

Quantitation of Total Protein Content in Some Common Edible Food Sources by Lowry Protein Assay

Laxminandan Satpathy ^{1,*} , Deependi Dash ¹ , Pritijyotsna Sahoo ² , Taslima Nasim Anwar ³ ,
Siba Prasad Parida ^{1*} 

¹ Department of Zoology, School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, 752 050 India; laxminandansatpathy@gmail.com (L.S.); dashdeependi98@gmail.com (D.D.); paridaspar@gmail.com (S.P.P.);

² Department of Biotechnology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, 751 004 India; pritijyotsna1@gmail.com;

³ Department of Zoology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh, 484 886 India; taslimacharmy@gmail.com;

* Correspondence: laxminandansatpathy@gmail.com (L.S.); paridaspar@gmail.com (S.P.P.);

Scopus Author ID 57197747626

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Abstract: The nutrient value or quality of different proteins varies due to amino acid composition, ratios of essential amino acids, vulnerability to hydrolysis during digestion, source, and the effects of transforming. To enhance the biological activity of proteins, better gripping is needed for the various correlated parameters that influence their nutrient value. This research article attempts to contribute to this effort. It analyzes the Lowry method used for protein quantity evaluation. Recent studies on the nutrient quality of 11 common protein sources including oyster mushroom (*Pleurotus ostreatus*), white button mushroom (*Agaricus bisporus*); chicken (*Gallus gallus domesticus*); black Bengal goat (*Capra hircus*); brown shrimp (*Metapenaeus monoceros*); Mola carplet (*Amblypharyngodon mola*), Rohu fish (*Labeo rohita*), Reba carp (*Cirrhinus reba*), slender rasbora (*Rasbora daniconius*), banded gourami (*Trichogaster fasciata*); and egg albumin are evaluated. The highest amount of protein present in brown shrimp, i.e., 85.982 mg/mL. This study is intended to trigger interest in the use of new protein sources for nourishing the booming world population.

Keywords: Analytical method; amino acid; edible protein sources; Lowry protein assay; UV- visible spectrophotometer.

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1. Introduction

The nutritional requirement depends upon numerous factors such as age, location, occupation, and health condition of an individual [1]. With changing habits and increasing food adulteration, cardio-vascular diseases, obesity, hypertension, diabetes, and cancer are increasing [1]. Doctors and nutritionists found that the intake of certain food items may prevent disorders [1]. Protein is one of the food-stuff that can prevent disorders. Protein was derived from the Greek word *proteios*, which means “primaries”, i.e., of first importance [2]. Proteins are complex nitrogen-containing organic molecules found in all animal and plant cells. It is the principal component of the living protoplasm [2]. It constitutes nearly one-half of the body's dry matter [2]. Many types of proteins present and they perform various functions like a catalytic activity for metabolic reaction in the form of enzymes; they help in muscle movement because actin and myosin are made up of protein molecules. Protein forms the structural

