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Determination of Protein and Nitrogen Fractions of Wild Edible Mushrooms

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The purposes of this study were to determine the crude protein content of mushrooms, as well as the amounts of water soluble nitrogen, trichloro acetic acid soluble nitrogen and phosphotungstic acid soluble nitrogen. Thirty wild edible mushrooms commonly collected in region of Erzurum in Turkey were analyzed for nitrogen contents, crude protein, water soluble-nitrogen (WSN), trichloro acetic acid-soluble nitrogen (TCA-SN) and phosphotungstic acid-soluble nitrogen (PTA-SN). The macronutrient profile in general revealed that the wild mushrooms were rich sources of protein. The investigated mushroom samples contained relatively high total protein content (18.32-64.70 %, based on dry weight). The highest protein concentration were found in *Polyporus squamosus* (64.70 %, based on dry weight). The highest values of nitrogen fractions was determined as follows: water soluble nitrogen (WSN/TN %) in *Marasmius oreades* (88.02 %), trichloro acetic acid-soluble nitrogen (TCA-SN/TN %) in *Marasmius oreades* (78.40 %), phosphotungstic acid-soluble nitrogen (PTA-SN/TN %) *Boletus chrysenteron* (50.16 %). The average values of total nitrogen, crude protein, water soluble nitrogen, trichloro acetic acid soluble nitrogen and phosphotungstic acid soluble nitrogen of musroom samples were found (5.68, 35.54, 63.45, 53.27 and 31.98 %), respectively. Mushrooms may be a valuable protein supplement for human diets although they are generally preferred for their flavour and taste. Most of the studies on mushroom protein fractions are to be limited on certain mushroom species.

Key Words: Mushrooms, Nitrogen, Protein, Nitrogen fractions.

INTRODUCTION

There are over 2500 different types of mushrooms in the world, but only a few are specifically cultivated for commercial purpose. Mushrooms have long been valued as highly tasty, delicious and nutritional foods by many societies throughout the world. Mushrooms are appreciated, not only for texture and flavour but also for their chemical and nutritional characteristics[1,2]. The consumption of wild edible mushrooms is increasing, even in the developed world, due to a good content of

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proteins as well as a higher content of trace minerals[3]. The increased proteins necessity in the population induced the investigations on natural products which would be an adequate substitution for the proteins of animal origin. Edible mushrooms have these kind of features, which classify them as protein source for the future, because the protein content in most of macrofungi species is higher than those of lots of vegetables. Also the cultivation of macrofungi takes shorter time and it is inexpensive than vegetables[1,4,5].

Protein in mushrooms consists of an high quality plant protein and is easily digested. Protein content can vary from 10-40 % of dry weight. The qualitative and quantitative analysis of their proteins revealed great intra and inter species differences. This variation can also be seen in their different nutritive parameters. The heterogeneity of the structural and physico-chemical properties of the mushroom proteins may be the main cause of such differences. However, there are only a few reports referring to this issue[4,6].

Wild mushrooms are a popular food source in Turkey[7-9]. *Morchella* sp., *Agaricus campestris* var. *campestris*, *Agaricus urinascens*, *Pleurotus eryngii* and *Lactarius deliciosus* is collected and consumed in Turkey[8-10]. Except for some cultivated mushrooms, little is known about the nutritional value of the edible wild species. Furthermore, many people, locally and internationally, believe that the protein quality of mushrooms could be higher than that of vegetable. Their great number enables the selection of those that are characterized by a high protein content and a high nutritional quality. In order to determine the quality of Turkey's mushrooms, it is desirable to be separated their proteins and studied the individual fractions in detail. The relative proportion of each fraction in mushrooms strongly affects the nutritional quality of the total mushroom protein[11-13]. Thus, the purposes of this study were to determine the crude protein content of mushrooms, as well as the amounts of water soluble nitrogen, trichloro acetic acid soluble nitrogen and phosphotungstic acid soluble nitrogen. No studies have been conducted on the protein and nitrogen fractions value, especially the protein quality of local types of mushroom.

