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Nutritional value and amino acids composition of the mushroom (*Agaricus bisporus*) at different stages of its development*

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Abstract

The study of the nutritional value and amino acid composition of the mushroom (*Agaricus bisporus*) was carried out with the Somycel 653 strain cultivated on a synthetic compost containing rye straw, broiler chicken manure and gypsum. In 4 developmental stages of the whole fruit-bodies and the caps and stipes the following were determined: dry matter, vitamin C, nitrates, total nitrogen, crude protein (N × 4.38) and amino acid composition. It was observed that in all stages of development the levels of total N and crude protein are significantly higher in the caps than in the stipes. From the amino acid composition it appears that the caps of stage 4 contain the highest amounts of essential and total amino acids as compared with caps, stipes and whole sporophores found in any developmental stage. This is correlated with the opening of the mushrooms in stage 4. It was observed that the nitrates level tends to be higher in the cap than in the stipe.

INTRODUCTION

There are many factors that can affect the nutritive value of the cultivated mushroom, *Agaricus bisporus*. One of the little known factors is the stage of the mushroom development. Therefore, a limited number of studies have been conducted on changes in the nutritional value and the amino acids composition of these mushrooms during the successive stages of their development. One of the earliest works on proteins was carried out by M ł o d e c k i et al. (1968).

They examined the content of total chitin and protein nitrogen in relation to the size of the cap diameter of the *Agaricus bisporus*. It was observed that the

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protein content of the mushrooms in the last stage of development (diameter of pileus 6-8 cm) decreased by 30% as compared with mushrooms in the first stage. Total nitrogen and protein nitrogen contents decreased also. In fact the chitin nitrogen increased during mushroom growth. Since the protein content decreased, the total amino acids amount was also reduced. Investigators found that mushrooms with cap diameter of 2 cm contained about 30% of free amino acids (as percentage of total amino acids content), and mushrooms with cap diameter of 6-8 cm contained about 12% of free amino acids.

S z y m c z a k (1972) investigated changes in the chemical composition of fruit bodies during three stages of mushroom development. He showed that crude fat content was highest in older fruit bodies and that palmitic acid increased in relation to linoleic acid.

H a m m o n d and N i c h o l a s (1976) reported changes in soluble carbohydrates and proteins during growth of the sporophore and mycelium of *Agaricus bisporus*. The protein content of sporophore tissues was measured to find, whether a change in protein level could be associated with an increase in mannitol in the stipe pileus as reported by R a s t (1965). An increase of protein content from 8.2 to 15.8% of dry sporophore weight between stages 1 and 7 was observed in one experiment, and a decrease from 20.6 to 16.6% was observed in another. These differences may have been due to variations in material. The protein extracted showed an overall decrease of 6 to 7% of dry weight in the stipe and pileus during sporophore development. The soluble protein content proved to be slightly higher in the pileus than in the stipe.

MATERIALS AND METHODS

The study of the mushroom nutritional values and amino acids composition at different stages of their development was carried out with a Somycel 653 strain cultivated on a synthetic compost prepared on a base of rye straw, broiler chicken manure, and gypsum. At harvest the sporophores were classified into one of four arbitrary stages of development.

I. Closed cap, approximate diameter of pileus < 15 mm;

II. Closed cap, approximate diameter of pileus 15-25 mm, velum visible and intact;

III. Closed cap, approximate diameter of pileus 25-40 mm, velum stretched sometimes starting to tear;

IV. Open cap, approximate diameter of pileus > 40 mm, velum torn, gills clearly visible.

The analyses of the mushrooms at each stage of development included the whole fruit-bodies, caps and stipes. The following determinations were made: dry

matter, vitamin C, nitrates and nitrites, total nitrogen, crude protein (N × 4.38), amino acids composition. In addition the whiteness value (Hunter L) was determined.

ANALYTICAL METHODS

Dry matter was determined by drying the samples at 70°C for 12 hours and next at 100°C to a constant weight.

Vitamin C (ascorbic acid) was determined by using the titration method with Tillmans reagent (2,6 dichlorophenolindophenol). The preparation of samples and the analytical procedure were described by Charłampowicz (1966).

Nitrates were determined by using a specific ion electrode for nitrates (Orion Model 93-07) in conjunction with a 407A specific ion metre (Orion Res. INc.) and a double junction reference electrode (Orion Model 90-02). Preparation of samples and determination of nitrates were conducted according to the Methods Manual 93 series electrodes (1978, Orion Res. In.).

