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Effects of Freezing and Icing on Proteins and Carbohydrate Contents in a Freshwater Catfish, *Clarias Gariepinus*

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Abstract: ***The present study analyzes the effects of icing and freezing, the most commonly used fish preservation methods in local fish markets of Ulhasnagar, on nutrient components especially the proteins and carbohydrates of muscle of a freshwater edible catfish, Clarias gariepinus. C. gariepinus, an air-breathing catfish is commonly sold alive in fish markets of the region hence preferred for the study. Irrespective of the sex, live C. gariepinus were brought to the laboratory, washed with plain tap water and divided into two groups each containing three (N=3) fish. In each group, the fish were marked with alphabets A, B and C and stored in ice and a freeze separately. The fish from both the groups were analyzed for total muscle proteins and carbohydrates using the standard protocols. In both icing and freezing methods of fish preservation there is no significant change in carbohydrate contents even after 48 hours (day 3) of preservation. It is perhaps due to the decreased or ceased glygenase activity of the muscle of C. gariepinus. The protein content however after an initial decrease increased significantly after 48 hours (day 3) in both icing and freezing methods. This significant increase in the protein contents after 48 hours (day-three) may be due to the protein aggregation and/or due to the loss in weight and moisture content of the fish. The findings of present study clearly indicate that the icing may be a suitable method of fish preservation in the fish markets where the fish are preserved for short-term duration.***

Keyword: *Clarias gariepinus*, nutrients, freezing, icing

1. Introduction

Fish are very delicate hence get quickly spoiled if not processed properly after the harvest. In our country, post-harvest fish loss is a major concern and is perhaps due to the improper fish handling, poor management and inadequate infrastructures. According to an estimate, there is an average post-harvest loss of 9.3% for marine fisheries and 9.84% for inland fisheries in the country. In a survey study conducted by Suraj et. al. 2020, it becomes apparent that the fisher men and women involved in fish procuring and fish selling activities are not trained enough to tackle the issues of post-harvest fish loss and therefore they need to be trained by the fishery institutions/ organizations located in the vicinity.

Fish spoilage occurs primarily due to overgrowth of microbes on fish body surface. The other reasons responsible for fish spoilage include enzymatic and autolytic degradation reactions. The fish spoilage may be prevented/minimized to a greater extent if standard post-harvest fish handling operations are followed.

Suraj et al. 2020 in his survey study also reveals that the most frequently used fish preservation methods in local fish markets are the icing and freezing. The fish sellers keep the fishes collected from the capture and culture sites into the varying sizes of thermocol boxes laden with the ice. They (fish sellers) are however unaware about the ratio of fish to ice into these thermocol boxes.

The catfish, *Clarias gariepinus* is a freshwater species found in Indian fresh water systems. The fish is air-breathing and very resistive in nature. Because of these properties, the fish is most preferred animal model for laboratory experiments (Singh et al. 2016; Janyani and Singh, 2019). Fish is voracious and feeds on variety of food organisms including the small fishes, molluscs and invertebrates as well as detritus and aquatic weeds. Fish has ability to survive both in water and on land due the gills and dendritic organs respectively. *C. gariepinus* is perhaps the only fish that is sold alive in the local fish markets of Ulhasnagar city. Anyone can easily identify *C. gariepinus* by its elongated cylindrical non-scaly body with a dark black/brown coloration on its dorsal surface.

In our country fish are often used as fresh by the fish eaters in the form of fish curry. Because of growing business of ready to eat food products, the fish are also being used as products and byproducts like fish chakli, fish kabab, fish soup and fish biscuits.

Nutrients are essential for an organism to live and grow. Nutrients are used to build and repair the tissues, regulate the metabolic processes and to liberate the energy so that organs can function. Carbohydrates although are very important and energy supplying substances for the fish, C. *gariepinus* are also important for the microbial growth. Fish spoiling bacteria get the energy from these substances and causes fish spoilage specially the early fish spoilage (Lone and Paw 2002).

Fish are well known for the proteins. Fish are said to have complete proteins with all essential amino acids. Proteins however make fishes very prone towards the enzymatic and autolytic degradation reactions. Due to this, fish become more perishable and get spoiled if not preserved properly hence need extra care. Temperature is an important factor that may regulate the fish spoilage processes by increasing the

bacterial growth, enzymatic activities and oxidation-reduction reactions (Nwaigwe, 2017). Freezing and icing methods of fish preservation lower the fish body temperature but by two different means. Storing fish into refrigerators is most conventional and requires less expertise but its high cost and unavailability make it a less preferred mechanism of fish preservation into the local fish markets. Icing on the other hand is most common technique used by fish sellers in the fish markets. Hence in present paper, study analyses the effect of freezing and icing on proteins, lipids and carbohydrates of fresh water catfish *C. gariepinus* and also to find out which fish preservation techniques is better for storing the raw fresh fishes in routine fish markets.

