PROTEIN DETERMINATION THROUGH BRADFORD'S METHOD OF NEPALESE MUSHROOM

Nina Pandey* and **Usha Budhathoki****
*Department of Botany, Patan Multiple Campus, Tribhuvan University, Lalitpur, Nepal.
**Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Abstract: The Wild edible mushrooms are one of the most important non timber forest products. Due to its vigorous growth in the rainy season, delicious taste and nutritional value the mycophagus group consume them. In this paper an attempt has been made to determine the protein of these 35 species through Bradford's method. Among them thirty three species of wild mushroom collected from different altitude (200m – 4200m), phytogeographical habitat of central Nepal and two species *Agaricus bisporous & Pleurotus sajorkaju* cultivated sample from Balambu farm. The highest amount of protein 1.576mg/ml in *Cantharellus subscibarius* and least 0.131 mg/ml in *Cordycep sinensis* were found.

Key words: Wild mushroom; Protein; Bradford's method.

INTRODUCTION

Protein is the most critical component contributing to the nutritional value of food. The determination of crude protein was done by the procedure known as Micro Kjeldahl's process (Sawhney, Singh, 2000). However, this process is both time consuming and sample consuming (Young, 1963).

Bradford's Method (Bradford, 1976) is a rapid, simple and sensitive method for estimation of proteins in a sample extract. The color development is virtually complete in 2 minutes and the color is stable for about 1 hour. Unlike Lowry's method (Lowry et.al.,1951) metal ions such as NH_4^+, Na^+, K^+, phenol and carbohydrate such as sucrose do not interfere in this assay. The procedure is based on interaction of a dye, Coosmassic Brillaint Blue with protein.

Wild edible mushrooms are one of the important minor forest products, which are locally traded in local market of the country. In Nepal the mushroom collection and consumption have been continuing since time immemorial by different ethnic groups. Due to the lack of scientific knowledge in ethnic groups, they only utilized few species that could be identified from their own traditional knowledge. Various mycophagus groups like Sherpa, Tamang, Gurung, Tharu, Danuwar, Chepang and Newar are directly concerned with the collection and consumption of mushrooms since long due to its delicious taste as well as vigorous growth during rainy season in appropriate habitat in the forest of the country. Due to their high content of vitamin, protein and mineral, mushrooms are considered as "Poor man's Protein"(Pandey 2004).

The Greeks and Romans described mushrooms as "Food for the Gods", and were served only on celebrations. Reference to mushrooms is found in Vedas (Chaube, 1995; Adhikari, 2000, 2004). Mushrooms can be used for the food to solve the malnutrition problem (Manandhar, 2003). Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets especially in some developing countries where animal protein may not be available and are expensive. The protein content of fresh mushroom is 3.7% stated by Food Agriculture Organization's publication in 1978. The edible and medicinal mushrooms can be used on human welfare in the 21st century (Chang, 1999).

Nowadays the marketing of wild mushroom is decreasing due to new policy of forest conservation applied by Nepal Government yet some delicious mushrooms were also supplied in the reputed hotels through different means. Wild edible mushrooms are also called "Rajashi Khana (Food for Royal Palace)" due to delicious taste (Pandey, 2004). More than twenty species of mushrooms have been cultivated commercially around the world (Manandhar, 1994).

OBJECTIVES

The main objective of this research is

- To collect and identify mushroom samples in the study area.
- To find out the amount of protein present in different species of mushrooms.

MATERIALS AND METHODS

The mushrooms needed for the research were collected from the Langtang, Kathmandu valley and adjoining area. Identification of different collected samples were done on

Author for Correspondence: Nina Pandey, Department of Botany, Patan Multiple Campus, Tribhuvan University, Lalitpur, Nepal. Email: ninapandey@yahoo.com.

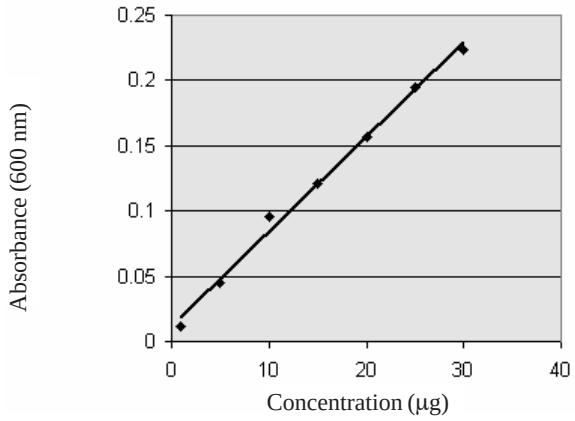


Figure 1: BSA Calibration curve for Bradford Method.

the basis of different important mushrooms characters and techniques such as habit and habitat, morphological structures, spore print color, microscopic observations, relevant monograph, recent literatures and books (Adhikari, 1990, 2000; Arora, 1986; Pandey, 2004; Pandey, 2006 a,b,c; Pacioni, 1981; Singer, 1986, Rea, 1922; Fries, 1838; Millor, 1984. Internet as well as cross checking with the voucher specimen. The proteins were determined by Bradford's method (Bradford, 1976).