material for plants, for example, cellulose and the structural proteins of animals are collagen and keratin [3]. A large number of proteins perform transportation activities inside the human body, i.e., hemoglobin in the blood and other protein molecules across the cell membrane. Many hormones are protein compounds, including human growth hormone (hGH), insulin, and erythropoietin. It helps in blood coagulation and immune response. Some proteins store materials like casein in milk and iron in the liver by ferritin. It also controls the regulation of gene expression [4]. A typical cell consists of about 9000 different proteins, and the entire human body contains 100,000 different proteins [4]. Proteins are made up of chains of amino acids linked by peptide bonds. Dietary proteins are hydrolyzed by proteases and peptidases to release amino acids, dipeptides, and tripeptides in the alimentary canal [5]. Amino acids provide nitrogen, sulfur, hydrogen, carbon, and oxygen, which are the essential components of an organism and cannot be replaced by any other nutrients like carbohydrates and lipids. It has enormous physiological importance. Therefore, amino acids are basic building blocks of proteins [6]. Over 300 amino acids are found in nature, out of which 20 constitute the monomer units of proteins [7]. Only L- α -amino acids occur in protein molecules [7]. So, we need twenty amino acids in order to be healthy. Nine out of the twenty amino acids are called essential because bodies cannot make them. These nine essential amino acids must come from the dietary supplements we eat [8]. Protein malnutrition results in stunted growth, physical weakness, under-weight, wasting, anemia, vascular dysfunction, edema, and immunity impairment [5]. Malnutrition of protein causes metabolic disorders, Kwashiorkor, and Marasmus in humans, mostly in children of developing nations [9]. Dietary protein deficiency also occurs in elderly humans of developed countries, which makes them susceptible to metabolic and infectious diseases [10]. On the other hand, over intake of dietary protein from meals and supplementation leads to hepatic dysfunction [11]. Based on studies, the Recommended Dietary Allowance of protein for a healthy adult with minimal physical activity is 0.8 g protein per kg body weight per day [5]. To meet the daily needs such as elevating skeletal muscle protein and physical strength, dietary consumption of 1.0, 1.3, and 1.6 g protein per kg body weight per day is recommended for individuals with minimal, moderate, and intense physical activity, respectively [5]. Long-term intake of protein at 2 g per kg body weight per day is safe for healthy adults, and the allowable upper limit is 3.5 g per kg body weight per day [5]. Prolong- high protein consumption of more than 2 g per kg body weight per day for adults may result in digestive, renal, and vascular abnormalities [5]. The quantity and quality of dietary proteins are the determinants of its nutritional values on humans. Dietary food that provides all the essential amino acids is known as high-quality proteins, and foods that do not provide all essential amino acids are called lower quality proteins [8]. Animal products such as meat, fish, poultry, and eggs are high-quality protein sources, whereas plant products like fruits and vegetables are low-quality protein sources [8]. Therefore, sufficient consumption of high-quality proteins from animal products is crucial for optimal growth, reproduction, development, lactation, health, and survival of human beings. Being such an essential component of the human diet, it is important to know the protein content in foods, and thus it is crucial to have a reliable analytical method [12]. Dietary protein analysis is not necessarily a direct procedure. This is partly due to foods being comprised of a wide range of different nutrients, such as lipids, carbohydrates, and a variety of macro and micronutrients. Composition, food structure, and interactions between the different nutrients may reduce the quality of the protein leading to underestimation of the protein content [12]. The main objective

of the study was to determine the protein content in common edible food. The chosen foods were mushroom, chicken, goat, shrimp, fishes, and egg.

Mushrooms were considered to be one of the most notable ingredients of cuisine throughout the world, particularly for their unique taste. There are more than 2,000 species of mushrooms found in nature, but only 25 are used as food, and few are commercially cultivated in the world [13-15]. Mushroom has significant medical properties, for example, hepatoprotective, detoxification, anti-diabetic, antioxidant, anti-allergic, anti-parasitic, anti-bacterial properties [13, 16-18]. Most of the vegetable proteins are extensively studied [19].

Chicken is commonly used as food in many cuisines throughout the world because of its low cost, adequately available, and reared easily. It is healthier than red meat because it contains a lower concentration of cholesterol and saturated fat [20].

Goat found throughout India. Black Bengal goat has higher prolificacy, resistance against common diseases, easily adapt to adverse environmental conditions, early maturity, high fertility rate, and tender meat with low intramuscular fat [21].

Shrimp has a short life span, high reproducibility. They spawn in deep water. Shrimp larvae feed on zooplankton and phytoplankton [22]. The concentration of total protein in the tissue of prawns, especially in the muscle increased with respect to age or size during growth, indicating that the quality of prawn muscle in terms of nutrient or protein accumulation improved from juvenile to sub-adult stages [23].

