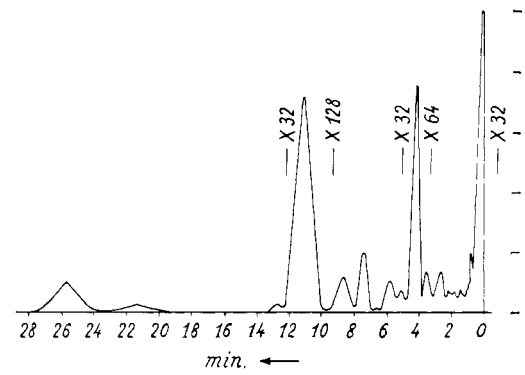


**Figure 1. Gas chromatographic analysis of fatty acid ethyl esters of *Agaricus bisporus* extract**

Peak identification, Table IV. Column packed with 20% poly(diethylene glycol succinate) on Celite 545 and operated at 200° C.

## CONCLUSIONS

A difference in response of mushroom culture to the nitrogen compound used in composting appears evident.

A close connection is not inferable because of the intense microbial activity occurring in composts and the consequent transformation of the nitrogen supplied.

Nevertheless, the availability of either urea or ammonium sulfate greatly affects the microbial metabolism, and in consequence, the mushroom composition—namely, the ash, urea, and amino acid content.

The increase in ash, total nitrogen, and amino acid nitrogen, and the decrease in urea and some individual amino acids following the succession of breaks can be considered as evidence of the altered metabolism of the mushroom.

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