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Protein and chitin nitrogen contents and protein content in *Pleurotus ostreatus* var. *columbinus*

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Proteins and chitin were extracted and purified from dried fruitbodies of *P. ostreatus* var. *columbinus* grown on wheat straw and grass hay. Water- and sodium chloride-extractable proteins were purified by dialysis and precipitated by 10% TCA. These proteins contained 15.2% nitrogen (Kjeldahl and amino acid methods). The protein/nitrogen ratio of 6.58 was found and recommended as the new conversion factor. Chitin was purified from acid- and alkali-insoluble residue by 2% $KMnO_4$ and 15% $NaHSO_3$. This chitin contained 5.9% nitrogen and its infrared spectrum was similar to that of commercial chitins. The chitinous nitrogen content was about 0.34% dry weight of fruitbodies. The protein content was calculated from the non-chitinous nitrogen content and the factor 6.58. It was 17.1 and 23.5% dry weight of fruitbodies on wheat straw and grass hay, respectively, rather than 18.4 and 24.4% (as total nitrogen × 6.25). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Much of the interest in mushrooms as a food source is centred on their high protein content and their relatively high nutritional value. Mushrooms have been considered for a long time as a protein source for developing countries. Protein content of food is generally evaluated as total Kjeldahl nitrogen multiplied by a suitable conversion factor. The conversion factor of 6.25 is generally used for foods such as eggs, peas, meat, beans, corn and mushrooms to express their protein contents from the total nitrogen contents. This factor is based on the presumption that proteins of those products contain 16% nitrogen.

The expected 16% nitrogen in protein was checked for some other foods of animal origin only. However, for mushrooms, the nitrogen content of purified proteins is unknown or different. For example, in *Agaricus campestris*, only 11.8% nitrogen was found in the purified proteins rather than the expected 16% nitrogen (Fitzpatrick *et al.*, 1946). In respect of this result, the conversion factor should rather be 8.48. Further, mushroom proteins are rarely 100% digestible like in animal products. A closer approximation of the crude protein of mushrooms is usually obtained using a conversion factor equal to (70% N×6.25) or (N×4.38), based on the fact that about 70% of mushroom nitrogen is proteinous (Chang and Miles, 1989). The indigestible nitrogen in mushrooms includes both protein and chitin.

In addition to the confusion surrounding the conversion factor, mushrooms contain a significant amount of non-protein nitrogen consisting of chitin and free amino acids. As chitin is indigestible, its nitrogen should not be used in the evaluation of crude protein. Fungal chitins contain 6.0–6.3% nitrogen (Proskuriakov, 1926). The chitin content of *Pleurotus* varies from 4.7 to 4.9% dry weight (Yoshida *et al.*, 1986).

Our objective was to determine the protein content of *P. ostreatus* var. *columbinus* Quélet apud Bresadola from the non-chitinous nitrogen content and a suitable conversion factor of nitrogen into protein.

MATERIALS AND METHODS

Fruitbody sample

Pleurotus ostreatus var. *columbinus* Quélet apud Bresadola MUCL 28785 from MUCL collection (Mycothèque de l'Université Catholique de Louvain) was cultivated on wheat straw and grass hay in a greenhouse. Dried and powdered mature fruitbodies were used as samples for extraction and purification of proteins and chitin.

Extraction and purification of proteins

A 100 g sample of dried and powdered fruitbodies of *P. ostreatus* var. *columbinus* was extracted into a 2000 ml