2. Methodology

Procurement of animal

Irrespective of the sex, live specimens of *C. gariepinus* (total six) were collected from a local fish market situated near railway station of Ulhasnagar city. The fish were brought to the laboratory, cleaned and divided into two groups each with total three fish.

Experimental design

The groups of fish were kept at two different temperatures, one in ice at 0 ^{0}C to 2 ^{0}C and other in a refrigerator at 2 ^{0}C -3 ^{0}C for total three days. Before keeping, the fish were measured for their size and weight using a scale and weighing balance. The fish were also tagged with the alphabets A, B and C for the purpose to identify them easily while collecting the muscle sample during the experiment tenure. The weight of fish A were 225 g, and length was 31 cm, Fish B weighed 180 g and length was 30 cm long, Fish C weighed 235g and length measured was 29 cm. The fishes which were kept in refrigerator also were named as A, B and C. The weight of fish A was 220 g and length were 29 cm, fish B was 195 g and length were 29 cm long and C was 175g and size 31 cm long (Table 1).

Tissue collection, processing and estimation of biochemical substances.

For every biochemical estimation, the tissue (muscle) was collected from the dorsal surface between the dorsal fin and lateral line without disturbing the fish. The skin was cut and removed with help of scalpel blade and a portion of muscle was take with help of scalpel blade and forceps. Muscle tissue was then processed for estimation of carbohydrates, proteins and lipids using the standard protocols.

Estimation of Carbohydrates

For carbohydrate estimation, collected muscle tissue was first weighed for 100 mg and then was transferred to a centrifuge tube containing 1 ml 30 % KOH solution. The tube was placed into a water bath at 90 ^{0}C for 30 minutes. After expiry of 30 minutes and when the tissue was completely digested, it was kept in a refrigerator for overnight. Next day, the tissue was taken out from the refrigerator and 1.0 ml 95% ethanol was added to it. Now it was centrifuged at 5000 rpm for 15 minutes, supernatant was decanted and the sediment was dissolved in 1.0 ml of distilled water for further estimation following the method of Carroll et al. 1956.

Estimation of Proteins

For protein estimation, 50 mg of muscle tissue was heat dissolved in 0.5N NaOH and the protein was estimated using the method of Lowery et. al. 1951.

3. Results and Discussion

The carbohydrates and proteins were analyzed in muscle of *C. gariepinus* preserved in ice and in a freeze after one-day (0 hour), two-day (24 hours) and three-day (48 hours) time periods. Icing is the most common method of fish preservation and is also cost effective. The fish stored in ice generally shows the temperature of 0 ^{0}C to 2 ^{0}C. The carbohydrates in fishes including *C. gariepinus* exist in very small amount as glycogen and free sugars (Mohapatra et al. 2025; Zheng et. al 2026). The carbohydrates in the muscle of *C. gariepinus* stored in ice showed slight decrease after two-day and three-day of preservation (Table 1 and Figure 1). Ice although slows down the bacterial and enzymatic degradation of carbohydrates but it does not stop completely. The glycogenases present in the muscle tissue act on the glycogen and start breaking down resulting in decrease in the carbohydrates at least for initial days of preservation. It is perhaps the reason of decrease in carbohydrate contents after one-day and two-day of ice preservation. The glycogen after its breakdown into the lactic acid lowers the muscle pH which in turn postpone further degradation of the carbohydrates. The carbohydrate contents in muscle tissue of *C. gariepinus* stored in a freeze was however almost constant as compared to zero-day (Table 1 and Figure 1) perhaps due to less or no glycogenase activity or any other factor(s) which were completely ceased at very low temperature.

Table 1: Table 1 shows the statistics of carbohydrate contents analyzed in muscle tissue of *C. gariepinus* at different days of icing and freezing methods of fish preservation. Note left-tailed distribution in both icing and freezing methods.

	Icing					
Fish/ Weight	Fish A (225g)	Fish B (180g)	Fish C (235g)	Mean	Std Error of Mean	Skewness (95% confidence limit)
Day 1 (0 h)	0.68	0.49	0.68	0.62	0.06	-1.73
Day 2 (24 h)	0.73	0.37	0.72	0.61	0.12	-1.73
Day 3 (48 h)	0.65	0.31	0.70	0.55	0.12	-1.62

	Freezing					
Fish/ Weight	Fish A (220g)	Fish B (195g)	Fish C (175g)	Mean	Std Error of Mean	Skewness (95% confidence limit)
Day 1 (0 h)	0.96	0.76	0.34	0.69	0.18	-0.99
Day 2 (24 h)	0.82	0.84	0.31	0.66	0.17	-1.72
Day 3 (48 h)	0.87	0.78	0.35	0.67	0.16	-1.53

