Changes in Amino Acids and Urea in the Cultivated Mushroom, *Agaricus bisporus*, as Influenced by Nutrient Supplementation of the Compost during the Growth Cycle

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Amino acids and urea in the cultivated mushroom, *Agaricus bisporus*, were studied as changes in chemical composition may influence the nutrition and alter the quality of the mushroom. Amino acids were assayed by column chromatography, while urea, total nitrogen, and dry matter were determined by conventional methods in alcohol extracts and fresh tissues. Where gelatin was added to the compost, the amounts of alanine, ammonia, and arginine decreased, but aspartic acid increased. With the addition of hydrolyzed casein, arginine decreased. In the extracts, both the sum of the nitrogen in the various compounds and the total nitrogen by the Kjeldahl method increased toward the end of the growing cycle. Calculations showed 79% to complete recovery of nitrogen in the alcohol extracts.

Mushrooms have for centuries been prized as a delicacy. They are known as a food of excellent nutritive value, low in calories and relatively high in proteins and amino acids.

Fitzpatrick, Esselen, and Weir (*5*) found that the total nitrogen content was 0.5% (fresh weight basis), of which 63% was in the form of protein. Workers at the University of Massachusetts investigated the composition of mushrooms (*Agaricus bisporus*, commonly known as *Agaricus campestris*). They determined a portion of the amino acids, carbohydrates, and vitamins. Esselen and Fellers (*4*) have summarized this work. They identified four amino acids qualitatively (phenylalanine, histidine, lysine, and threonine) and measured quantitatively six amino acids (arginine, isoleucine, leucine, methionine, tryptophan, and valine) which are known to be essential to man. In 1953, Block *et al.* (*2*), using two-dimensional paper chromatography, found 12 amino acids or amino acid derivatives in *Agaricus bisporus*. In 1953 Hughes, Lynch, and Somers (*8*) found 23 amino acids or amino acid derivatives in *Agaricus bisporus* using one- and two-dimensional paper chromatography, and in 1959 Hughes (*6*) found 30 amino acids or amino acid derivatives using paper and column chromatography. Hughes (*7*) further compared certain free amino acids in *Agaricus bisporus* which were harvested at three successive times during the growth period. His results showed that proline and histidine increased while ornithine, tyrosine, and phenylalanine decreased during the growth period.

There are, however, nitrogen-containing compounds in mushrooms other than amino acids, amino acid derivatives, and proteins; urea and chitin are also found. There is a need to study these nitrogenous substances with newer technology in order to attain a more complete accounting of the nitrogenous phase of mushrooms. Ivanoff (*9*) reported finding varying amounts of urea in the fruit bodies of mushrooms (*Agaricus bisporus*), from a trace to as high as 13% of the dry weight, or up to 50% of the total nitrogen of the mushroom. Ivanoff attributed differences in urea content found in mushrooms, of the same age and grown in a similar type of compost, to variations in available nitrogen. More recently, Schisler and Sinden (*11*, *12*) reported increased yields of mushrooms supplemented with protein hydrolyzate or cottonseed meal during the mushroom growth cycle. This practice is gaining interest among the growers. Since such practice may alter the composition of mushrooms, an investigation appears appropriate.

This study is concerned with the changes of amino acids and urea in the cultivated mushroom (*Agaricus bisporus*) as influenced by nutrient supplementation of the compost during the growth cycle. It further serves to evaluate the free amino acids and urea in protein-supplemented mushrooms and to account more completely for the nitrogenous phase of mushrooms.

Materials and Methods

The supplementations of gelatin and casein hydrolyzate were made in duplicate in a commercial mushroom house where the compost bed after spawning was divided by boards into 6-square foot sections. A preliminary experiment was carried out with commercial grade gelatin, which was dissolved in hot water, cooled, and spread evenly on top of the bed just before casing in amounts corresponding to 20 grams of gelatin per square foot. Harvests of treated mushrooms and controls were made for sampling at the beginning and end of the 60-day growth period, which included several breaks. A second experiment used 10 and 20 grams of casein hydrolyzate (acid-hydrolyzed casein from Nutritional Biochemical Corp., Cleveland, Ohio) per square foot; this was applied by spreading the powdered casein hydrolyzate evenly on top of the bed just before casing. Four harvests for sampling were made at regular intervals during the growth period.

