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Effect of Frozen Storage (-18±1^{O}C) on Bio-chemical Properties and Textural Hardness of Whole Gutted Chocolate Mahseer (*Neolissochilus hexagonolepis*)

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Abstract—*Neolissochilus hexagonolepis, commonly known as Chocolate Mahseer, is affluently available in the rivers of Kalimpong, West Bengal. Recently initiation is taken for application of aquaculture and technology enlighteningly in this region to exploit its potential fully. The success of this novel initiative will lead to its optimum production, thus, enhancing the acceptability of the cold-water species and cold chain establishment for distribution of produce to a wide periphery. Frozen storage has been largely used to retain the freshness of fish. In the present experiment, the Chocolate Mahseer randomly collected from farms of Kalimpong were graded according to size, small (<50g), medium (51-150g) and large fish (>150g). The moisture content was found to be 75.64±2.83, 76.89±0.56 and 76.07±3.35 % for small, medium and large size grades, whereas, the protein content was found to be 17.44±0.56, 19.14±4.43 and 16.14±3.21 % respectively. The ash content mean values of small, medium and large fish were found to be 1.060±0.08, 0.83±0.04 and 1.1±0.16 % respectively and that of lipid content were found to be 5.864±0.31, 5.98±2.83 and 8.18±0.58 % respectively. A decrease in protein, lipid, moisture and ash content was observed during 120 days of frozen storage in -18±1^{0}C, with significant ($p<0.05$) lowering in lipid and ash content. The quality indices viz. total volatile base nitrogen (TVB-N), peroxide value (PV) and pH were well within the limit of acceptability till the end of storage period with significant ($p<0.05$) increase in pH and PV. The significant decrease ($p<0.05$) in hardness values may be due to the weakening of the muscle fibers under frozen storage (-18±1^{O}C). Thus, this species is a good candidate for aqua-culture and the produce is marketable to various geographical locations adapting cold chain especially frozen storage (-18±1^{O}C) where it is considered as a delicacy.*

1. INTRODUCTION

Fish has been playing an important role in addressing nutritional and livelihood security of people in the developing countries. Fish provides 20% of animal protein intake to about 2.6 billion people globally and at least 50% of animal protein intake for over 400 million in Asia and Africa. But, in developed countries, it provides only 13% of animal protein intake [1]. Fish is one of the most highly perishable commodities and the public has always required continuous reassurance about its quality. Fish contains all the essential amino acids in their right proportion, other nitrogenous compounds, water, lipids, carbohydrates, minerals and vitamins. Fish diet has high commercial and medicinal values [2].

Neolissochilus hexagonolepis (McClellend, 1839) (Family-Cyprinidae) is commonly known as Chocolate Mahseer. The species is recorded in the basins of rivers of Nepal such as Gandaki, Trisuli, Koshi, Karnali and Mahakali [3], Bangladesh, Pakistan, Myanmar, Thailand, Malaysia (Peninsular), China and Indonesia (Sumatra) [4]. Menon [5], reported that the species is distributed in small numbers through the eastern Himalayas in India (Assam), Bangladesh and Myanmar. In India, this species is naturally available in Sikkim (river Teesta), Assam, Arunachal Pradesh (district East Kameng mostly), Meghalaya and in the rivers of Nagaland such as Arachu, Dhansiri, Dikhu, Doyang, Intangki, Likhimro, Meguiki, Milak, Tesuru, Tizit, Tizu and Tsurang. In West Bengal, the rivers of sub-himalayan region are affluently flooded with chocolate mahseer (*Neolissocheilus hexagonolepis*), popularly called "Katli".

