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Sushant Karki

Faculty of Fishery Sciences,
West Bengal University of
Animal and Fishery Sciences,
Chakgaria, Kolkata, West
Bengal, India

Supratim Chowdhury

Faculty of Fishery Sciences,
West Bengal University of
Animal and Fishery Sciences,
Chakgaria, Kolkata, West
Bengal, India

Swarnadyuti Nath

Faculty of Fishery Sciences,
West Bengal University of
Animal and Fishery Sciences,
Chakgaria, Kolkata, West
Bengal, India

Krushna Chandra Dora

Faculty of Fishery Sciences,
West Bengal University of
Animal and Fishery Sciences,
Chakgaria, Kolkata, West
Bengal, India

Corresponding Author:**Sushant Karki**

Faculty of Fishery Sciences,
West Bengal University of
Animal and Fishery Sciences,
Chakgaria, Kolkata, West
Bengal, India

Freezing effects on yield, quality, and microbial load of farmed fish *Neolissochilus hexagonolepis* (Chocolate Mahseer)

Sushant Karki, Supratim Chowdhury, Swarnadyuti Nath and Krushna Chandra Dora

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Abstract

Locally known as “Katli” in Kalimpong and commonly known as Chocolate Mahseer, *Neolissochilus hexagonolepis* has been introduced into the aquaculture system in the region. A ray of hope for the species as several of the larger species have suffered severe declines, and are now considered threatened. The culture is not only important for the region but also can be marketed to the regions where the fish is considered as a delicacy. Fresh fish samples were randomly harvested from the farms in Kalimpong district, West Bengal India during the year 2016-2017, frozen at $-40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for 6 hrs, packed in a LDPE laminated film bag of 15X20 cm dimension and stored at $-18\pm 1^{\circ}\text{C}$. Subsequently, samples were randomly drawn once in 30 days and its yield, quality and microbial load were studied for a period of 180 days. A significant change ($p < 0.05$) in TPC during freezing and frozen storage was seen, increasing from an initial value of 3.26 ± 0.11 log cfu/g to a final value of 3.75 ± 0.12 log cfu/g. Figures crossed the suggested limit of cook loss of 30% on 180 days reaching a final value of $31.34 \pm 1.41\%$. A significant change in the drip loss and texture parameters was also seen except for cohesiveness, springiness and resilience during the period.

Keywords: Chocolate Mahseer, freezing, yield, texture, TPC

Introduction

Fish has been playing an essential role in addressing the nutritional and livelihood security of people in developing countries. In developed countries, it provides 13% of animal protein intake [1]. The main sources of energy reserves in fish are protein and lipid. The relative contributions of lipids content and amino acids to energy production in fish depends on a number of factors such as the species involved, environmental conditions, stage of maturity of the gonads, nutritional state, and age [2].

Mahseer is the common name used for the genera *Tor*, *Neolissochilus*, and *Naziritor* in the family Cyprinidae [3]. *Neolissochilus hexagonolepis* (Family-Cyprinidae) is locally known as “Katli” in Kalimpong and it is commonly known as Chocolate Mahseer. The fish intake is increasing every day in the Kalimpong regions, which are either fulfilled by the fishes coming from Andhra Pradesh or from Kolkata. Thus the demand for the local fresh fish is always high which is unfortunately not met in the region due to various setbacks. One ray of light for the demand of the local fish related matter is that the fish “Katli” has been introduced into the aquaculture system in the region. The culture is not only important for the region but also can be marketed to the regions where the fish is considered as a delicacy.

The present work has been designed to generate information on the changes in textural changes and cooking attributes and microbiological composition of the raw muscle of a fish (*Neolissochilus hexagonolepis*) available in the ponds in the Kalimpong region of the Darjeeling Himalayas, stored in frozen conditions ($-18\pm 1^{\circ}\text{C}$). This study has immense importance to satisfy consumer’s query relating to and how long fish muscle can be stored without any deterioration in the domestic refrigerator and their safety for the betterment of the public health.

