Influence of different storage conditions on the biochemical composition of muscle in *Clarias batrachus* (linnaeus, 1758)

Tara Rani*

Department of Zoology, Ganga Singh College, J. P. University, Chapra, Bihar, India

***Author for correspondence**: Email: ranichaurasia48@gmail.com

Abstract: Fish is a highly perishable commodity subjected to oxidative and microbial deterioration. Storage at low temperature can delay the process of deterioration up to some extent. In the present study, fish *Clarias batrachus* was used to investigate the effects of storage condition and duration of storage on the protein and glycogen level in the muscle. The fish were randomly divided into 4 groups; I: whole fresh fish stored in chilled condition, II: whole fresh fish stored in super- chilled condition, III: potassium sorbate treated fish stored in chilled condition, IV: potassium sorbate treated fish stored in super- chilled condition. The fish were stored in tea- chest boxes insulated with thermocol sheets. Ice and fish were arranged in alternate layers. Muscle of the stored fish were estimated for total protein and glycogen level on days 4, 8, 12, 16 and 20 using standard laboratory protocols and were compared with those at day "0" of storage. The experiment was conducted separately in summer and winter seasons. A gradual decline in the level of protein and glycogen can be observed in all the groups with increasing days of storage. However, the decline was comparatively slower in winter season. Preservative treated fish showed lesser decline in the biochemical composition of fish muscle, when compared with whole fresh fish. Fish stored in super- chilled condition showed inhibition of loss of muscle protein and glycogen than that in chilled condition. Storage of fish using combination of safe food preservative and super- chilled condition can extend the shelf life of the fish with delayed deterioration of nutritive biochemical components.

Keywords: *Clarias*, Potassium sorbate, Super- chilled, chilled, storage, glycogen.

1. INTRODUCTION

Fish are considered as one of the prime sources of animal protein and are popularly known for their high commercial as well as medicinal values [1]. The proteins of fish muscle contain adequate amount of essential amino acids, have a high biological value and is easily digestible [2]. They also provide a good source of various micro and macronutrients [3]. However, they are highly perishable commodity and are susceptible to oxidative damage and microbiological deterioration. So, they need efficient storage strategies to increase their shelf life without compromising the nutritional quality [4, 5]. The preservation of fish is necessary not only to meet the demands of consumers, but also to mitigate the post-harvest loss of the fish [6]. Handling and storage of fish greatly influence the preservation of nutritional quality [7]. Recently there have been many advancements in the fish storage technologies, such as, high pressure processing, pulsed light technology, microwave processing, irradiation, pulsed electric fields, modified atmosphere and vacuum packing etc. [5, 7]. Despite the emergence of these advanced technologies, storage using chilling and freezing are still the most widely used preservation methods. Super chilling technology can be applied in fish chilling to delay the deterioration process [5]. Storage using ice or refrigeration is easily accessible and manageable for local people in rural and urban India. Although the storage techniques extend the shelf life of the fish, quality of fish is gradually lost due to changes in muscle integrity, lipids and proteins [1, 8]. In the present study, an effort has been done to investigate the efficiency of different storage conditions in preserving the biochemical composition of the fish, *Clarias batrachus*.

2. MATERIALS AND METHODS

2.1. Experimental animal

Healthy and live individuals of *Clarias batrachus* (Clariidae, Siluroidae) weighing 130-150 g were procured from local fish market in Chapra, Bihar. For analysis of parameters in summer season fish were brought in the month of April to June, whereas for winter season they were collected in the months of December to February. The carried fish were treated with 0.2 % aqueous $KMnO_4$ solution to get rid of any dermal infections.

2.2. Experimental design

For experimentation, fish were thoroughly washed with normal tap water twice or thrice before transferring them to different storage conditions.

2.2.1. Grouping of animals:

The fish were randomly divided into 4 different groups.