Amino Acids. Five mushrooms with cap diameters of approximately 3.5 cm. were chosen at random from each harvest for sampling. One thin slice was removed radially from each mushroom cap, yielding about 5 grams of fresh cap tissue. These samples were extracted immediately by homogenizing the fresh sample in 50 ml. of boiling isopropyl alcohol for 2 minutes using a Servall Omni Mixer, followed by filtering through a Whatman No. 1 filter paper and washing the residue with two successive 25-ml. portions of boiling isopropyl alcohol. The volume of the extract was made up to 100 ml. after cooling to room temperature. Twenty-five milliliters of this extract were evaporated to dryness under partial vacuum with low heat in a Roto Vac, and the residue was taken up in about 8 ml. of distilled water. This water extract was adjusted to pH 1 using 0.1*N* HCl and the total volume was made up to 10 ml. at room temperature. One-half milliliter of this extract was analyzed for amino acids using column chromatography, essentially according to the method of Moore and Stein (*10*). A Technicon Auto-Analyzer was used in conjunction with a water-jacketed borosilicate glass column 150 cm. long and 0.6 cm. in inside diameter, packed with Dowex 50W (8% cross linkage) having a particle size of 20 to 40 microns. Amino acid fractions from the column were assayed with ninhydrin reagent at 570 mμ and the results were recorded on an automatic recorder. Each compound

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was determined quantitatively by comparing with a standard calibrated chromatogram, except for urea which was determined individually by a method described below. The amino acids and urea are listed in Tables I and III in the order in which they appear on the chromatograms and they represent individual runs. The nitrogen in the extracts was calculated and is shown in Tables II and IV.

Urea. A method for determination of urea in blood by Ceriotti and Spandrio (*3*) was modified for the determination of urea in mushrooms by omitting the deproteinizing step prior to the determination of urea in blood. For each determination a 0.1-ml. sample of the alcohol extracts was used as prepared for the amino acids. Determinations were made in duplicate.

Total Nitrogen by Kjeldahl. A composite sample of approximately 5 grams of fresh tissue from five caps as described for the determination of amino acids was used in duplicate to determine total nitrogen according to the AOAC (*1*) method. Also, the various isopropyl alcohol extracts were analyzed in duplicate for total nitrogen by this method, using 25-ml. portions and evaporating most of the isopropyl alcohol with low heat.

Total Dry Matter. A composite sample of approximately 3 grams of fresh tissue from five caps as described previously was dried to constant weight at 110° C. Dry matter determinations were made in duplicate.

Results and Discussion

The results of the gelatin supplementation experiments are shown in Tables I and II. The sum of the nitrogen present in each compound found in the extracts as compared to the total nitrogen of the extracts, by Kjeldahl, provides a check on the recovery. The recoveries in Table II show that it was possible to account for almost all of the total nitrogen found in the extracts. It is noticeable in Table I that the amount of alanine, ammonia, and arginine apparently decreased following the supplementation with gelatin, while aspartic acid showed a pronounced increase. Table II shows that the nitrogen contents of the extracts of the mushrooms increased substantially at the last break and that ammonia, alanine, and urea were the major contributors to this increase. From a practical viewpoint, although gelatin is a very rich source of organic nitrogen, its application as a nutrient supplement to the compost during the growth period of the mushrooms was considered not desirable because of its viscous nature and tendency to become gelatinous.