Materials and Methods

Sample Collection: Fresh fish samples were randomly harvested from the farms in Kalimpong district, West Bengal India during the year 2016-2017.

After weighing the fishes were 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 washed with ice cold potable water, deiced, frozen at $-40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for 6 hrs, packed in a LDPE laminated film bag of 15X20 cm dimension and stored at $-18\pm 1^{\circ}\text{C}$ [4]. Subsequently, samples were randomly drawn once in 30 days, i.e., D0, D30, D60, D90, D120, D150, D180, and the changes during frozen storage were evaluated. All the values obtained are a mean of triplicates.

Total plate count: Total Plate Count (TPC) was determined according to standard American Public Health Association method [5]. Physiological saline was prepared and 10g sample was mixed into it. Nutrient agar was used to make the media. Serial dilution process was done and pour plate technique was followed. Plates were incubated at 37°C for 24 hrs.

Textural Analysis: Texture analysis of fish flesh was

$$\text{Drip loss (\%)} = \frac{100 \times (\text{Weight of meat before freezing} - \text{Weight of thawed meat after freezing})}{\text{Weight of meat before freezing}}$$

Determination of cooking loss: Cooking yield was determined according to Bigelow and Lee with modifications [8]. Each sample was placed on a grid and cooked at $95\text{-}100^{\circ}\text{C}$ for 15 min in a pre heated conventional steam oven. After

$$\text{Cooking yield (\%)} = \frac{\text{Weight of meat cooked} \times 100}{\text{Weight of meat uncooked}}, \text{ cooking loss was then estimated as}$$

$$\text{Cooking loss (\%)} = 100 - \text{cooking yield (\%)}$$

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 and R software.

Results and Discussion

Total plate count: A significant change ($p < 0.05$) in TPC during freezing and frozen storage was seen, increasing from an initial value of 3.26 ± 0.11 log cfu/g to a final value of 3.75 ± 0.12 log cfu/g (Table 1). There was a decrease in TPC after 30 days of frozen storage followed by a gradual increase up to 120 days of frozen storage reaching a value of 3.90 ± 0.19 log cfu/g. This may be due to a sudden cold shock resulting initial decrease in population, followed by growth of psychrophiles. Again a sudden decrease was recorded after 120 days of storage which may be due to the depletion of nutrients. Similar results were obtained in silver carp (*Hypophthalmichthys molitrix*) frozen storage study wherein the counts increased from 9.50×10^3 to 1.10×10^4 cfu/g during 180 days [9]. A decrease in TPC after 120 days in this study fairly relates to the findings of Magnusson and Martinsdottir [10], who suggested that with increasing storage time in the freezer, the reductions in bacterial numbers were greater. In another study of frozen raw muscles of *Wallago attu*, the values for TPC increased from 3.44 log cfu/g to 8.55 log cfu/g on 30th day of frozen storage [11]. On the contrary a decrease in TPC was seen during a period of 90 days from 2.57×10^6 to 8.2×10^5 cfu/g in Nile Tilapia [12]. The

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, springiness, cohesiveness, chewiness, gumminess and resilience. Gel preparation for the analysis was carried out with minor modifications [6]. Gels were equilibrated at room temperature ($25^{\circ}\text{C}\text{-}27^{\circ}\text{C}$) before analysis. Gels were cut in cylinders of 18 mm diameter x 18 mm length and were compressed vertically in two consecutive cycles of 50% compression, 5 seconds apart using a flat plunger (SMS-P/75) and a heavy-duty platform [7].

Determination of drip loss: Drip loss of frozen whole meat was determined according to Bigelow and Lee with modifications [8]. Frozen fillets sample were put on plastic pallets with small sized holes and thawed at 4°C . Thawed fillets were removed from the pallet, left to drip on the plastic film on the top of pallet, and the drip was left on the plastic film.

cooking, samples were cooled down at room temperature for 8 min, and then reweighed. The cooking yield was calculated as follows:

values of TPC in the present study is at least 2.25 log cycles less than the limit of acceptability (< 6 log cfu/g) [13] suggesting it to be fit for human consumption till 180 days.