One of the setbacks for the species is that it is not an aquaculture variety. This restricts its availability only from the natural sources and makes its sustainable exploitation caged. Recently initiation is taken for application of aquaculture and technology enlighteningly in this region to exploit its potential fully. The success of this novel initiative will lead to its optimum production as well as establishment of a cold chain so as to distribute the produce to a wide periphery where it is considered as a delicacy. Moreover, it can also be made available throughout the country, thus, enhancing the acceptability of the cold-water species. Present day consumer preference for fresh food leads to the use of frozen storage and cold chain from the farm to table. Freezing and frozen storage have been largely used to retain the freshness of the fish which can be reflected by assessing the sensory and nutritional properties [6]. During frozen storage fish muscle may, however, lead to denaturation due to formation and accretion of ice crystals and aggregation of especially myofibrillar proteins. These changes result in altered functional properties, changed textural attributes and reduced water holding capacity and juiciness. Thus, the objective of the study is to determine the effect of frozen storage on bio-chemical properties and textural hardness of whole gutted chocolate mahseer to assess its shelf life in frozen storage (-18±1^{O}C).

2. METHODOLOGY

2.1. Sample collection

Fresh fish samples were randomly harvested from the farms in Kalimpong district weighed, gutted and engilled. The fishes were then kept in cold ice boxes and transported to the laboratory of Fish Processing Technology, Faculty of Fishery Sciences, WBUAFS, Kolkata. On arrival at the laboratory the fishes were deiced, graded into three size grades viz, small (<50g), medium (51-150g) and large fish (>150g). the fishes were then frozen at -40^{O}C±2^{O}C and stored at -18±1^{O}C. Evaluation of the various physical and biochemical parameters was carried out on fresh fish and after 30, 60, 90 and 120 days kept under frozen condition at (-18±1^{O}C).

2.2. Proximate composition

Proximate composition of fresh fishes belonging to different size grades were done initially to assess the difference in composition among the grades. Changes in proximate composition and other biochemical and textural properties during frozen storage were done using the medium size grade as this size grade was abundant in the harvest. Determination of moisture content was done by the method described by the AOAC (1995) [7]. Protein content (%) was determined by Kjeldahl method in a Kjeltec Auto sampler system (N x 6.25) [7]. Estimation of fat was done by the method described by Bligh and Dyer [8], by using Soxhlet apparatus. The ash content was measured by the method of AOAC [7]. All the results were expressed on wet weight basis.

2.3. Quality parameters

Total TVB-N (Total Volatile Basic Nitrogen) was estimated by the method described by AOAC [7], using Conway units. Peroxide value (PV) was estimated according to the method described by Egan et al. [9]. The fat extraction was achieved according to Folch et al. [10]. The pH value was recorded using a pH meter according to the method of Benjakul et al. [11].

2.4. Textural Analysis

Hardness of fish flesh was performed at ambient temperature with TA-XT plus texture analyzer (Stable Micro System, Surrey, UK) and a 50 kg load cell. The attributes evaluated was hardness. Gel preparation for hardness analysis was carried out as described by Rawdkuen et al. [12], with minor modifications. Frozen dressed whole fish was randomly selected from medium size grade and thawed at 4^{0}C for 4 to 5 hours until the core temperature reached 0^{0}C, the meat was then picked and blended with 2.5% w/w NaCl and chopped for 4 min in a walk-in cold room at 4^{0}C, to obtain the homogeneous paste. The paste was then stuffed into polyvinylidine chloride casing with a diameter of 2.0 cm and both ends of the casing were sealed tightly. It was then incubated at 40^{0}C for 30 minutes, followed by heating at 90^{0}C for 20 minutes. After heating, all gels were immediately cooled in iced water for 30 minutes and stored overnight at 4^{0}C prior to analysis. Gels were equilibrated at room temperature (25^{0}C-27^{0}C) before analysis. Gels were cut in cylinders of 18 mm diameter x 18 mm length and was compressed vertically in two consecutive cycles of 50% compression, 5 seconds apart using a flat plunger (SMS-P/75) and a heavy-duty platform. This test was done according to the specifications used by Mao et al. [13]. The adopted test settings for this experiment were pre-test speed of 1.5 mm/second, test speed of 0.5 mm/second, post-test speed of 1.5 mm/second, strain at 50% compression, interval (time) of 5 seconds, Trigger type was auto (Force) and trigger force was 25 gm.

2.5. Statistical Analysis

All of the data were checked for normal distributions with normality plots prior to one-way analysis of variance (ANOVA), to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of Microsoft excel.