The results of the hydrolyzed casein supplementation experiments are shown in Tables III and IV. From about 79% to complete recovery of the nitrogen was attained from the extract analysis (Table IV). The nitrogen content of the whole mushroom caps by Kjeldahl may be noted in Table IV. There appears to be a pronounced increase in aspartic acid, threonine, serine, proline, leucine, tyrosine and, to some degree, urea with the growth breaks, while taurine, glutamic acid, and arginine decreased with the breaks throughout the growth period. The values for taurine were far greater in the first breaks than in any of the succeeding breaks. Arginine decreased following the supplementation with hydrolyzed casein as compared with the controls. The urea content was greatest in the extracts of mushroom caps from the last break, which was treated with 20 grams of hydrolyzed casein per square foot, as compared with the treatment with 10 grams of hydrolyzed casein per square foot and the controls. The nitrogen in urea comprised from 8 to 14% of the total nitrogen of the whole mushroom cap. The results in Table IV further show that, in general, the total nitrogen

Table I. Effect of Supplementing Mushrooms during the Growing Cycle with Gelatin on Amino Acids and Urea in Extracts of the Mushroom Caps

(Expressed as milligrams per 100 grams of dry matter)

Amino Acids and Urea	Control		Gelatin Supplement (20 G./Sq. Ft. Bed)	
	First break	Last break	First break	Last break
Cysteic acid	20.7	27.3	6.0	40.3
Taurine	13.8	3.4	0.2	5.5
Urea[a]	1333.0	2577.0	1460.0	2824.0
Aspartic acid	29.7	19.6	69.8	97.0
Threonine	208.2	238.1	162.7	259.3
Serine	94.3	144.8	82.3	260.5
Glutamic acid	209.2	185.3	152.1	432.7
Proline	663.9	835.2	756.5	404.4
Glycine	55.0	48.7	47.3	76.2
Alanine	594.0	876.0	407.3	812.1
Valine	250.7	193.6	1173.5	168.9
Cystine	78.3	31.7	9.8	119.8
Isoleucine	170.8	117.6	142.7	102.0
Leucine	271.9	215.8	262.1	190.2
Tyrosine	270.7	250.6	278.5	275.9
Phenylalanine	284.5	320.2	267.4	355.6
Ammonia	74.3	140.7	60.5	85.9
Ornithine	45.5	13.2	11.3	17.3
Lysine	10.5	8.5	6.0	8.0
Histidine	32.6	3.6	29.0	16.2
Arginine	613.8	586.1	71.3	310.0

[a] Determined separately, not by column chromatography.

Table II. Nitrogen Contents of Amino Acids and Urea Extracted from Mushroom Caps Supplemented with Gelatin during Growing Cycle

(Expressed as milligrams of nitrogen per 100 grams of dry matter)

Amino Acids and Urea	Control		Gelatin Supplement (20 G./Sq. Ft. Bed)	
	First break	Last break	First break	Last break
Cysteic acid	1.7	2.3	0.5	3.3
Taurine	1.6	0.4	Trace	0.7
Urea[a]	621.8	1202.3	681.1	1317.4
Aspartic acid	3.1	2.1	7.3	10.2
Threonine	24.5	28.0	19.1	30.5
Serine	12.6	19.3	11.0	34.7
Glutamic acid	19.9	17.6	14.5	41.2
Proline	80.8	101.6	92.1	49.2
Glycine	10.3	9.1	8.8	14.2
Alanine	93.4	137.7	64.0	127.7
Valine	30.0	23.2	20.8	20.2
Cystine	9.1	3.7	1.1	14.0
Isoleucine	18.2	12.6	15.2	10.9
Leucine	29.0	23.1	28.0	20.3
Tyrosine	20.9	19.4	21.5	21.3
Phenylalanine	24.1	27.2	22.7	30.2
Ammonia	61.2	115.9	49.8	70.7
Ornithine	9.7	2.8	2.4	3.7
Lysine	2.0	1.6	1.2	1.5
Histidine	8.8	1.0	7.9	4.4
Arginine	197.4	188.5	22.9	99.7
Total	1280.1	1939.4	1091.9	1926.0
Nitrogen in extracts (Kjeldahl)	1262.4	2122.3	1252.0	1954.0
Recovery (compared to Kjeldahl), %	101.4	91.4	87.2	98.6
Nitrogen in caps (Kjeldahl)	8601	9330	8540	8720

[a] Determined separately, not by column chromatography.