EXPERIMENTAL

Thirty macrofungi samples were collected during field trips in Erzurum province between 1997 and 2000. During fields studies, colour slides of the macrofungal specimens were taken in their natural habitats. After relevant notes were taken on their morphological and ecological features, they were put in specially prepared boxes and brought to the laboratory. Their spore prints were taken and spore dimensions were measured using an ocular micrometer. Then, dried specimens were placed in locked polyethylene bags and kept in deep freezer at -20 ºC to protect against parasites.

Identification of the specimens was performed with the help of several researchers[14-22]. All specimens are kept in Herbarium of Yüzüncü Yil University, Department of Biology (VANF) (Table-1).

TABLE-1
SPICES OF WILD EDIBLE MACROFUNGI COLLECTED FROM ERZURUM REGION OF TURKEY

No	Class, Family and species of macrofungi	Habitat	VANF no:
	Ascomycetes		
	Morchellaceae Rchb.		
1.	*Morchella vulgaris* (Pers.) Boud.	Erzurum, Senkaya, Gaziler village, in mixed wood	1145
2.	*Morchella esculenta* (L.) Pers	Erzurum, Senkaya, Sindiran village, in pine forest	1277
	Helvellaceae Fr.		
3.	*Helvella lacunosa* Afzel.	Erzurum, Senkaya, Gaziler village, under *Salix* sp.	1247
	Basidioomycetes		
	Agaricaceae Chevall.		
4.	*Agaricus campestris* L., var. *campestris*	Erzurum, Hinis, Çiçekdagi village, steppe	1544
5.	*Agaricus urinascens* (Jul. Schäff. & F.H. Møller) Sing. var. *urinascens*	Erzurum, Senkaya, Yukari Gözebasi village, steppe	1290
6.	*Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson	Erzurum, Karayazi, Çelikli village, on *Populus* sp. Stumps	1532
7.	*Macrolepiota procera* (Scop.) Singer var. *procera*	Erzurum, Oltu, Ormanagzi village, in pine forest	1374
8.	*Leucoagaricus nympharum* (Kalchbr.) Bon	Erzurum, Ispir, along Ispir-Rize highway, in conifer forest	1826
	Boletaceae Chevall.		
9.	*Boletus chrysenteron* Bull.	Erzurum, Senkaya, Gaziler village, in mixed wood	967
10.	*Leccinum scabrum* (Bull.) Gray var. *scabrum*	Erzurum, Senkaya, Gaziler village, in mixed wood	1302
	Bolbitiaceae Singer		
11.	*Agrocybe dura* (Bolton) Singer	Erzurum, Tekman, 10 km along the Tekman-Erzurum highway, steppe	1549
	Cantharellaceae J. Schröt.		
12.	*Cantharellus cibarius* Fr. var. *cibarius*	Erzurum, Oltu, Ormanagzi village, steppe	963
	Lycoperdaceae Chevall.		
13.	*Handkea utriformis* (Bull.) Kreisel	Erzurum, Senkaya, Gaziler village, in mixed wood	967
14.	*Lycoperdon perlatum* Pers.	Erzurum, Senkaya, Sindiran village, in mixed wood	967
15.	*Vascellum pratense* (Pers.) Kreisel	Erzurum, Ispir, Çamlikaya village, in conifer forest	1451
	Marasmiaceae Roze ex Kühner		
16.	*Marasmius oreades* (Bolton) Fr.	Erzurum, Pasinler, in garden	1609
	Pleurotaceae Kühner		
17.	*Pleurotus eryngii* (DC.) Gillet	Erzurum, S enkaya, Yukari Gözebasi village, steppe	1289
18.	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Erzurum, Horasan, Azapsuyu picnic area, on *Salix* sp., stumps	1502
	Pluteaceae Kotl. & Pouzar		
19.	*Volvariella gloiocephala* (DC.) Boekhout & Enderle	Erzurum, Karayazi, Daragut village, weadow	1531
	Polyporaceae Fr. ex Corda		
20.	*Lentinus tigrinus* (Bull.) Fr.	Erzurum, Karayazi, Çelikli village, on *Populus* sp. Stumps	1534
21.	*Polyporus squamosus* (Huds.) Fr.	Erzurum, Karayazi, Çelikli village, on *Populus* sp.	1533

