4. METERIALS AND METHODS

4.1 Marketing Process and Livelihood Study

4.1.1 Marketing Process

Different market places were selected to study about the marketing process and seasonal marketing transaction, where every day a huge amount of transactions were carried-out. There were three "Marketing Zones" among the south western part of West Bengal were selected. The selected three "Marketing Zones" were **:-**

Midnapore Town Zone - Rail gate Market, Miya Market, Kotwali Market, School Market, Raza Market.

Bankura Zone - Bankura Sadar Market, Sonamukhi Market, Bishnupur Market, Patrasayar Market, Balsi Market, Khatra Market

Purulia Zone - Purulia Town Market, Punchar Market, Lalpur Market, Man Market.

According to the protocol of "Private Distribution System" (Gongongopadhyay,2001), the whole marketing process of *Bellamya bengalensis* was considered. In the broad sense, private distribution system referred about the product flow system, here product may reach from collectors to consumers, either direct or through the several types of middlemen. That's why, in other word it was attributed as "Branch system distribution process". Where each middleman performs some work for bringing the product to the ultimate consumers, here the ultimate consumers were placed at the end point of the channels. (Gongongopadhyay, 2001).

Here people are called "Collectors" who engage in the collection of *B.bengalensis*, are belonging in the group "D" ("D" means "Division"). In this branch system distribution process, three divisions were made from group "D", from the collectors to

the ultimate consumers. Firstly, the people who were belonging in the group of D-I, brought the products from the collectors and sell it door to door either whole (fleshy part covered with shell) or flesh (only fleshy part excluding shell part). Secondly, group D-II was referred, when product was sold by wholesaler from collectors and then the product was canalized to the ultimate consumers through different branch system, either in flesh or whole form. Group D-IIA was referred, when product moved to the consumer (either in flesh or whole form), directly through wholesaler. And in group D-IIB, where the product (only in whole form) moved to the consumer, through wholesaler and then retailer. Thirdly, other groups of people (collectors), who collected the products from several collection ground and sold their items to the consumers directly, either moving door to door of houses or placed within the market, this group was attributed as D-III (Fig. 1). In this study collection place, collection time etc. also were taken into account.

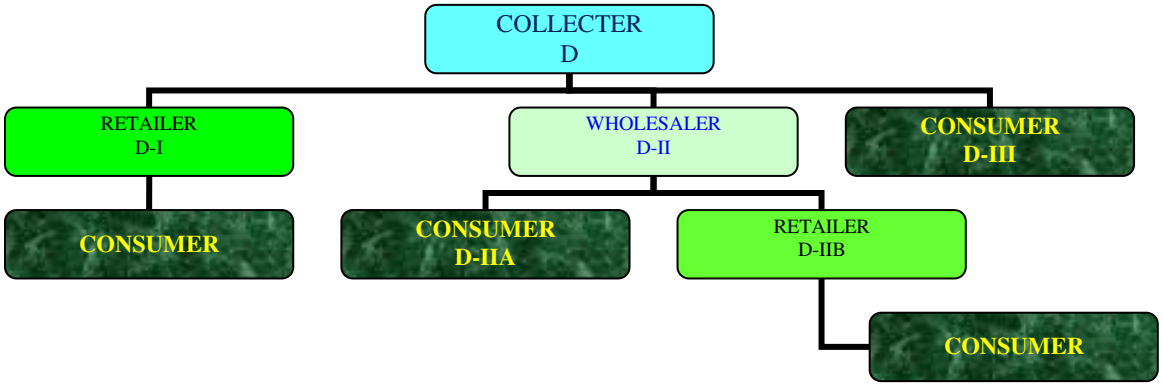

Fig. : 1

- For seasonal market survey three successive season were chosen, which were as follows:
  - Pre-monsoon (February to May)
  - Monsoon (June to September)
  - Post Monsoon (October to January).

- This survey work was done from February,2010 to January, 2013.
- In each season of a year, 12 times market sampling was measured, for every market (3 times in each months. i.e. starting , middle and end part of any month). So total 36 numbers of sampling were done in every year.
- For statistical analysis mean, mode, standard deviation and t-test paired two sample for means (MS Office Excel, 2007) were applied to show the significance differences, where $p < 0.05$.

4.1.2 Livelihood study

Livelihood study was done, to know about the socio-economical conditions of those people, who engaged directly with the profession of selling *Bellamya bengalensis*. Through this livelihood study we should able to know about the life style and family background of those people, who depends on this profession (snail marketing) directly as their main source of livelihood, since the immemorial time being.

In this study two villages were chosen, where a large number of people were actively involve with this profession. The selected two villages were Pakui and Chowksahapur. These villages were under Jalimonder Grampanchayet (near Shyamchak Rail Station) and Debra Block of West Midnapur District. There were over 100 nos of families from each villages, were recorded. But among the 100 families (approx.), only 25 numbers of families, from each villages were taken for sampling. Because these 50 families (aggregating two village-samples families) were actively involve with these profession (Table 2).