The extraction and determination of nitrites were conducted according to Backer (1965). The analytical procedure was based on the red reaction product formed when nitrites react with the Griess reagent (mixture of naphthylamine and sulfanilic acid). The colour was determined by means of Bausch and Lomb Spectronic 20 at 525 mm wavelength.

Amino acids composition was determined by hydrolysing mushroom powder (containing 6% of moisture) in a closed ampoule with 6 N hydrochloric acid for 4 hours at 145°C (McKellar and Kohrman, 1975). Before hydrolysis a few drops of 5% phenol as reducing agent were added to the ampoule in order to prevent oxidation of phenylalanine, tyrosine and histidine. After hydrolysis, the mixture was filtered and the solution was evaporated to dryness at 50°C under vacuum. The residue was extracted with 0.1 N HCl and then filtered. The amino acids were determined in an automatic Amino Acid Analyzer Hd-1200E. The standard solution of amino acids was prepared by mixing free amino acids purchased from the Pierce Chemical Company.

The whiteness of the mushrooms was determined by using the Hunter Color and Color Differences Meter Model D 25 D2. The reflectance plate used for standardization of the instrument was a standard white plate with Hunter values: $L = 91.9$; $a = -1.2$; $b = -0.3$.

The total nitrogen content was determined by the Kjeldahl method. Crude protein was calculated from the nitrogen content, as determined by Kjeldahl analysis, using the conversion factor N × 4.38 (FAO, 1970, 1972; Watt and Merril, 1963). Statistical limit values were evaluated by the Dean and Dixon (1951) test.

RESULTS AND DISCUSSION

NUTRITIONAL VALUES

At all stages of development, the dry matter content does not change significantly in the total sporophore, cap and stipe (Table 1). It can be observed from Table 1 that the level of vitamin C in the total sporophore, cap and stipe tissues is highest at stage II. The cap usually contains a higher amount of vitamin C than the stipe, the averages being respectively: 7.53 and 6.11 mg per 100 g of fresh weight. There are some differences in the nitrate content (Table 1). The total sporophore at stage III has a higher amount of nitrates than in stages II and IV. There is a tendency (significant, however, only at stage IV) to a higher level of nitrates in the cap than in the stipe. There is also a significant difference in the content of nitrites in the total sporophore tissue at stage II in comparison with stages III and IV (Table 1). The whiteness of the smallest caps is lower than in the larger ones (Table 1). The content of total nitrogen and crude protein calculated

Tab

Contents of dry matter, vitamin C, nitrates, tissues at different stages

Component	I. Closed cap			II. Closed cap		Stage of spo
	whole sporophore	cap	stipe	whole sporophore	cap	stipe
Dry matter* (%)	9.40 ± 0.20	8.80 ± 0.33	9.70 ± 0.68	9.20 ± 0.18	8.80 ± 0.42	10.1 ± 0.82
Vitamin C* (mg/100g fresh weight)	6.52 ± 0.25	6.99 ± 0.17	5.80 ± 0.25	7.85 ± 0.25	8.26 ± 0.38	6.52 ± 0.25
Nitrates* (mg/100g fresh weight)	4.14 ± 0.36	3.91 ± 0.74	4.86 ± 2.43	3.30 ± 0.75	2.96 ± 0.33	4.28 ± 1.35
Nitrites* (mg/100g fresh weight)	–	–	–	0.125 ± 0.003	0.091 ± 0.023	0.082 ± 0.004
Whiteness value** (Hunter L)	–	81.1 ± 1.2	–	–	83.9 ± 1.90	–

*Average from three independent replicates ± confidence limits at p = 0.95 evaluated according to Dean and Dixon test.
**Averages from 20 mushrooms ± confidence limits at p = 0.95 evaluated according to Dean and Dixon test.

as total N × 4.38 is significantly lowest at stage IV, both in the total sporophore and the cap tissues (Table 2). For the stipe, however, a higher percentage of total N and crude protein can be observed at stages I and II in comparison with stages III and IV. At all stages of development the level of total N and crude protein is significantly higher in the cap than in the stipe (Table 2).

AMINO ACID COMPOSITION

The contents of individual amino acids are vary, depending on the stage of development (Table 3). The following differences were found:

Lysine: In the sporophore tissue the highest amounts were found in stages I and II. In the stipe a higher content was observed in stage I in comparison with all other stages. Only at stage I did the caps contain a significantly higher amount than the stipes.