No	Class, Family and species of macrofungi	Habitat	VANF no:
	Psathyrellaceae Vilgalys		
22.	*Psathyrella candolleana* (Fr.) Maire	Erzurum, Olur, Begendik village, on *Populus* sp. Stumps	1351
	Russulaceae Lotsy		
23.	*Lactarius piperatus* (L.) Pers.	Erzurum, Olur, Begendik village, mixed forest	1354
24.	*Russula delica* Fr.	Erzurum, Senkaya, Gaziler village, mixed forest	962
	Strophariaceae Singer & A.H. Sm.		
25.	*Stropharia coronilla* (Bull.) Fr.	Erzurum, Çat, steppe	1590
	Suillaceae Besl & Bresinsky		
26.	*Suillus granulatus* (L.) Roussel	Erzurum, Senkaya, Gaziler village, in conifer forest	1296
27.	*Suillus luteus* (L.) Roussel	Erzurum, around Abdurrahman Gazi Türbesi, *Pinus slyvestris* nursery	1220
	Tricholomataceae R. Heim ex Pouzar		
28.	*Clitocybe gibba* (Pers.) P. Kumm.	Erzurum, Senkaya, Sindiran village, in conifer forest	1258
29.	*Lepista nuda* (Bull.) Cooke	Erzurum, Senkaya, Sindiran village, in conifer forest	1253
30.	*Lepista personata* (Fr.) Cooke	Erzurum, around Abdurrahman Gazi Türbesi, *Pine* nursery	1581

Determination of protein content: The crude protein content of the samples was estimated by the Kjeldhal method[23,24], in which the sample was digested with a known quantity of acid in the digestion apparatus. The digested material was distilled after the addition of alkali. The released ammonia was collected in 4 % boric acid in the automatic distiller. The resultant boric acid, which now contained the ammonia released from the digested material, was then titrated against 0.1 N HCl, manually. The total nitrogen content determined was multiplied by a factor of 6.25 to arrive at the amount of crude protein.

Determination of nitrogen fractions: Water-soluble nitrogen (WSN) was determined by the Kjeldahl method as described follows; 5 g of sample was homogenized with 100 mL distilled water and filtered. The nitrogen content of the mushroom extracts was expressed as percentage of total nitrogen (WSN/TN, %). Trichloroacetic acid soluble nitrogen (TCA-SN/TN, %) was determined in the same mushroom extract described below. Water-soluble extract (10 mL) was added to equal volume of 24 % (w/v) TCA (Sigma-Aldrich) and after mixing it was incubated at room temperature for 2 h. Then, precipitate was filtered through Whatmann filter paper no. 40. Filtrate of the nitrogen was determined according to the Kjeldahl method and TCA-SN was expressed as a percentage of total nitrogen (TCA-SN/TN, %). Water-soluble extract (15 mL) was mixed with 10.5 mL of 3.95 M H_2SO_4 (Merck) and 4.5 mL 33 % (w/v) phosphotungstic acid (PTA; Sigma-Aldrich). The mixture was held at room temperature for 3 h and then filtered through Whatmann filter paper no. 40. Nitrogen content of filtrate was determined according to the Kjeldahl method and PTA-SN was expressed as percentage of total nitrogen (PTA-SN/TN, %)[24-26].

Statistical analysis: Statistical data processing was carried out on SPSS for Windows Base System User's Quide Release 9.00[27].