Textural analysis: Santana reported that gelation properties are responsible for foods, texture, particularly its breaking pattern [14]. Springiness, gumminess, chewiness, fracture force, cohesiveness are also important in terms of texture profile parameters [15]. The values of hardness exhibited a significant degree ($p < 0.05$) of lowering from an initial value of 2607.56 ± 343.92 g to 1608.81 ± 424.10 g over a period of 180 days (Figure 1). During storage in ice, some myofibrillar protein degrades and fish muscle generally becomes softer [16]. It has also been reported that the reduction in textural properties is attributed to the weakening of connective tissue and the Z-lines completely extinguishes after storage [17]. As in the present study, similar lowering in hardness figures was reported for whole ungutted and gutted catfish with significantly lower ($p < 0.05$) in whole ungutted catfish than that in gutted [18].

A similar trend was observed in case of gumminess values with a gradual change ($p < 0.05$) in values from 1406.05 ± 49.22 to 851.89 ± 166.35 (Figure 2). Changes in cohesiveness (Figure 3) was however insignificant ($p > 0.05$) reaching a final value of 0.54 ± 0.05 . Cohesiveness slightly decreased from an initial value of 0.289 to 0.223 and 0.221 in whole and gutted sutchi catfish on 22nd and 26th days of storage indicating that there was not much change in the internal bonding of fish muscle during storage [18]. A significant ($p < 0.05$) lowering of chewiness values was observed over 180 days with an initial value of 1240.71 ± 17.67 reaching

702.82 ± 123.91 (Figure 4). A decrease in chewiness indicates that the fish muscle becomes soft during storage. Springiness (Figure 5), which indicates the elasticity of muscle that can be stretched and returns to its original length, exhibited non-significant ($p>0.05$) changes from 0.88 ± 0.04 to 0.83 ± 0.06 . Springiness is the elastic or recovering property of the fish muscle during compression. A decreasing trend was observed for springiness values in whole as well as gutted fish over the storage period which is similar to the present finding [18]. Resilience (Figure 6) changed from 0.20 ± 0.03 to 0.19 ± 0.03 over a period of 180 days ($p>0.05$). The changes in various parameters of texture profile analysis may be due to the fact that some myofibrillar proteins degrade during frozen storage [15].

Cooking loss: Cooking can decrease the moisture content so as to adversely affect consumer acceptance. Therefore, the study of the effect of cooking on the frozen product is important which is most often measured in terms of cook loss or cooking yield. In fact estimation of cooking loss or otherwise cooking yield is very significant in determining the extent of protein denaturation and reduction in the water holding capacity of the fish muscle under frozen storage. In the present study, the cooking loss increased steadily ($p<0.05$) over the entire period of storage from an initial value of $21.60 \pm 1.40\%$ reaching a final value of $31.34 \pm 1.41\%$ (Table 2). This indicates that increased degradation of the muscle with storage time had negative effects on water-holding properties of the muscle during heating [19]. In another study where changes in frozen common carp fillets were investigated, the cooking loss of -20°C samples showed a tendency to rise in the first 4 months and decline during 5-6 months of storage [20]. In the mince meat of oil sardine (*Sardinella longiceps*) cook loss increased by about 30% of the initial value during 150 days of frozen storage ($-20\pm 1^{\circ}\text{C}$) and significantly increased during the early period of storage [21]. The fishes studied crossed the suggested limit of cook loss of 30% [22] on 180 days of storage concluding that the shelf life of the whole gutted Chocolate Mahseer under frozen storage ($-18\pm 1^{\circ}\text{C}$) is 180 days.