GROUPS	STORAGE CONDITIONS
I	Whole fresh fish stored in chilled condition
II	Whole fresh fish stored in super chilled condition
III	Potassium sorbate treated fish stored in chilled condition
IV	Potassium sorbate treated fish stored in super chilled condition

For chilled condition, the fish and ice were layered alternately and stored in tea- chest boxes internally insulated by thermocol sheets. For super chilled condition, 375 g of local normal salt (Tata refined) were mixed with 20 kg of ice and fish were layered alternately. For chemical treatment of the fish, ice was prepared using water containing 5 % of potassium sorbate. Total protein and glycogen content in the muscle of fish of different group were analyzed at an interval of 4, 8, 12, 16 and 20 days. For control, these parameters were studied in the muscle of fresh fish also and designated as day "0" of storage. The experiment was conducted in summer and winter seasons.

2.2.2. Estimation of protein:

For estimation of protein 100 mg of muscle was homogenized in 2 ml of double distilled water and was repeated with residue. 1 ml of homogenate was mixed with 8.75 ml of 30 % sodium hydroxide solution and was left for 20 minutes. Further, 0.25 ml of 20 % aqueous copper sulphate solution was added. The solution was mixed and optical density was measured at 530 nm using colorimeter. Total protein (%) was calculated using following formula:

Total protein (%) = O.D. of sample × K^{-1} protein × 4.

Where, K^{-1} is constant and is 48.661 mg/ml

4 is the number of total homogenates of 100 mg muscle

2.2.3. Estimation of glycogen:

For estimation of glycogen, 100 mg of muscle was homogenized in 5 ml of ice cold 10 % trichloroacetic acid. The homogenate was centrifuged for 20 minutes at 1500 rpm. 2 ml of supernatant was mixed with 6 ml of Conc. sulfuric acid and was boiled for 6 and half minutes using water bath. It was cooled to room temperature and optical density was read at 530 nm. Percent of glycogen in the muscle was calculated using standard curve obtained from OD of samples having known concentration of glycogen.

2.2.4. Statistical analysis

The data obtained were represented as mean ± standard error. The data were statistically analyzed using two- way ANOVA and considered significant at $p<0.05$ and $p<0.01$.

3. RESULTS

3.1. Effects on total protein content

Fig 1 and 2 showed the effects of different storage conditions and days of storage on total protein content of muscle in the experimental fish in summer and winter seasons, respectively. In case of whole fresh fish, a gradual decline in the total protein content in the muscle of the fish can be observed in the summer as well as winter season. However, the decline was rapid in the summer season. While comparing the storage conditions, whole fresh fish stored in super chilled condition (Group II) showed a decline of 3.56 % from day "0", whereas fish stored in chilled condition (Group I) showed a decline of 4.09 % in the protein level of muscle of whole fresh fish in summer season (Table 1). During winter season, whole fresh fish showed a decline of 2.87 % in chilled condition and 1.88 % in super chilled condition (Table 2). In case of chemically treated fish, a decline of 4.61 % and 2.62 % in muscle protein content of fish was observed when stored in chilled (Group III) and super chilled condition (Group IV), respectively, during summer season (Table 1). In winter season, chemically treated fish stored in chilled and super chilled conditions showed a decline of 1.62 % and 1.56 %, respectively, after 20 days of storage (Table 2). The change was significant among all the groups in summer season, whereas in winter, only the whole fresh fish stored in chilled condition showed significant decline in the muscle protein content (Table 1 & 2).

3.2. Effects on total glycogen content

Fig 3 and 4 showed the effects of storage conditions and duration of storage on the muscle glycogen content in fish during summer and winter seasons, respectively. In case of whole fresh fish, a decline of 34.54 % was observed in the chilled condition (Group I) and the decline was 22.73 % in super chilled condition (Group II) during summer season (Table 3). During winter season, whole fresh fish stored in chilled and super chilled condition showed a decline of 19.30 % and 18.42 % in glycogen content of muscle, respectively (Table 4). In case of chemically treated fish, a decline of 21.82 % and 15.45 % in glycogen content was observed in fish stored in chilled (Group III) and super chilled (Group IV) conditions, respectively, during summer season (Table 3). During winter season, chemically treated fish stored in chilled condition showed a decline of 19.30 %, whereas those stored in super chilled condition showed a decline of 16.67 % in muscle glycogen content of fish (Table 4). The decline in glycogen content was significant in group I, II and III during summer season, whereas during winter season, the decline from day "0" was significant in group I and III only (Table 3 & 4).