Fish contains high-quality protein with different varieties of vitamins and minerals such as vitamin A, vitamin D, phosphorus, magnesium, selenium, and iodine. So, it has a high nutritional value. Fish protein is easily digestible. Studies show that a small quantity of fish protein can compensate for the essential amino acids necessary for humans [24].

Eggs are low cost and consist of essential lipids, vitamins, minerals, and trace elements like zinc and calcium. It offers a moderate amount of calories, i.e., about 140 kcal/100 g. Egg white and egg yolk are highly concentrated in proteins. On average, the concentration of proteins is 12.5 g per 100 g of a whole raw fresh egg. In comparison, egg yolk, with its vitelline membrane contains 15.9 g protein, and egg white contains 10.90 g protein per 100 g [25].

2. Materials and Methods

2.1. Raw materials.

The raw materials like an oyster mushroom, white button mushroom; chicken liver, chicken leg muscle, chicken breast; black Bengal goat liver, black Bengal muscle; brown shrimp; Mola carplet, Rohu fish, Reba carp, slender rasbora, banded gourami; and egg albumin were collected from a local market.

2.2. Chemicals and reagents.

The imperative chemicals and standard reagents used in the experiments were purchased from Sigma-Aldrich unless otherwise mentioned.

2.3. UV-Visible spectrometry.

A number of methods have been used to determine protein concentration, which is based on UV-visible spectroscopy. These methods use either the natural characteristic of proteins to scatter light in the UV-visible region of the electromagnetic spectrum, or they

physically or chemically modify the proteins to make them absorb light in the UV-visible region. First, a curve of absorbance (Abs) versus protein concentration is prepared by using a series of protein solutions of known bovine serum albumin (BSA) concentrations, i.e., 0, 200, 400, 600, 800 and 1000 μg per mL (Figure 1). Then the absorbance of the solution being analyzed, compared, and measured at the same wavelength of 750 nm, and the unknown protein concentration was determined from the calibration curve.

2.4. Lowry protein assay.

Lowry protein assay, also known as the Lowry method, has been the most widely used method to estimate the quantity of proteins in biological samples [26]. It is a combination of the biuret reagent with the Folin-Ciocalteau phenol reagent, which interacts with the tryptophan and tyrosine residues present in protein [26]. This gives the solution a bluish color which can be read at 750 nm [26]. This method is more sensitive to low concentrations of proteins than the biuret and UV-visible spectroscopy method.

2.5. Estimation of standard bovine serum albumin (BSA) solution.

A standard curve was prepared as follows. Bovine serum albumin (BSA) standard solution was prepared using BSA powder dissolved in distilled water (DW), and it is diluted to a concentration of 100mg/100mL, which is then stored at 4°C. A series of dilutions (0, 2, 4, 6, 8, and 10 mg/test tube) were made in duplicates (Table 1). The final volume in each of the test tubes was 2 mL. For the protein solutions, 4 mL of alkaline copper sulfate reagent was added. Then the solution was mixed properly by using Tarson 3020 Spinix vortex shaker. The solution was incubated at room temperature for 10 minutes. Then 0.5 mL of folin phenol reagent was added to each tube and mixed properly by using Tarson 3020 Spinix vortex shaker. Then these solutions were incubated for 30 minutes at room temperature. By using blank, the spectrophotometer was set at zero, and the absorbance was taken at 750 nm. The absorbance was plotted against protein concentration to get a standard calibration curve (Figure 1).

2.6. Preparation of protein sample.

Fresh samples of common edible proteins oyster mushroom (*Pleurotus ostreatus*), white button mushroom (*Agaricus bisporus*); chicken (*Gallus gallus domesticus*); black Bengal goat (*Capra hircus*); brown shrimp (*Metapenaeus monoceros*); Mola carplet (*Amblypharyngodon mola*), Rohu fish (*Labeo rohita*), Reba carp (*Cirrhinus reba*), slender rasbora (*Rasbora daniconius*), banded gourami (*Trichogaster fasciata*); and egg albumin were collected from the market. Each sample was cut into small pieces and homogenized by using mortar and pestle. Then weigh 1g of protein was taken by the help of Citizen MP300 digital weighing scale and mixed with 9 mL of sucrose solution. Then this homogenized mixture was transferred to the centrifuge tube for centrifugation in a table-top cooling centrifuge (REMI C24BL) at 4,000 rpm for 6 minutes. The supernatant was collected and stored at 4 °C.