An amount of 2 gm of mushroom samples were cut into pieces with a scissor and grinded in mortar with 5ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant of different mushroom samples were put in separate tubes. The volume of all of the samples in tubes were then made equal by adding phosphate buffer solution and the extraction were stored in the refrigerator at 4^0c for further analysis.

After extraction, 30µl of different mushroom samples were taken out in separate tubes and were mixed with 70µl of distilled water separately. In all of these separate sample tubes 2.9 ml of Coosmassic Brillaint Blue solution was then added and mixed thoroughly. The Total volume now was 3ml in each tube. All these tubes were incubated for 5 minutes at room temperature and absorbance at 600(595) nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (µg) of protein was plotted as shown in Figure 1.

Protein content in the extracted samples was determined from the standard curve and the amount of protein in mg/ml was calculated. The results were illustrated in the Table1.

RESULTS

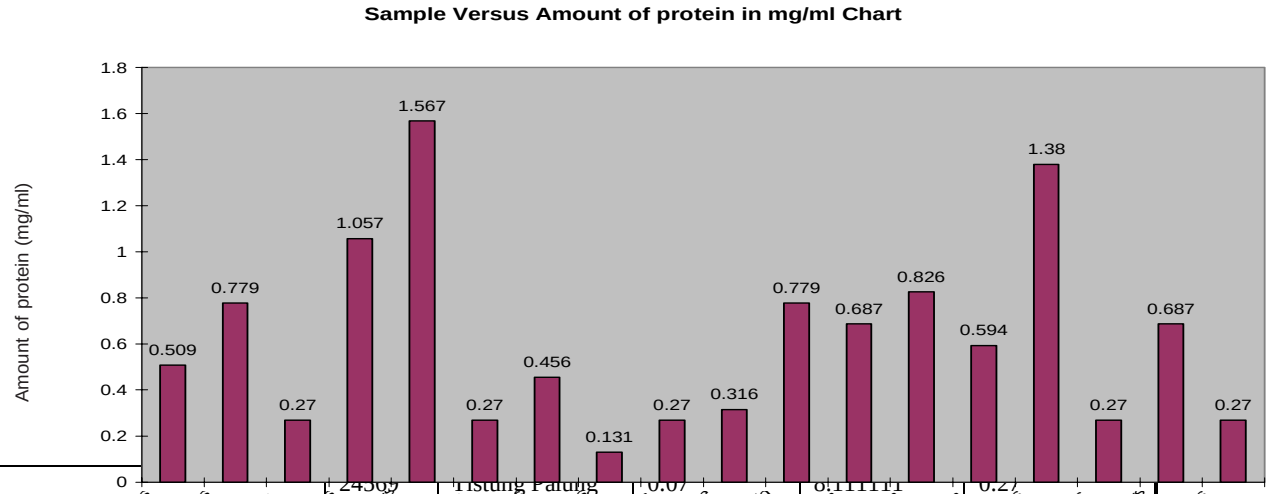
The highest amount of protein was observed in *Cantharellus subcibarius* 1.567 mg/ml followed by *Russula Virescens* 1.427 mg/ml, *Lactarius piperatus* 1.38 mg/ml and least amount of protein was found in *Cordycep sinemsis* 0.131 mg/ml. (Table 1)