Histidine: In the sporophore tissue, histidine was significantly higher in stage I than in stage IV. In all developmental stages, no differences were observed for

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nitrites and whiteness value in sporophore of development (strain 653)

rophore development

III. Closed cap			IV. Open cap		
whole sporophore	cap	stipe	whole sporophore	cap	stipe
8.50 ± 1.37	8.90 ± 0.52	10.40 ± 0.74	8.70 ± 1.29	9.20 ± 2.52	1.10 ± 1.40
6.74 ± 0.36	7.54 ± 0.25	6.27 ± 0.38	6.01 ± 0.36	7.31 ± 0.38	5.83 ± 0.24
4.14 ± 0.09	2.68 ± 0.92	3.14 ± 0.36	2.95 ± 1.01	3.14 ± 0.62	4.42 ± 0.56
0.065 ± 0.026	0.093 ± 0.022	0.109 ± 0.012	0.085 ± 0.27	0.082 ± 0.027	0.097 ± 0.00
–	86.6 ± 1.40	–	–	84.8 ± 1.60	–

Table 2

Contents of total nitrogen protein (expressed as dry weight) in sporophore tissues at different stages of development (strain 653)*

Sporophore tissue	Stage of development							
	I. Closed cap		II. Closed cap		III. Closed cap		IV. Open cap	
	total N %	crude protein N × 4.38 (%)	total N %	crude protein N × 4.38 (%)	total N %	crude protein N × 4.38 (%)	total N %	crude protein N × 4.38 (%)
Whole	6.03 ± 0.31	26.41 ± 1.36	6.27 ± 0.32	27.46 ± 1.40	5.97 ± 0.34	26.15 ± 1.49	5.20 ± 0.31	22.78 ± 1.36
Cap	5.83 ± 0.17	25.54 ± 0.74	5.46 ± 0.36	23.91 ± 1.58	5.45 ± 0.20	23.87 ± 0.88	4.83 ± 0.22	21.16 ± 0.96
Stipe	4.74 ± 0.10	20.76 ± 0.44	4.77 ± 0.29	20.89 ± 1.27	3.97 ± 0.55	17.39 ± 2.41	4.09 ± 0.17	17.91 ± 0.74

*Average from three independent replicates ± confidence limits at p = 0.95 evaluated according to Dean and Dixon test.

the caps. For the stipe, however, stage IV showed a significantly higher amount as compared with stage II. There were significant differences between the cap and the stipe at stages II and IV.

Arginine and aspartic acid: At all development stages, they did not change significantly in the whole sporophore, cap and stipe.

Threonine: Threonine was highest in the sporophore tissue at stage III. The caps and stipes contained a significantly higher amount at stage III than stage II.

Serine: A significantly lower amount of serine was observed in the sporophore at stage I than at stages III and IV. Similar observations were made concerning the caps, which contained a lower amount at stage II in comparison with III and IV.

Glutamic acid: Definitively the highest amounts of glutamic acid in the sporophore tissue and the stipe were observed at stage I. When comparing the stipe and cap, significantly higher amounts were found in the cap in stages II and IV.

Proline: In the sporophore tissue, the lowest amount was found at stage I, and for the cap and stipe the highest amounts were found at stage III. No differences befween the cap and stipe were noticed at any stage of development.

Glycine: There were some significant differences between the cap and the stipe at different stages of development, but none in the whole sporophore were observed.

Alanine: The ony significant differences were found in the whole sporophore at stages I and IV. A higher amount of alanine was found at stage IV. Similar differences for the cap were observed. In the cap and stipe, the amounts of alanine were not significantly different.

Valine: No significant differences for the cap and stipe were noticed. The total sporophore was characterized by a significantly lower amount of valine at stage IV in comparison with stages I and II, but only at stage IV did the cap contain a higher amount than the stipe.

Methionine: No significant differences.

Isoleucine: The only significant difference was observed in the cap, where at stage IV the highest amount was noted. At this stage, the cap had a higher amount than the stipe.

Leucine: The same observations as for isoleucine were noted. The leucine content in the caps increased steadily throughout maturation. The amount of leucine was almost twofold higher at stage IV than at stage I.

Tyrosine: The whole sporophore was characterized by a higher amount of tyrosine at stage III than at stage II. In the caps, at this stage, there was a higher content of tyrosine in comparison with stages I and II. No significant differences were observed between the cap and stipe at any developmental stage.