2.7. Estimation of total proteins content by Lowry method for common edible protein samples.

1 mL of protein samples were taken in each test tube. 1 mL of distilled water was taken before taking the protein sample to prevent the degeneration of the sample protein. To these solutions, 4 mL of alkaline copper solution was added. Then the mixtures were mixed thoroughly by using Tarson 3020 Spinix vortex shaker. The solutions were incubated at room

temperature for 10 minutes. Then 0.5 mL of Folin-Ciocalteau phenol was added to these solutions and mixed properly by using vortex Shaker. Then these solutions were incubated for 30 minutes at room temperature and in dark condition. Duplicates test tube were prepared for each sample. The reading of the absorbance of unknown samples was taken at 750 nm (Table 3).

| Test tube number | Volume of BSA (mL) | Concentration of BSA (mg) | Volume of distilled water (mL) | Alkaline copper solution (mL) | Incubate (minute) | Folin-Ciocalteau Phenol reagent (mL) | Incubate in dark (minute) | Absorbance (Abs) at 750nm |
|------------------|--------------------|---------------------------|--------------------------------|-------------------------------|-------------------|--------------------------------------|---------------------------|---------------------------|
| 1 | 0.0 | 0 | 2.0 | 4 | 10 | 0.5 | 30 | 0 |
| 2 | 0.2 | 200 | 1.8 | 4 | 10 | 0.5 | 30 | 0.034 |
| 3 | 0.4 | 400 | 1.6 | 4 | 10 | 0.5 | 30 | 0.096 |
| 4 | 0.6 | 600 | 1.4 | 4 | 10 | 0.5 | 30 | 0.131 |
| 5 | 0.8 | 800 | 1.2 | 4 | 10 | 0.5 | 30 | 0.178 |
| 6 | 1.0 | 1000 | 1 | 4 | 10 | 0.5 | 30 | 0.224 |

Table 1. Absorbance of different bovine serum albumin (BSA) concentration.

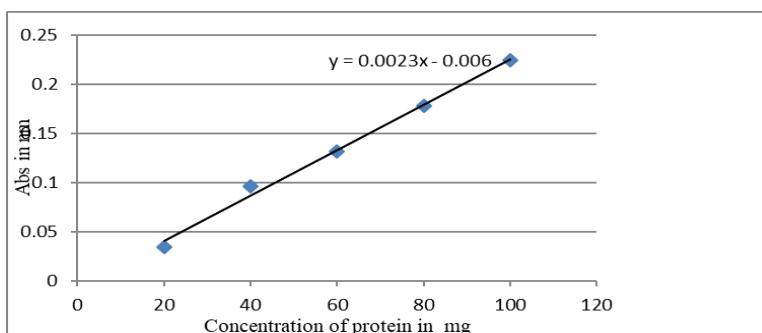


Figure 1. Bovine serum albumin standard curve (absorbance was plotted against protein concentration) using the Lowry method.

3. Results and Discussion

3.1. Calculation.

As BSA is taken as a standard solution, 100 mg of protein (BSA) is added in 100mL of distilled water that is 1 mg in 1 mL or 1000 μ g in 1 mL of sample.

Hence 1mL contains 1000 μ g or 1 mg protein.

For 1mL of BSA sample absorbance = 0.224

At absorbance 0.224, concentration of BSA = 1000 μ g or 1 mg

At absorbance 1, concentration of protein = 1/0.224 mg

At absorbance X concentration protein = 1/0.224 \times X mg

At absorbance 'X' concentration of sample protein = 1 mL = 1/0.224 \times X \times 1/1

But 1g sample was homogenized by using 9 mL of sucrose so that the dilution factor will be 10.

