Journal of Advanced Zoology
ISSN: 0253-7214
Volume 42 Issue 2 Year 2021 Page 340-346

Effect Of Refrigerating Temperature On Protein Compositional Level In Body Muscle Of *Labeo Bata* (Hamilton)

Prasanjit das[1*]

[*1]*Department of Zoology, Sikkim University, Gangtok, Sikkim*

***Corresponding author:** Prasanjit das
* mail: prasanjitdas500@gmail.com*

Abstract

A 14 days experiment was conducted to analyze the changes of protein level in *Labeo bata* (Hamilton 1822) due to refrigeration. The result indicates that the proportion of protein in the muscle of fish decreased with the increased number of days the fish in the refrigerator. Due to refrigerating temperature the muscle got tighter with the number of days increased and the protein content decreased. Protein composition was classified into five fractions based on solubility, including water-soluble proteins, NPN, salt-soluble proteins, alkali-soluble proteins and alkali-insoluble proteins. Effects of heating cooling on the content of each fraction With the decrease of temperature, significant changes in content of water-soluble proteins, salt-soluble proteins and alkali-soluble proteins were observed, and little changes for NPN and alkali-insoluble proteins.

Keyword: Labeo bata, protein, refrigeration, fish muscle

1. Introduction

Fish is a good source of high quality protein, vitamin and essential minerals. India is one of the largest producers of freshwater fish in the world. *Labeo bata* is one of the major species produced in India and Bangladesh and has a unique taste and texture, and is very popular among consumers especially in Assam, west Bengal and Odisa, as well as other part of the country. Protein is the main nutritional ingredient of fish muscle (meat) and the main supporting composition of muscle structure, and the changes of protein will directly affect the structure, textural and sensory properties of fish meat.

Infrigidation is one of the major steps in preservation of fish. Difference chilling temperature can cause varying degrees of degeneration or degradation of protein in the muscle, leading to the differences in organizational characteristics, texture and sensory quality (Palka and Daun, 1999). It is reported that the stability of protein during chilling is close related to its structure, changes in texture, flavor, color and other sensory qualities, also the protein solubility and collagen content of various muscles, including beef, pork, chicken, rabbit, shrimp, sea fish, etc. few studies involved in the changes of protein composition in fresh water fish chilled at different temperatures. The results obtained from different specie muscles could have something to do with the muscle type and structure, and cooling properties of protein, such as the protein compositions and solubility, as well as the content and nature of the intramuscular collagen.

The effects of cooling temperature on the texture, color of *Labeo bata* muscle were studied, and the objective of this paper was to discuss the effects of chill treatments on the protein components of fish muscle, and further understand the relationship between the texture and protein composition. In addition, the biochemical characteristics changes of collagen in *Labeo bata* carp during chilling were studied by Bradford Reagent.

Labeo bata is predominantly a bottom feeder and herbivorous fish (Chondar, 1999).Datta et al. (1986) reported that Labeo bata can grow up to 44-67g in 10-12 months in paddy field with other carps. Datta et al. (1996) also

observed the growth of *Labeo bata* from 0.09-0.2 g to 23-38 g in 6-10 months under mono culture with sewage water as input.
Minor carp *Labeo bata* (pictures: 1) is a freshwater subtropical species which is commonly known as 'Bangon Bata'. This fish is commercially important and target species for commercial small- and large-scale fishers in Bangladesh, India and Pakistan. It is also used by both culture and capture fisheries nowadays. L. bata is in great demand in the market because of its high nutritional value and good taste (Bhuiyan, 1964). This fish contains about 15.42% of protein and 3.73% of lipid (Ahmed et al., 2012).

2. Materials and methods

Sample treatments Labeo bata weighing between (35-47gm) was bought from Nagaon Bara Bazar (since the fish was live and brought in a small glass fish pot). Then it was transferred to the laboratory, the fish were beheaded, gutted and washed. Before it was killed muscle of that live fish was isolated and then the fish killed and the skin was removed and the muscles were trimmed of visible fat and connective tissue. The muscles were cut with the fibers parallel to the longest axis. The fish blocks were individually weighed and packed within the centrifuged tube and stored in the laboratory fridge.

