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Estimation of protein content in muscle tissues of some dry fish species

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Abstract:
Proteins are the building blocks of life. Their role as a building block of cells or as enzymes for metabolism or as hormones for body control or as signaling molecule for specific signaling etc., is very crucial to sustain a life. Adequate intake of protein is essential for higher organisms as they are unable to produce all the standard amino acids (only produce nonessential amino acids). Current situation says, only terrestrial farming is not enough to maintain the hunger of this growing population. So, to get a better option in order get food, the culturing system pervaded into aquaculture. The availability of enough quantity of fishes, crabs, prawns, shrimps, etc., fulfilled the hunger as well as maintained the economic and social status of human being. Presence of high digestible proteins in fishes provided more nutrition and, hence, a good healthy life. According to need and supply, convenient approaches came into aquaculture. Fishes with high protein content, as high preservation time, ruled the aquaculture market. Locally found fishes (fresh water) of Balangir, like ***Lepidocephalusthermalis&Chela laubuca*** *contains ~ 23.17% & ~16.54% of proteins in 1 gram of their muscle tissue, respectively. Likewise,* ***Mystusvittatus*** *with ~ 13.97%,* ***Parluciosomadaniconius*** *with ~ 12.82% and* ***Mastacembeluspancalus*** *with ~ 9.64% of proteins in their 1gm muscle tissue, are adhering their role economically.*

Keywords:*Lepidocephalusthermalis, Chela laubuca, Mystusvittatus, Parluciosomadaniconius, Mastacembeluspancalus, Dry fish protein content.*

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I. INTRODUCTION

From a population perspective, the 20th century was resembled: we started the century with fewer than 2 billion people and ended with 6 billion and counting. The UN Population Division suggests that our numbers may reach 8.9 billion by the middle of the century- a 50% increase in 50 years. Virtually all of the increase will be in the developing centuries which are already densely populated and straining to meet the needs of their people for food, water, health care, shelter and employment. Under the leadership of the Food and Agricultural Organization (FAO) of the United Nations representatives and heads of states from 100 countries met during November 13-17,1996, called the world food summit, the meeting was the result of 2 years of planning and negotiation. The objective was to bring about a renewed commitment around the world to eradicate hunger and malnutrition and to promote conditions leading to food security for individuals, families and countries everywhere. The meet for this high-level attention was emphasized by new data from the FAO indicating that 840 million people, or 18% of the population of the developing world, are malnourished or hungry. In light of continuing increase in population, rising cost of grain and declining per capita grain production, the summit was a unique opportunity for world leaders to take a new look at the meaning of sustainability. To maintain such quantity of food demand, the culturing system must have to expand its wings to various aspects. Thus, aquaculture also made its grip in modern day. Export and import of fishes are fulfilling both hunger as well as maintaining the economic development. Dry fishes contain less water constituent and allow them for long term preservation [24]. This has increased the demand of dry fishes in aquaculture.

1.1 ROLE OF PROTEINS IN BODY

Proteins have a major role in the growth and maintenance of the human body and are, along with carbohydrates and lipids, the energy giving nutrients in diet. In addition, proteins also pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes [9].

In order to maintain these important functions, it is essential to provide the body with good quality proteins through diet. Inadequate intake of dietary proteins containing essential amino acids results in increased turnover of muscular proteins, leading to reduced growth and loss of muscle mass, impaired immunity, as well as reduced hormonal and enzymatic activity may subsequently follow. [25]

It has been recognized since the early 1960s that, in the absence of nutritional intake, muscle protein serves as the principal reservoir to replace blood amino acids taken up by other tissues. In the fasting state, blood amino acids serve not only as the precursor for the synthesis of proteins but also as precursors for hepatic gluconeogenesis. [25]

1.2 FISH AS A SOURCE OF PROTEIN

Fishes are the wide spread organism across the world. So, they stand as a better source of food. Even the regions those are covered with ice throughout the year, where land culture is impossible, there also fishes are being used a cheap source of food. According to marketing perspective, fishes can be divided into two categories;

1. Wet fish
2. Dry fish

Wet fishes are having the high-water content in their body, if they are stored for long, enzymatic reactions inside decays the body. They have a very low preservation rate. In contrast, dry fishes are those, which are having high-protein content. If they are stored for long, they don't show such body decomposition.

Fish and all see foods products have rich nutritional value. They have a greater number of proteins, lipids, and carbohydrates as well as micronutrients. Fish and see foods also provide vitamins, iron, and zinc to human body. In-preagricultural times, some other foods are consumed by humans are like, fruits, vegetables, nuts, that containing higher amount of n-3 PUFA (Polyunsaturated fatty acids), and lower amounts of n-6 PUFA than the modern foods. An increasing number of evidences suggest that due to its high content of polyunsaturated fatty acids (PUFA) fish flesh and fish oils are beneficial in reducing the serum cholesterol (Huynh et al., 2007). Fresh fish meet provides good source of human diet, about 90-95% of fish proteins are assimilated by humans (Memon et al.,2011). Aquatic animals' foods have a lower caloric density, and have a high content of omega-3 long chain poly unsaturated fatty acids as compared to land living animals (Tacon and Metian,2013). Fish consumption is of growing importance because it provides the high content of health significant omega-3-PUFAs, particularly eicosapentaenoci acid (20:5n-3, EPF) and dicosahexaenoic acid (22:6n-3, DHA) (Elvevoll et al., 2000).