All the collected data (both two villages) were interpreted in result as whole number percentage format.

Date of Sampling:
Name of Block:
Name of Village:
No. of Male..…… No. of Female…….. Total family members……

Table 2 : The format represent about the livelihood study

Age Group	Name	Age	Male/ Female (M/F)	Literate/ Illiterate (L / IL)	Mode of work		Monthly Income			Children		Others
					Main Work	Additional Work	Main income	Addi-tional Income	Total	NO.	L/ IL	
6-16												
17-45												
46-70												

NO.-Number, L-Literate, IL-Illiterate.

4.2 Study on the availability of Bio-Chemical parameters

4.2.1 Selection of parameters regarding bio-chemical estimation of *B. bengalensis*

The bio-chemical compositions such as "Protein, Carbohydrate, Fat, Moisture and Ash" were selected, side by side qualitative and quantitative "Amino Acid, Fatty Acid and Vitamin" composition in *B. bengalensis* were also selected to analysis.

Biochemical composition in *B. bengalensis* i.e. protein, carbohydrate, fat, moisture and ash contents were done by using both laboratories i.e. Office of the Deputy Director of Fisheries (Microbiology & Parasitology), Dept. of Fisheries, Govt. of West Bengal, Pailan, (P.O. Pailan Hut, Kolkata-700 104),West Bengal as well as Fisheries Research Laboratory, Dept. of Aquaculture Management and Technology, Vidyasagar University,(Midnapore-721102, West Midnapore), West Bengal. Whereas, amino acid and fatty acid contents were analysed by using the laboratory, Department of Chemical

Technology, University College of Science and Technology, University of Calcutta, (92, Acharya Prafulla Chandra Road, Kolkata - 700 009), West Bengal. Vitamin contents were analysed by using the laboratory of M/S Effrac Pvt. Ltd., (P.O.: Nilgunj Bazar Barasat,Kolkata 700 121, N-24 pgs),West Bengal.

For statistical analysis mean, mode and standard deviation were applied, where $p < 0.05$.

4.2.2 Preparation of samples for biochemical estimations

4.2.2.1 Sample collection

Adult Samples were collected at random from the different market places of Kolkata, West Bengal.

4.2.2.2 Sample preparation and preservation

At first collected *B. bengalensis* samples were properly washed with the double distill water. Immediately after collection, the collected sample were kept in an insulated box with sufficient ice and transferred to the laboratory. The collected sample was properly washed with double distill water. Then length and weight were measured separately. Flesh portion was carefully removed from the shell, using cleaned, grease free scissors, forceps and scalpels and thus the edible fleshy parts were separated. After separation of flesh, removing of unwanted matters, proper washing was taken place with double distill water. The flesh was dried in hot air oven (Plate No. 3) at 50^0C. The oven dried samples were grounded finely with cleaned grease free pestle and mortar. The powdered form of sample was preserved sufficiently in sterilized bottles (Tarsons made, amber coloured wide mouth bottle of 60 ml capacity, product code: 581310) for analysis. The sample vials were then preserved in desiccators.

4.2.3 Proximate analysis of biochemical contents in muscle tissue of *B. bengalensis*

4.2.3.1 Estimation of "Protein" content (Kjeldahl Technique)

Protein content was estimated by using Kjeltech machine made of "Pieces", model name "KJELPRO" (Plate No.2). According to the methodology of Gupta *et.al.* (1992), this estimation was carried out.

Procedure

Digestion:

- After taking 2 gm of sample, it was then transferred into Kjeldahl flasks.
- After adding, 7 gm of digestion mixture and 12 ml of con. H_2SO_4, they were properly mixed with sample.
- After placing the tube in digestion unit, it was heated until the sample colour turned from brown to greenish blue. After proper digestion the flasks were removed from digestion unit, followed by proper cooling.

Distillation:

- Digested samples were transferred into volumetric flask and the sample volume was made up to 100 ml with distilled water.
- The volumetric flask were transferred into distillation chamber.
- After addition of 50% NaOH into volumetric flask, trapping flask were taken containing boric acid and mixed indicator for subsequent volumetric flask.
- Distillation process was continue the until the colour of the trapping flask was turn in to light green.
- Then the trapping flasks were taken for titration.

Titration:

- The titration was done using N/100 H_2SO_4 untill the colour of the trapping flask solution turns from light green to baby pink.

Calculation:

% of Nitrogen = V X 0.00014 X D X 100 / W X A

% of crude protein= **% of Nitrogen X 6.25**

V= Burette reading

D= Dilution factor (volume made in volumetric flask).

W= Weight of sample.