Table III. Effect of Supplementing Mushrooms during the Growing Cycle with Hydrolyzed Casein on Amino Acids and Urea in Extracts of Mushroom Caps from Different Breaks

(Expressed as milligrams per 100 grams of dry matter)

Amino Acids and Urea	Control				Hydrolyzed Casein							
					10 G./Sq. Ft. Bed				20 G./Sq. Ft. Bed			
	Break				Break				Break			
	1	2	3	4	1	2	3	4	1	2	3	4
Cysteic acid	11.2	14.3	23.4	18.1	18.9	9.7	19.2	11.6	17.4	15.4	18.0	19.3
Taurine	36.9	8.6	6.4	14.0	28.5	3.0	5.4	13.7	54.1	6.3	6.6	2.2
Urea[a]	1699.7	2167.5	1770.2	1996.9	1728.4	1244.0	1604.7	2018.6	1572.6	1966.8	2135.5	2728.8
Aspartic acid	56.7	57.4	59.3	89.6	57.8	82.3	50.7	60.9	27.2	56.6	52.1	85.4
Threonine	93.6	121.6	199.0	249.8	101.6	134.3	149.0	197.0	132.3	185.3	200.1	236.4
Serine	91.6	103.7	123.2	148.0	85.4	105.0	123.8	136.6	81.4	122.6	118.8	144.2
Glutamic acid	493.0	352.6	293.3	290.2	461.8	489.0	304.0	226.9	400.0	371.5	283.7	243.0
Proline	670.9	543.5	1447.8	1398.7	596.9	713.0	1633.6	885.9	501.7	513.3	1632.9	1585.1
Glycine	63.4	55.0	58.3	65.4	63.3	59.0	65.5	69.3	55.2	56.3	65.8	68.2
Alanine	364.6	422.2	478.8	393.4	320.3	390.3	416.8	364.0	332.2	477.2	458.1	436.1
Valine	101.1	103.6	117.5	115.7	90.8	83.4	96.1	128.6	87.0	105.4	127.6	122.2
Cystine	72.1	64.9	76.4	52.9	78.6	52.9	74.4	45.3	61.2	62.0	68.2	55.3
Isoleucine	73.5	80.1	81.5	86.7	66.5	80.4	65.6	79.6	630.2	70.4	71.3	75.8
Leucine	110.7	117.3	115.6	153.4	106.7	91.6	102.5	139.3	75.9	103.8	95.7	147.3
Tyrosine	127.7	149.3	125.8	214.1	106.7	100.5	141.9	226.6	113.7	141.1	158.5	197.8
Phenylalanine	303.2	306.0	250.4	243.7	268.6	227.7	240.0	380.9	278.2	248.4	260.6	284.9
Ammonia	53.0	52.0	49.9	40.4	56.5	41.3	45.9	32.5	58.4	54.8	43.4	23.7
Ornithine	14.7	19.0	28.4	23.6	21.1	34.0	8.6	24.9	16.4	15.2	18.8	29.6
Lysine	21.3	6.8	10.3	7.4	7.1	17.0	5.5	9.2	7.2	13.1	7.1	6.0
Histidine	26.2	30.0	34.2	55.4	25.3	25.7	23.0	74.5	28.2	30.7	55.8	140.3
Arginine	692.3	260.4	138.1	232.4	477.5	135.6	144.6	136.7	354.7	156.4	185.2	143.6

[a] Determined separately, not by column chromatography.