So at absorbance X concentration of sample protein of 1mL = 1/0.224 \times X \times 10

Table 2. Analysis of different edible samples of protein by Lowry method.

| Serial number | Sample | Absorbance (Abs) at 750nm | Amount of protein (mg) |
|---------------|-----------------------|---------------------------|------------------------|
| 1 | Oyster mushroom | 1.011 | 45.133 |
| 2 | White button mushroom | 1.209 | 53.973 |
| 3 | Chicken liver | 1.762 | 78.660 |
| 4 | Chicken leg muscle | 1.806 | 80.625 |

| Serial number | Sample | Absorbance (Abs) at 750nm | Amount of protein (mg) |
|---------------|-----------------------------|---------------------------|------------------------|
| 5 | Chicken breast | 1.920 | 85.714 |
| 6 | Black Bengal goat liver | 1.832 | 81.785 |
| 7 | Black Bengal goat muscle | 1.226 | 54.732 |
| 8 | Brown shrimp muscle | 1.926 | 85.982 |
| 9 | Mola carplet fish muscle | 1.852 | 82.678 |
| 10 | Rohu fish muscle | 1.403 | 62.633 |
| 11 | Reba carp fish muscle | 1.581 | 70.580 |
| 12 | Slender rasbora fish muscle | 1.641 | 73.258 |
| 13 | Banded gourami fish muscle | 1.548 | 69.107 |
| 14 | Egg albumin | 1.703 | 76.026 |

Table 3. Absorbance of different edible samples of protein using UV- visible spectrophotometer.

| Serial number | Test tube number | Sample in mL | Distilled water in mL | Alkaline copper solution in mL | Folin-Ciocalteau phenol reagent in mL | Absorbance (Abs) at 750 nm | Mean |
|---------------|-----------------------------|--------------|-----------------------|--------------------------------|---------------------------------------|----------------------------|-------|
| 1 | Blank 1 | 0 | 1 | 4 | 0.5 | 0.000 | 0 |
| | Blank 1' | 0 | 1 | 4 | 0.5 | 0.000 | |
| 2 | Oyster mushroom 2 | 1 | 1 | 4 | 0.5 | 1.019 | |
| | Oyster mushroom 2' | 1 | 1 | 4 | 0.5 | 1.003 | 1.011 |
| 3 | White button mushroom 3 | 1 | 1 | 4 | 0.5 | 1.235 | |
| | White button mushroom 3' | 1 | 1 | 4 | 0.5 | 1.183 | 1.209 |
| 4 | Chicken liver 4 | 1 | 1 | 4 | 0.5 | 1.818 | |
| | Chicken liver 4' | 1 | 1 | 4 | 0.5 | 1.707 | 1.762 |
| 5 | Chicken leg muscle 5 | 1 | 1 | 4 | 0.5 | 1.804 | |
| | Chicken leg muscle 5' | 1 | 1 | 4 | 0.5 | 1.808 | 1.806 |
| 6 | Chicken breast 6 | 1 | 1 | 4 | 0.5 | 1.926 | |
| | Chicken breast 6' | 1 | 1 | 4 | 0.5 | 1.914 | 1.920 |
| 7 | Black Bengal goat liver 7 | 1 | 1 | 4 | 0.5 | 1.801 | |
| | Black Bengal goat liver 7' | 1 | 1 | 4 | 0.5 | 1.769 | 1.832 |
| 8 | Black Bengal goat muscle 8 | 1 | 1 | 4 | 0.5 | 1.218 | |
| | Black Bengal goat muscle 8' | 1 | 1 | 4 | 0.5 | 1.235 | 1.226 |
| 9 | Brown shrimp muscle 9 | 1 | 1 | 4 | 0.5 | 1.974 | |
| | Brown shrimp muscle 9' | 1 | 1 | 4 | 0.5 | 1.879 | 1.926 |
| 10 | Mola carplet fish muscle 10 | 1 | 1 | 4 | 0.5 | 1.892 | |
| | | | | | | | 1.852 |

