*Chemistry of Natural Compounds, Vol. 35, No. 3, 1999*

# AMINO ACID AND FRACTIONAL COMPOSITIONS OF THE PROTEIN FRACTIONS OF THE BIOMASS OF MICROMYCETES AND THEIR BIOLOGICAL VALUE

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UDC 582.28:581.19

*On the basis of the results of an investigation of cellulolytically active micromycetes the full value and balanced nature of their protoplasmic proteins have been shown.*

The albumin fractions of micromycetes are the most balanced in relation to amino acid composition. In addition to tryptophan, they contain all the essential amino acids, among which lysine, valine, cystine+methionine, isoleucine, and threonine are the most abundant. The protoplasmic proteins of the biomass — albumins and globulins — predominate in the cultures investigated and together amount to from 57.67 to 90.26% of the total protein content of the biomass, while the reserve proteins — prolamines and glutelins — amount to from 23.28 to 39.07%.

The nutritional and biological value of products of microbiological origin is determined by the amount of protein and its composition. Biological efficacy can be judged from the amino acid composition in comparison with aminograms of standard proteins, from the qualitative and quantitative proportions of essential amino acids, and from the calculation of amino acid indices. The most balanced and, from the physiological point of view, accessible proteins are considered to be the protoplasmic varieties — albumins and globulins — and the less balanced are the reserve proteins — prolamines and glutelins [1].

In this connection, we have studied the fractional and amino acid compositions of micromycete proteins. We found that the micromycetes investigated formed a considerable amount of biomass: *T. harzianum*, 20 g/liter; a protoplast culture of *T. harzianum*-19/28-30, 22 g/liter; and a hybrid culture of *T. harzianum*-G-26, 25 g/l, the maximum amount of protein being found in the biomass of the hybrid culture — 38.20% (Table 1).

TABLE 1. Biochemical Characteristics of Mycelial Fungi Grown on a Synthetic Medium

| Culture | Biomass | Protein | Fat | Nucleic acid | Vitamins of the B group, μg/g | | | Ash composition, % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | thiamine | biotin | inositol | P | Ca | Mg |
| *T.harzianum* | 20.20 | 30.20 | 2.10 | 0.78 | 20.00 | 5.30 | 12.80 | 2.16 | 1.12 | 0.33 |
| *T.harzianum-19/28-30* | 22.60 | 35.60 | 2.70 | 0.84 | 25.50 | 3.90 | 18.60 | 2.32 | 1.24 | 0.39 |
| *T.harzianum-G-26* | 25.20 | 38.20 | 2.90 | 0.82 | 28.50 | 6.20 | 29.60 | 2.75 | 1.63 | 0.45 |

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 385—389, May-June, 1999. Original article submitted January 4, 1999.

0009-3130/99/3503-0347$22.00 

TABLE 2. Amino Acid Composition of the Protein from the Biomass of Mycelial Fungi

| Amino acid | FAO norm, g/100 g of protein | Content of essential amino acids (a) and of amino acids in the biomass (b), % | | | | | |
|---|---|---|---|---|---|---|---|
| | | a | b | a | b | a | b |
| | | *T.harzianum* | | *T.harzianum*-19/28-30 | | *T.harzianum*-G-26 | |
| Lysine | 5.5 | 4.50 | 4.50 | 4.63 | 4.63 | 9.20 | 9.20 |
| Histidine | | - | 9.40 | - | 8.60 | - | 8.00 |
| Arginine | | - | 13.20 | - | 7.85 | - | 6.80 |
| Aspartic acid | | - | 9.20 | - | 10.60 | - | 6.60 |
| Threonine | 4.0 | 5.05 | 5.05 | 4.42 | 4.42 | 4.70 | 4.70 |
| Serine | | - | 10.40 | - | 9.30 | - | 8.40 |
| Glutamic acid | | - | 12.52 | - | 14.00 | - | 17.90 |
| Glycine | | - | - | - | 6.18 | - | 5.90 |
| Alanine | | - | 7.42 | - | 5.82 | - | 4.56 |
| Valine | 5.0 | 6.20 | 6.20 | 5.00 | 5.00 | 5.40 | 5.40 |
| Cystine+methionine | 3.5 | 5.00 | 5.00 | 5.70 | 5.70 | 5.50 | 5.50 |
| Isoleucine | 4.0 | 6.00 | 6.00 | 7.40 | 7.40 | 5.82 | 5.82 |
| Leucine | 7.0 | 6.02 | 6.00 | 5.50 | 5.50 | 5.32 | 5.32 |
| Tyrosine + phenylalanine | 6.0 | 5.05 | 5.05 | 5.00 | 5.00 | 5.86 | 5.86 |
| Without taking tryptophan into account | | | | | | | |
| Sum of essential amino acids | | 37.90 | | 37.65 | | 41.80 | |
| Total sum of biomass amino acids | | | 100.0 | | 100.0 | | 100.0 |
| FAO norm | 35.0 | | | | | | |
| Biological value | | 108.28 | | 107.50 | | 119.42 | |