Table IV. Nitrogen Contents of Amino Acids and Urea Extracted from Mushroom Caps Supplemented with Hydrolyzed Casein during Growing Cycle

(Expressed as milligrams of nitrogen per 100 grams of dry matter)

Amino Acids and Urea	Control				Hydrolyzed Casein							
					10 G./Sq. Ft. Bed				20 G./Sq. Ft. Bed			
	Break				Break				Break			
	1	2	3	4	1	2	3	4	1	2	3	4
Cysteic acid	0.9	1.2	1.9	1.5	1.6	0.8	1.6	1.0	1.4	1.3	1.5	1.6
Taurine	4.4	1.0	0.8	1.7	3.4	0.4	0.6	1.6	6.5	0.8	0.8	0.3
Urea[a]	792.9	1011.1	825.8	931.6	806.3	580.3	748.6	941.7	733.6	917.5	996.7	1273.0
Aspartic acid	6.0	6.0	6.2	9.4	6.1	8.7	5.3	6.4	2.9	6.0	5.5	9.0
Threonine	11.0	14.3	23.4	29.4	12.0	15.8	17.5	23.2	15.6	21.8	23.5	27.8
Serine	12.2	13.8	16.4	19.7	11.4	14.0	16.5	18.2	10.9	16.3	15.8	19.2
Glutamic acid	46.9	33.5	27.9	27.6	44.0	46.5	29.0	21.6	38.1	35.4	27.0	23.1
Proline	81.7	66.2	176.2	170.2	72.6	86.8	198.8	107.8	61.1	62.5	198.7	192.9
Glycine	11.8	10.3	10.9	12.2	11.8	11.0	12.2	12.9	10.3	10.5	12.3	12.7
Alanine	57.3	66.4	75.3	61.8	50.4	61.4	65.5	57.2	52.2	75.0	72.0	68.6
Valine	12.1	12.4	14.1	13.8	10.9	10.0	11.5	15.4	10.4	12.6	15.3	14.6
Cystine	8.4	7.6	8.9	6.2	9.2	6.2	8.7	5.3	7.1	7.2	8.0	6.4
Isoleucine	7.9	8.6	8.7	9.3	7.1	8.6	7.0	8.5	6.7	7.5	7.6	8.1
Leucine	11.8	12.5	12.3	16.4	11.4	9.8	11.0	14.9	8.1	11.1	10.2	15.7
Tyrosine	9.9	11.5	9.7	15.7	8.3	7.8	11.0	17.5	8.8	10.9	12.3	15.3
Phenylalanine	25.7	26.0	21.2	20.7	22.8	19.3	20.4	32.3	23.6	21.1	22.1	24.2
Ammonia	43.6	42.8	41.1	33.3	46.6	34.0	37.8	26.8	48.1	45.1	35.8	19.5
Ornithine	3.1	4.0	6.0	5.0	4.5	7.2	1.8	5.3	3.5	3.2	4.0	6.3
Lysine	4.1	1.3	2.0	1.4	1.4	3.3	1.1	1.8	1.4	2.5	1.4	1.2
Histidine	7.1	8.1	9.3	15.0	6.9	7.0	6.2	20.2	7.6	8.3	15.1	38.0
Arginine	222.7	83.8	44.4	74.7	153.6	43.7	46.5	44.0	114.1	50.3	59.6	46.0
	1381.5	1442.4	1342.5	1476.6	1302.3	982.6	1258.6	1383.7	1172.0	1326.9	1545.2	1823.5
Nitrogen in extracts (Kjeldahl)	1357	1667	1499	1796	1248	1241	1555	1665	1418	1517	1659	2151
Recovered (compared to Kjeldahl), %	101.8	86.4	89.7	82.2	104.3	79.2	80.9	83.1	82.7	87.5	93.1	84.8
Nitrogen in caps (Kjeldahl)	6300	7730	8850	8410	6820	7270	8880	8360	6820	7970	9000	8950

[a] Determined separately, not by column chromatography.

in the extracts of mushrooms increased with the breaks and that the increase was greatest in the extracts prepared from mushrooms supplemented with 20 grams of hydrolyzed casein per square foot. Tables II and IV show that 15 to 25% of the total nitrogen in the mushroom caps was removed for assay by the various extracts.