2.1 Chemical preparation

The Bradford reagent consists of the dye Coomassie Brilliant Blue G in phosphoric acid and methanol or ethanol.
Brilliant Blue G forms a complex with proteins in solution resulting in a shift in the absorption maximum of the dye from 465 to 595nm. The absorption is proportional to the amount of protein present. This reagent can be used to quantify proteins in the concentration range from 0.1 to approximately1.4mg/ml. The Bradford assay is compatible with reducing agents such as dithiothreitol; however it is only compatible with very low concentrations of detergents. If the samples contain detergent then the BCA assay is recommended. It is always advisable to prepare the standard in the same buffer as the sample to minimize any interference effects. The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues. Compared to "average" proteins, the Bradford assay is more sensitive to bovine serum albumin, by a factor of about two Immunoglobulin G (IgG - gamma globulin) is therefore the preferred protein Standard.

2.2 Materials required

Bradford Reagent
Suitable tubes to hold and mix 3.1ml samples
Plastic disposable cuvettes (Jenway 060 084)
Standard protein solution of known concentration (2Mg/ml)
Method
Preparation of the Bradford reagent
The Bradford reagent is available commercially from a number of different sources. However it can be
Prepared as follows:
1. Dissolve 100mg Coomassie Brilliant Blue G-250 in50ml 95% ethanol; add 100ml 85% (w/v) Phosphoric acid.
2. Once the dye has completely dissolved, dilute to1 litre with deionised water.
3. Filter through Whatman #1 paper just before use.

2.3 **Flow Chart Showing The Process The Process Of Extraction**

From fish 1gm tissue is weight and weight and prepared (1gm tissue in 2ml of distilled water
From the above solution 1ml solution is taken
1ml cold 10% TCA is added
Solution is then centrifuged at about 4000rpm for 5 -10 minute
Supertant is then decanted
2.5 ml of 5%TCA is added to it and centrifuged
Precipitated is then washed with 2.5 ml of alcohol and centrifuged
Again wash with alcohol ether (3:1 and centrifuged
Then after tissue is inverted in a stand having a tissue paper below.
After 3o minutes 3 ml of o.1 N NaoH is added and left in water bath at 37° FOR 3-4 hours
Supertant is warmed up