Phenylalanine: The content in the whole sporophore was higher at stage I

Tab

Amino acids composition (expressed in
in sporophore tissues of different

	Stage of sporo					
	I. Closed cap			II. Closed cap		
	whole sporophore	cap	stipe	whole sporophore	cap	stipe
Lysine*	8.12 ± 0.20	6.50 ± 0.73	7.58 ± 0.12	7.64 ± 0.47	6.70 ± 0.26	5.52 ± 1.18
Histidine*	2.27 ± 0.10	2.26 ± 0.61	2.30 ± 0.65	2.19 ± 0.25	1.95 ± 0.10	1.56 ± 0.29
Arginine*	4.96 ± 0.29	4.73 ± 0.60	4.64 ± 0.42	4.35 ± 0.26	4.53 ± 0.17	4.03 ± 0.21
Aspartic acid	9.50 ± 1.18	9.46 ± 0.72	9.15 ± 0.79	10.22 ± 0.10	10.03 ± 1.31	9.28 ± 0.08
Threonine*	3.37 ± 0.07	3.75 ± 0.22	3.86 ± 0.23	3.47 ± 0.86	3.40 ± 0.31	3.72 ± 0.62
Serine	3.23 ± 0.26	3.53 ± 0.92	3.97 ± 0.30	4.37 ± 0.96	3.40 ± 0.05	4.05 ± 0.07
Glutamic acid	21.65 ± 0.44	20.27 ± 1.34	20.94 ± 2.86	21.66 ± 2.42	21.94 ± 1.05	17.40 ± 0.18
Proline	6.01 ± 0.09	7.74 ± 0.25	7.15 ± 0.64	7.83 ± 1.16	8.11 ± 0.34	6.67 ± 1.94
Glycine	4.22 ± 0.26	4.40 ± 0.44	4.48 ± 0.34	4.51 ± 0.73	4.48 ± 0.74	3.83 ± 0.59
Alanine	5.17 ± 0.40	5.33 ± 0.38	5.94 ± 0.99	5.66 ± 0.69	6.01 ± 0.43	6.23 ± 0.52
Valine*	4.59 ± 0.39	4.34 ± 0.42	4.58 ± 0.21	4.71 ± 0.46	4.62 ± 0.55	4.31 ± 0.22
Methionine*	1.16 ± 0.09	1.13 ± 0.05	1.17 ± 0.10	1.06 ± 0.08	0.58 ± 0.031	0.99 ± 0.25
Isoleucine*	3.55 ± 0.65	3.29 ± 0.17	3.48 ± 0.35	3.44 ± 0.21	3.37 ± 0.64	3.44 ± 0.22
Leucine*	5.68 ± 1.66	5.68 ± 0.34	5.70 ± 0.57	6.80 ± 1.48	6.65 ± 1.14	5.76 ± 0.18
Tyrosine*	3.03 ± 0.21	2.95 ± 0.20	2.96 ± 0.12	2.38 ± 0.31	2.71 ± 0.72	2.86 ± 0.92
Phenylalanine*	4.59 ± 0.81	4.23 ± 1.30	4.64 ± 0.86	4.56 ± 1.30	3.54 ± 0.96	3.34 ± 0.35
Total essential amino acids*	36.33	33.93	36.27	36.25	33.82	31.50
Total amino acids	91.05	89.39	92.54	94.85	92.32	82.99

*Essential amino acids.

than at stage IV. The cap at stage IV contained a significantly higher amount than at stages II and III. For the stipe, a higher amount of phenylalanine was found at stage IV than at stages I and II.

The amino acids composition at all developmental stages was characterized by a relatively high content of glutamic acid, aspartic acid, proline and lysine. The lowest content of methionine found in this study (Table 3) confirmed the data presented by Doesburg and Meijer (1965) and FAO (1970) indicating that methionine is a limiting amino acid in mushrooms.

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grams per 16 grams of nitrogen (g/16 g N))
stages of development (strain 653)