Erlenmeyer flask with 1000 ml of distilled water (or 5% sodium chloride) and stirred by a magnetic shaker for 1 h. The suspension was centrifuged (5000 rpm for 15 min at 5°C) and the supernatant liquid filtered on Whatman No. 1 filter paper. Proteins were isoelectrically precipitated from the filtrate with 10% trichloroacetic acid (TCA). The precipitate was dispersed in cold potassium phosphate–sodium chloride buffer at pH 7.6 consisting of 0.0325 M dipotassium hydrogen orthophosphate (K_2HPO_4), 0.0026 M potassium dihydrogen phosphate (KH_2PO_4), 0.4 M sodium chloride (NaCl) and 0.01 M β-mercaptoethanol (Roberts and Briggs, 1965). The protein solution was dialysed for 3 days at 4°C against the same potassium phosphate buffer through a semipermeable membrane (Wolf *et al.*, 1966). Dialysis tubings (Visking 101/2″) were first washed with 50% ethyl alcohol, 10 mM sodium carbonate, 10 mM EDTA and distilled water. Proteins precipitated from dialysate by the addition of 10% trichloroacetic acid were recovered by centrifugation and filtration. They were washed with distilled water and 50% acetone and dried overnight at 40°C. The yield of purified proteins was calculated by the following formula:

$$\text{protein yield}(\text{g kg}^{-1}) = \frac{\text{weight of purified proteins}}{\text{weight of fruitbody sample}} \times 100. \quad (1)$$

Extraction and purification of chitin

Chitin was extracted by successive treatments with 1 N hydrochloric acid and 2 N sodium hydroxide according to Hackman (1954). About 100 g of dried and powdered fruitbodies of *P. ostreatus* var. *columbinus* was constantly agitated in 1000 ml of 2 N HCl for 48 h at room temperature. The acid-insoluble cell wall residue was collected by centrifugation, washed with water and extracted twice with 2 N NaOH for 12 h at 100°C. The alkali-insoluble cell wall residue was collected by centrifugation (5000 rpm, 15 min) and washed with distilled water (until obtaining neutral reaction). Then it was suspended in 2% $KMnO_4$ for 8 h and 15% $NaHSO_3$ for 2 h. The remaining material was collected by filtration through Whatman No. 1 filter paper, washed with 1 N HCl, distilled water, ethanol and ether. Finally, it was dried overnight at 4°C. The yield of purified chitin was calculated as described above for proteins.

General chemical analyses

Three samples of purified proteins were subjected to nitrogen and amino acid determination, two representing water- or sodium chloride-extractable proteins of *P. ostreatus* var. *columbinus* on wheat straw and one representing water-extractable proteins of *P. ostreatus* var. *columbinus* on grass hay. Two commercial purified proteins from animal products (casein C2244, Merck, Darmstadt, Germany, and albumin 4542, UCB, Belgium) were included to determine their nitrogen content along with purified proteins of *P. ostreatus* var. *columbinus*.

Commercial chitin (C22730, Fluka Chemie AG, Buchs, Switzerland) and chitosan (C3645, Sigma Chemical Co., St. Louis, Missouri) from crab shells were analysed for comparison of their nitrogen contents and infrared spectrum to those of purified chitin from *P. ostreatus* var. *columbinus*. The total nitrogen (TN) was determined by the Kjeldahl procedure (AOAC, 1980). The nitrogen content of purified proteins or chitin was calculated taking into account their ash content as follows:

$$\text{nitrogen content (\% dry weight)} = \frac{(V_s - V_b) \times F_c \times N_{HCl} \times 14.01 \times 10}{(W - A) \times DW} \quad (2)$$

where V_s = volume of hydrochloric acid used by sample; V_b = volume of hydrochloric acid used by blank; F_c = correction factor of the normality of hydrochloric acid solution; N_{HCl} = normality of hydrochloric acid solution; W = sample weight; A = ash weight in sample; and DW = dry weight of sample.

The ash content of purified proteins and chitin was estimated after drying protein and chitin samples overnight at 55°C. The weight of ash in the protein sample subjected to nitrogen determination was calculated by the following formula:

$$\text{ash weight in sample}(\text{g}/100\text{ g}) = \frac{\text{sample weight} \times \text{ash content in sample}}{100}. \quad (3)$$

The weight of ash in chitin was calculated by this same formula.