A= Aliquot taken

4.2.3.2 Qualitative and quantitative analysis of "Amino acid" content

Amino acid profile was determined, according to methodology of Alaiz *et.al.,*(1992) by using HPLC (High-performance liquid chromatography) technique, made of Waters 2487 with UV detector, column Milford, MA, USA, Nova-Pak C_{18}, 3.9 X 150 mm) (Plate No. 3).

Procedure

- At first the samples (50 mg) were hydrolyzed in 6 N HCL.
- Sealed containers were kept into "Hot Air Oven" at the temperature of 105^0C for overnight.
- Hydrolysis of samples were taken place to made it acid free with 6N NaOH.
- Derivatisation of amino acid, a necessary step for their detection and quantification in HPLC.

- Derivatisation was done by addition of 70 µl of AccQ Flour Borate Buffer into 10 µl sample containing vial. After this the vials were vortexed for several second. AccQ Flour reagent (6-amino-N-Hydroxysuccinimidyl Carbamate) was added into it and again the vials were vortexed briefly.
- After keeping the vials for 1 minutes in room temperature the mixture was heated for 10 min in 55^0C.
- Amino acid profile was determined by injecting 20 µl of sample solution.

4.2.3.3 Estimation of "Lipid" content

Procedure

- This estimation were done by using protocol of "Folch" (1957),
- Sample was taken in Methanol & Chloroform Mixture (1:1).
- After this sample mixture was centrifuged and supernatant was taken for initial weight.
- Final weighing of supernatant solution was done after 2 to 2:30 hr., up to when the weight of that solution become constant.

Calculation:

$$\% \text{ of Lipid} = \frac{\text{Initial weight of Petri plate - Final weight of Petri plate}}{\text{Weight of sample}} \times 100$$

4.2.3.4 Qualitative and quantitative analysis of "Fatty acid" content

Fatty Acid profile was determined according to the methodology, AOCS (1991), Mukhopadhyay *et.al.* (2003) and Pal *et.al.* (2011), by using the technique of "Gas Cromatograph (made of Agilent, 6890A, Beijing, China)".The instrument was equipped with a flame ionization detector (FID) and capillary DB-wax column (30 m X 0.25 mm id).

Procedure

- About 200 mg of sample oil was hydrolyzed with 0.5 N NaOH in methanol for 1 hr at reflux temperature. Then BF 3 -methanol reagent was added and boiling was done for one more minutes.
- After cooling saturated NaCl solution was added. Hexen solution of methyl-esters at the top was extracted and transferred into test tube with anhydrous Sodium sulfate.
- The dry hexane solution was injected directly (1µl) into gas chromatograph. Fatty Acid profile was determined by "Gas Cromatograph" where N 2, H 2 and air flow rate was maintained at 1 mL/min, 30 mL/min and 300 mL/ min respectively.
- Inlet detector temperature was kept at 250^0C and the oven temperature was programmed as 150-190-230^0 C with increase rate of 15 ^{0} C/min and 5 min hold, up to 150^0 C and 4^0C/min with 10 min hold up to 230^0C.
- Quantitative data were corrected for differences in detector responses through analysis of authentic standards of each reported fatty acid.

4.2.3.5 Estimation of "Carbohydrate" content

Carbohydrate content was analysed by using Hedge (1962) methodology.

Reagent:

1. Anthron Reagent: Addition of 760 ml of con. H_2SO_4 to 330 ml of water with stirring and cooling. Addition of 1 gm if Thiourea and Anthrone and stiring was done until it dissolve. It was then stored in refrigerator.
2. Glucose Stock Solution: Anhydrous D(+)-Glucose was dissolved 100 mg in 100 ml water. Working standard—10 mL of stock diluted to 100 mL with distilled water. Store refrigerated after adding a few drops of toluene

Procedure:

- Weighted 100 mg of sample kept into a boiling tube. It was hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature.
- Neutralised it with solid sodium carbonate until the effervescence ceases. It was then made up the volume to 100 ml and centrifuge. Collection of the supernatant was done and it was taken at 0.5 and 1 ml aliquots for analysis.
- Preparation the standards was carried out by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank. Making up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water.
- Then 4 ml of anthrone reagent was added. It was then heated for eight minutes in a boiling water bath and cooled rapidly. It was read the green to dark green colour at 630 nm.

- Standard graph was plotted by making concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculation was made about the amount of carbohydrate present in the sample tube.

Calculation:

Amount of carbohydrate present in 100 mg of the sample =

mg of glucose / Volume of test sample X 100

4.2.3.6 Estimation of "Moister" content

According to the methodology of Gupta *et.al.* (1992), this estimation of moister content was carried out.

Procedure

- At First the Aluminum disc were kept in oven at 135±2^{o}C. The Disc were kept in Desiccators, after this Aluminum disc were weighted.
- Taking 2.00 gm of muscle samples of *B. bengalensis*. Thereafter, oven dry were taken place at 135±2 ^{o}C until the weight of the sample became constant.
- It was done in triplicate determinations method.
- This process took time approximately 2 hours.
- Then the samples were cooled in desiccator (about 1 hour) and final weight was taken.