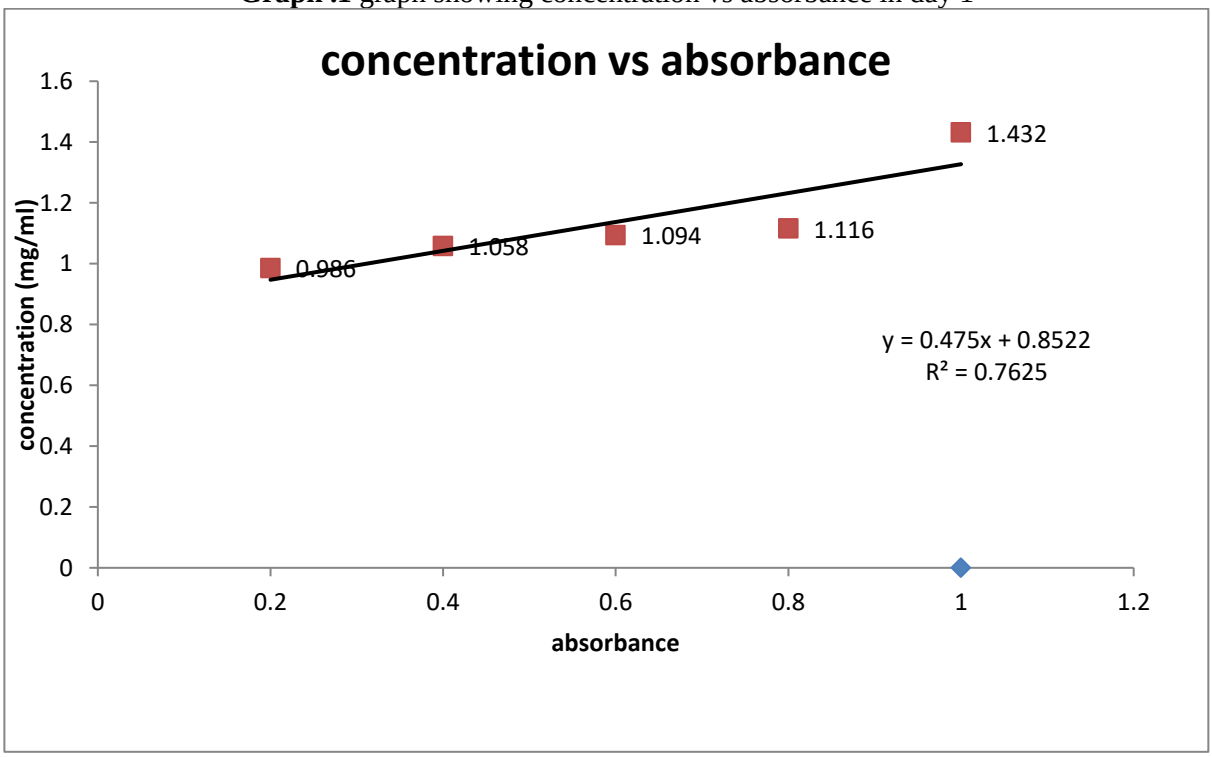
20µl(10µg),40µl(20µg),60µl(30µg),80µl(40µg) of 0.1 mg/ml
BSA were taken out separate cuvettes and made the volume 100µl with distilled water 100µl tissue extracted is taken as unknown. The cuvette with no protein standard in it served as blank
1.5 ml of Bradford reagent is added to each cuvette
Cuvette are covered with plastic paraffin film
0.1% Stock solution and mixed well
Then the cuvette were incubated at room temperature for 1o minutes
Absorbance of cuvette is measured at 595ηm
Absorbance of each BSA standard as function of its theoretical concentrated plotted
Centrifugation of unknown is calculated from the graph

3. Result

Table. 1 showing concentration and absorbance parameters in day 1

sample	Concentration(mg/ml)	Absorbance$_{595nm}$
Blank	**1ml**	**0.00**
S1	**0.2(mg/ml)**	**0.986**
S2	**0.4(mg/ml)**	**1.058**
S3	**0.6(mg/ml)**	**1.094**
S4	**0.8(mg/ml)**	**1.116**
S5	**1.0(mg/ml)**	**1.432**
Unknown	**Unknown(mg/ml)**	**1.673**