Drip loss: In the present study, the drip loss increased steadily ($p<0.05$) over the entire period of frozen storage of

180 days at $-18\pm 1^{\circ}\text{C}$ from an initial value of $4.97\pm 0.92\%$ reaching a final value of $16.00 \pm 0.23\%$ (Figure 7). The results are in accordance with Gang (2014), who stated that the drip loss in fillets has a positive correlation with storage time ($p<0.01$) [23]. The fish stored at -20°C , had a gradual increase in its drip loss along the entire storage period, in case of frozen fillets of common carp [20]. A similar results was observed when frozen storage study of salmon was investigated [24]. The above results suggests that the drip loss during the thawing process increased with an increase in the storage time. Upon thawing, there is a loss of fluid from the flesh of any fish product, which is explained by the denaturation of protein during the freezing process, which causes the protein to lose its water-binding capacity. Again during frozen storage, partial degradation and breakdown of the muscle by bacteria and enzymes are also reported [25].

Table 1: Total Plate Count of Chocolate Mahseer flesh stored under frozen storage ($-18 \pm 1^{\circ}\text{C}$)

Days	TPC (log cfu/g)
0	3.26 ± 0.11
30	3.13 ± 0.09
60	3.61 ± 0.01
90	3.78 ± 0.16
120	3.90 ± 0.19
150	3.72 ± 0.02
180	3.75 ± 0.12

*Results are mean of three determinations (n=3) with s.d.

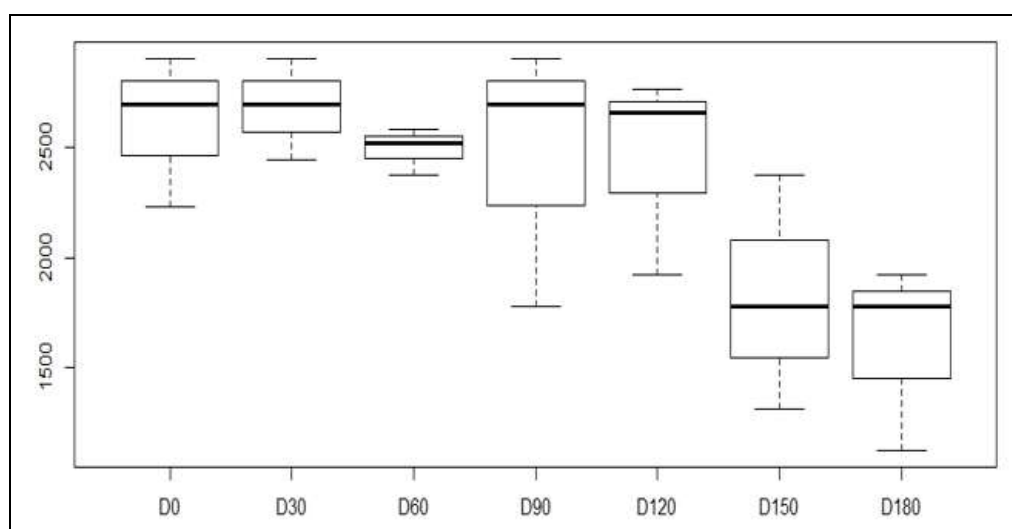
Values of TPC vary significantly ($p<0.05$) between the days

Table 2: Cook loss in Chocolate Mahseer flesh stored under frozen storage ($-18 \pm 1^{\circ}\text{C}$)

Days	Cook Loss (%)
0	21.60 ± 1.40
30	24.77 ± 3.36
60	26.17 ± 3.73
90	27.62 ± 1.42
120	28.61 ± 1.01
150	29.49 ± 0.86
180	31.34 ± 1.41

* Results are mean of three (n=3) determinations with SD.