| Serial number | Test tube number | Sample in mL | Distilled water in mL | Alkaline copper solution in mL | Folin-Ciocalteau phenol reagent in mL | Absorbance (Abs) at 750 nm | Mean |
|---------------|---------------------------------|--------------|-----------------------|--------------------------------|---------------------------------------|----------------------------|-------|
| | Mola carplet fish muscle 10' | 1 | 1 | 4 | 0.5 | 1.812 | |
| 11 | Rohu fish muscle 11 | 1 | 1 | 4 | 0.5 | 1.422 | 1.403 |
| | Rohu fish muscle 11' | 1 | 1 | 4 | 0.5 | 1.385 | |
| 12 | Reba carp fish muscle 12 | 1 | 1 | 4 | 0.5 | 1.638 | 1.581 |
| | Reba carp fish muscle 12' | 1 | 1 | 4 | 0.5 | 1.525 | |
| 13 | Slender rasbora fish muscle 13 | 1 | 1 | 4 | 0.5 | 1.681 | 1.641 |
| | Slender rasbora fish muscle 13' | 1 | 1 | 4 | 0.5 | 1.602 | |
| 14 | Banded gourami fish muscle 14 | 1 | 1 | 4 | 0.5 | 1.522 | 1.548 |
| | Banded gourami fish muscle 14' | 1 | 1 | 4 | 0.5 | 1.716 | |
| 16 | Egg albumin 16 | 1 | 1 | 4 | 0.5 | 1.754 | 1.729 |
| | Egg albumin 16' | 1 | 1 | 4 | 0.5 | 1.703 | |

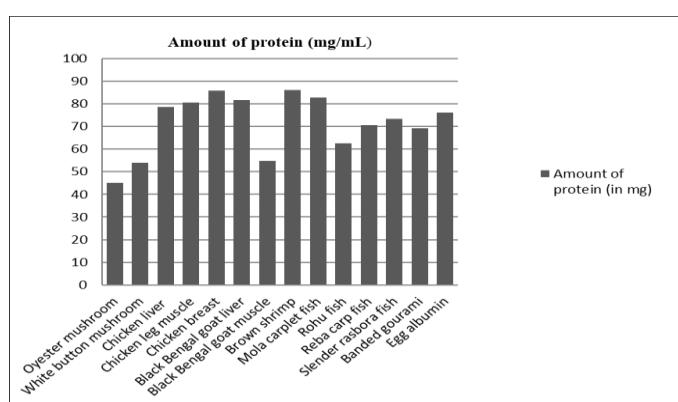


Figure 2. Different protein concentrations present in common edible sources.

The protein concentration of oyster mushroom is 45.133 mg, white button mushroom is 53.973 mg; chicken liver is 78.660 mg, chicken leg muscle is 80.625 mg, chicken breast is 85.714 mg; black Bengal goat liver is 81.785 mg, black Bengal goat muscle is 54.732 mg; brown shrimp muscle is 85.982 mg; Mola carplet fish muscle is 82.678 mg, Rohu fish muscle is 62.633 mg, Reba carp fish muscle is 70.580 mg, slender rasbora fish muscle is 73.258 mg, banded gourami fish muscle is 69.107 mg, and eggs albumin is 76.026 mg (Table 2). Protein concentration is found to be highest in brown shrimp muscle and lowest in oyster mushroom (Figure 2).

4. Conclusions

From the result of this experiment, it was found that brown shrimp had more protein content than chicken, fishes, goat, and egg albumin. Further detailed and comprehensive analysis is suggested to be undertaken on these samples taking protein concentration into consideration because these edible foods are easily available in the local market, which would be very helpful to fight against protein deficiency, malnutrition, food for the fast-growing world population.

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Conflicts of Interest

The authors declare no conflict of interest.

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