

Nutritive Analysis of Wild Edible Mushroom *Boletus edulis* Bull ex. Fries Colleted from North West Himalayas

Renu Rana

Associate Professor, Department of Botany, RKMV Shimla, Himachal Pradesh, India

ABSTRACT: *Boletus edulis* is also known as 'the king bolete'. It has many names such as king, cep, porcini, steinpilz, penny bun and many others. It is highly variable in coloration. *Boletus edulis* is a large mushroom with a greasy to tacky, bald, brown cap and a meaty, swollen stem that features fine reticulation. The pore surface is initially white, with "stuffed" pores but as the mushroom matures the pores become more visible and the pore surface becomes greenish yellow. The flesh does not change color when the mushroom is sliced, and its surfaces do not bruise on handling. To estimate the nutritive value of *Boletus edulis* fruit bodies were dried in an oven at 38°C until a constant weight was obtained. The sample was powdered and used for nutritive analysis. The aim of the study was to determine the crude protein content, crude fiber content and total free amino acids according to standard methods mentioned under materials and methods. Crude protein, crude fiber and total free amino acids contents were found to be 31.86%, 8.9% and 25.29 mg/g (d. w.) respectively.

KEYWORDS: Crude protein, crude fiber, total free amino acids composition, *Boletus edulis*

I. INTRODUCTION

Edible mushrooms, have been part of human culture for thousands of years as a food in the most important civilizations throughout history. Mushrooms are high valued source of nutrition and mineral constituents which are of paramount importance in the present age. The mushrooms are reported to be a good source of proteins, carbohydrates, vitamins and minerals. According to Gokce and Yucel (2004), mushrooms are a healthy, low cholesterol food consisting of 90.7% water, 3.5% proteins, 0.3% fats 4.5% carbohydrates and 1.0% minerals based on fresh weight. These components are contributing to nutritional value of food. Mushrooms are recommended as an unconventional alternative source of qualitative proteins. Whereas, low amount of fat signifies its importance in diet for the sufferers of diabetes, obesity, atherosclerosis and high blood pressure. Further, proteins are made up of amino acids. Mushrooms consist of complete set of eighteen amino acids that a human being needs for his growth. Ten of these are classified as essential amino acids because they are not synthesized in human body and must, therefore, be present in diet. These are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, cystine and tyrosine. The amount of methionine in mushrooms is relatively in large amounts. These two essential amino acids are generally lacking in cereals and vegetables. Approximately 25-30% of total amino acids occur as free amino acids and the remaining being combined in proteins. In addition to common amino acids and amides, less common or unusal amino acids and related nitrogenous compounds have also been detected in various mushroom species. These are methionine, sulphoxides, cysteic acid, hydroxyproline, hydroxylysine, amino adaptic acid, aminobutyric acid, pipercolic acid, phosphoserine, cystathione, canavalline, creatinine, citrulline, ornithine, glucosamine and ethanalamine (Altamura *et al.*, 1967; Hatanaka and Terakawa, 1968; Hughes *et al.*, 1958). Carbohydrate content of mushrooms is in appreciable amount and mainly consists of glycogen, chitin and hemicellulose. The fat content is also low and contains free fatty acids, mono, di and triglycerides, sterols, sterol esters and phospholipids.

II. MATERIALS AND METHODS

Estimation of nitrogen and crude protein by micro-kjeldahl method Jackson, (1967).

Nitrogen is one of the major elements found in living organisms. It is an essential constitute of a number of compounds such as amino acids, amides, proteins, nucleotides, coenzymes, vitamins, hormones etc. Proteins are the major source of nitrogen.

Principle

In most proteins, nitrogen constitutes nearly 16% of the total composition and hence, the total nitrogen content of the sample is multiplied by 6.25 to calculate the crude protein content. However, the conversion factor varies with the amino acid composition of protein. The micro-Kjeldahl method is suitable for estimating the total nitrogen content of food material, agricultural and clinical samples. It is also used to estimate the non-protein nitrogen of a sample, after precipitating out the proteins with ice cold 10% TCA.