Fish drying is an age-old practice and was adopted as a practical method of preserving fish that have not been immediately consumed or sold in the fresh market. Improved fishing techniques and infrastructure resulted in increased fish catch, better marketing, processing and curing facilities. However, drying still remains the cheapest and popular mode of fish preservation. Dried products are in great demand both within and outside the country and form an important source of protein rich food in various forms. Fish drying over the years, has grown from a subsistence kind of occupation to a full-fledged flourishing business. Dried fish now caters to different sectors such as quality fish/prawns for human consumption, and low value fishes for the preparation of fish feed as well as poultry feed. [3]

The match between dry fish supply and nutrient needs a vital to support the health and wellbeing of the increasing human population. The depleting fisheries resources also suggests a corrective measure to define accurately the amount and quality of nutrient supplied by dry fish. Dry fish is increasingly becoming a vital factor in providing high quality proteins, healthy fats (including long chain omega-3 fatty acids like eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), and a unique source of essential nutrient such as iodine, zinc, copper, selenium and calcium. [17]

Fishes are highlighted as an important source of Vitamin A, D and E. There are large number of literatures reporting the significance of fish in brain development, and learning in children in protecting vision and eye health, decreasing incidence of breast cancer, rheumatoid arthritis, multiple sclerosis, asthma, psoriasis, inflammatory bowel disease and regulation of prostaglandin synthesis (Dhaneesh et al., 2012).

1.3 FISH MUSCLE PROTEIN

The relative amount of muscular tissue is higher in fish than in domestic animals or in man. Fish and marine invertebrates constitute an important part of the human diet worldwide, their muscles are the major edible parts. The muscles are a rich source of proteins, which in other terms determines the nutritional value and the quality of sea food products. Though the fish skeletal muscle proteins share many similarities with their mammalian counterparts, there exist great differences in their biological activity and structural stability. While the muscles of shell fish or aquatic invertebrates share many properties and general structural features with their vertebrate counterparts, they have unique characteristics. Based on their structure and function, muscles are classified into striated and smooth muscles, and the former invertebrates is further categorized into skeletal and cardiac muscles. The skeletal muscle is further categorized into fast and slow skeletal muscles. The muscles in the fish correspond to nearly 50% of the total weight to the general composition of the fish muscle. Depending on the species, fish muscle tissue contains 12.2-21.79% protein, 0.0813.1% fat, and 67.3-86.7% of water. The

energy value of the muscle tissue of different fish species, which depend on the proportion of its basic components, ranged from 210.7-797.5 KJ/100g. The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment and season [21].

1.4 STRUCTURE OF MUSCLE PROTEIN

Fibrillar proteins play an important role in contributing textual quality to the flesh food. Our knowledge of muscle proteins is mainly derived from the work on rabbit muscle proteins. The chief contractile protein has been identified as actomyosin which is composed of actin and myosin. The properties and structure of these proteins and their sub-units have been extensively studied in rabbit muscle. Striated muscle consists of two sets of filaments one containing myosin and the other containing actin. The A-bands (A for anisotropic) contain filaments of 100 Å diameter spaced about 45 Å apart. These bands correspond exactly to the length of myosin filaments and each filament spanning an A band contains 200-400 myosin molecules. A second array of filaments extends on either side of the Z line through the I-bands (I for isotropic) terminating at the edges of the H zones. These thinner filaments contain actin [21].

The proteins in fish muscle tissue can be divided into the following 3 groups.

(1) Structural proteins: - Actin, myosin, tropomyosin and actomyosin, which constitutes 70-80% of the total protein content (compared with 40% in mammals). These proteins are soluble in neutral salt solution of fairly high ionic strength.
(2) sarcoplasmic proteins: - It constitutes proteins like myoalbumin, globulin and enzymes, which are soluble in neutral salt solutions of low ionic strength. This fraction constitutes 25-30% of the protein.
(3) Connective tissue proteins: - It constitutes collagen, which constitutes approximately 3% of the protein in teleostei and about 10% in elasmobranchii (compared with 17% in mammals). Actin constitutes about 20% of the total amount of myofibrillar proteins in fish muscle. Actin is easily extracted. However, this characteristic presents a problem when pure myosin is to be isolated, because the extracted actin spontaneously forms actomyosin complex in the solution and hinders isolation of pure myosin. Therefore, actomyosin is the main form of salt-soluble fish muscle proteins.