Calculation:

$$\% \text{ of Moisture} = \frac{\text{Wt. of original sample - Wt. of dried sample}}{\text{Wt. of original sample}} \text{ x } 100$$

4.2.3.7 Estimation of "Ash" content

According to the methodology of Gupta *et.al.* (1992), this estimation of ash content was carried out.

Procedure

- At first Porcelain crucible was oven dry at 600±2^{0}C for 1 hr. in Muffle furnace. After this the crucible was cooled in desiccators. After this its weight was recorded.
- 2.0 gms of muscle samples of *B. bengalensis* was taken in silica crucible.
- It was done in triplicate determinations method.
- Drying was done in an oven at 600±2^{0}C for 2 hrs until the residues were uniformly tuned from their natural colour to ashy-white colour
- Then the samples were cooled in desiccators for 1 hr and there after final weight was recorded.

Calculation:

$$\% \text{ of Ash} = \frac{\text{Wt. of residue (ash)}}{\text{Wt. of original sample}} \times 100$$

4.2.3.8 Estimation of Vitamin (A,D,B_5 , B_{12}) content

4.2.3.8.1 Sample collection and preparation

Healthy matured samples of *B. bengalensis* were collected at random from the nearby market places of Kolkata, West Bengal. Immediately after collection, the collected sample were kept in an insulated iced box containing with sufficient ice pack and transferred to the laboratory. The collected sample was properly washed with chilled double distill water. After proper cleaning the length and weight of collected *B. bengalensis* were measured separately (in iced condition). Then flesh portion was

carefully removed from the shell, using cleaned, grease free scissors, forceps and scalpels and thus the edible fleshy parts were separated (in iced condition). After separation of flesh, removing of unwanted matters, again proper washing was taken place with chilled double distill water. Next, the well-preserved fleshy parts were transferred to the laboratory for analysis. Vitamin contents were analysed by using the laboratory of M/S Efrac Pvt. Ltd., Nilgunj Bazar, Barasat, Kolkata 700 121,West Bengal.

4.2.3.8.2 Analysis of Fat soluble vitamin A and D

Procedure

- At first fat soluble vitamins were extracted. In this process About 30 gms of tissue was homogenized in ice cooled chloroform-methanol mixture (2:1). Followed by mixing of water and kept it for separation about 24 hr.
- Chloroform part was collected by passing through anhydrous Sodium sulphate. Oil containing chloroform was evaporated to make it at 5/10 ml. After refluxing (adding NaOH), followed by again refluxed with BF_3- Methanol.
- Separation was done by using petroleum ether in separating funnel. Finally, collection of petroleum ether part was done by passing through $NaSO_4$. .
- After concentration, the sample was prepared by dissolving in acetonitrile solution prepared for high performance liquid chromatography (HPLC) with UV detecting technique. Vitamin A and D were determined by HPLC (Waters, Column; C-18 , with size 15 cm x 3.9 mm, inject volume: 5 µl.) with UV-detection.

- The mobile phase was employed for vitamin A was MeOH-Water (v/v) working at a flow-rate of 1 ml/min in isocratic mode at wavelength 325nm. While the mobile phase was employed for vitamin D was MeOH-Water (v/v) working at a flow-rate of 1 ml/min in isocratic mode at wavelength 264 nm.

4.2.3.8.3 Analysis of water soluble vitamin B_5 and B_{12}

Procedure

- Only 250 mg from the total sample was transferred into a 50 ml volumetric flask.
- Approximately 40 ml of 0.5% oxalic acid solution was added and the sample stirred.
- After 20 min treatment in an ultrasonic bath, the sample solution was cooled down and the volume adjusted to 50 ml with 0.5% oxalic acid.
- Before injection, the sample was filtered through a 0.45 µm syringe filter. Vitamin B_5 was analysed by HPLC (Waters, Column; C-18 , with size 15 cm x 3.9 mm, inject volume: 5 µl.).
- The mobile phase employed for Vitamin B_5 was MeOH-Phosphate Buffer (v/v) working at a flow-rate of 1 ml/min in isocratic mode at wavelength 275 nm.
- The mobile phase employed for Vitamin B_{12} was-ACN- Formic acid (v/v) working at a flow-rate of 0.3 ml/min in gradient mode. This vitamin B_{12} was analysed by using LC-MS/MS methodology.

4.2.3.9 Seasonal occurrence of nutritional parameters of *B. bengalensis*

Another set of experiment was made to notice about the seasonal availability or change in naturally occurring nutritional parameters such as, protein, carbohydrate and lipid content in the muscle tissue of *B bengalensis.*

4.2.3.9.1 Preparation of sample

The sample was collected at random,from the different fresh water fish culture ponds under the Office of the Deputy Director of Fisheries (Microbiology & Parasitology), Dept. of Fisheries, Govt. of West Bengal, Pailan, West Bengal. The sample preparation was done as per point no. **4.2.2** .