4. DISCUSSION

The study showed a gradual decline in the muscle content of protein and glycogen in all the experimental storage conditions (group I to IV) during both summer and winter seasons. However, the loss was comparatively slow in winter season. On comparing the storage condition, super chilled condition showed greater prevention in the loss of protein and glycogen content in the muscle of fish. On the other hand, chemically treated fish showed lesser loss of nutrients (Table 1-4). Potassium sorbate ($C_6H_7O_2K$) is an antimicrobial food preservative used widely for many foodstuffs. It has been classified as "generally recognized as safe" by Hazardous Substances Data Bank (HSDB) [9]. Storage with 5 % potassium sorbate extended the shelf life of frozen fish fillets [10]. However, potential health risk of food preservatives cannot be sidelined [11].

A decline in protein content of muscle reported in the present study was in accordance with the study conducted by Mazrouh M M., 2015 [1]. They reported a significant decline in the protein content of muscle of *Saurida undosquamis*, brushtooth lizard fish, frozen for 21 days. They suggested that a loss in protein content may be due to denaturation, loss of gelatin caused by extended period of freezing or may be due to induction of microbial proteolysis [1]. The protein content of tuna fillet was decreased significantly, when stored frozen for 60 days [12]. A gradual decline in protein content of frozen fish may be due to loss of water-soluble sarcoplasmic proteins. These proteins might lose with drip- loss of ice-melting water during freezing [12]. Denaturation and changes in the chemical composition can also be the reasons for loss of protein [13]. Similar trend of loss of muscle protein content was reported in rohu and rainbow trout during preservation using freezing [6]. At low temperature, biochemical processes are slowed down, but they do not stop. Some phosphorus compounds and glycogen decompose in the process of freezing and give rise to phosphorus and lactic acids [14].

5. CONCLUSION

The condition and duration of storage had significant impact on the biochemical composition of the stored fish. Maintenance of comparatively low temperature in winter season and super- chilled condition was effective in preservation of fish up to longer duration keeping the nutritive values intact. Use of food preservative can provide a better alternative for high maintenance and costly preservation techniques used for fish and fish products.

6. ACKNOWLEDGMENT

Author is thankful to Prof. Ashok Kumar, Retd. Professor, J. P. University, Chapra, Bihar for his valuable guidance in successful completion of the experiment and analysis of data. The author is grateful to the authority of the University for providing infrastructure to conduct the experiment.

REFERENCES

1. Mazrouh MM. (2015). Effects on freezing storage on the biochemical composition in muscles of *Saurida undosquamis* (Richardson, 1848) comparing with imported frozen. Int. J. Fish. Aquat. Stud. 3(2): 295- 299.
2. Amin HF, El- Lahamy AA, Mohamed HR, et al. (2023). Effect of frozen storage on fish quality and fishery products: a review. Mediterr. Aquac. J. 10 (2): 25-35.
3. Famurewa JAV, Akise OG & Ogunbodede T. (2017). Effect of storage methods on the nutritional qualities of African catfish *Clarias gariepinus* (Burchell, 1822). Afr. J. Food Sci. 11 (7). 223- 233. https://doi.org/10.5897/AJFS2016.1514.
4. Mehta NK, Elavarasan K, Reddy AM & Shamasundar. (2014). Effect of ice storage on the functional properties of proteins from a few species of freshwater fish (Indian major carps) with special emphasis on gel forming ability. J Food Sci Technol. 51 (4): 655-663. https://doi.org/10.1007/s13197-011-0558-y.
5. Duarte AM, Silva F, Pinto FR, et al. (2020). Quality assessment of chilled and frozen fish- mini review. Foods. 9: 1739. https://doi.org/10.3390/foods9121739.
6. Pelden Y, Wangchuk K & Jambay J. (2025). Impact of preservation techniques and storage durations on nutritional quality of rohu and rainbow trout. B J A S. 9(1): 46-59.
7. Sampels S. (2014). The effects of storage and preservation technologies on the quality of fish products: a review. J. Food Process. Preserv. https://doi.org/10.1111/jfpp.12337.