In the biomass of the cultures studied the fat content ranged from 2.10 to 2.90% and the nucleic acid content from 0.78 to 0.82%. As can be seen from Table 1, the culture synthesized group B vitamins: thiamine ($B_1$), biotin ($B_7$), and inositol ($B_8$). The most active producer of these vitamins was the *T. harzianum*—G-26 fungal culture, forming the largest amounts of thiamine and inositol in the biomass — 28.50 and 29.60 μg/g, respectively.

A study of the ash composition of the fungal mycelium showed that the largest amounts of phosphorus, calcium, and magnesium were also contained in the *T.harzianum*—G-26 hybrid culture — 2.75, 1.63, and 0.45%, respectively.

A study of the amino acid composition of the protein of the biomass of *T. harzianum* showed that all the cultures produced the same number of amino acids (Table 2).

The *T. harzianum*—G-26 hybrid culture formed 51% of essential amino acids on the total amount of biomass protein. The amount of lysine in *T. harzianum* was 4.50%, in the protoplast culture of *T. harzianum*-19/28-30 it was 4.63%, and in the *T. harzianum*—G-26 hybrid culture 9.20%. The amounts of cystine+methionine were, respectively, 5.00, 5.70, and 5.50% of the total sum of the amino acids of the protein biomass. So far as concerns the sum of the essential amino acids, all the cultures investigated exceeded the FAO norm.

It is known that the ratio of protoplasmic and reserve proteins, together with the amino acid composition, determines the biological value of proteins to a considerable degree [2]. The biological values of the proteins of *T. harzianum* and of the protoplast and hybrid cultures of the fungus amounted to 108.28, 107.50, and 119.42, respectively. As can be seen from Table 3, the protoplasmic proteins (albumins and globulins) predominated in the biomass proteins of all the micromycetes studied and amounted in total to from 57.67 to 90.26% of the total biomass protein.

TABLE 3. Amino Acid Compositions of the Protein Fractions of the Biomasses of Mycelial Fungi (%) and their Biological Values