Graph .1 graph showing concentration vs absorbance in day 1

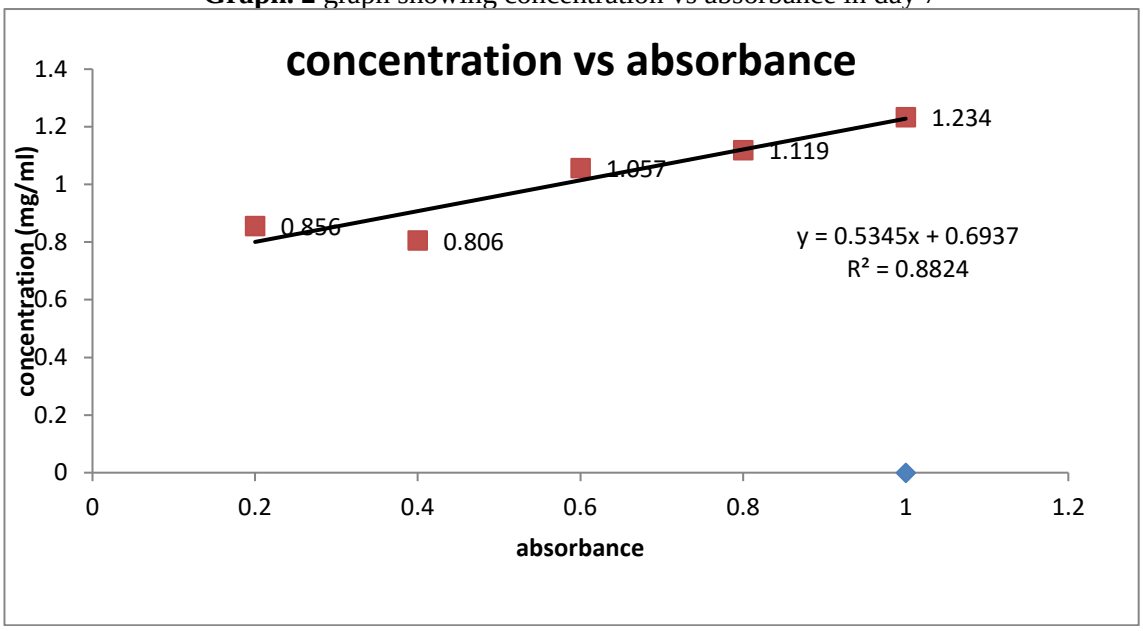


Calculation
Unknown absorbance=1.637
Unknown concentration (mg/ml) =1.42
From the equation $y = 0.475x + 0852$

Table. 2 showing concentration and absorbance parameters in day 7

sample	Concentration(mg/ml)	Absorbance$_{595nm}$
Blank	1ml	0.00
S1	0.2(mg/ml)	.856
S2	0.4(mg/ml)	.806
S3	0.6(mg/ml)	1.057
S4	0.8(mg/ml)	1.119
S5	1.0(mg/ml)	1.234
Unknown	Unknown(mg/ml)	1.437

Graph. 2 graph showing concentration vs absorbance in day 7



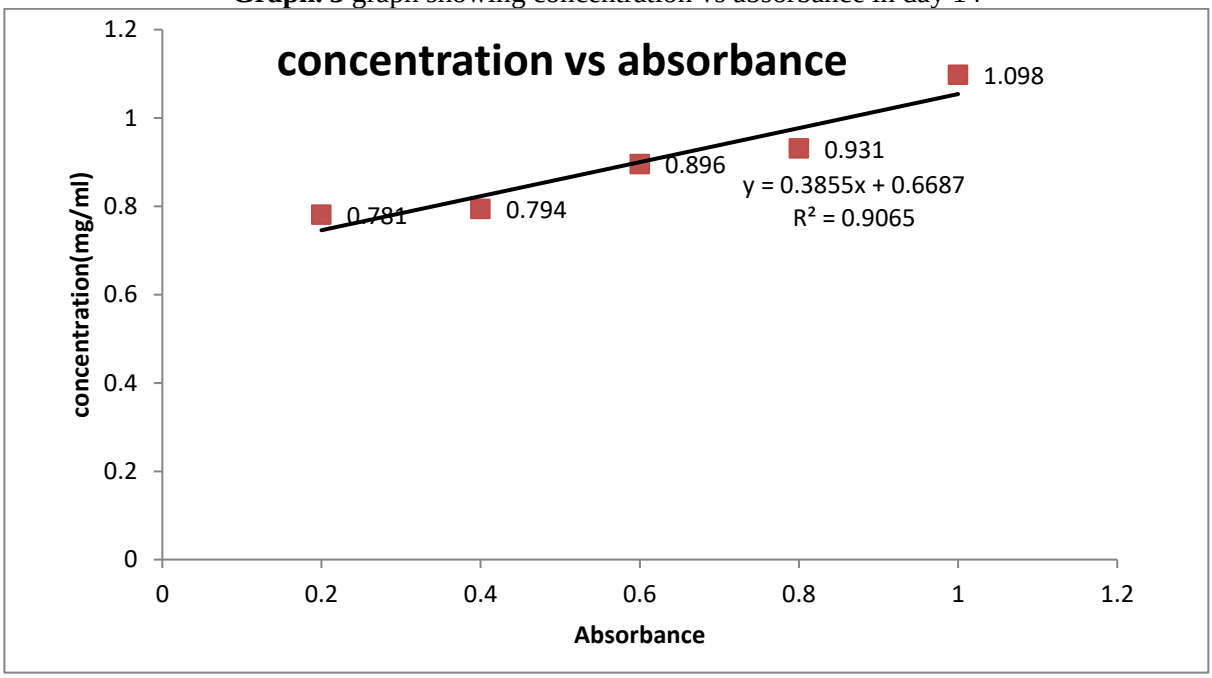
Calculation
Unknown absorbance=1.437
Unknown concentration (mg/ml) =1.29
From the equation $y = 0.534x + 0.693$
Therefore x =1.117mg/ml