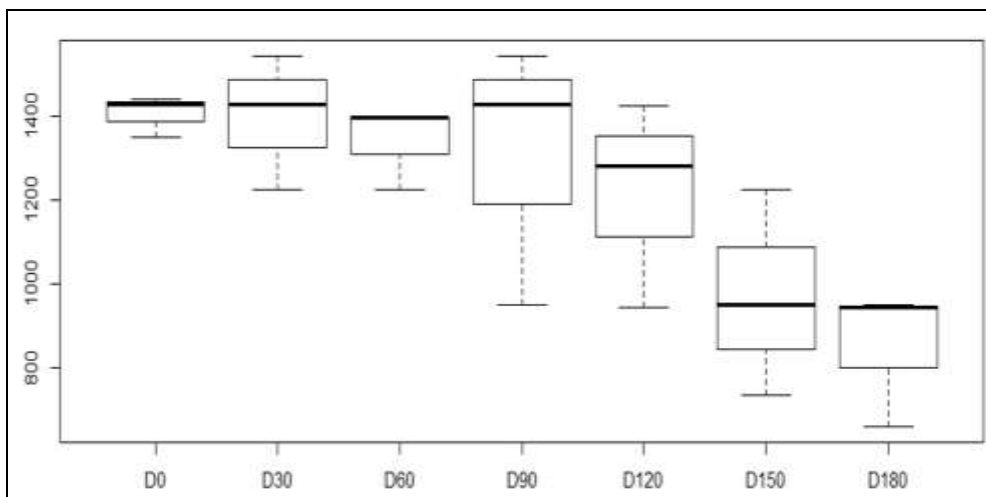
Values of cook loss gumminess vary significantly ($p<0.05$) between the days.



* Results are mean of three (n=3) determinations with SD

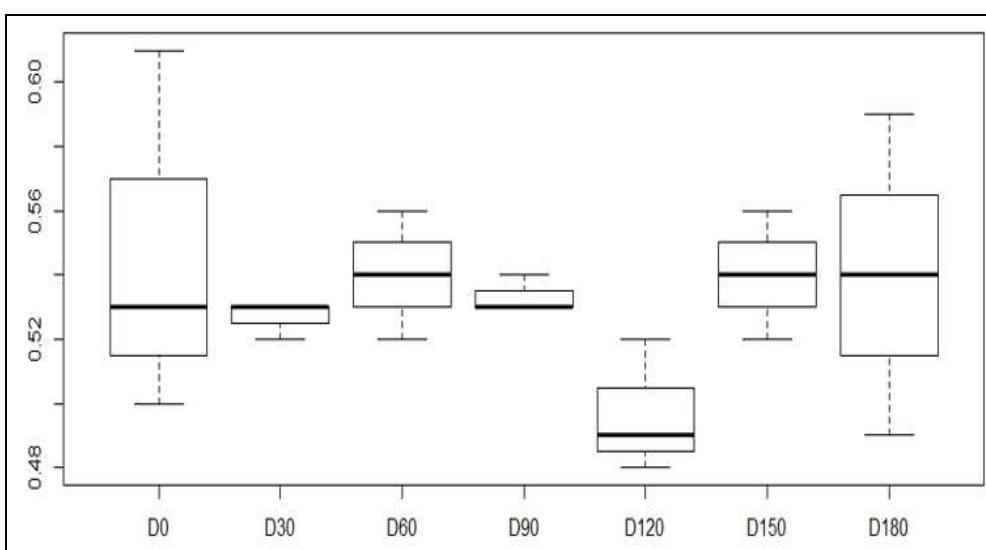
X-axis depicts days and Y-axis depicts Hardness values.