phore development

III. Closed cap			IV. Open cap		
whole sporophore	cap	stipe	whole sporophore	cap	stipe
6.02 ± 0.36	5.73 ± 0.72	6.66 ± 1.31	6.45 ± 0.81	6.55 ± 0.87	6.35 ± 1.11
2.21 ± 0.36	1.93 ± 0.23	1.85 ± 0.57	1.74 ± 0.22	1.77 ± 0.31	2.48 ± 0.18
4.31 ± 0.08	4.61 ± 0.65	4.48 ± 0.40	4.33 ± 0.38	4.41 ± 0.68	4.79 ± 0.00
9.56 ± 0.39	9.91 ± 0.87	9.20 ± 0.03	10.16 ± 0.51	8.94 ± 1.38	9.08 ± 0.56
4.71 ± 0.08	3.74 ± 0.68	4.34 ± 1.22	3.87 ± 0.30	4.10 ± 0.33	4.66 ± 0.08
4.58 ± 0.88	3.95 ± 0.40	3.32 ± 0.10	4.16 ± 0.64	4.25 ± 0.35	4.94 ± 0.22
20.41 ± 1.82	19.97 ± 1.53	20.92 ± 2.80	17.34 ± 0.22	18.60 ± 1.66	14.73 ± 0.64
7.06 ± 1.40	11.28 ± 1.38	9.69 ± 0.91	7.91 ± 0.73	8.02 ± 2.42	7.94 ± 0.70
4.58 ± 0.74	5.02 ± 0.09	5.24 ± 0.10	4.90 ± 0.09	4.96 ± 0.86	3.70 ± 0.16
5.63 ± 1.40	6.99 ± 0.56	6.14 ± 0.20	6.75 ± 0.81	6.48 ± 0.65	5.97 ± 0.49
4.43 ± 0.51	4.80 ± 1.22	4.47 ± 1.11	3.93 ± 0.09	4.82 ± 0.31	4.28 ± 0.07
1.24 ± 0.42	1.37 ± 0.46	1.52 ± 0.43	1.23 ± 0.17	1.16 ± 0.17	1.18 ± 0.14
3.39 ± 0.92	4.41 ± 0.77	3.40 ± 0.65	3.54 ± 0.49	5.20 ± 0.53	3.46 ± 0.51
6.67 ± 0.09	7.56 ± 1.13	6.63 ± 1.29	6.15 ± 0.39	1.89 ± 1.37	6.68 ± 0.42
3.12 ± 0.38	3.39 ± 0.51	2.90 ± 0.44	2.80 ± 0.55	4.69 ± 0.89	4.53 ± 1.03
3.70 ± 0.44	3.74 ± 0.77	3.33 ± 0.94	3.37 ± 0.38	5.29 ± 0.53	4.21 ± 0.65
35.49	36.57	35.10	33.08	45.47	37.83
92.02	98.30	94.09	88.63	101.13	88.98

CONCLUSION

1. The total nitrogen and crude protein contents are lowest at developmental stage IV. At all stages of development the levels of total N and crude protein are significantly higher in the cap than in the stipe.

2. Analyses of sixteen amino acids in the sporophore, at different developmental stages, do not indicate any major differences. Remarkably high amounts of the following are present at stage I, lysine and phenylalanine; at stage II, lysine;

at stage III, treonine and proline; at stage IV, glutamic acid, alanine, tyrosine and phenylalanine.

3. One of the peculiarities of the amino acids composition of the various developmental stages is the fact that the mushroom caps of stage IV contain the highest amounts of essential and total amino acids among the caps, stipes and whole sporophores found in any developmental stages. This is correlated with the opening of the mushrooms in stage IV.

4. The dry matter content does not change significantly in the whole sporophore, cap and stipe at any developmental stage. It can be observed that the level of nitrate tends to be higher in the cap than in the stipe.

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Wartość odżywcza i skład aminokwasowy pieczarek (*Agaricus bisporus*) w różnych stadiach rozwojowych

Streszczenie

Badania wartości odżywczej i składu aminokwasowego grzybów (*Agaricus bisporus*) przeprowadzono na pieczarkach rasy Somycel 653, uprawianych na podłożu syntetycznym zawierającym słomę żytnią, nawóz kurzy i gips. Przeprowadzono analizy zawartości suchej masy, witaminy C,

azotynów, azotanów, azotu ogółem, białka surowego (N × 4.38), aminokwasów w całych owocnikach, trzonkach, kapeluszach, w czterech stadiach rozwojowych pieczarek. Zaobserwowano, że we wszystkich stadiach rozwojowych zawartość azotu ogółem i białka surowego jest istotnie wyższa w kapeluszach niż w trzonkach. Zawartość aminokwasów egzogennych i aminokwasów ogółem w kapeluszach pieczarek w stadium IV jest najwyższa w porównaniu ze składem aminokwasowym kapeluszy, trzonków i całych owocników pieczarek z pozostałych trzech badanych stadiów rozwojowych, co skorelowane jest z otwieraniem się kapeluszy w stadium IV. Zaobserwowano również, że zawartość azotanów w kapeluszach jest wyższa niż w trzonkach.