Amino acid analysis was performed after hydrolysis with 6 M HCl at 100°C for 24 h by ion-exchange chromatography according to Moore and Stein (1963). The sulphur amino acids were analysed after performic acid oxidation to reduce losses during acid hydrolysis. Tryptophan was not determined. The nitrogen contents of individual amino acid residues of purified proteins were summed together and compared with the Kjeldahl nitrogen content. The quantification of proteins by the summation of amino acid nitrogen was done according to Horstmann (1979). The amount of glutamine and asparagine was presented as a half of the estimated amount of glutamic and aspartic acid, respectively, because amide changes into acid after hydrolysis (Tkachuk, 1966).

The infrared spectrum of chitin was measured by infrared spectrometry in potassium bromide (KBr) disc (Mol and Wessels, 1990). A 100 μl sample of the 8% chitin suspension in calcium fluoride (containing 10% alcohol) was sprayed onto a KBr disc and dried for 5 min. The infrared spectrum of pressed disc was recorded by a MIDAC spectrophotometer from 500 to 2200 cm^{-1}.

The chitin content was determined colorimetrically at 650 nm according to Ride and Drysdale (1972) after

hydrolysis with 6 M HCl at 80°C for 16 h. Chitin (C3641, Sigma Chemical Co., St. Louis, Missouri) from crab shells was used for the preparation of standards.

Protein content

The protein content was calculated by multiplying the nitrogen content of the fruitbody sample by a suitable conversion factor. The conversion factor of nitrogen into protein was calculated as the ratio of protein to nitrogen by the following formula:

$$\text{conversion factor} = \frac{\text{weight of purified proteins}}{\text{weight of nitrogen in purified proteins}} \quad (4)$$

The chitinous nitrogen was calculated by the following formula:

$$\text{chitinous nitrogen content (\% dry weight)} = \frac{\text{chitin content} \times \text{nitrogen in chitin}}{100} \quad (5)$$

As total nitrogen from mushrooms includes chitin nitrogen, the chitinous nitrogen must be subtracted from the total nitrogen. The protein content was expressed as non-chitinous nitrogen multiplied by the new factor as follows:

$$\text{protein content (\% dry weight)} = (\text{total nitrogen} - \text{chitinous nitrogen}) \times F \quad (6)$$

where F = conversion factor of nitrogen into protein.

RESULTS AND DISCUSSION

The nitrogen content in purified proteins of *P. ostreatus* var. *columbinus* on both substrates was 15.2% dry weight as presented in Tables 1 and 2 for Kjeldahl and amino acid methods, respectively. It is as high as nitrogen contents of commercial proteins (casein and albumin) from animal products.

These results showed that purified proteins from *P. ostreatus* var. *columbinus* contained at least 15.2% of nitrogen rather than the accepted 16%. This value is in the range of 11.3–32.5% nitrogen reported for purified proteins from other food products (Tristram and Smith, 1963). For mushrooms, the nitrogen content of purified proteins was determined in *Agaricus campestris* (Fitzpatrick *et al.*, 1946) to be 11.8%, which appears low compared to that of proteins of *P. ostreatus* var. *columbinus*. It is evident that purified proteins of *P. ostreatus* var. *columbinus* were highly purified because of their high nitrogen content (15.2% dry weight) and low ash content (0.5% dry weight). They were as pure as certain commercial proteins such as casein which contained 15.0 and 2.4% of nitrogen and ash, respectively.

Table 1. Kjeldahl nitrogen content of *P. ostreatus* var. *columbinus* and commercial proteins

Samples	Yield[a]	Ash[b]	Nitrogen[b]
Pleurotus proteins[c]	17.7 ± 1.8	0.5 ± 0.08	15.2 ± 0.2
Casein	ND	ND	15.0 ± 0.2
Albumin	ND	ND	13.8 ± 0.3

Data are means of three replicates ± SE.
[a]Protein yield (g kg^{-1} dry weight of fruitbodies).
[b]Ash or nitrogen content of purified proteins (as % dry weight).
[c]Mean of water- or sodium chloride-extractable proteins of *Pleurotus* grown on wheat straw or grass hay.