4.2.3.9.2 Selection of seasons

This experiment was studied by segregating three different seasons in a year which was discussed as follows**:**

- Pre-monsoon (February to May).
- Monsoon (June to September).
- Post-Monsoon (October to January).

4.2.3.9.3 Process of Analysis

In every month laboratory analysis of Protein, Carbohydrate and Fat contents were done in *B bengalensis* at fortnight interval. Each time 10 samples were taken for analysis of each parameters. This experiment had been continued from February'2011 to January'2014. Estimation of mentioned biochemical compositions were done at the Office of the Deputy Director of Fisheries (Microbiology & Parasitology), Dept. of Fisheries, Govt. of West Bengal, Pailan, West Bengal. For statistical analysis mean, mode, standard were applied,where $p < 0.05$.

4.3 Study about the behavioral pattern of *Bellamya bengalensis*

Behavioral pattern of *B. bengalensis* were observed by keeping them within captive aquatic device. This experiment was done at the Office of the Deputy Director of Fishery (Microbiology & Parasitology), Dept. of Fisheries, Govt. of West Bengal, , Pailan, West Bengal. Latitude and Longitude of that area was calculated $22^0$26'01.52"N and $88^0$17'28.35"E respectively. This process had been continued from February 2010 to January 2013. This experiment was done by using following steps.:

4.3.1 Preparation of model for setting of experimental tanks

- Twelve numbers of "Earthen tanks" (local Bengali name-Majla) were taken for keeping the snails to observe their behavioral pattern. The surface area & volume of the tanks were 9.45 ft^2 (avg.) and 2.10 ft^3 (avg.) respectively. Among these twelve numbers earthen tanks, three tanks were selected for first set of brooder stock culture. Among the rest of nine numbers of tanks, three were selected as first set of juvenile culture tank, where they attained maturity. And rests of six numbers tank were selected as second set of culture tank. When First set of juveniles attained their maturity they were transferred to the second sets of brooder tank. Rest of three numbers of tanks selected as second set of juvenile keeping tank. All those process was made regarding to avoid over crowding.
- Side by side, among the twelve numbers of tanks eight number tanks were set for manuring and liming. And rests of four tanks were set as control tanks, where manure and lime application were not taken place. Except, mud application and water filling, which was done for rest of previous eight tanks also.
- The whole previous process is described schematically where two numbers of Brooder Tank (BT), where manure (M) and lime (L) application was taken place.

The first set (Set-1) of this type of tanks named as "MLBT1" and "MLBT2" respectively and one number of control (C) was setup named "CBT". Juvenile tanks for "MLBT1" and "MLBT2" were "MLJT1" and "MLJT2" respectively. For control juvenile tank, it was named as "CJT".

- Tank preparation of Set -2 was started almost 20-25 days prior to juveniles' maturity of Set-1 and they were transferred to the "MLBT1a","MLBT2a" and "CBTa" from "MLJT1","MLJT2" and "CJT" respectively. Whereas "CBTa" is act as control. Juvenile tanks for "MLBT1a" and "MLBT2a" were "MLJT1a" and "MLBJ2a" respectively. For control juvenile tank, it was named as "CJTa". After the preparation of tanks same amount of manuring and liming were done only to the "MLBT1a" and "MLBT2a" .