Myosin Myofibrillar protein gels formed in muscle foods are generally heat induced. Two types of gels can be produced from myofibrillar proteins-the myosin gel and the mixed myofibrillar protein gel. Myosin forms filaments at ionic strengths close to the physiological condition. Hence, it can form a somewhat brittle gel at low concentration of salt.

Tropomyosin and troponin regulate muscle contraction. The molecular weight of tropomyosin is 68 KDa and it has 2 subunit chains. Tropomyosin is the most heat stable muscle protein and is easily purified. Troponin is a necessary protein for tropomyosin to act as a relaxation factor during muscle contraction. Water solubility of myofibrillar protein varies depending on the temperature, pH, and ionic strength. Extreme pH and high temperature cause protein denaturation resulting in low solubility (Suzuki, 1981).

II. MECHANISM BEHIND PROTEIN EXTRACTION

This experiment is solely based on LOWRY MECHANISM. There are two distinct steps which leads to the final colour with protein:

2.1 REACTION WITH COPPER ALKALI

- The colour obtained in the absence of copper is probably attributable entirely to the tyrosine and tryptophan content and this is not greatly increased by alkaline pre-treatment.
- In the presence of copper, alkaline treatment of protein results in a 3-15 folds increase in colour, but in contrast, the presence of copper has only a small effect on the colour obtained with free tyrosine and tryptophan.
- The reaction with copper, although not instantaneous, is nearly complete in 5 or 10 minutes at room temperature under the prescribed conditions. Heating to 100° C or increasing the concentration of alkali accelerates the reaction with copper without changing the final colour.
- Pre-treatment with alkali alone does not alter the subsequent reaction with copper in alkali solution. Prolonged heating with strong alkali will, however, decrease the final colour. [8]

2.2 REDUCTION OF FOLIN REAGENT

- It is the reduction of the phosphomolybdic-phosphotungstic reagent by the copper treated protein.
- When the Folin reagent is added to the copper treated protein, maximum colour results if the reduction occurs at about pH 10.
- At this pH the reagent is only reactive for a short time. It is for this reason that even a few seconds delay in complete mixing will lessen the amount of colour. The decrease in reactivity of the reagent appears to be a function of the disappearance of the original yellow colour of phosphomolybdate (half time of

8secs) and is presumably due to dissociation of the phosphate from molybdate. Surprisingly, the colour with protein continues to develop for a number of minutes after the reagent itself has become unreactive to freshly added protein. Possibly the primary reduction product rearranges, since the absorption spectrum changes in shape between 3mins and 30mins.

- During the first minute or so after the addition of the Folin reagent, extra acid is liberated, which also may result from the dissociation of the phosphomolybdate. Therefore, for maximum colour the solution must be rather well buffered. It was found that a mixture of NaOH, sufficient to neutralize the excess phosphoric acid, and Na_2CO_3, to buffer the mixture near pH 10, gives more colour than any amount of either reagent alone. [8]

III. ESTIMATION OF PROTEIN

It may fall under 2 sub-steps to perform the experiment.

1. Sample Collection
2. Protein Quantification

3.1 SAMPLE COLLECTION

Locally available dry fishes were taken into consideration. These are; *Lepidocephalusthermalis* (local name- Thuro), *Parluciosomadaniconius* (local name- Dano), *Mastacembeluspancalus* (Baenri), *Chela laubuca* (local name- Jardaa), *Mystusvittatus* (local name- Tengnii).

3.2 PROTEIN QUANTIFICATION

The quantification process is further may divided into two sub-procedures. First one is the plotting of the standard graph, and second is the extraction of protein from the sample to be tested, followed by absorbance reading and comparison with the standard graph.

(1) Preparation of standard graph
(2) Estimation of Fish muscle protein

3.2.1 PREPARATION OF STANDARD GRAPH

To make the standard graph following solutions were made freshly.

Solution-A: 0.1 N NaOH solution was prepared. 2% of Na_2CO_3 then added to 0.1 N NaOH.

Solution-B: 1% Sodium-Potassium Tartrate solution was prepared.

Solution-C: 0.5% $CuSO_4.5H_2O$ solution was freshly prepared.

Reagent- I: This is a mixture of Solution-A, Solution-B and Solution-C in the following proportions:

[48 ml. of Solution-A + 1 ml. of Solution-B + 1 ml. of Solution-C]

(This is also known as Lowry solution.)

Reagent- II: Folin-Ciocalteu's Phenol Reagent (2N) and Distilled water added in equal proportions i.e., 1:1 ratio.

The stock BSA solution and dilution was prepared for the standard curve (Table 1, freshly prepared).

Test tube no.	*Volume of stock BSA solution (in ml)*	*Volume of distilled water (in ml)*	*Final concentration (in ml)*
1	*0*	*1*	*1*
2	*0.2*	*0.8*	*1*
3	*0.4*	*0.6*	*1*
4	*0.6*	*0.4*	*1*
5	*0.8*	*0.2*	*1*
6	*1*	*0*	*1*

[Table 1. Dilution from the BSA stock solution]

- To 1 ml. standard solution, then 4.5 ml. of reagent-I was added.
- The incubation then done for about 10 min at room temperature. After incubation 0.5 ml. of reagent-II was added followed by 30 min incubation.
- By the help of spectrophotometer, the O.D. was then taken at 680 nm. The values were noted down.