Table 1

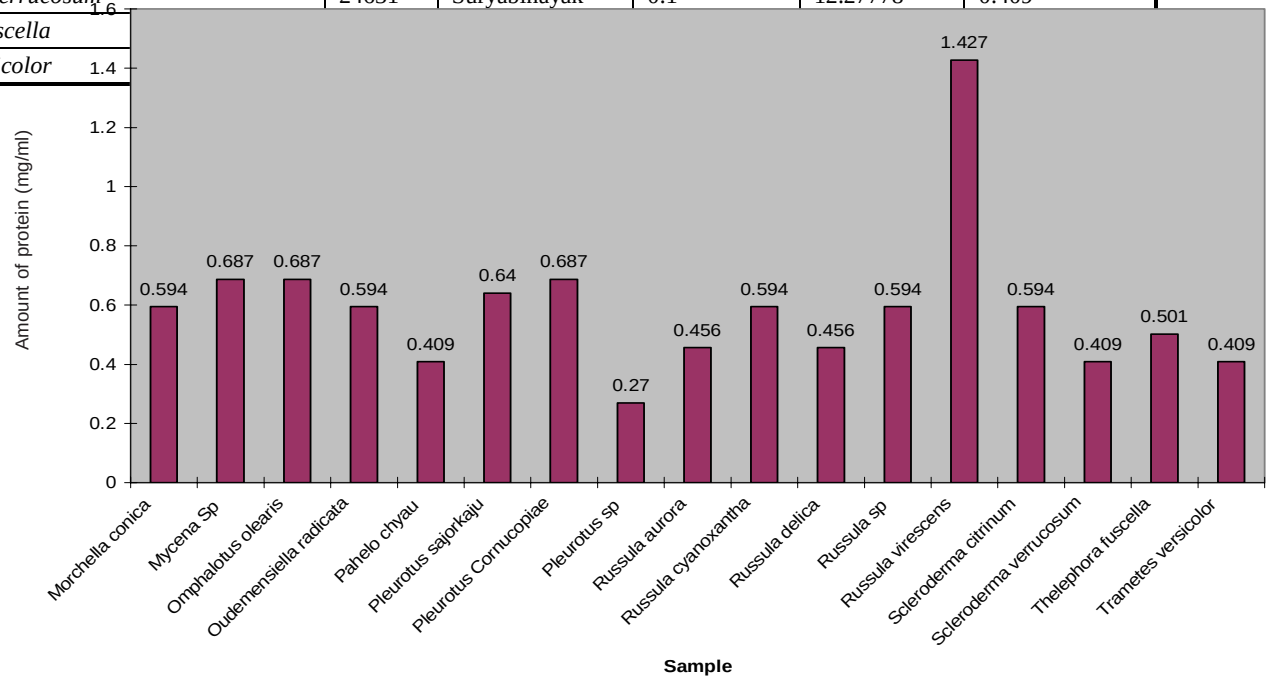
Sample	Coll.No	Place of coll.	Abs(600nm)	Conc. in 30µl	protein mg/ml
Agaricus bisporous	Market	Baneshwor	0.1	19.22222	0.509
Agaricus campestris	24536	Kirtipur	0.18	23.38889	0.779
Amanita vaginata	24609	Matatirtha	0.1	12.27778	0.27
Cantharellus cibarius	24630	Suryabinayak	0.24	31.72222	1.057
Cantharellus subcibarius	24606	Matatirtha	0.35	47	1.567
Clavaria rosea	24584	Sundarijal	0.07	8.111111	0.27
Coprinus comatus	21108	Langtang	0.11	13.66667	0.456
Cordycep sinensis	23455	Langtang	0.04	3.944444	0.131
Coriolus hirsitus	25645	Matatirtha	0.07	8.111111	0.27
Ganoderma tsugae	23440	Langtang	0.08	9.5	0.316
Hypholoma capsonoid	25653	Langtang	0.18	23.38889	0.779
Laccaria laccata	25629	Suryabinayak	0.16	20.61111	0.687
Laccaria laccata	24598	Matatirtha	0.19	24.77778	0.826
Laccaria laccata	24526	Godawari	0.14	17.83333	0.594
Lactarius piperatus	22158	Suryabinayak	0.31	41.44444	1.38
Lactarius volemus	24570	Tistung Palung	0.09	10.88889	0.27
Laetiporous sulphureus	24541	Langtang	0.16	20.61111	0.687
Marasmius maximus	24572	Tistung Palung	0.09	8.111111	0.27
Morchella conica	23461	Langtang	0.14	17.8333	0.594
Mycena Sp	25651	Langtang	0.16	20.61111	0.687
Omphalotus olearis	24576	Baneshwor	0.16	20.61111	0.687
Oudemensiella radicata	24617	Baneshwor	0.14	17.8333	0.594
Pahelo chyau	24573	Baneshwor	0.1	12.27778	0.409
Pleurotus sajorkaju	Market	Tistung Palung	0.15	12.27778	0.64
Pleurotus Cornucopiae	23469	Matatirtha	0.16	20.61111	0.687

Abbreviations: Sp = Species, Conc = Concentration, Coll = Collection, No = Number.

Sample Versus Amount of protein in mg/ml Charts:



Pleurotus sp	24567	Tisting Palung	0.07	8.111111	0.27
Russula aurora	24601	Matatirtha	0.09	13.66667	0.456
Russula cyanoxantha	24605	Matatirtha	0.32	42.83333	0.594
Russula delica	24571	Tisting Palung	0.11	13.66667	0.456
Russula sp	24604	Matatirtha	0.14	17.83333	0.594
Russula virescens	25681	Dakchinkali	0.32	42.83333	1.427
Scleroderma citrinum	23412	Nuwakot Tigaon	0.14	17.83333	0.594
Scleroderma verrucosum	24631	Suryabinayak	0.1	12.27778	0.409
Thelephora fuscella					
Trametes versicolor					



DISCUSSION AND CONCLUSION

Large amount of protein is found in mushrooms. Hence it is regarded as an ideal protein source for vegetarian as well as for old age people who are unable to chew meat. It was found that the amount of protein varies from species to species in the same genus as for example two different species of genus *Cantharellus*, i.e., *Cantharellus cibarius* contents 1.057 mg/ml of protein while *Cantharellus subcibarius* contents 1.567 mg/ml of protein. Similarly the three different species of the genus *Pleurotus*, four species of the genus *Russula* and two species of the genus *Scleroderoma* have different protein concentration as shown in the Table1.

In our research, wild edible mushrooms *Laccaria laccata* collected from three different places namely Godawari, Matatirtha and Suryabinayak were found to have 0.594, 0.862 and 0.687 mg/ml amount of protein. Clearly, it was seen that the amount of protein in mg/ml is different even in the mushrooms of the same species collected at the three different places. Hence it was also concluded from the research that the amount of protein varied even in the same species according to phytographical condition.

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