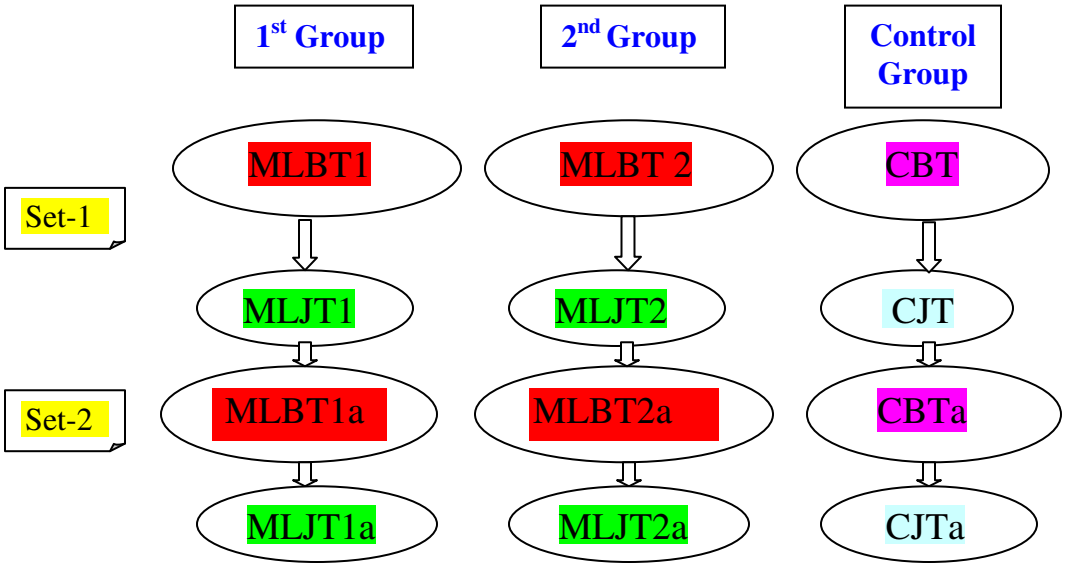

Fig. : 2

- Blue and green colour represented about the manuring and liming of experiential tanks, where as pink and sky blue colour represented about the controled experiential tanks without manuring and liming (Fig. 2).

4.3.2 Tank Preparation

- At first tanks were filled with mud about five inch from its bottom. Then the mud was made free from stone, plastics and other unwanted objects.
- After this the tanks were filled with pond water up to its neck. Water carrying capacity of each tanks was about 45±0.5 lit. At this stage, it was kept for two days (Plate no. 5).
- After removing the suspended impurities, muddy scum from water manuring was done.
- Manuring was done by mixing of DAP (Di-Ammonium Phosphate) with 5 to 7 days old cow dung in 1:2 (15g : 30g) ratio. Mixing of this manure was done properly to be sure, that the manure was mix with water and mud (Plate no. 6 to 8).
- Day after application of manure, lime application was done. Taking tank water in a mug 15 gm of lime was mixed with the water and this water was gradually mixed throughout the water. Vigorously disturbing of muddy bed of tank bottom was done, to ensure the proper mixing of lime, with soil and water (Plate no. 9 and 10).
- These course of work were done at day time (when the environment temperature was above 27^0C or near to 30^0C or more).

4.3.3 Stocking of *B. bengalensis*

- After 20 to 22 days of lime application, when algal growth was appear around the periphery of the tank, at that time mature snails were applied. Before stocking water quality were checked.
- The collection of snail, *B. bengalensis* was done by picking up the coconut tree branches from the water. After this sorting was done size wise (Length and Weight) by hand and placing them in the earthen pot (Mahata,2012). i.e. "MLBT1","MLBT2" and "CBT".
- All the selected species were adult in nature, their avg. length and avg. weight were 3.5±0.31 cm and 5.1±0.82 gm. respectively.
- Stocking density was 50 no. of snails for each brooder tanks including brooder control tank.
- After stocking daily observation was done on each snail (Adult and Juvenile).
- Stocking was done in February 2010.
- Generated algae of the earthen pots ware sent to BSI for identification.

4.3.4 Analysis of water quality parameters

- Temperature, pH, D.O, Alkalinity, Nitrate, Phosphate, Ammonium, total Hardness-these parameters were chosen as required water quality parameters within a captive condition (Tanveer, 1992 and Mahata,2012).
- The whole water quality parameters were analysed by using freshlu bought "Compact laboratory water testing kit" made of Merck (Model no. 1.11151.0001).
- Pre-stocking and post-stocking water quality analysis was done.

- Every fortnight period water quality parameters were analysed for each culture tank including control tank.

4.3.5 Sex Determination and Biometric Data

- Shell length and width were measured with the aid of "Venire calipers".
- Snails were weighed with the aid of electronic balance, made of Mettler-Toledo (model name: AB 104-S, model no.: SNR 1119063069). Female snails were counted and classified as immature (without ova), mature (with ova), gravid (ova with uterine young).
- Dissection was done to obtain a direct count of uterine young. This permitted increased accuracy in the assessment of female reproductive output. In every month of each years, 10 samples were taken to study.
- All the statistical calculations were represented by applying the methodology of mean, mode and standard deviation, where $p<0.05$.
- Microscopic Picture was taken with the aid of stereo microscope, made of Olympus, model no. **:** SZ40.

4.4 Preparation of soup powder using *B.bengalensis*

This ready to cook, edible soup powder (material subjected for cooking before consumption) was manufacture from *B.bengalensis*. It was made with an objective that in our fast life style we always search for those food items which should be easy to make, not time killing process, can provide a great nutritious value without spending too much cost etc. This snail soup powder can execute all of those stated criteria. Here procedure of making soup powder is discussed as follows.