8. Walayat N, Tang W, Wang X, et al. (2023). Quality evaluation of frozen and chilled fish: a review. eFood. 4 (e67). https://doi.org/10.1002/efd2.67.

9. National Center for Biotechnology Information (2025). PubChem Compound summary for CID 23676745, Potassium sorbate. https://pubchem.ncbi.nlm.nih.gov/compound/Potassium-Sorbate.

10. Gupta V & Gandotra R. (2019). Studies on the effect of potassium sorbate treatment on fillets of *Tor tor*. International conference on "ETAHBS'. 118-121.

11. Dehghan P, Mohammadi A, Mohammadzadeh- Aghdash H, Nazhad Dolatabadi JE. (2018). Pharmacokinetic and toxicological aspects of potassium sorbate food additive and its constituents. Trends in Food Science & Technology. https://doi.org/10.1016/j.tifs.2018.07.012.

12. Chakma S, Rahman Md. A, Mali SK, et al. (2022). Influence of frozen storage period on the biochemical, nutritional and microbial quality of Skipjack tuna (*Katsuwonus pelamis*) collected from the Bay of Bengal coast of Bangladesh. F C A. 100139. https://doi.org/10.1016/j.focha.2022.100139.

13. Malik IA, Elgasim EA, Adiamo OQ, Ali AA & Mohamed Ahmed IA. (2021). Effect of frozen storage on the biochemical composition of five commercial freshwater fish species from River Nile, Sudan. Food Sci. Nutr. 9(7): 3758- 3767. https://doi.org/10.1002/fsn3.2340.

14. Puchala R, Bialowqs H & Pilarczyk M. (2005). Influence of cold and frozen storage on carp (*Cyprinus carpio*) flesh quality. Pol. J. Food Nutr. Sci. 14/55 (1): 103-106.

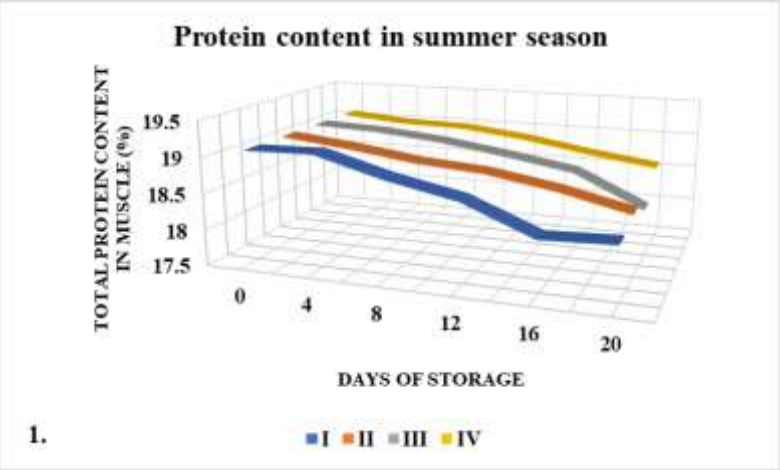
List of figures:

Fig 1: Graph showing time- dependent change in total protein content in muscle of fish stored at different conditions in summer season.

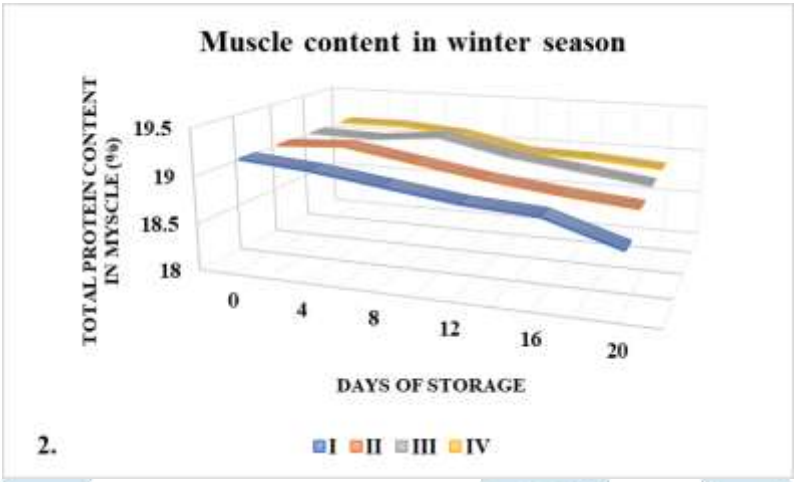
Fig 2: Graph showing time- dependent change in total protein content in muscle of fish stored at different conditions in winter season.