The sample is digested with conc. H_2SO_4 in the presence of a catalyst to convert nitrogen in protein or any other material to ammonium sulphate. By steam distillation of this salt in the presence of a strong alkali, ammonia is liberated and collected in boric acid solution as ammonium borate which is estimated against a standard acid. By an average most proteins have 16% nitrogen in their composition. In other words, 1mg nitrogen equals 6.25 mg protein. Thus, by finding out the amount of ammonia formed from a known amount of sample, one can calculate the amount of protein present.

(Note: 1ml of 0.1 N acid= 1.04/mg N).

Reagents

1. Conc. H_2SO_4 (sp. gr. 1.84, N-free)
2. Mercuric oxide
3. Potassium sulphate
4. Sodium hydroxide-sodium thiosulphate solution: Dissolve 600g of NaOH and 50 g $Na_2SO_3 \cdot 5H_2O$ in water and make up to 1 litre.
5. 0.02 N Standard HCl or H_2SO_4
6. 4% Boric acid solution: Dissolve 4 g of Boric acid (H_3BO_3) in warm water and dilute to 100 ml.

Mixed indicator solution: Mixed 2 parts of 0.2% methyl red in ethanol with one part of 0.2 % methylene blue in ethanol (or mix 1 part of 0.2% methyl red in ethanol) with 5 parts of 0.2% bromo-cresol green in ethanol.).

Estimation Method

1. Weighed about 100 mg of finely powered homogenous sample into a 30 ml digestion flask.
2. Added 2 g K_2SO_4 and 100 mg of H_2O 20ml of conc. H_2SO_4 and mixed well.
3. Flask was left for digestion over digestion rack approximately 40 min at $370^\circ C$ till the solution of flask was clear white.
4. Final volume was made 100 ml with distilled water.
5. 10 ml of this solution 1 aliquot was transferred in distillation flask of distillation apparatus.
6. Placed a marked 100 ml conical flask containing 5 ml of boric acid solution with a few drops of mixed indicator in such a way that the tip of the condenser dipping inside the solution.
7. Added 10ml of sodium hydroxide solution to the aliquot in distillation apparatus through the funnel.
8. Distilled and collected the ammonia in boric acid flask. The colour changed from violet to green is an indication of ammonia absorbed, collected at least 20 ml of distillate.
9. Rinsed the tip of the condenser with water and titrated the distilled sample against the standard H_2SO_4 0.01 Norn/100 until the appearance of original violet colour as the end point. Reading at the end point was taken.
10. Ran a blank digested similarly with an equal volume of water after washing the distillation apparatus by back suction with excess of water.

Percentage of nitrogen was then calculated as under:

$$\% \text{ age of nitrogen in the sample} = \frac{0.00014 \times T \times V \times 100}{S \times W}$$

Whereas V = Total volume of aliquot prepared.
T = ml of N/100 (0.01N) H_2SO_4 used.
S = ml of aliquot taken.
W = weight of the sample.
1ml of N/100 H_2SO_4 = 00014g of N.
.1N acid = 1.40/mg N
.01 acid=.1401 mg N or .0001401 g N.

The total crude protein was estimated by multiplying the percent nitrogen content with conversion factor 6.25 on the assumption that only 70% of total nitrogen is proteinaceous, rest 30% being mostly from chitin and thus indigestible (Crisan and Sands, 1978).

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Estimation of crude fiber content: (A.O.A.C., 1980)

The crude fiber content is commonly used as a measure of the nutritive value, and in the analysis of various foods and food products to detect, quality, quantity and adulteration. The crude fiber largely consists of cellulose and lignin and some mineral matter.

Principle

Crude fiber is the organic residue that remains after the food sample has been treated standardized condition with petroleum ether, boiled with dilute sulphuric acid, dilute sodium hydroxide and alcohol. The dried, powdered mushroom sample is first made fat free and then digested successively with acid and base. During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content.