Fig 1: Box plot for hardness value of different months of Chocolate Mahseer flesh stored under frozen storage ($-18 \pm 1^{\circ}$)



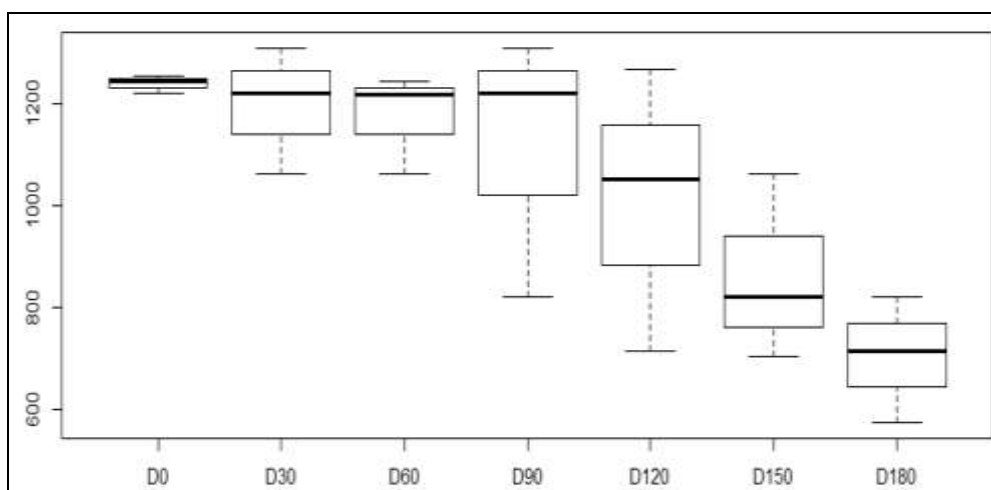
* Results are mean of three (n=3) determinations with SD.
X-axis depicts days and Y-axis depicts Gumminess values.

Fig 2: Box plot for gumminess value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)



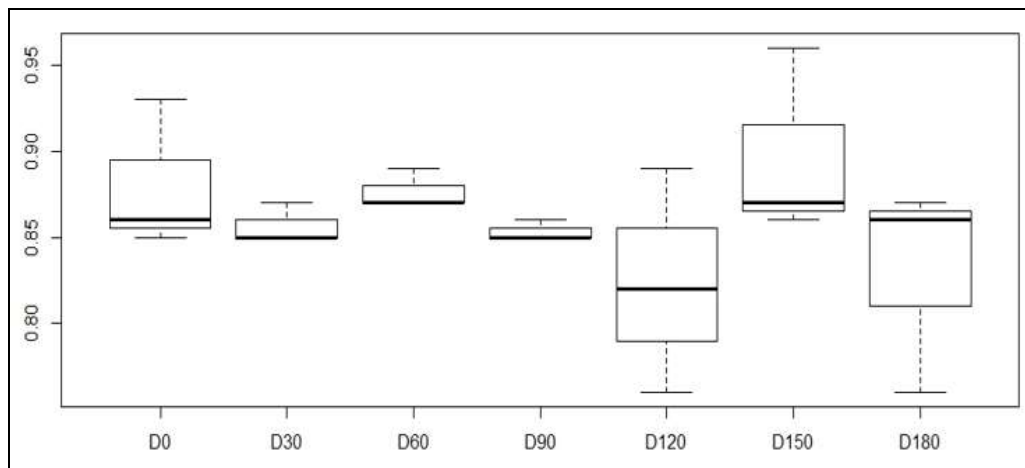
*Results are mean of three (n=3) determinations with SD
X-axis depicts days and Y-axis depicts Cohesiveness values

Fig 3: Box plot for cohesiveness value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)