Table 3: Requirement of "Ingredients and Machineries" to make snail soup powder

Sl. No.	**Name of ingredients**	**Amount (g)**
1.	Snail	300 **(w/w)**
2.	Sunflower Oil	15 ml
3.	Cumin	30 **(d/w)**
4.	Pest of Ginger	45 **(w/w)**
5.	Pest of Garlic	45 **(w/w)**
6.	Black Pepper	30 **(d/w)**
7.	Sugar	75 **(d/w)**
8.	Milk Powder	120 **(d/w)**
9.	Salt	60 **(d/w)**
10.	Onion	150 **(w/w)**
11.	Flour	180 **(d/w)**
12.	Vitamin C	15 **(d/w)**
13.	Vinegar	150-200 ml
14	Sodium benzoate	1.0 **(d/w)**
Sl. No.	**Name of Machineries & Others**	**Details**
1.	Oven	Locally available
2.	Hot Air Oven	I.C.T
3.	Impulse Sealer with N_2 Cylinder	I.J.T
4.	Grinder Machine	Prestige
5.	Aluminum foil	Local market available
6.	Weighing balance	Local market available

4.4.1 Procedure of Cooking

- At fast matured *B. bengalensis* was collected from local fish market (Haridevpur Bazar, Kolkata, West Bengal). The individual weight ranging from 3.50 to 4.5 g (approx.)
- After collection of matured snail (*B. bengalensis*), the fleshes were taken off from the shell with aid of needle.
- Non edible parts like its intestine, gastric portion etc were removed from the flesh (Plate no. 12).

- The flesh were cleaned at first by normal edible water and then 10% of saline water by keeping them for 10-15 min. Finally cleaned with vinegar about 10 min..
- At first ginger-garlic, onion, cumin were mixed with sunflower oil. After 10 to 15 min snail was added to it (Plate no. 11 and 13).
- Next gradually sweet, salt, black paper, flower were added (Plate no. 13)..
- After closing of oven when its temperature came down the vitamin C was added to it.

4.4.2 Drying and Packaging

This cooked material was dried by keeping it, in hot air oven within the temperature of 50-55^{0}C, until it became into dried condition. In other process, this cooked material can be kept in open air under the shed to get dry. After dried up of cooked soup powder, the material was kept in "Aluminum foil" at a certain weight (20 gm). Packaging was done by incorporating of Nitrogen Gas. Nitrogen gas were used for packaging because, it doesn't react with food like oxygen does, so foods stay fresher longer and it doesn't affect the flavor or texture of the food. Since the nitrogen fills up the bag, it helps to protect the delicate foods inside, inhibit from rancidity, discoloration and the food spoilage. The packages were kept in clean, cool & dry place at room temperature. This soup powder was attributed as "Snail soup powder". In "Bengali language" this snail soup powder was attributed as "Kambaji Sudha".

4.4.3 Preparation of snail soup powder

This snail soup was prepared by adding 5 gm of soup powder into 150 ml of boiled water (Shakila and Nambudiri, 2012). Finally, it was catered by garnishing with black paper, green chili, few drops of lemon and coriander leaves (Plate No. 42).

4.4.4 Biochemical Analysis of snail soup powder

Protein , Carbohydrate, Fat, Ash and Moisture content of were analysed as stated under the point of 4.2.3 by taking dried preserved soup powder sample. Here biochemical study of soup powder was done at first initial stage and it was continued up to six months, regarding to observed any significant changes in the biochemical composition. At the initial stage, analysis was carried out by taking the sample before packaging and in every time up to 6 (six) months the sample was taken randomly from packaged soup powder sample.

4.4.5 Quality assessment and Self Life estimation of snail soup powder

Quality and shelf life assessment are very important due to increasing consumer demand for consumption. This processes were executed by using some standard methods (Beaty and Gibbons,1937, Iyer *et. al.,* 2000, Chacko *et. al.,* 2005, Masniyom ,*et al*,.2005, Yola and Trimurti, 2012, Abouel-Yazeed, 2013). Several methods were considered to estimate its (soup powder) physico-chemical and microbiological characteristic features, to identify the changes during 6 (six) months packaging condition. Biochemical, Microbiological and Sensory/Organoleptic methods were used to assess the freshness and quality of snail soup powder during storage condition. All the biochemical, microbiological and organoleptic tests were done by randomly taking soup powder samples, in each and every month to evaluate the condition of self life of this snail soup

powder (Beaty and Gibbons,1937; Chacko *et. al.,* 2005; Yola, 2012).This study was continued for six months.

4.4.5.1 Estimation of pH

The pH measurement of soup powder was carried out using the methodology of Masniyom *et. al.,*(2005).Fish samples (2 g) was homogenized thoroughly with 10 ml distilled water and the homogenate was subjected to determination of pH with aid of pH meter pH-meter of Extech Instrument (Model name : EC500).

4.4.5.2 Estimation of free fatty acid (FFA)

The estimation of free fatty acid was carried out by using the methodology of Anonymous, (2001).After weighing of sample it was taken in a flask and after addition of 50 ml Benzene it was kept for 30 min due to extraction of free fatty acid. There after, 5ml of extraction was taken in a flask and 5 ml of benzene and 10 ml of alcohol were added into extraction with phenolphthalein as indicator. Then this mixture was titrated against 0.02 (N) KOH until light pink colour was disappeared.