Reagents

1. 0.255 ± 0.005 N standard H₂SO₄
2. 0.313 ± 0.005 N standard NaOH

Estimation Method

1. 2 gm of powdered sample was extracted with petroleum ether to remove fat.
2. Added 200 ml of H₂ SO₄ in a flask immediately connected the digestion flask with condenser and heated for 30 minutes.
3. Filtered through muslin cloth and washed with boiling water until washings are free of acid.
4. Boiled the residue with 200 ml of NaOH for 30 minutes.
5. Filtered through muslin cloth again and washed with 25ml of H₂SO₄, 50 ml portions of water and 25 ml of alcohol.
6. Removed the residue and transferred to pre weighed ash dish ('W'₁g).
7. Dried the residue for two hours at 130±2⁰C, in a desiccators and weighed ('W'₂g).
8. Ignited for 30 minutes at 600±15⁰C.
9. Cooled residue in a desiccators and reweighed ('W'₃g)

Calculations

Loss in weight = (W₂-W₁) - (W₃-W₁)

$$\% \text{ crude fiber content} = \frac{\text{Loss in weight noted}}{\text{Weight of the sample (s)}} \times 100$$

where W₁(in gm) = weight of empty crucible
W₂ (in gm) = weight of crucible + weight of the sample residue after drying at 130⁰C
W₃ (in gm) = weight of crucible + weight of the sample residue after drying at 600⁰C.
S (in gm) = weight of the sample.

Colorimetric estimation of total free amino acids: (Moore and Stein, 1948)

The amino acids are the basic building blocks of all proteins. These amino acids which exist in free form in tissues and not bound to proteins are called as free amino acids. Normally, during diseased conditions in plants, there will be a change in total free amino acid composition. Hence, estimation of total free amino acids gives an indication about the physiological and health condition of plants. Moore and Stein (1948) introduced the ninhydrin method and it is widely used for estimation of total free amino acids.

Principle

Ninhydrin (Tri-ketohydrindene hydrate) a powerful oxidizing agent reacts with x-amino acid between pH 4 and 8 and decarboxylates to give an intensely bluish purple coloured compound which is measured colorimetrically at 570 nm. The amino acids proline and hydroxyproline gives a yellow colour.

Reagents

1. 80% ethanol.
2. 0.5 M citrate buffer, pH 5.0
3. Ninhydrin reagent: Dissolve 0.8 g of stannous chloride (SnCl₂.H₂O) in ninhydrin in 500 ml of methyl cellosolve (2-methoxy ethanol).
4. Diluent solvent: Mix equal volumes of water and n-propanol.
5. Stock standard leucine solution: 50mg/50ml of water
6. Working standard leucine solution: Dilute 10ml of stock leucine solution to 100 ml with water.

Sample Extraction

1. Weighted 100 mg of dried, powdered mushroom sample.
2. Added 5 ml of 80% ethanol and centrifuged.
3. Repeated the extraction twice and pooled all the supernatants used this extract for the estimation of total free amino acids.

Estimation Method

1. To 200 µl of extract added 3.8 ml of mixture containing Ninhydrin reagent, propanol and citrate buffer in (5:12:2) ratio.
2. Heated in a boiling water bath for 20 minutes.
3. Cooled the tubes at room temperature.
4. Read the absorbance of purple colour against a reagent blank prepared by taking ethanol instead of extract at 570 nm.
5. Calculated the amount of total free amino acids using standard curve prepared by pipetting out 0.1-1.0 ml (10-100 µg range) of working standard leucine solution.
6. Expressed the result as percentage equivalent of leucine.

III. OBSERVATIONS

Fruit bodies of *Boletus edulis* were analyzed for their contents of crude protein, crude fiber, and total free amino acids following standard methods of analysis (as described in materials and methods). Protein values were calculated estimated with the conversion factor equal to Nx6.25 on recent assumption that only 70% of total nitrogen is proteinaceous, rest 30% being mostly from chitin and thus indigestible (Crisan and Sands, 1978) treating the sample with acid and alkali. The concentration of total free amino acids has been estimated by colorimetric technique proposed by Moor and Stein (1948).