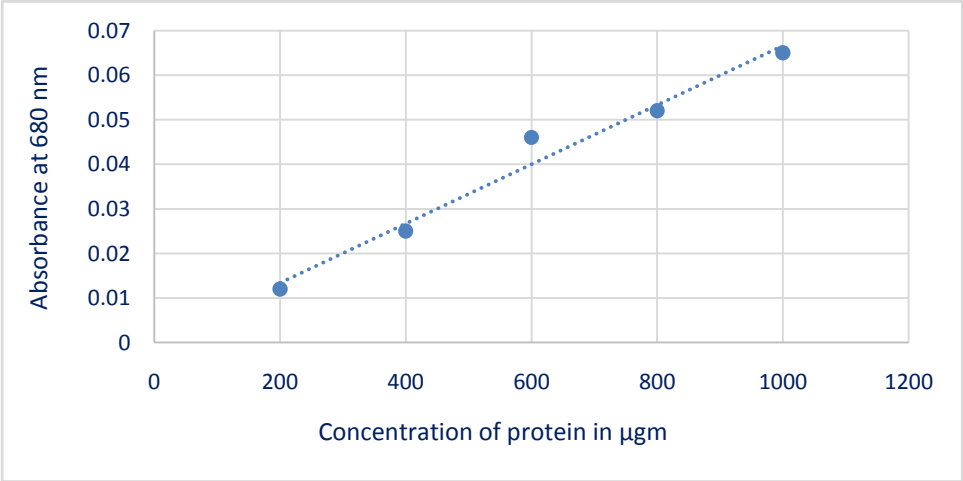
Test tube no.	***Volume of Stock Solution (BSA) in ml***	***Concentration of stock solution (µgm/ml)***	***Distilled water added (in ml.)***	***Reagent-I (in ml.)***	***I N C U***	***Folin-Ciocalteu's Phenol reagent added (in ml.)***	***I N C U***	***Absorbance at 680 nm***

1	0	0	1	4.5	***BATION 10 MIN***	0.5	***BATION 30 MIN***	0
2	0.2	200	0.8	4.5		0.5		0.012
3	0.4	400	0.6	4.5		0.5		0.025
4	0.6	600	0.4	4.5		0.5		0.046
5	0.8	800	0.2	4.5		0.5		0.052
6	01	1000	0	4.5		0.5		0.065

[Table 2. Different chemical concentration and absorbance at 680 nm]

Standard Graph Plotting:

- A standard curve, also known as a calibration curve, is a type of graph used as quantitative research technique.
- Multiple samples with known properties are measured and graphed, which then allows the same properties to be determined for unknown samples by interpolation or extrapolation on the graph.
- The samples with known properties are the standards and graph, the standard curve. The concentration of the unknown may be calculated from the mass in the assay.
- For drawing a standard graph, the values of protein concentration were taken in the x-axis and their absorbance value in the y-axis. A straight line was drawn through the points that will represent the value of absorbance drawn in the paper.



[THE STANDARD GRAPH]

3.2.2 ESTIMATION OF FISH MUSCLE PROTEIN

- One gram of fish muscle was taken and homogenized with 5ml of 5% TCA.
- It was transferred to a centrifuge tube and centrifuged for 10 minutes with 4000 rpm.
- The supernatant was discarded and pellet is treated with 5ml of 5% chilled TCA. It was again centrifuged at 4000 rpm for 5minutes. Again, supernatant was discarded and pellet get washed with absolute alcohol. It was again centrifuged at 4000 rpm for 5 minutes and the supernatant was discarded.
- The remaining pellet was then treated with diethyl ether, same as the amount of pellet. Then it was centrifuged at 4000 rpm for 5 minutes. Again, supernatant was discarded and pellet was treated with 5ml of 20% TCA and underwent a steam bath at 90°C for 30 mins.
- It was then allowed to cool down, it took 15-20 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded again and pellet was treated with 1N NaOH**,** amount equal to that of the pellet. It was stirred well, left for some time to dissolve properly and reaction to happen. Then, again it was centrifuged at 4000 rpm for 5 minutes.
- The proteins got separated from other macromolecules and are found in the supernatant area. The supernatant was collected and volume made up to 10 ml with the help of distilled water. 0.1 ml of that extract was then taken in a test tube and 4ml of protein/Lowry reagent (i.e., the reagent-I) was added to it. Then 0.5 ml of Folin reagent was added to it. It was kept for 20 mins for the chemicals to react properly.

- This extract was then taken into the cuvette and absorbance was taken at 680nm.