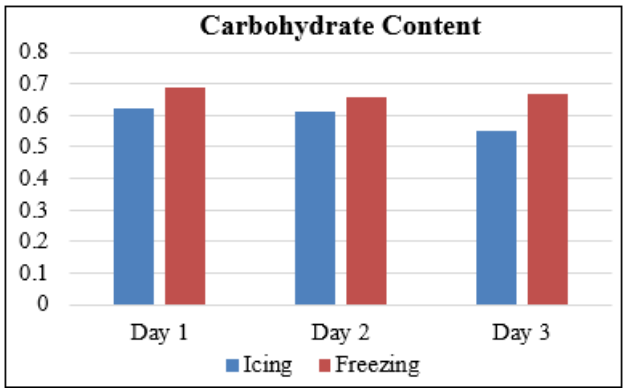

Figure 1: Figure 1 shows the carbohydrate contents in muscle tissue of *C. gariepinus* at different days of icing and freezing methods of fish preservation. Note there is no significant change in carbohydrate contents in freezing method of fish preservation.

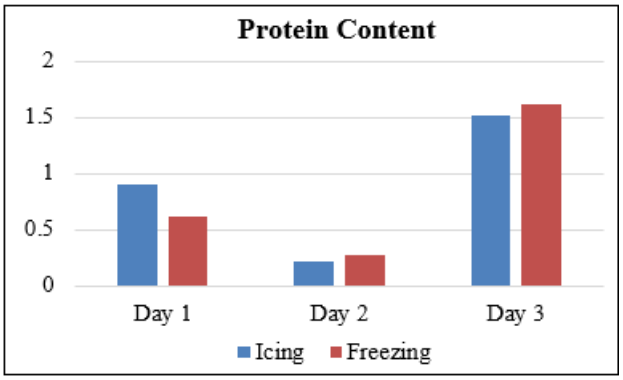

Figure 2: Figure 2 shows the protein contents in muscle tissue of *C. gariepinus* at different days of icing and freezing methods of fish preservation. Note there is a significant increase in protein content after day 3 (48 hours) of preservation.

The protein contents in both icing and freezing methods of fish preservation after an initial decease, increased significantly after the day-three (48 hours) of preservation (Table 2 and Figure 2). The proteins are temperature sensitive and hence need proper treatment for their preservation and storage especially after the death of the animals including the fish. The initial decrease in the proteins is perhaps due to the early autolysis and microbial degradation reactions (Mehta et. al. 2025; Liu et.al. 2026). The endogenous protein digesting enzymes present in the muscle remains active at least at initial stages of fish preservation and break down the structural and other muscle proteins into the smaller ones. Another reasons for decrease in the muscle protein after 24 hours of preservation, especially in the refrigerated fish may be due to the leakage of fluids and the proteins.

Table 2: Table 2 shows the statistics of protein contents analyzed in muscle tissue of *C. gariepinus* at different days of icing and freezing methods of fish preservation

	Icing					
Fish/ Weight	Fish A (225g)	Fish B (180g)	Fish C (235g)	Mean	Std Error of Mean	Skewness (95% confidence limit)
Day 1 (0 h)	0.89	0.93	0.90	0.90	0.01	1.29
Day 2 (24 h)	0.26	0.20	0.21	0.22	0.02	1.55
Day 3 (48 h)	1.52	1.55	1.50	1.52	0.01	0.59

	Freezing					
Fish/ Weight	Fish A (220g)	Fish B (195g)	Fish C (175g)	Mean	Std Error of Mean	Skewness (95% confidence limit)
Day 1 (0 h)	0.86	0.12	0.84	0.61	0.24	-1.73
Day 2 (24 h)	0.29	0.25	0.27	0.27	0.02	-1.27
Day 3 (48 h)	1.58	1.60	1.64	1.61	0.02	-0.02

The new proteins synthesis in the fish is however not possible during the preservation. The increase in the protein content after day-three of fish preservation may be due to the protein aggregation (Tolstorebrov et.al. 2015; Bhat et al. 2022) and due to the loss in weight and moisture content of the fish. Freezing-concentration effect may be another reason for the increase in the total muscle proteins of *C. gariepinus* after 48 hours of fish preservation. According to the research studies, these effects are however more prone in fish preserved in the refrigeration.

4. Conclusion

Both icing and freezing techniques are very effective for fish preservation and are suitable for short-term and long-term preservation respectively. Icing keeps the fish near 0 ^{0}C temperature and the fish remain moist too. Present study reveals that the carbohydrates are very much stable and are least affected by microbial and autolytic degradation in *C. gariepinus* preserved in ice and freeze. The proteins being more susceptible to the temperature change are heavily affected and hence show the fluctuations. Icing is thus more suitable and convenient method for fish preservation in local fish markets where the fish are stored for short duration. Icing however needs extra care and expertise which are often the problems with the fisher men and women. This can be achieved through the short-term training activities to the fisher men and women by the nearby fishery institutions and government/non-government organization.

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