3. RESULTS AND DISCUSSION

Fish is an important part of a healthy diet because they are considered to be an excellent source of high value protein and essential nutrients. The chemical composition of fish varies greatly from species to species as well as individual to individual depending on the starvation and intensive food intake periods [14], feeding habit of fish or absorption capability and conversion potentials of essential nutrients [15], and external factors such as temperature and salinity [16]. Variations in percentage of fat should be reflected in the percentage of water, and these two normally constitute around 80% of the fillet [14]. The proximate composition of different sizes of Chocolate Mahseer is shown in the Fig. 1.

In the present experiment, the protein content of small (<50g), medium (51-150g) and the large (>150g) sized chocolate mahseer were found to be 17.44±0.56, 19.14±4.43 and 16.14±3.21 % respectively. The variation in protein content could be due to the variation in species, feed availability, sexual maturity, spawning, season of catching and processing methods [17]. Protein and lipid are the major nutrients in fish and their levels help to define the nutritional status of the particular organism. The fat/lipid content of small (<50g), medium (51-150g) and the large (>150g) fishes were found to be 5.864±0.31, 5.98±2.83 and 8.18±0.58 % respectively. In the present study, the ash content mean values of small (<50g), medium (51-150g) and the large fish (>150g) were found to be 1.060±0.08, 0.83±0.04 and 1.1±0.16 % respectively. The variations maybe attributed to the feed availability and the size of the fish.

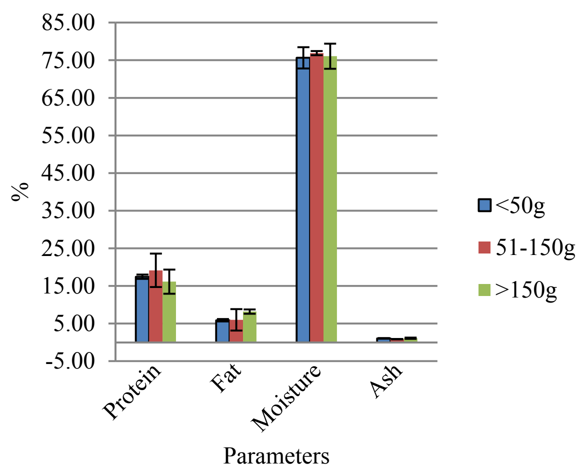

Fig. 1: Proximate composition of different sizes of Chocolate Mahseer

Generally, fish has been widely accepted as a good source of protein and other elements necessary for the growth, development and maintenance of healthy body [18]. Inadequate storage techniques would imply a substantial shortfall in fish availability thereby affecting the animal protein intake of the people in the tropics whose protein intake from fish ranges between 17.5-50% [18].

The maximum and minimum values of total protein content were recorded to be 19.16±1.08 and 18.38 ±1.28% respectively (Fig. 2) after 120 days of frozen storage in -

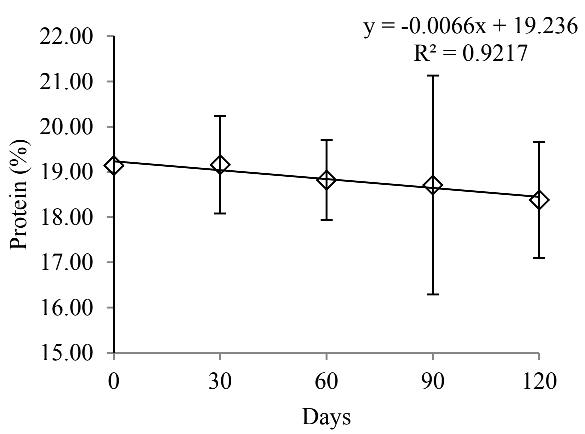

Fig. 2: Protein content of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

18±1^{0}C, although no significant changes ($p>0.05$) observed over the storage period. On the contrary, Siddique et al. [19], reported significant decrease in protein content in *Puntius sp.* during frozen storage at -50^{0}C over a storage period of 21 days. The decrease in protein content may be due to denaturation and loss in gelatin caused by extended frozen storage as well as proteolysis induced by enzymatic activities of psychotropic microbial growth [2].