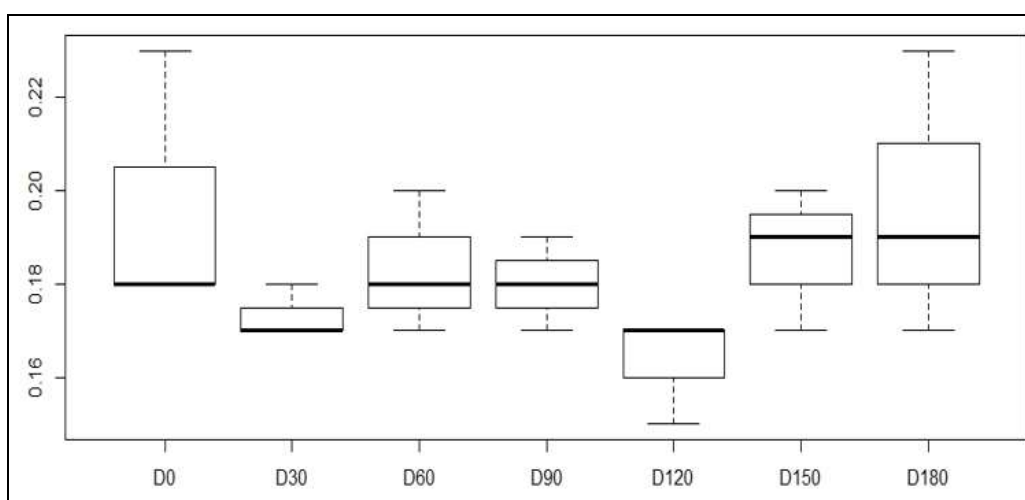
*Results are mean of three (n=3) determinations with SD.
X-axis depicts days and Y-axis depicts Chewiness values

Fig 4: Box plot for chewiness value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)



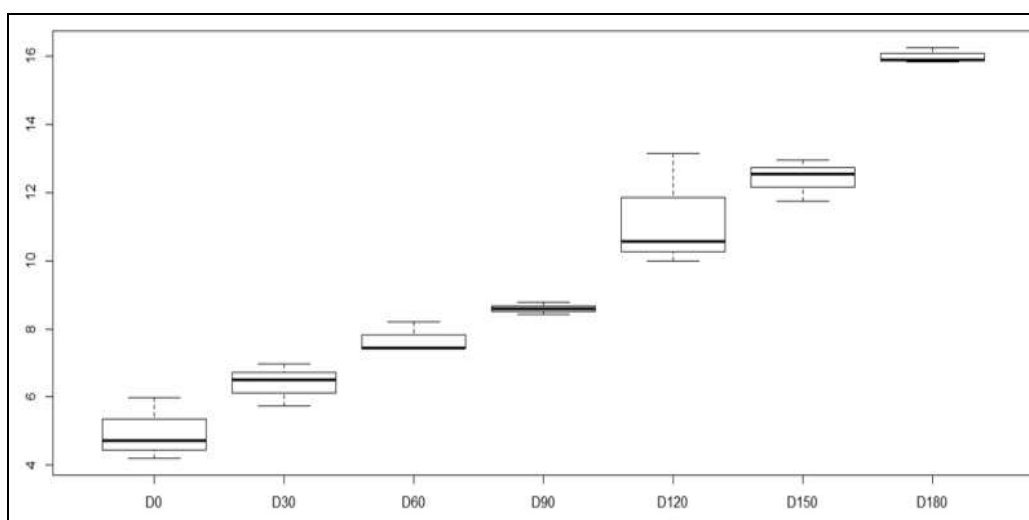
* Results are mean of three (n=3) determinations with SD.
X-axis depicts days and Y-axis depicts Springiness values

Fig 5: Box plot for springiness value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)



* Results are mean of three (n=3) determinations with SD.
X-axis depicts days and Y-axis depicts Resilience values

Fig 6: Box plot for resilience value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)



* Results are mean of three (n=3) determinations with SD
X-axis depicts days and Y-axis depicts Drip Loss values

Fig 7: Box plot for drip loss value of different months of Chocolate Mahseer flesh stored under frozen storage (-18 ± 1°C)

Conclusion

Moreover, the fish is in good condition even after the frozen storage period of 180 days at -18±1°C within the limits of acceptability except for cooking loss. This renders it fit for consumption even after the storage period, which opens a

window of opportunity for the establishment of cold chain and its distribution to other places throughout the country where it is considered as a delicacy and thus increasing the acceptability of coldwater fishes within the nation.

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