The same procedure was done for all the 5 sample of fish tissue we have chosen and their absorbance was noted on the copy. The absorbance data of the proteins from different fish samples are as follows:

Sl no.	Fish species	Absorbance at 680 nm
1	*Lepidocephalusthermalis*	1.543
2	*Parluciosomadaniconius*	0.853
3	*Mastacembeluspancalus*	0.627
4	*Chela laubuca*	1.045
5	*Mystusvittatus*	0.680

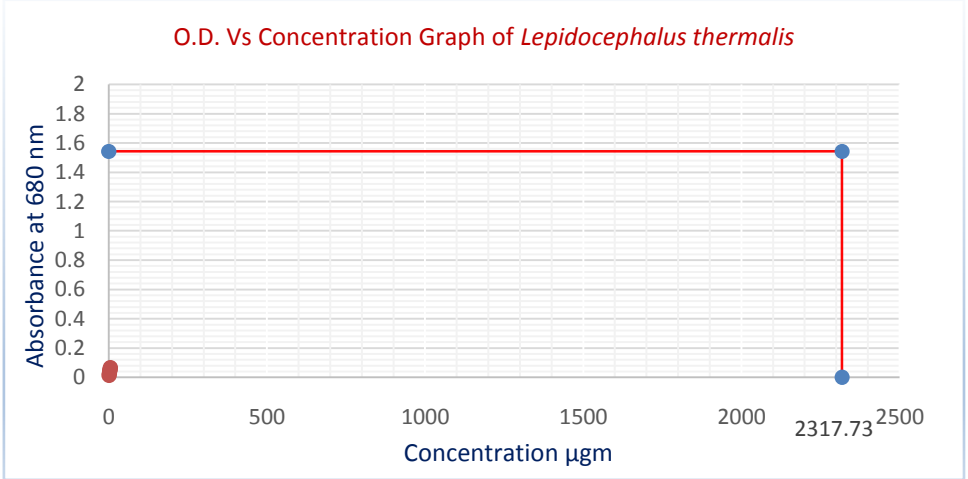
[Table 3. Absorbance taken of different fishes at 680 nm]

IV. RESULTS & DISCUSSION

- As the value of standard curve is very small in respect to obtained data, the computational help is needed. For this purpose, Microsoft excel has provided a great value in graph plotting and data interpretation.
- Values of different protein content is described below, by the help of standard graph:

4.1 FOR *Lepidocephalusthermalis*

- Optical Density obtained is **1.543**
- The standard curve interpreted, **slope (M) values to 0.000665714** and **intercept (C) is 4.7619E-05.**
- We have the formula,
  y = mx + c (for a straight line).
- Thus, the value of X will be,
  x = (y-c)/ m
- So, the value of X is,
  = (1.543-4.7619E-05)/ 0.000665714
  = 2317.73



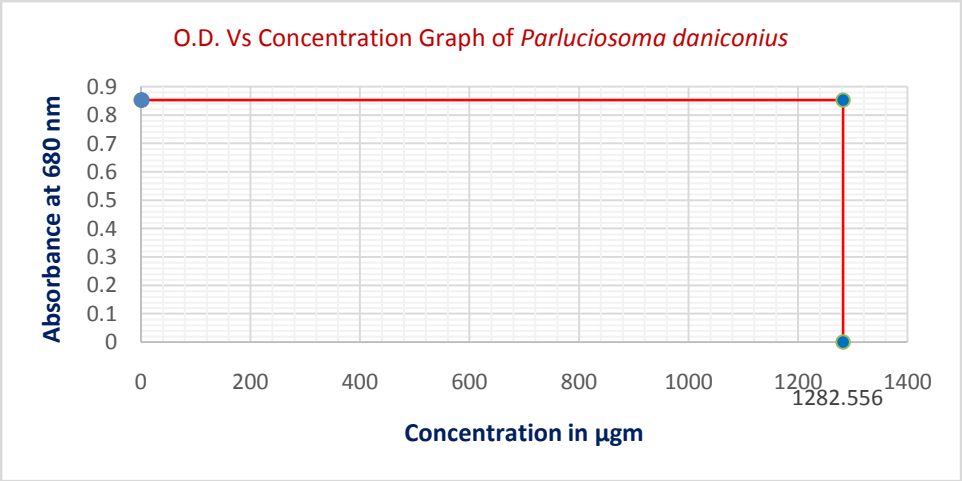
- The obtained protein content in 0.1 ml sample is 2317.73 µmg.
- When we covert µmg into mg, its value will be,
  2317.73 µgm / 1000 = 2.3177mg
- 0.1ml of protein sample contain 2.3177 mg of protein.
- So, 1ml of protein sample contain,
  = 2.3177×10 = 23.177 mg
- For 10 ml (equivalent to 1 gm) will be,
  = 23.177 mg × 10 = 231.77 mg
- Hence, we found that 1gm of muscle tissue of fish *Lepidocephalusthermalis* contain **231.77** mg of protein.