RESULTS AND DISCUSSION

Table-2 presents data on the relative and average amounts of total nitrogen, crude protein, water soluble nitrogen, tricloroacetic acid soluble nitrogen and phosphotungstic acid soluble nitrogen in wild edible mushrooms. Total nitrogen varies from 2.93 to 10.35 % (on the dry basis) and, in particular, *Pleurotus eryngii* shows

TABLE-2
AMOUNTS OF TOTAL N, CRUDE PROTEIN AND NITROGEN FRACTIONS WILD EDIBLE MUSHROOMS (%)

No	Total N	Crude Protein	WSN/TN	TCA-SN/TN	PTA-SN/TN
1.	9.68	60.48	73.00	56.50	38.02
2.	7.60	47.60	87.37	73.86	46.93
3.	3.03	19.07	46.29	45.47	26.10
4.	4.35	27.21	45.02	43.74	21.44
5.	7.02	43.93	56.59	44.00	14.54
6.	6.83	42.71	71.09	67.55	38.93
7.	3.11	19.48	75.17	64.04	30.51
8.	7.00	43.75	59.35	49.08	29.84
9.	8.44	52.72	74.33	69.44	50.16
10.	8.82	55.13	55.86	49.61	40.43
11.	3.94	24.68	47.95	44.93	18.81
12.	4.60	28.86	78.68	66.30	41.30
13.	6.19	38.73	78.10	61.42	33.70
14.	4.49	28.13	77.40	72.77	41.05
15.	4.37	27.64	68.98	61.45	35.27
16.	3.66	22.89	88.02	78.40	46.30
17.	2.93	18.32	35.28	34.22	20.81
18.	6.43	40.30	62.52	56.14	31.18
19.	5.33	33.36	45.25	38.03	17.95
20.	4.64	29.03	69.55	51.75	41.81
21.	10.35	64.70	56.62	27ç62	17.81
22.	4.22	26.45	38.42	35.62	15.24
23.	8.31	52.04	42.04	31.13	30.77
24.	8.69	54.01	71.16	59.47	48.88
25.	4.22	26.47	82.11	68.14	36.04
26.	6.52	40.73	64.35	54.14	33.53
27.	4.97	31.16	56.68	38.28	22.83
28.	2.95	18.51	54.65	35.72	13.43
29.	3.99	25.01	75.44	61.14	40.56
30.	3.69	23.14	66.19	58.08	35.25
Average	5.68±0.40	35.54±2.46	63.45±2.68	53.27±2.54	31.98±1.99

TN = Total Nitrogen, WSN = Water soluble nitrogen, TCA-SN = Tricloroacetic acid soluble nitrogen (NPN), PTA-SN = Phosphotungstic acid soluble nitrogen, ± Standard Error of Mean.

the lowest nitrogen content[1] was reported that total nitrogen varies from 3.47 to 7.93 % (on the dry basis). This large variability can be ascribed, as already reported in the literature[1,28,29], to the large genetic variation.

Protein is an important component of dry matter of mushrooms. Protein ranged from 18.32 to 64.70 % in mushrooms (Table-2). Protein and nitrogen fraction contents were changeable. The average protein content for all mushrooms was *ca.* 35.54 %. Protein content of some mushroom species, such as *Polyporus squamosus, Morchella vulgaris* and *Leccinum scabrum* are higher than those of other species (Table-2). However, protein content of *Pleurotus eryngii*, *Clitocybe gibba* and *Helvella lacunosa* are lower than those of other species (Table-2). The results obtained from the nitrogen fraction have indicated some variations between the same and different species from different origins in all fractions, which is probably due to genetic and environmental factors[4,7,8,30,31]. The protein contents of the mushrooms were close to those reported[4], in which the author obtained for *Cantharellus cibarius* (16.19 %), *Marasmius oreades* (40.19 %), *Pleurotus ostreatus* (24.69 %) and *Suillus granulatus* (24.68 %). Macedonian mushroom samples contained relatively high total protein content (14.0-52.1 %, dry weight)[4]. Edible fungi are highly valued as a good source of protein and their protein contents usually range from 19 to 35 % of dry weight[28], from 15.4 to 26.7 %[32] or from 14.6 % to 22.3 %[33]. In this study, protein contents of 30 mushroom taxa ranged from 18.32 to 64.70 %. This meant that some mushroom contained much higher protein than common mushrooms. More variability was observed in the protein and nitrogen fractions content of mushrooms (Table-2). It is known that the protein and nitrogen fraction contents of mushrooms are affected by a number of factors, namely the type of mushrooms, the stage of development, the part sampled, level of nitrogen available and the location[34,35]. Thus, a higher protein content in the mushrooms could not be attributed to a higher trichloro acetic acid-soluble nitrogen and other nitrogen fraction content (Fig. 1).