| Amino acid | *T. harzianum* | | | | | *T. harzianum*-19/28-30 | | | | | *T. harzianum*-G-26 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | total | I | II | III | IV | total | I | II | III | IV | total |
| Lysine | 2.76 | 0.44 | 0.12 | 1.08 | 4.4 | 3.04 | 0.51 | 0.18 | 1.24 | 4.97 | 4.2 | 0.71 | 0.22 | 1.74 | 6.87 |
| Histidine | 1.04 | 0.16 | 0.08 | 0.48 | 1.76 | 1.26 | 0.22 | 0.16 | 0.64 | 2.28 | 1.74 | 0.44 | 0.24 | 0.98 | 3.40 |
| Arginine | 0.88 | 0.22 | 0.11 | 0.76 | 1.37 | 0.96 | 0.34 | 0.22 | 0.88 | 2.40 | 1.24 | 0.74 | 0.34 | 1.44 | 3.76 |
| Aspartic acid | 3.02 | 0.96 | 0.26 | 2.06 | 6.3 | 3.48 | 1.08 | 0.44 | 2.44 | 7.44 | 4.54 | 2.02 | 0.84 | 3.98 | 11.39 |
| Threonine | 2.34 | 0.58 | 0.33 | 0.74 | 3.98 | 3.04 | 0.76 | 0.28 | 0.95 | 5.03 | 5.02 | 1.02 | 0.62 | 1.24 | 7.97 |
| Serine | 1.76 | 0.32 | 0.18 | 1.02 | 3.28 | 2.04 | 0.46 | 0.26 | 1.48 | 4.23 | 3.24 | 0.86 | 0.44 | 1.95 | 6.49 |
| Glutamic acid | 10.20 | 2.04 | 0.56 | 3.18 | 15.98 | 13.04 | 2.54 | 0.74 | 3.96 | 21.28 | 16.04 | 4.02 | 0.96 | 7.86 | 28.86 |
| Proline | 1.34 | 0.22 | 0.11 | 0.42 | 2.09 | 1.74 | 0.44 | 0.19 | 0.76 | 3.13 | 2.04 | 0.56 | 0.26 | 1.03 | 3.89 |
| Glycine | 3.14 | 0.78 | 0.24 | 1.44 | 5.60 | 3.72 | 0.94 | 0.42 | 1.56 | 6.64 | 4.22 | 1.02 | 0.32 | 2.98 | 8.54 |
| Alanine | 4.02 | 0.54 | 0.11 | 1.04 | 5.71 | 5.34 | 1.02 | 0.18 | 2.42 | 8.46 | 6.34 | 1.42 | 0.44 | 3.54 | 11.74 |
| Valine | 3.42 | 0.41 | 0.08 | 1.08 | 4.99 | 4.54 | 0.96 | 0.28 | 1.34 | 7.12 | 5.24 | 1.22 | 0.32 | 1.74 | 8.52 |
| Methionine+cystine | 0.84 | 0.16 | 0.05 | 0.47 | 1.52 | 0.96 | 0.22 | 0.16 | 0.42 | 1.76 | 1.24 | 0.44 | 0.24 | 1.94 | 3.86 |
| Isoleucine | 3.04 | 0.36 | 0.07 | 1.56 | 5.03 | 4.56 | 0.72 | 0.12 | 0.82 | 6.25 | 5.46 | 0.92 | 0.22 | 1.18 | 7.78 |
| Leucine | 3.76 | 0.78 | 0.31 | 1.84 | 6.69 | 4.41 | 0.92 | 2.24 | 2.24 | 7.81 | 5.56 | 1.22 | 0.28 | 1.04 | 8.38 |
| Tyrosine | 0.84 | 0.44 | 0.12 | 0.22 | 1.62 | 1.24 | 0.64 | 0.22 | 1.48 | 2.58 | 2.04 | 0.75 | 0.12 | 1.54 | 4.45 |
| Phenylalanine | 1.32 | 0.44 | 0.14 | 0.04 | 1.91 | 1.96 | 0.92 | 0.31 | 1.74 | 3.98 | 2.48 | 1.56 | 0.44 | 1.08 | 5.56 |
| Essential | 18.32 | 3.61 | 1.13 | 7.03 | 30.08 | 23.75 | 5.65 | 1.79 | 12.24 | 33.43 | 31.30 | 8.84 | 2.46 | 11.50 | 54.10 |
| Nonessential | 25.40 | 5.34 | 1.98 | 10.4 | 40.81 | 31.58 | 7.04 | 2.61 | 14.19 | 55.86 | 39.04 | 11.08 | 3.84 | 23.70 | 78.00 |
| Without taking tryptophan into account | | | | | | | | | | | | | | | |
| Biological value | 52.34 | 10.31 | 3.22 | 20.08 | 85.94 | 68.85 | 16.14 | 5.11 | 35.00 | 95.51 | 89.42 | 25.25 | 7.02 | 32.85 | 154.70 |

I - albumins; II - globulins; III - prolamines; IV - glutelins.