4.2 FOR *Parluciosomadaniconious*

- Optical Density obtained is **0.853**
- According to formula x = (y-c)/m

x = (0.853-4.76E-05)/ 0.000665714

= 1282.556



- The obtained protein content in 0.1 ml is 1282.556 µgm.
- When we covert µmg into mg, its value will be,

1282.556 µgm/1000 = 1.282 mg

- 0.1ml of protein sample contain 1.282 mg of protein
- So, 1ml of protein sample contain,

= 1.282 mg × 10 = 12.82 mg

- For 10 ml (equivalent to 1 gm) will be

= 12.82 mg × 10 = 128.2 mg

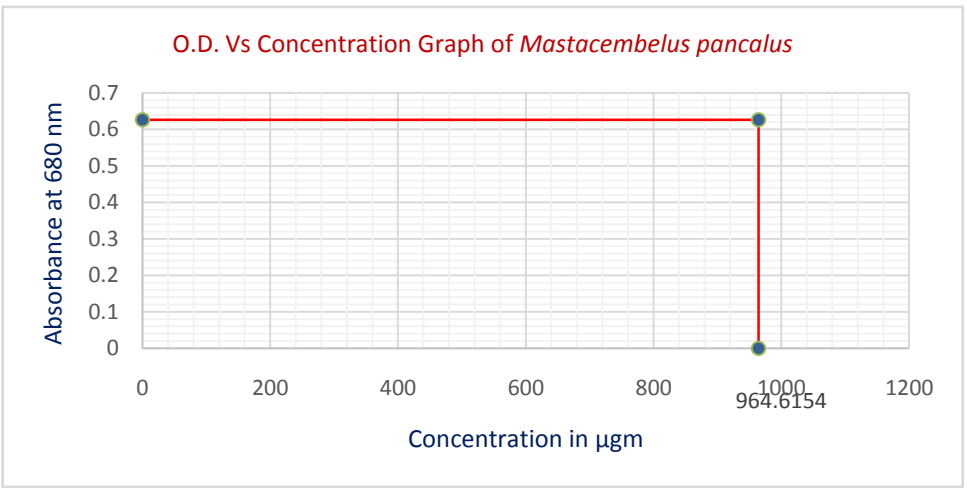
- Hence, we found that 1gm of muscle tissue of fish *Parluciosomadaniconius* contain **128.2** mg of protein.

4.3 FOR *Mastacembeluspancalus*

- Optical Density obtained is **0.627**
- According to formula x = (y-c)/M

x = (0.627-4.76E-05)/ 0.000665714

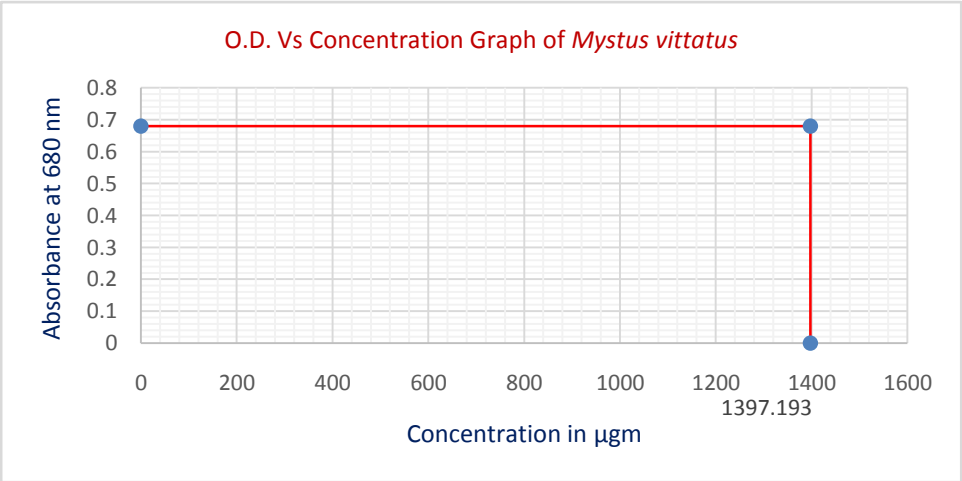
= 964.6154



- The obtained protein content in 0.1 ml is 964.6154 µgm.
- When we covert µmg into mg, its value will be,
  964.6154 µgm/1000 = 0.9646 mg
- 0.1ml of protein sample contain 0.9646 mg of protein.
- So, 1ml of protein sample contain,
  = 0.9646×10 = 9.646 mg
- For 10 ml (equivalent to 1 gm) will be,
  = 9.646 mg × 10 = 96.46 mg
- Hence, we found that 1gm of muscle tissue of fish *Mastacembeluspancalus* contain **96.46** mg of protein.