Table 2. Nitrogen content of purified proteins of *P. ostreatus* var. *columbinus* calculated from nitrogen content of amino acids

Amino acid	Nitrogen/amino acid ratio	Amino acid content (as % dry weight)	Nitrogen content (as % dry weight)
Aspartic acid	0.106	6.5 ± 0.4	0.69 ± 0.04
Asparagine	0.214	6.5 ± 0.4[a]	1.39 ± 0.08
Glutamic acid	0.096	5.4 ± 0.3	0.52 ± 0.03
Glutamine	0.174	5.4 ± 0.3[a]	0.94 ± 0.05
Alanine	0.158	6.6 ± 0.3	1.04 ± 0.05
Arginine	0.322	5.9 ± 0.2	1.90 ± 0.08
Cystine	0.116	1.0 ± 0.1	0.12 ± 0.01
Glycine	0.188	5.5 ± 0.3	1.03 ± 0.05
Histidine	0.271	2.7 ± 0.1	0.73 ± 0.03
Isoleucine	0.108	6.7 ± 0.7	0.72 ± 0.04
Leucine	0.108	7.3 ± 0.7	0.79 ± 0.04
Lysine	0.193	6.7 ± 0.3	1.29 ± 0.05
Methionine	0.095	1.1 ± 0.1	0.10 ± 0.01
Phenylalanine	0.085	5.4 ± 0.2	0.46 ± 0.02
Proline	0.123	5.5 ± 0.9	0.68 ± 0.01
Serine	0.134	5.5 ± 0.3	0.74 ± 0.04
Threonine	0.118	6.8 ± 0.4	0.80 ± 0.05
Tyrosine	0.078	5.2 ± 0.2	0.41 ± 0.01
Valine	0.120	7.3 ± 0.3	0.88 ± 0.02
Total nitrogen			15.23 ± 0.3

Data are means of three replicates ± SE.
[a]Glutamine and asparagine amounts were calculated as half of the glutamic and aspartic acid concentrations, respectively.

Unfortunately, the protein yield was low (approximately 17.7 g kg^{-1}). Generally it varies from 1.7 to 3.6% for other foods (Jones and Gersdorff, 1923). The low yield can be attributed to either a high content of non-protein nitrogenous substances or losses during the purification procedure (incomplete extraction or partial hydrolysis of proteins). *Pleurotus* mushrooms may contain up to one-fifth of nitrogenous substances as free amino acids (Bano and Rajarathnam, 1988). Water-insoluble glycoproteins were reported in *Pleurotus* mushrooms (Zhang *et al.*, 1994) and might not be extracted by water and sodium chloride.

The chitin from *P. ostreatus* var. *columbinus* is intermediate between commercial chitins from crab shells (Fig. 1). The three spectra appear similar but an unknown peak was observed at 1720 cm^{-1} in the spectrum of the chitin of *P. ostreatus* var. *columbinus*. The peak at 1720 cm^{-1} might be associated with the vibration of a C=O group bonded to hydroxyl when chitin is partially deacetylated. This peak is not characteristic of chitin nor chitosan because the C=O··H–O bonds were not found in either polymers (Pearson *et al.*, 1960).

The chitin purified from *P. ostreatus* var. *columbinus* contained 5.9% nitrogen which approaches the range of 6.0–6.4% reported for fungal chitins (Proskuriakov, 1926). The low nitrogen of fungal chitin can be attributed to the fact that chitin is associated with other organic compounds in fungal cell walls: β-glucans and pigments (Pont-Lezica and Quesada-Allué, 1990). The chitin yield was about 27.3 g kg^{-1} of the dry weight of fruitbodies (Table 3). Generally, the alkali-resistant cell wall material from fungi comprises 20–44% of their dry weight. But most of this is non-chitinous and can be destroyed by oxidation with potassium permanganate. The chitin yield from fleshy fungi generally ranged from 3 to 5% (Foster and Webber, 1960). The ash content of *P. ostreatus* var. *columbinus* being 0.5% of the dry weight was lower than the 5% indicated in chitosan from crab shells.

A ratio of proteins to nitrogen of 6.58 was found and is recommended as a conversion factor. This factor differs from the factors 6.25 and 4.38 used for the evaluation of protein content of mushrooms. It is slightly higher than the factors reported for non-fungal foods that varied between 5.18 and 6.38 (Lillevik, 1970).