Fig 3: Graph showing time- dependent change in total glycogen content in muscle of fish stored at different conditions in summer season.

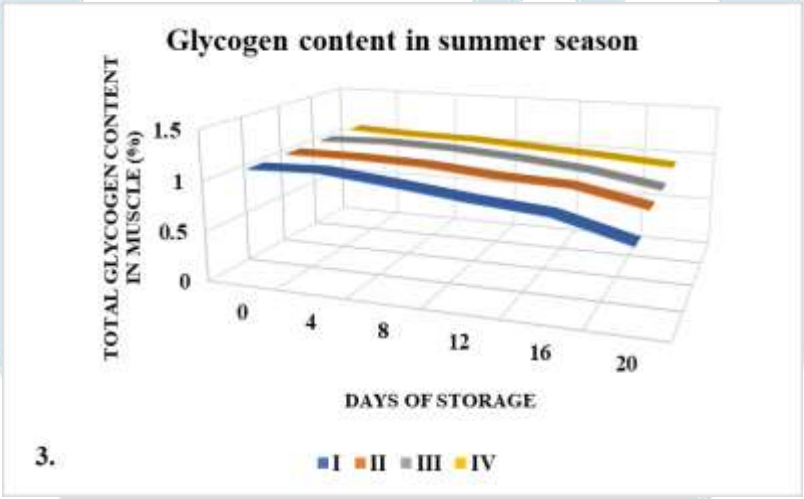
Fig 4: Graph showing time- dependent change in total glycogen content in muscle of fish stored at different conditions in winter season.



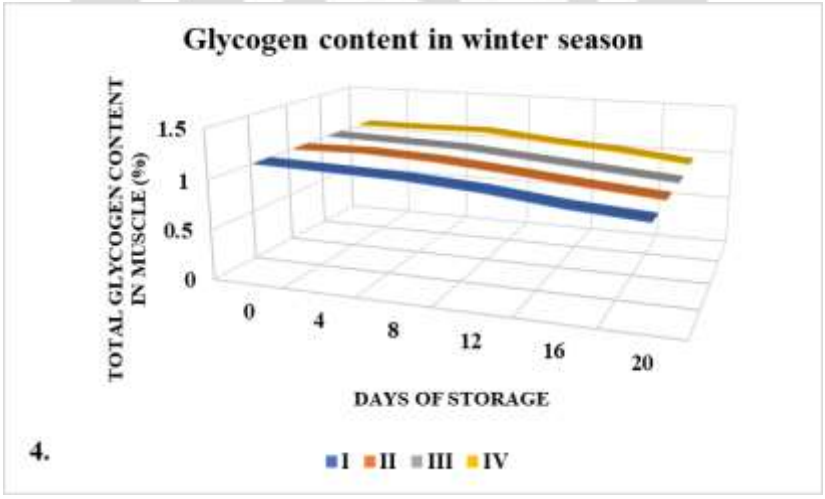
1.



2.



3.



4.

List of tables:

Table 1: Effect of storage conditions and days of storage on protein content in muscle of *Clarias batrachus* in summer season.

Table 2: Effect of storage conditions and days of storage on protein content in muscle of *Clarias batrachus* in winter season.

Table 3: Effect of storage conditions and days of storage on glycogen content in muscle of *Clarias batrachus* in summer season.

Table 4: Effect of storage conditions and days of storage on glycogen content in muscle of *Clarias batrachus* in winter season.

Table 1.