4.4 FOR*Mystusvittatus*

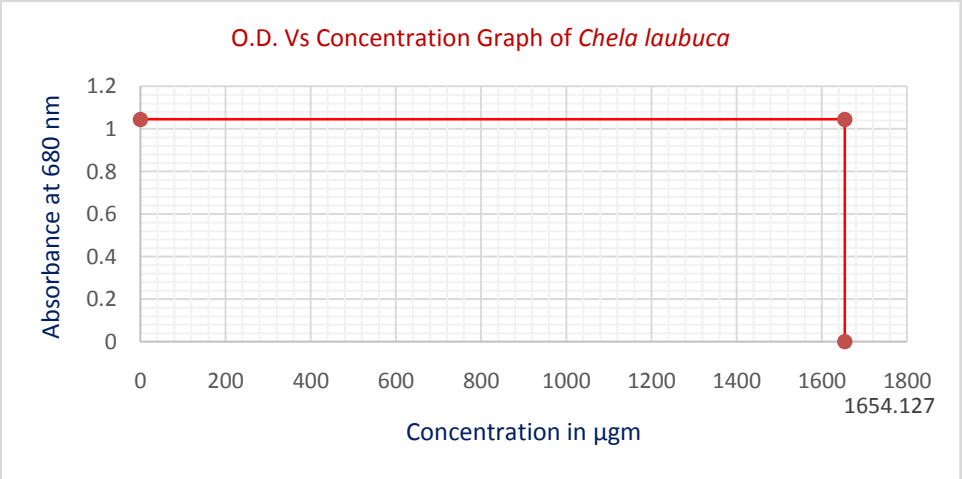
- Optical Density obtained is **0.680**
- According to formula x = (y-c)/M
  x = (0.680-4.76E-05)/ 0.000665714
  = 1397.193



- The obtained protein content in 0.1 ml is 1397.193 µgm.
- When we covert µmg into mg, its value will be,
  1397.193 µgm/1000 = 1.3971 mg
- 0.1ml of protein sample contain 1.3971 mg of protein.
- So, 1ml of protein sample contain,
  = 1.3971 ×10 = 13.971 mg
- For 10 ml (equivalent to 1 gm) will be
  = 13.971 mg × 10 = 139.71 mg
- Hence, we found that 1gm of muscle tissue of fish *Mystusvittatus* contain **139.71** mg of protein.

4.5 FOR *Chela laubuca*

- Optical Density obtained is **1.045**
- According to formula x = (y-c)/M
  x = (1.045-4.76E-05)/ 0.000665714
  = 1654.127



- The obtained protein content in 0.1 ml is 1654.127 µgm.
- When we covert µmg into mg, its value will be,

  1654.127 µgm/1000 = 1.6541 mg
- 0.1ml of protein sample contain 1.6541 mg of protein.
- So, 1ml of protein sample contain,

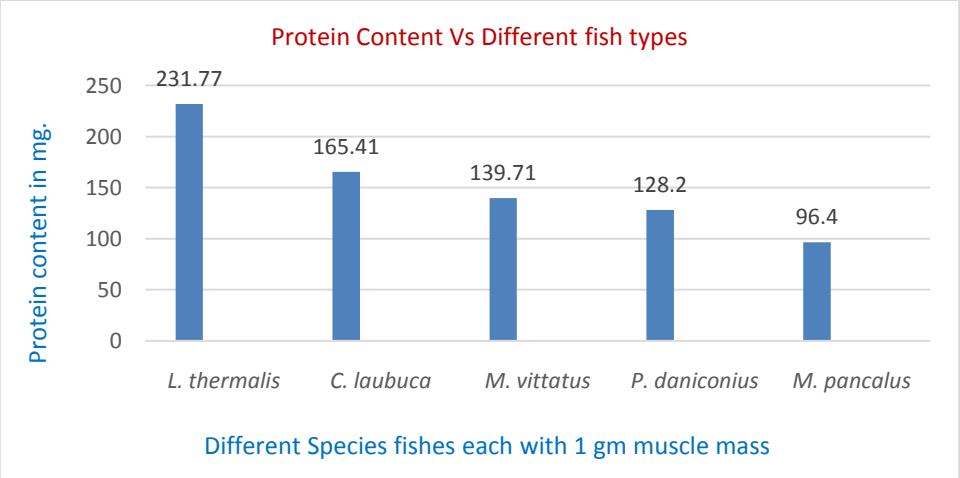
  = 1.6541 mg ×10 = 16.541 mg
- For 10 ml (equivalent to 1 gm) will be

  = 16.541 mg × 10 = 165.41 mg
- Hence, we found that 1gm of muscle tissue of fish *Chela laubuca* contain **165.41** mg of protein.

V. CONCLUSION

From the observation of the above data obtained from different fish species is that, different fish species contain difference in protein content in their muscle. Some fishes contain high amount of protein, whereas some contain low amount of protein. From our experiment the species *Lepidocephalusthermalis* muscle contain the highest amount of protein i .e., 231.7 mg/gm (~23.17%). *Mastacembeluspancalus* has the lowest amount of protein in its muscles i .e., 96.46 mg/gm. (~ 9.64%), *Chela laubuca*, *Mystusvittatus*, *Parluciosomadaniconius* species contains 165.41 mg. (~ 16.54%), 139.71 mg. (~ 13.97%) and 128.20 mg (~12.82%) of protein in decreasing order, respectively**.**

From the estimation we concluded that, *L. thermalis* has highest amount of protein, so it can be preserved in dry form for a longer period. More to it, it has high dietary value. So, if there is high market rate for this fish, it is justifiable among the other given species. Likewise, for other four species, the amount of protein will determine their preservation time, their taste and hence, will determine their market price.