Table. 3 showing concentration and absorbance parameters in day 14

sample	Concentration(mg/ml)	Absorbance$_{595nm}$
Blank	1ml	0.00
S1	0.2(mg/ml)	.781

S2	0.4(mg/ml)	.794
S3	0.6(mg/ml)	.896
S4	0.8(mg/ml)	.931
S5	1.0(mg/ml)	1.098
Unknown	Unknown(mg/ml)	1.112

Graph. 3 graph showing concentration vs absorbance in day 14



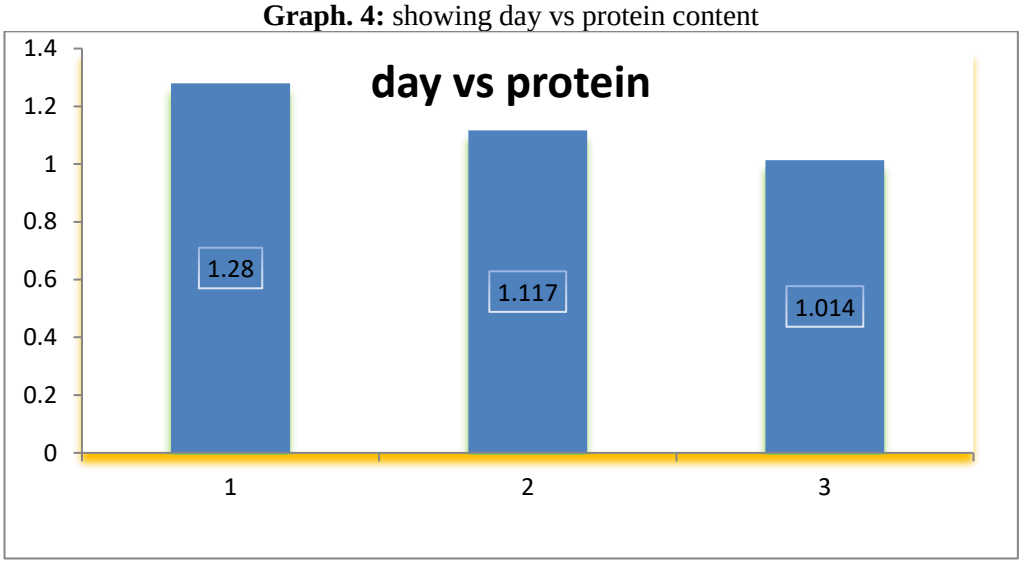
Calculation
Unknown absorbance=1.112
Unknown concentration (mg/ml) =1.098
From the equation $y = 0.385x + 0.668$
Therefore x =1.116 mg/ml

4. DISCUSSION
As shown in the above table -1, 2, and 3 variation of total protein content (mg/ml) in body the muscles of selected fishes (*Labeo bata*) collected from Nagaon Bara Bazar, dated 12.03.2020.The total protein content in body muscle of selected fish ranges from 1.280±1.114(mg/ml).
The highest protein content in the selected fish was 1.280±.314 and the lowest was 1.114±.093.