Days of storage	Total protein content (%) in muscle during summer season			
	Groups			
	I	II	III	IV
0	19.08 ± 0.13	19.08 ± 0.13	19.08 ± 0.13	19.08 ± 0.13
4	19.10 ± 0.11	18.98 ± 0.10	19.04 ± 0.13	19.00 ± 0.11
8	18.84 ± 0.10	18.85 ± 0.12	18.95 ± 0.10	18.98 ± 0.12
12	18.65 ± 0.12	18.78 ± 0.10	18.81 ± 0.09	18.87 ± 0.11
16	18.26 ± 0.11*	18.62 ± 0.13	18.65 ± 0.13	18.71 ± 0.12
20	18.30 ± 0.09**	18.40 ± 0.06**	18.20 ± 0.14**	18.58 ± 0.11*
% change on day "20" from day "0"	(-) 4.09 %	(-) 3.56 %	(-) 4.61 %	(-) 2.62 %

Values were represented as mean ± SE. *p<0.05, **p<0.01.

Table 2.

Days of storage	Total protein content (%) in muscle during winter season			
	Groups			
	I	II	III	IV
0	19.16 ± 0.11	19.16 ± 0.11	19.16 ± 0.11	19.16 ± 0.11
4	19.10 ± 0.14	19.20 ± 0.15	19.14 ± 0.13	19.16 ± 0.14
8	19.00 ± 0.11	19.08 ± 0.13	19.21 ± 0.15	19.10 ± 0.12
12	18.90 ± 0.12	18.96 ± 0.10	19.05 ± 0.13	18.94 ± 0.13
16	18.85 ± 0.14	18.87 ± 0.12	18.95 ± 0.11	18.92 ± 0.09
20	18.61 ± 0.13*	18.80 ± 0.11	18.85 ± 0.13	18.86 ± 0.15
% change on day "20" from day "0"	(-) 2.87 %	(-) 1.88 %	(-) 1.62 %	(-) 1.56 %

Values were represented as mean ± SE. *p<0.05, **p<0.01.

Table 3.

Days of storage	Total glycogen content (%) in muscle during summer season			
	Groups			
	I	II	III	IV
0	1.10 ± 0.03	1.10 ± 0.03	1.10 ± 0.03	1.10 ± 0.03
4	1.12 ± 0.05	1.10 ± 0.06	1.12 ± 0.04	1.07 ± 0.06
8	1.05 ± 0.06	1.08 ± 0.05	1.10 ± 0.04	1.06 ± 0.05

12	0.97 ± 0.05	1.02 ± 0.06	1.05 ± 0.04	1.02 ± 0.06
16	0.91 ± 0.05*	0.99 ± 0.06	0.98 ± 0.07	0.98 ± 0.05
20	0.72 ± 0.06**	0.85 ± 0.06*	0.86 ± 0.07*	0.93 ± 0.06
% change on day "20" from day "0"	(-) 34.54 %	(-) 22.73 %	(-) 21.82 %	(-) 15.45 %

Values were represented as mean ± SE. *p<0.05, **p<0.01.

Table 4.

Days of storage	Total glycogen content (%) in muscle during winter season			
	Groups			
	I	II	III	IV
0	1.14 ± 0.05	1.14 ± 0.05	1.14 ± 0.05	1.14 ± 0.05
4	1.12 ± 0.06	1.15 ± 0.07	1.12 ± 0.06	1.14 ± 0.06
8	1.10 ± 0.04	1.12 ± 0.06	1.10 ± 0.07	1.15 ± 0.08
12	1.05 ± 0.05	1.06 ± 0.05	1.04 ± 0.05	1.08 ± 0.07
16	0.97 ± 0.04	0.99 ± 0.06	0.98 ± 0.07	1.03 ± 0.06
20	0.92 ± 0.07*	0.93 ± 0.09	0.92 ± 0.06*	0.95 ± 0.06
% change on day "20" from day "0"	(-) 19.30 %	(-) 18.42 %	(-) 19.30 %	(-) 16.67 %

Values were represented as mean ± SE. *p<0.05, **p<0.01.