REFERENCES

[1]. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.
[2]. Chauhan, B. S. (1947). Fish and fisheries of the Patna State, Orissa.
[3]. Das, M., Rohit, P., Maheswarudu, G., Dash, B., & Ramana, P. V. (2013). Overview of dry fish landings and trade at Visakhapatnam Fishing Harbour. Marine Fisheries Information Service; Technical and Extension Series, (215), 3-7.
[4]. Dhaneesh, K. V., Noushad, K. M., & Ajith Kumar, T. T. (2012). Nutritional evaluation of commercially important fish species of Lakshadweep archipelago, India.
[5]. Elvevoll, E. O., & James, D. G. (2000). Potential benefits of fish for maternal, foetal and neonatal nutrition: a review of the literature. Food, nutrition and agriculture, (27), 28-39.
[6]. Huynh, M. D., & Kitts, D. D. (2009). Evaluating nutritional quality of pacific fish species from fatty acid signatures. Food Chemistry, 114(3), 912-918.
[7]. Kumar, B. S., &Mohanta, R. (2014). A preliminary study on community based traditional fish drying activities of important marine fishes: Ganjam coast, Odisha and Improved scenario using modern technique. Int. J. Res and Innov. Earth. Sci, 1(1), 2394-1375.
[8]. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193, 265-275.
[9]. Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen IJ. Protein Determination-Method Matters. Foods. 2018 Jan 1;7(1):5. doi: 10.3390/foods7010005. PMID: 29301260; PMCID: PMC5789268.
[10]. Memon, N. N., Talpur, F. N., Bhanger, M. I., &Balouch, A. (2011). Changes in fatty acid composition in muscle of three farmed carp fish species (Labeorohita, Cirrhinusmrigala, Catlacatla) raised under the same conditions. Food Chemistry, 126(2), 405-410.
[11]. Mishra, S., Gouda, R., Nayak, L., &Panigrahy, R. C. (1999). A check list of the marine and estuarine fishes of South Orissa, east coast of India. Records of the Zoological Survey of India, 97(3), 81-90.
[12]. Naik, D. (2001). An economic analysis of marine fish marketing in Orissa. Research Project submitted to Orissa University of Agriculture and Technology, Bhubaneshwar, Orissa.
[13]. Parvathy, U. (2018). Drying and salting of Fish. ICAR-Central Institute of Fisheries Technology.
[14]. Rej, R., & Richards, A. H. (1974). Interference by Tris buffer in the estimation of protein by the Lowry procedure. Analytical biochemistry, 62(1), 240-247.
[15]. Remya, S., Renuka, V., Sreelakshmi, K. R., & Joseph, T. C. (2018). Hygienic drying and packing of fish..
[16]. Sahu, N. C., Issac, A., & Bali, S. K. (2009, January). Economy of the fisherwomen in Ganjam District of Orissa: conditions for environmental governance and sustainable development. In Fifth Biennial Conference of the Indian Society for Ecological Economics (INSEE) on Environmental Governance, Ahmedabad, India (pp. 21-23).
[17]. Siddhnath, Ranjan, A., Mohanty, B. P., Saklani, P., Dora, K. C., & Chowdhury, S. (2020). Dry fish and its contribution towards food and nutritional security. Food Reviews International, 1-29.
[18]. Soumyashree Rath, Siba Prasad Parida, "Comparative Studies of Protein Estimation in Different Fresh Water Fishes", International Journal of Science and Research (IJSR), https://www.ijsr.net/search_index_results_paperid.php?id=SR20415184446, Volume 9 Issue 4, April 2020, 1408 - 1409
[19]. Suzuki, T. (1981). Fish and krill protein: Processing technology. London: Applied Science Publishers.
[20]. Tacon, A. G., &Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285(1-4), 146-158.
[21]. Warrier, S. B. K., Gore, M. S., &Kumta, U. S. (1975). Fish muscle structural proteins. Fishery Technology, 12(1), 1-15.
[22]. Wilson, K., & Walker, J. (Eds.). (2010). Principles and techniques of biochemistry and molecular biology. Cambridge university press.
[23]. Wolfe, R. R. (2006). The underappreciated role of muscle in health and disease. The American journal of clinical nutrition, 84(3), 475-482.
[24]. Wright, R. T. (2004). Environmental science: Toward a sustainable future. Upper Saddle River, NJ: Pearson/Prentice Hall.
[25]. Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J., & Wu, Z. (2014). Amino acid nutrition in animals: protein synthesis and beyond. Annu. Rev. Anim. Biosci., 2(1), 387-417.