TABLE 4: showing protein content in three different days

Sample	day	protein
1	1	1.28
2	7	1.117
3	14	1.014

Graph. 4: showing day vs protein content



5. CONCLUSION

Total protein content in body muscles of *Labeo bata* was higher on the 1st day of experiment and lower in the last of experiment i.e. on 14th day. At the very first day the fish muscle content highest amount of protein i.e. ±1.28 (mg/ml) and then the fish was kept under observation for 7 days and the same process repeated and protein content of the fish was calculated. On the 7th day the fish muscle contain slightly less protein then the 1st day i.e. ±1.117 (mg/ml). On the 14th day same process was repeated and protein content was measured at ±1.116 (mg/ml).

6. REFERENCES

1. Ahmed, S., Rahman, A. F. M. A., Mustafa, M. G., Hossain, M. B., Nahar, N. (2012): Nutrient Composition of indigenous and exotic fishes of rain fed waterlogged paddy fields in Lakshmipur, Bangladesh. World Journal of Zoology, 7, 135-140.
2. Ahmed, Z. F., Smith, C., Ahmed, F., Hossain, M. Y. (2007): Growth and reproduction of the Indian river Shad Gudusia chapra (Clupeidae). Folia Zoologica, 56, 429-439. Bhuiyan, A. L. (1964): Fishes of Dacca. Asiatic Society of Pakistan, Dacca, 148 pp.
3. Association of Official Analytical Chemists (2000). AOAC. Official methods of analysis, 17th ed.AOAC: Gaithersburg, Maryland.
4. Bradford, M.M. A rapid and sensitive for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254, (1976).
5. Cambero, M.I., Jaramillo, C.J., Ordoñez, J.A., Cobos, A., Pereira-Lima, C.I., and García de Fernando, G.D. (1998). Effect of cooking conditions on the flavour compounds and composition of shrimp (Parapenaeus longirostris) broth. Eur. FOOD Res. Technol.206, 311-322
6. Dahanukar, N., Raut, R., Bhat, A. (2004): Distribution, endemism and threat status of freshwater fishes in the Western Ghats of India. Journal of Biogeography, 31, 123-136.
7. Datta, A.K., Roy, A.K., Mukhopadhyay, S.K. and Bhattacharyya, G.B. 1986. Performance of *Labeo bata* (Ham.) in mixed culture in sewage fed paddy plot. Proceedings of the National Symposium on Fisheries and Environment, 11-14thDec, 1986, Hardwar, India pp. 139-144 (Abstr.)
8. file:///D:/15_chapter4.pdf.pdf(Estimation of Total Protein content (mg/g) in body muscles of selected fishes WALLAGO ATTU and SPERATA (MYSTUS SEENGHALA collected from selected habitats in different seasons (reservoirs) by Lowry's method (1951)
9. http://www.ruf.rice.edu/~bioslabs/methods/protein/bradford.html
10. https://indiabiodiversity.org/species/show/232611
11. https://info.gbiosciences.com/blog/bid/201326/spectrophotometry-and-its-application-in-protein-estimation
12. https://ribarstvo.agr.hr/articles/74993_THREATENED_FISHES_OF_THE_WORLD_Labeo_bata_(Hamilton_1822)_(Cypriniformes_Cyprinidae)_en.pdf
13. https://www.fws.gov/fisheries/ANS/erss/uncertainrisk/ERSS-Labeo-bata_Final.pdf

14.https://www.ias.ac.in/public/Volumes/secb/049/06/0377-0385.pdf
15.https://www.ijcmas.com/6-8-2017/Kamal%20Bijalwan,%20et%20al.pdf
16.https://www.ijpbs.com/view.php?iid=1061
17.https://www.researchgate.net/publication/284409743_Evaluation_of_Crude_Protein
18.https://www.sciencedirect.com/science/article/abs/pii/S0044848618313930