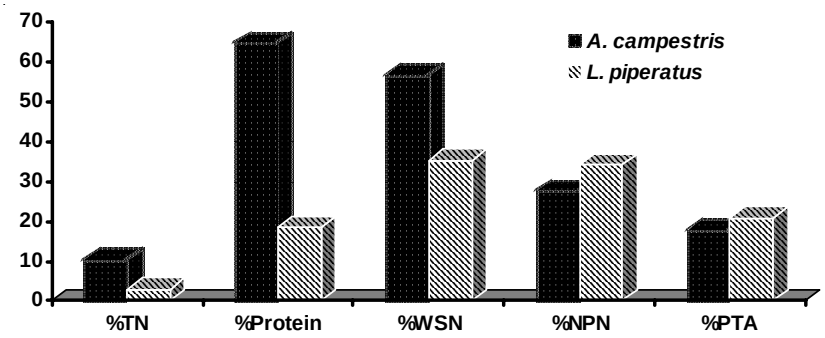

Fig. 1. Contents of the highest and lowest protein of two mushrooms and nitrogen fractions

Globular proteins are water-soluble and include the globulins and albumins. Water soluble nitrogen content of some mushroom species, such as *Marasmius oreades* (88.02 %), *Morchella esculenta* (87.37 %) and *Stropharia coronilla* (82.11 %)

are higher than those of other species (Table-2). The reported that[4], for most of the mushrooms samples, albumins were the highest, followed by globulins, whereas glutelin-like substances and the prolamine-like material gave the lowest values in most of the investigated samples.

Apart from protein compounds, free amino acids, chitin, amines, nucleic acids and urea can also be found in mushrooms. TCA-SN is known to be an indication of the amount of small peptides (< 20 amino acid residues) and amino acids present in mushroom. PTA-SN ratio is known as tri-dipeptides and the free amino acids soluble in this fraction. The highest and lowest PTA-SN was found 50.16 % in *Boletus chrysenteron* and 13.43 % in *Clitocybe gibba*, respectively. Average value of trichloro acetic acid soluble nitrogen and phosphotungstic acid soluble nitrogen ratio was found as 53.27 and 31.98 %, respectively in all the investigated samples. The protein fraction is effective on the taste of mushrooms. The taste of edible mushrooms is primarily due to the presence of several small water soluble substances, including nucleotides, free amino acids, some other elements, such as nitrogen, phosphorus, potassium, sulphur, iron and zinc and also on the autooxidation of ansaturated fatty acids and soluble sugars and polyols[36-39].

Mushrooms may be a valuable protein supplement for human diets although they are generally preferred for their flavour and taste. Most of the studies on mushroom protein fractions are to be limited on certain mushroom species. However, the present results indicate that economically important and edible mushrooms contain significant amounts of valuable protein. Therefore, studies should be performed on protein and nitrogen fractions content of other economically important and edible mushrooms. Also, a careful study and popularization of the more nutritious species of wild mushrooms from Turkey is necessary to realize their full nutritional potentials as food supplements. In conclusion, mushrooms, in spite of the great variability observed in the present study, represent an interesting food item.

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