Conclusions

The results of this study should be of value as a basis for further investigations of the influence of nutrient supplementation of the compost during the growth cycle on the chemical composition of the cultivated mushroom. However, the changes of the nitrogenous compounds observed in the mushrooms may not be the direct result of nutrient utilization by the mushroom but may be indirectly

attributed to microbiological metabolism. This study should also serve as a basis for further investigations on the chemical composition of amino acids and urea of the mushroom. For the first time, an attempt has been made to account for a nitrogen balance sheet of the soluble nitrogens obtained from cultivated mushrooms (*Agaricus bisporus*).

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BARLEY SILICA

Relation of Silicon in Barley to Disease, Cold, and Pest Resistance

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The silica content of the various parts of five varieties of barley was determined both in the fall and, again, in the spring when the plants were heading. The content varied from an average of 0.51% for developing heads to 5.8% in spring roots. On the average, leaves and roots had twice as much silica in the spring as in the fall. The silica content of barley differed from silica contents previously reported for wheat and oats. By means of spodograms, the actual patterns of silica deposits in the various parts of barley plants were determined. Index of refraction studies showed the silica to be opal. The results showed no direct relationship between total silica content and resistance to greenbugs, cold, or diseases.

SILICA IN BARLEY (*Hordeum vulgare*) has been of interest because of its nutritional value and its relationship to disease. Brenchley, Maskell, and Warington (*1*) made a considerable study of the relationship of silica to phosphate in the growth of barley plants. They found that significant increases in dry weight of barley plants occurred in water culture with addition of silica if available phosphate was quite low. They believed that silica might act within the plant by releasing phosphate from relatively quiescent parts of the plant and enable phosphate to be transferred to assimilation and growth regions.

Toth (*11*) reported definite increases in barley yield when either calcium or magnesium silicate was added to the soil. Barley markedly absorbed silica when grown in these silicated soils. Okawa (*9*) reported that silica was a nutrient of young barley plants, and that they appeared to be protected from cold injury when colloidal silica was present in the culture solution.

Germar's (*2*) research showed that cereals, well supplied with silica, were more resistant to mildew infections, apparently because deposition of silica in the epidermis makes the latter more resistant to attack by enzymes secreted by the fungus hyphae. Resistance to fungi that enter through stomata was not increased by silica. In laboratory experiments, Wagner (*12*) found that the amount of mildew infection in barley was related inversely to available silica.

None of the workers mentioned studied the silica content of individual parts of the barley plant or depositional patterns, as Lanning and coworkers have done for sorghum (*7, 8*), rice (*5*), and wheat (*6, 8*). Jones, Milne, and Wadham (*3, 4*) studied silica content of various parts of the oat plant rather completely, and reported the percentages of silica: leaf blade 5.34%, leaf sheath 4.55%, root 1.84%, and seed 0.12%.

This study was initiated to obtain more complete data on silica deposition in various parts of barley plants and to relate the silica to insect, disease, and cold resistance.

Materials and Methods

The barley plants (*Hordeum vulgare*) studied were grown in experimental plots on the Agronomy Farm of Kansas State University. Available silica content of the soil was high, approximately 20 mg. per 100 grams of soil (*7*). The pH of the soil was 5.2 at 1 to 1 dilution. The five varieties studied were Hudson (CI 8067), Dicktoo (CI 5529), Meimi (CI 5136), Chase (CI 9581), and Will (CI 11652). Dicktoo and Will resist attack by greenbugs, and Will resists mildew and loose smut also. Dicktoo is the most winter hardy and Hudson the least hardy. The Hudson variety winterkilled 50% while none of the other varieties were affected. The first samples were collected December 6, 1964; the second, from the same plots April 13, 1965, when all varieties except Hudson were heading. The plants were separated into roots, stems, leaves, sheaths, and heads. All were washed, thoroughly and then, dried at 110° C.

Silica and ash contents of plant materials were determined by classical gravimetric techniques. The material was ashed at about 600° C. After being weighed, the ash was treated repeatedly with 6*N* hydrochloric acid to remove other mineral impurities. The silica