There is a gradual decrease ($p<0.05$) in the total lipid content, over a period of 120 days of frozen storage at -18±1^{0}C reaching a final value of 5.34±0.14% while the initial was 5.98±2.83% (Fig. 3). Similar trend was reported by Arannilewa et al. [18], where 25.92% decrease in total lipid content was observed in Tilapia after storing it in freezer for 60 days. The loss of lipid occurred may be due to losses in triglyceride fraction and due to the oxidative rancidity [2].

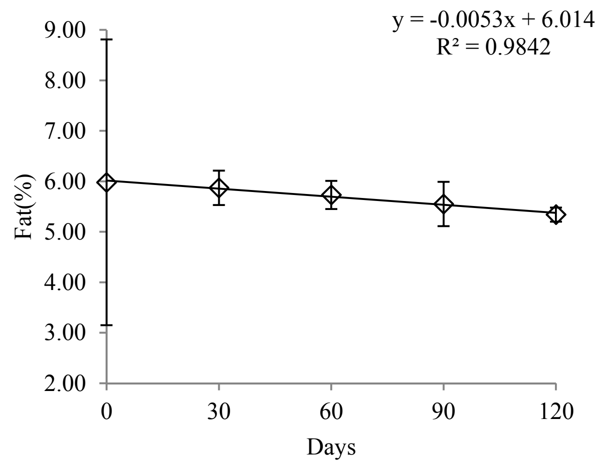

Fig. 3: Fat content of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

The decrease in moisture content from 76.89±0.56% to 71.40±1.67% (Fig. 4) over a period of 120 days of frozen storage in -18±1^{0}C reveals no significant ($p>0.05$) change in moisture content. Roopma et al. [20], also reported a decreasing trend in total moisture content in muscle samples of *Mystus seenghala* stored at two different temperatures, 4^{0}C chilled and -12^{0}C frozen, which may be due to sublimation of surface water of meat in the freezer. On the contrary, Siddique et al. [19], found an increasing trend in moisture content in *Puntius sp*. whereas, Kirschnik et al. [21], observed constant moisture content in samples of tail meat of the giant river prawn (*Macrobrachium rosenbergii*) throughout a period of 14 days of storage without direct contact in ice.

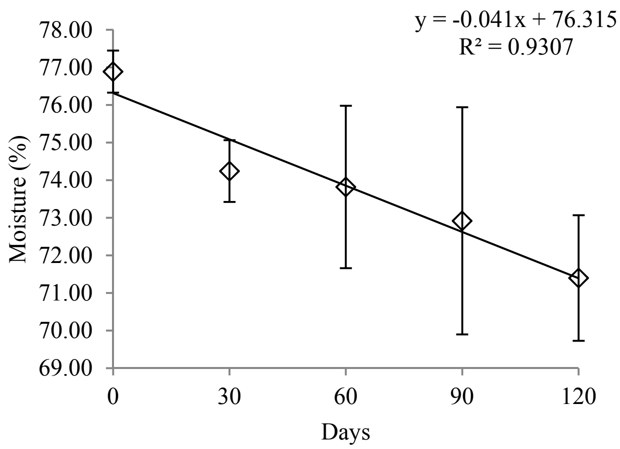

Fig. 4: Moisture content of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

The ash content significantly decreased ($p<0.05$) from 0.83 ±0.04% to 0.65±0.03% (Fig. 5) over a period of 120 days of frozen storage in -18±1^{0}C. Similar decreasing trend in ash content during eight weeks of freezing were reported by Roopma et al. [20], and Mariam [22], with the storage samples of *Mystus seenghala* (4±1^{0}C and -12±2^{0}C) and Tilapia respectively. The decrease in ash content may be due to the exudates flowing from the flesh as a result of protein degradation and even thaw loss which contained various minerals.