The albumin fractions were the most balanced in amino acid composition, as well. They contained all the essential amino acids, among which lysine, threonine, valine, leucine, and isoleucine were present in the largest amounts. Of the nonessential amino acids, high levels of glutamic and aspartic acids and glycine and alanine were noted. The reserve proteins contained a smaller amount of essential amino acids (see Table 3).

The biological values of the protein fractions of the micromycetes studied, calculated from aminograms, were as follows: in *T. harzianum* the protoplasmic proteins amounted to 10.31—52.34%, in a protoplasmic culture of *T. harzianum*-19/28-30, to 16.14—67.85%, and in the *T. harzianum*—G-26 hybrid to 25.25—89.42% of the total amount of biomass protein. The biological values of the reserve proteins of the above-mentioned cultures were, respectively, 3.22—20.08, 5.11—35.00, and 7.02—32.85% of the total sum of the amino acids of the biomass proteins.

The largest amount (62.40%) of albumins was contained in the *T. harzianum*—G-26 hybrid culture (Table 4). The ratio of the total protoplasmic water- and salt-soluble proteins to the total amount of protein in the biomass shows that the maximum amount of albumins was present in the hybrid culture. This is probably connected with the source of nutrition and with the synthesis of a more active enzyme complex.

It was observed that in all the cultures studied the water-, salt-, and alkali-soluble protein fractions predominated. The alcohol-soluble fractions of the biomass formed the highest percentage of the total sum of the soluble proteins, the difference in their amounts being insignificant and ranging from 2.40 to 2.90%. The protoplasmic proteins of the biomass — the albumins and globulins — predominated in the micromycetes investigated and amounted in sum to from 61.60 to 66.60%, while the reserve proteins amounted to from 32.40 to 38.40% of the total biomass protein.

TABLE 4. Fractional Composition of the Biomass Proteins of Mycelial Fungi, %

| Culture | Albumins | Globulins | Prolamines | Glutelins |
|---|---|---|---|---|
| *T. harzanum* | 52.40 | 9.20 | 2.90 | 35.5 |
| *T. harzanum*-19/28-30 | 56.40 | 7.20 | 2.40 | 34.0 |
| *T. harzanum*-G-26 | 62.40 | 5.20 | 2.40 | 30.0 |

Thus, the full value and balanced nature of the protoplasmic proteins of micromycetes with respect to amino acid composition permit the proteins studied to be considered as products of high biological value.

## EXPERIMENTAL

Cellulolytically active micromycetes were investigated. A culture of *Trichoderma harzianum* was isolated from the soil of the Tashkentskaya oblast; a protoplasmic culture of *T. harzianum*-19/28-30 was obtained by the protoplastizing and regeneration of protoplasts of the mutant strain *T. harzianum*-19/28; and the hybrid culture *T. harzianum*—G-26 was obtained by the interspecies hybridization of *T. harzianum*.

The material for sowing was prepared in Czapek-Dox liquid nutrient medium. Fermentation was conducted by the deep cultivation method in Erlenmeyer flasks containing 100 ml of fermentation medium at a cultivation temperature of 28°C on a shaking machine at 220 rpm. The time of cultivation was 7 days. We used 5% of sowing material on the volume of the fermentation medium. The fermentation medium used was modified Czapek-Dox medium containing as the source of carbon comminuted wheat straw in an amount of 2% on the volume of the fermentation medium. After the end of the time of cultivation of the micromycetes, the biomass was collected by filtration and was dried at 50°C to constant weight. The fractional compositions of the proteins in samples were determined by the method of [3].

Fat and ash contents were determined by the generally adopted methods of GOST[State Standard]-23637-79, vitamins by the method of [4], and the amino acid composition of the proteins fraction of the micromycete biomass by the methods of [5] and [6]. The quantitative determination of amino acids was carried out on an AAA-881 amino acid analyzer.

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