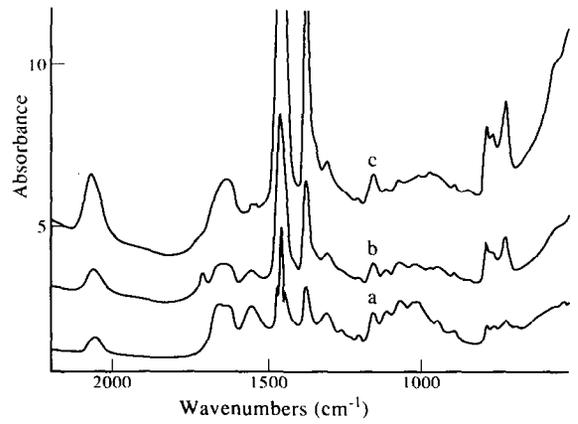

Fig. 1. Infrared absorption spectra: (a) chitin from crab shells, (b) chitin from *P. ostreatus* var. *columbinus*, (c) chitosan from crab shells.

Table 3. Nitrogen content of *P. ostreatus* var. *columbinus* and commercial chitins

Samples	Yield[a]	Ash[b]	Nitrogen[c]
Pleurotus chitin[c]	27.3 ± 1.7	0.5 ± 0.05	5.9 ± 0.09
Chitin of crab shells	ND	ND	6.9 ± .0.01
Chitosan of crab shells	ND	ND	6.4 ± 0.05

Data are means of three replicates ± SE.
[a]Chitin yield (g kg^{-1} dry weight of fruitbodies).
[b]Ash or nitrogen content in purified chitin (as % dry weight).
[c]Mean of chitins of *Pleurotus* grown on wheat straw and grass hay.

Table 4. Nitrogen and chitin contents of *P. ostreatus* var. *columbinus* (as % dry weight)

Samples	Total nitrogen (TN)	Chitin	Chitinous nitrogen (CN)
Wheat straw	2.9 ± 0.3	5.2 ± 0.2	0.31 ± 0.01
Grass hay	3.9 ± 0.3	6.2 ± 0.1	0.37 ± 0.01

Data are means of three replicates ± SE.

The chitin of *P. ostreatus* var. *columbinus* contained 5.9% nitrogen (Table 3). The chitin content was about 5.2 and 6.2% of the dry weight of fruitbodies on wheat straw and grass hay, respectively (Table 4). The chitinous nitrogen was 0.31 and 0.37% of the dry weight of fruitbodies on wheat straw and grass hay, respectively (Table 4). To calculate non-chitinous nitrogen, the mean of the chitinous nitrogen was subtracted from the total nitrogen.

The protein content expressed as non-chitinous nitrogen multiplied by the new factor 6.58 was lower than that obtained from total nitrogen and factor 6.25. It was 17.1 and 23.5% of the dry weight of fruitbodies on wheat straw and grass hay, respectively (Table 5). But when using the factor 6.25 and total nitrogen, the protein content was higher, being 18.4 and 24.4% on wheat straw and grass hay, respectively. The difference between both calculations depends on protein and chitinous nitrogen contents. However that may be, the factor 6.58 is based on the real nitrogen content of proteins of *P. ostreatus* var. *columbinus* and is established following the same concept as that used for non-fungal food proteins. So, the present investigation should be

Table 5. Protein content of *P. ostreatus* var. *columbinus* (as % dry weight)

Samples	Protein content (TN×6.25)	Protein content (nCN×6.58)
Wheat straw	18.4 ± 1.9	17.1 ± 2.0
Grass hay	24.4 ± 1.6	23.5 ± 1.7

Data are means of three replicates ± SE. nCN is non-chitinous nitrogen (TN–CN).

continued in order to establish a common conversion factor of nitrogen into proteins for edible mushrooms. This investigation has its merit in the fact that the protein content of food, including mushrooms, is often calculated by the Kjeldahl method, an easy and inexpensive technique currently used in developing countries.

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