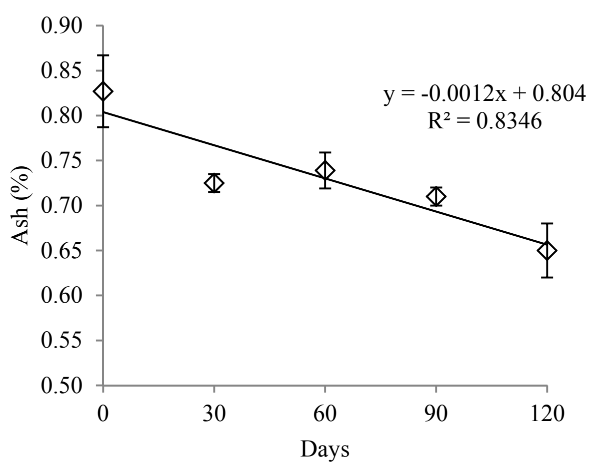

Fig. 5: Ash content of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

Post mortem spoilage of food products can be caused by chemical, enzymatic or microbial activities and is accompanied by the formation of compounds responsible for changes in odour, flavour and texture of fish meat. One of the chemical markers of spoilage in fish is the total volatile basic nitrogen, including ammonia, trimethylamine (TMA) and dimethylamine (DMA), the concentrations of which increase with spoilage by either bacterial or enzymatic degradation. The total volatile basic nitrogen is produced during degradation of proteins and non-protein nitrogenous compounds, mainly as a result of microbial activity [23]. The concentration of 30–35 mg TVB-N/100 g flesh is considered the limit of acceptability for ice-stored cold water fish [23]. Oxidative rancidity is another important factor that determine the acceptability of the fish during processing and storage. Balachandran [24], stated that the Peroxide Value is a measure of the first stage of oxidative rancidity. Lakshmanan [25], recommended a level of PV in seafoods is 10 – 20 meq O_2/kg of fat as a limit of acceptability. The pH is an important intrinsic factor related to post-mortem changes of fish flesh. In the present study, pH showed significant ($p<0.05$) gradual increase from an initial value of 6.1±0.12 to 6.4±0.22 (Fig. 6) over a period of 120 days under frozen storage (-18±1^{0}C). This value of pH fairly correlates with the TVBN recorded initially 3.4±0.48 mg% and reaching a final value of 25.04±1.67 mg% (Fig. 7), although the increase in TVBN values were not significant ($p>0.05$) over the period of 120 days of frozen storage in -18±1^{0}C. The increase in pH may be attributed to production of volatile basic components such as ammonia, trimethylamine and total nitrogen by fish spoilage bacteria [26]. A gradual significant ($p<0.05$) increase in PV was also observed reaching a final Fig. of 8.71±0.52 meqO_2/kg of fish (Fig. 8). All values of these indices were well within the limit of acceptability till the end of storage period.