Calculation:

$$\% \text{ FFA} = \frac{228 \textbf{ X } 0.02\text{N KOH } \textbf{X} \text{ ml of alkali used}}{1000 \textbf{ X } \text{Wt. of original sample taken}} \textbf{ X } \text{dilution factor } \textbf{X } 100$$

4.4.5.3 Estimation of Total Volatile Base-Nitrogen (TVB - N) and Tri-Methyl Amine- Nitrogen (TMA-N)

The estimation of total volatile base-nitrogen (TVB - N) and tri-methyl amine-nitrogen (TMA-N) were carried out by using the methodology of Qingzhu,2003 and Sallam,2007. 100 g of snail soup powder sample was added in 200 ml of 7.5% (v/v) aqueous trichloroacetic acid (TCA) solution. The extraction was prepared by

homogenizing method with homogenizer for 1 min at high speed. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant liquid was then filtered through Whatman filter paper, No. 1. TVB-N was measured by steam-distillation of the TCA-soup powder extract. Here 25 ml of the filtrate were loaded into a Kjeldahl-type distillation tube, followed by 5 ml of 10% (w/v) aqueous NaOH solution. Steam-distillation was performed using a vertical steam-distillation unit, and the distillate was received into a beaker containing 15 ml of 4% (v/v) aqueous boric acid solution with indicator (0.016 g methyl red and 0.083 g bromocresol green in 100 section of ethanol) up to a final volume of 50 ml. The titration was allowed to run against aqueous 0.02 N sulphuric acid to reach the onion skin color.

The same experimental procedure of TVB-N was used for the TMA measurement. The only difference was the addition of 20 ml of 35% (v/v) formaldehyde to the distillation tube to block the primary and secondary amines, whilst leaving only the tertiary amines to react. Steam-distillation was performed using a vertical steam-distillation unit, and the distillate was received into a beaker containing 15 ml of 4% (v/v) aqueous boric acid solution with indicator (as described above) up to a final volume of 50 ml and finally titration was done with 0.02 N sulphuric acid.

The amount of TVB-N and TMA were calculated from the volume of 0.02 (N) sulphuric acid used for titration and the results were expressed in mg nitrogen /100 g of sample.

Calculation: $14 \times a \times b = M$ mg/ ml of extract

$$C = 100 \times M$$

14 = Molecular Wt. of Nitrogen

a = Normality of H_2SO_4

b = Volume of H_2SO_4 used

C = mg % value

4.4.5.4 Microbiological testing

Microbiological Testing was done on Total plate count method (by making serial dilution up to 10^6), *Escherichia coli,* Coliform, Salmonella tests were done by using the methodology of APHA,(1992).

4.4.5.5 Sensory evaluation

This was study done to make sensory evaluation, by making 6 panel members of the Office of the Deputy Director of Fisheries (Microbiology & Parasitology), Dept. of Fisheries, Govt. of West Bengal, Pailan,West Bengal. Panelists were asked to evaluate the overall acceptability with regard to appearance, odour intensity, flavour and aftertaste, etc by using 9 point of "Hedonic scale" (Amerine *et.al.,*1965, Chacko *et. al.,* 2005, Sallam,2007 and Yola, 2012). 1-9 point Hedonic scale is described as follows :

1. Dislike extremely
2. Dislike very much
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like very much
9. Like extremely

All the data obtained rating of sensory attributes of the soup powder were analysed. Statistical analysis was done by using mean, mode and standard deviation. All analysis were carried out in triplicate, $p < 0.05$ was considered as level of significant.

All the photographs for this Ph. D. thesis work (here attributed as "Plate") was capture with the camera, made of Sony, model name : Sony cyber shot, model no. : Sony crop. DSC HX 300, with 20.4 megapixel and 50 X optical zoom.

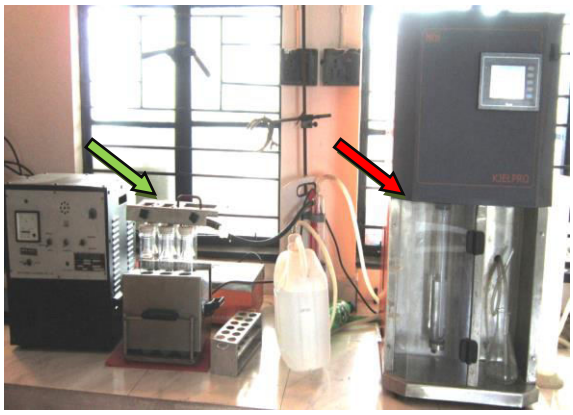

Plate No. 2

Plate No. 3

Plate No. 4

Plate No. 5

Plate No. 6

Plate No. 7

Plate No. 8

Plate No. 9

Plate No. 10

Plate No. 11

Plate No. 12

Plate No. 13