Crude proteins, crude fibers and total free amino acid composition

Crude protein content, fiber content and total free amino acid composition was found to be 31.86%, 8.9% and 25.29 mg/g (d.w.) respectively.

Proximate composition of crude protein, crude fiber and total free amino acids contents are given in the Table 1.

Table 1: Proximate Composition of Crude Protein, crude fiber and total free amino acids of wild edible mushroom *Boletus edulis*:

Name of the mushroom	Crude protein %	Crude fiber %	Total free acids of some wild edible (mg/g)
<i>Boletus edulis</i>	31.86	8.9	25.29

IV. DISCUSSION

Mushrooms are the macro fungi that have been used as food since the time immemorial. Nutritionally, they are a valuable source of healthy food, which is low in calories and rich in carbohydrates, proteins, essential amino acids, fibers, important vitamins and minerals. Mushrooms have been used in medicines for the centuries in the orient but their potential as health potentates and elicitors of immune system is recent. In the last two decades, there has been an upsurge on the use of mushrooms as nutraceutical. Therefore, many edible species of mushrooms have been thoroughly investigated and authenticated for medicinal use. The active constituents found in mushrooms are polysaccharides, dietary fibers, oligosaccharides, tetraterpenoids, peptides, proteins, alcohols, phenols and mineral elements. There are more than 5,000 species of mushrooms recorded from all over the world, out of which around 2000 are identified as edible. A large number of the species are yet to be analysed for their nutritional, nutraceutical and medicinal potential (Lakhanpal and Rana, 2005).

Taking into consideration the nutritional value of mushroom, the investigation of the chemical composition is very important. The first step in the evaluation of nutritive value of any food stuff is the determination of its chemical components. In the presence of definite nutritional studies, chemical composition serves as a relative measure for comparison with other food stuffs of established nutritive values.

Crisan and Sands (1978), Purkayastha and Chandra (1985), Bislaria *et al.*, (1987), Khanna and Garcha, (1984), Singh and Rajaratham (1977) Bano *et al.*, (1981), Goyal *et al.*, (2006), YuJie *et al.*, (2007), Gyar *et al.*, (2008), Ingale A and

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Ramteke A (2010), Akyoz and Krbag (2010), Jonathan and Adeoyo (2011) and so many other researchers have discussed in detail the nutritive value of several edible fungi taking examples from available literature. Hence, in the present investigation, comparison of chemical composition of wild edible mushrooms under study is being done mainly with the work of earlier researchers.

Boletus edulis possessed the crude protein content ranging from 31.86% on d. w. basis. These values are in accordance with the range given by Crisan and Sands (1978), Purkayastha and Chandra (1985), Maggioni *et al.*, (1968), Chang and Miles (1987). Similarly, protein content in *Agaricus bisporus* has been reported 23.9%, 27.8%, 27.95% and 37% respectively by Bernas *et al.*, (2006). Akyuz and Kirbag (2010), Beluhan and Rangojec (2010), also reported 25% and 27% protein content in *A. bisporus* on dry weight basis. According to recent research on nutritional aspects of various *Pleurotus* spp. protein content reported 29.5 by Akyuz and Kirbag (2010). While *Pleurotus florida*, *P. flabattus*, *P. subcervinus*, *P. cervinus* and *P. eous* has been reported to contain protein 24.69%, 30.3%, 29.01%, 46% and 26.40%, respectively by YuJie *et al.*, 2007; Gyar *et al.*, 2008; Ingale and Ramateke 2010 and Olasupo *et al.*, 2012. The most explored mushroom *Pleurotus sajor-caju* (Fr.) singer has been found to contain 24.34%, and 24.39 protein by Oyetayo and Akindahunsi 2004; Olasupo *et al.*, 2012, respectively. Crisan and Sands 1978 and Wahid *et al.*, (1988) assessed protein content to be 28.5% in *Russua delica*, 18.5% in *Russula vesca* and 32.4% in *Boletus edulis*, 44.65% in *Boletus aestivalis* and 17.4% in *Cantharellus cibarius*.