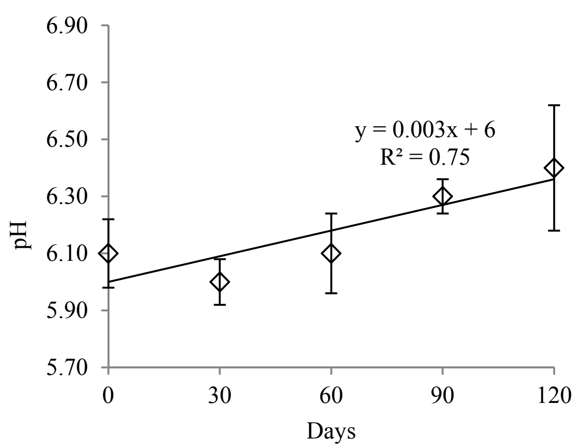

Fig. 6: pH of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

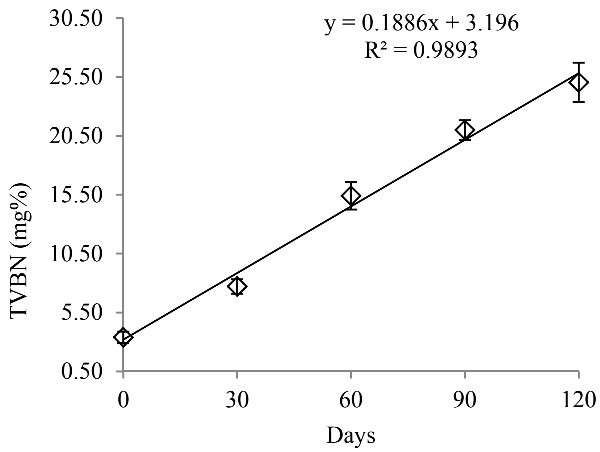

Fig. 7: TVBN content of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

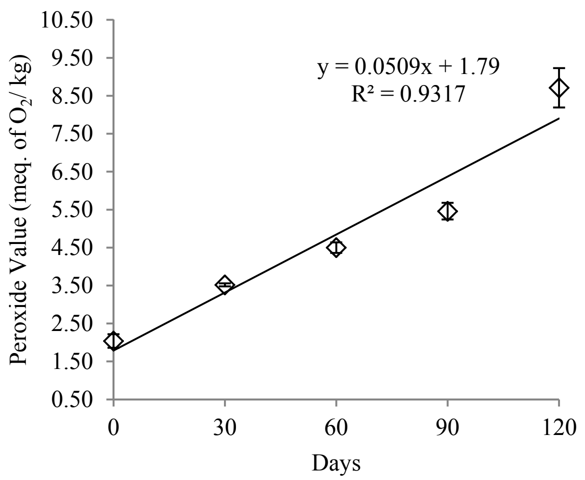

Fig. 8: PV of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

Texture is one of the most important parameters that determine the overall quality perception [27]. Soft flesh leads to reduced acceptability by the consumers [28]. The varying chemical composition and physical structure throughout the fillet affects the textural properties, so the sampling location should be emphasized during measuring the textural properties [29]. Gelation is a property of protein aggregation, resulting in a well-balanced tertiary network structure, which traps within a large quantity of water [30]. Santana et al. [31], reported that gelation properties are responsible for foods' texture, particularly its breaking pattern. There was a significant decrease ($p<0.05$) in hardness values under frozen storage (-18±1^{O}C) reaching a final value of 2445.86±457.08 (Fig. 9). This may be due to denaturation of protein and weakening of the muscle fibres in the frozen condition over the 120 days' storage period.

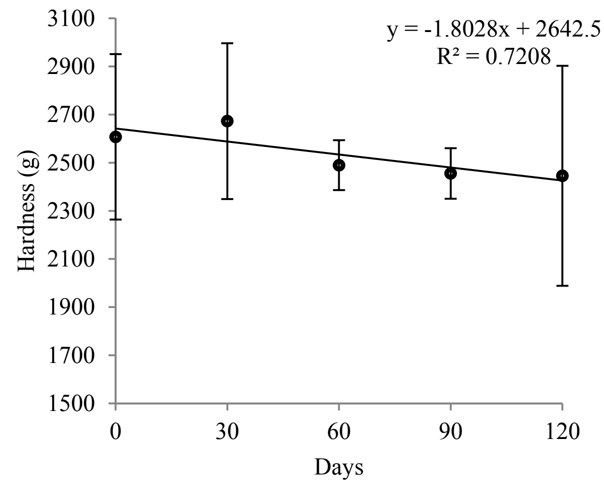

Fig. 9: Hardness of whole gutted chocolate mahseer during frozen storage (-18±1^{0}C)

4. CONCLUSION

The proximate analysis along with chemical indices of whole gutted Katli (*Neolissochilus hexagonolepis*)under frozen storage (-18±1^{O}C) showed that all inspected marks were within limits specified by National regulations over the storage period and with satisfied qualities, in spite for the frozen storage which have no or little significant effect on the quality of the samples. Considering its protein content, the fish (*Neolissocheilus hexagonolepis)* can be considered as a good protein source for the consumers and can be established as an important aquaculture species with commendable marketing strategies, thus, enhancing the acceptability of the cold-water species. This provides a strong base for the aqua-cultured produce to be marketable to various geographical locations adapting cold chain especially frozen storage (-18±1^{O}C)where it is considered as a delicacy.

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