The value recorded for fiber content during present study for *Boletus edulis* (8.9%), varies slightly from values reported by Crisan and Sands (1978), Bano and Rajarathnam (1982) in different species of *Pleurotus* ranging from 7.5% to 27.6%. Li and Chang (1982) have found crude fiber content to vary from 5.0% to 15.0% in different developmental stages of *Volvariella volvacea*, while Crisan and Sands(1978) reported fiber content 6.6% to 19.4% *Agaricus bisporus*, 6.3% to 11.9% in *Volvariella volvacea* and 7.5% 14.33% in *Pleurotus ostreatus*. Recent research by Oyetayo and Akindahunsi, (2004), LiQiong *et al.*, (2007), ShuYao *et al.*, (2007), Rai *et al.*, (2007), YuLing *et al.*, (2009), and Beluhan and Ranogajec revealed fiber content in *Pleurotus sajor-caju* (16.32%), *Pleurotus ferulae* *P. nebrodensis*, *P. dyamor* and *P. sapidus* (11.2-17.2%), *Agaricus blazei* (17.7%), *Armillariella mellea* 15.8+or-1.20% and *Cantharellus cibarus* (13.98%) respectively. In general, the range of fiber content recorded during present study for other species is also in accordance with the values reported by above mentioned workers.

The total free amino acid amount in the present study is (25.29 mg/g). The values reported from *Agaricus bisporus* (42.8 mg/g), *Coprinus comatus* (9.94 mg/g), *Lentinus edodes* (6.05 mg/g), *Pleurotus sajor-caju* (46.87 mg/g), *Cantharellus cibarius* (18.1 mg/g), *Agaricus blazei* (14.91 mg/g), *Fistulina hepatica* (14.9 mg/g), *Armillariella mellea* (11.08 mg/g) and *Hypsizygus marmoreus* (46.87 mg/g) by Shuyao *et al.*, (2006), respectively have been found in accordance with the values reported in the present investigation.

It is clear from this study that chemical composition of *Boletus edulis* is not much different from gilled members of Basidiomycotina. The nutritive contents of presently investigated wild edible mushroom *Boletus edulis* is at par with the commercially cultivated mushrooms.

Protein values recorded in the present investigation are higher than those found in pulses. In this respect, those mushrooms can serve as a supplementary source of protein. Supplementation of mushrooms with cereal and legume protein to make up the deficiency of essential amino acids is advocated to serve as a balanced diet for certain section of population in India (Kuppuswamy *et al.*, 1956). But legume protein lacks certain amino acids (tryptophan, lysine; proline and serine) Therefore, the supplementation of cereal diet with mushrooms may help to overcome lysine, tryptophan, and serine in our country where nutrition is predominantly cereal based high fiber content leads to the retention of dietary minerals present in a diet. Much lower level of sugar content of mushrooms advocates their use as a safe diet for diabetic patients. The low energy level of mushrooms is useful in low calorific diets. The potential of mushrooms depends upon the nutritive medicinal and nutraceutical composition. The present investigation on nutritional potential of wild edible mushrooms species has been compared favourably with other commercially accepted mushrooms as *Agaricus bisporus*, *Agaricus blazei*, *Lentinus edodes*, *Pleurotus ostreatus*, *P. sajor-caju* etc. Wild edible mushrooms are a better source of polysaccharides, crude fiber, protein, essential amino acids, minerals especially, Se, K, Zn, Cu, Na, and Ca etc. and vitamins A, B₁, B₂, C, D & K. Mushrooms especially morels are almost free of cholesterol. All these properties of wild edible mushrooms prove them to be of good nutritional use.

V. CONCLUSION

In conclusion, it would be appropriate to popularize the utilization of the mushrooms as unconventional source of protein, crude fiber, minerals and vitamins. Such food sources to supplement the traditional cereal based diet are aimed at combating, particularly the problems of protein malnutrition in India as well as other developing and underdeveloped

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countries. The researchers also believe that these nutrient rich mushrooms may prove useful in the formulation of different food products. Utmost need is to exploit them commercially and make them popular among the common people like other cultivated mushrooms.

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