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Quality changes in the muscles of *Wallago attu* during frozen storage (-12±2ºC) conditions

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Abstract

The present study was aimed to investigate the effect of frozen storage on the proximate, biochemical and microbial profile of the muscle of a silurid cat fish (Wallago attu). The fish muscle was subjected to the frozen storage for a period of one month and the analysis was carried out at an interval of 10 days. It was observed that proximate composition viz. protein, lipid, moisture and ash content decreased significantly (P<0.05) with increase in the duration of frozen period. The fresh (unfrozen) samples revealed the highest values for all i.e.15.45±0.2% for protein, 4.02±0.04% for lipid, 81.66±0.03 for moisture and 1.48±0.1% for ash while the least values were observed at the end of one month frozen storage period i.e.10.14±0.015%, 2.36±0.03%, 74±0.05% and1.33±0.02% for protein, lipid, moisture and ash respectively. pH of fish ranged between 5.9 to 7.1 while the biochemical parameters viz.,Free Fatty Acids (FFA) and Thiobarbituric acid (TBA) showed an increasing trend with increase in frozen storage period. Similarly, the microbial count for Total Plate Count (TPC), Coliform Count (CC) and Psychrotrophic Count (PC) increased gradually from 2.18±0.02 log cfu/g, 2.02±0.04 log cfu/g and 2.43±0.03 log cfu/g on day 0 to 6.87±0.1 log cfu/g, 5.25±0.2 log cfu/g and 5.99±0.02 log cfu/g on day 30th respectively. Thus, considering the importance from consumer point of view, these studies reveal that a significant loss is observed in fish during frozen storage. However, it could be implied that fish could be kept under frozen conditions when preservation is of utmost importance, so as to retain its taste and nutrition.

Keywords: Frozen period, proximate, biochemical, microbial, *Wallago attu*.

Introduction

Fish as a whole is considered to have a great potential for food and thus can be expected to provide relief from malnutrition. Also, it is a very good source of protein, has a high content of water and lipid soluble vitamins, minerals and polyunsaturated fatty acids (PUFAs) of n-3 family. It is believed that Omega-3 fatty acids from fish can lower body triglycerides thus reducing the chances of heart diseases. Fish also reduces blood pressure by small but significant amounts and improve blood clotting regulation[1]. Fish, however, is very susceptible to microbial and chemical deterioration like lipid oxidation, hydrolysis and protein denaturation etc. Moreover, the high ambient temperature of our country favours the rapid microbial growth, hence there is a need to properly handle and preserve the fish, so as to increase its shelf life. Shelf life extension can be achieved by various preservation methods, viz. salting, brining, smoking, icing, glazing, refrigeration and freezing. Since, in our country, fish in a fresh state is not always available due to seasonal fishing and the far location of major fishing grounds from cities and consuming centres, the freezing of fish becomes an update method of long term preservation. Refrigeration and freezing provide low temperature thus, inactivating the microbial growth and there by reducing enzymatic and chemical deterioration. Although many damaging processes are inhibited by such low temperature storage methods, but the undesirable reactions associated with lipids and proteins are shown to occur, leading to the detrimental changes in nutritional and sensory properties. Some disadvantages of frozen storage include freezer burn, product dehydration, rancidity and drip loss and this deterioration increases as duration of storage increases. Hence, the objective of this study is to determine the changes in nutritional, biochemical and microbial quality in the muscles of Silver carp, stored in frozen storage conditions (12±2ºC).

Material and Methods

Collection of fish samples: Fresh samples of *Wallago attu* were purchased from local market of Jammu city. They were immediately brought to the lab in polythene bags along with crushed ice. The viscera of fish was removed and the fish was washed with large amount of water. The fish was cut into pieces and these pieces were immediately wrapped in aluminum foil, kept in air tight plastic container and stored at -12±2°C (frozen storage). Analytical procedures for biochemical and microbiological changes were done on 0, 10th, 20th and 30th day of storage.

Analysis: The proximate composition (protein, lipid, ash and moisture) of the fish samples were evaluated using the standard AOAC procedure[2]. The protein content was determined using the Lowry *et al*[3] method. Fat content was determined using Folch *et al*[4] method. Thiobarbituric acid value of fish muscle during frozen storage was determined by using the method of

Witte *et al* (1970)[5]. Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick)[6]. Extract Release Volume (ERV) was determined as per the method of Strange *et al.* (1977)[7]. The pH of fish muscle was determined by the method of Keller *et al.* (1974)[8]. The microbiological profile was determined according to APHA method[9]. Data was expressed as mean ± SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

Statistical Analysis: Mean and standard errors were calculated for different parameters. The data analyses were performed using SPSS software (12.0 for Windows). Differences between treatments were analyzed using independent-measures one-way ANOVA. Post-hoc comparisons were conducted using Duncan's test. The values were expressed as mean ± SE. p values <0.05 were considered as significant and p values <0.001were considered as highly significant.

Results and Discussion

Proximate Composition: Protein content: Perusals of table-1 revealed that the highest protein content (15.45±0.2%) was recorded for fresh (unfrozen) fish samples and the least protein content (10.14±0.015%) was recorded for fish samples stored for 30 days under frozen conditions. A significant percental decrease ($p \leq 0.05$) was found in total protein content i.e.8.67%, 20.71% and 34.3% on 10th, 20th and 30th day of frozen storage respectively table-2. In support with the present studies, Keyvan *et al* (2008)[10] in Caspian white fish (*Rutilus frisi kutum*), El-deen and El-shamrey (2010)[11]in Gahsh (*Lethrinus elongates*) and Aberoumand(2013)[12] in various Iranian fishes also found a protein loss during frozen storage. They related this protein loss to the denaturation of proteins and loss of nitrogen as volatile bases and nitrogenous substances formed by bacterial decomposition that escaped from tissue during frozen storage.

Lipid content: The results shown in table-1 show that the lipid content decreased significantly ($p \leq 0.05$) from day 0 i.e. 4.02±0.04% to 2.36±0.03% on day 30th. There was 14.42%, 25.37% and 41.29% decrease on 10th, 20th and 30th day of frozen storage, table-2. Arannilewa *et al*[13] in Tilapia, Keyvan *et al*[10] in Caspian sea white (*Rutilus frisi kutum*), Siddique *et al*[14] in *Puntius* sps. and Aberoumand[12] in various Iranian fishes also found a decrease in total lipid content during frozen storage. They opined that this protein loss must be due to oxidation and hydrolysis of lipids during frozen storage.

Table-1
Change in Proximate composition of *Wallago attu* during frozen storage.

Days of storage	0 day	10th day	20th day	30th day
Protein (%)	15.45 [a] ±0.2	14.11 [b] ±0.05	12.25 [c] ±0.03	10.14 [d] ±0.015
Lipids (%)	4.02 [a] ±0.04	3.44 [b] ±0.02	3.00 [c] ±0.07	2.36 [d] ±0.03
Moisture (%)	81.66 [a] ±0.03	79.2 [b] ±0.4	76.94 [c] ±0.1	74 [d] ±0.05
Ash (%)	1.48 [a] ±0.1	1.41 [b] ±0.5	1.38 [c] ±0.02	1.33 [d] ±0.02

Table-2
Percent decrease in proximate composition of fish muscle of *Wallago attu* under frozen storage

Days	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
0-10	8.67	14.42	3.01	4.72
0-20	20.71	25.37	5.78	6.75
0-30	34.3	41.29	9.3	10.13

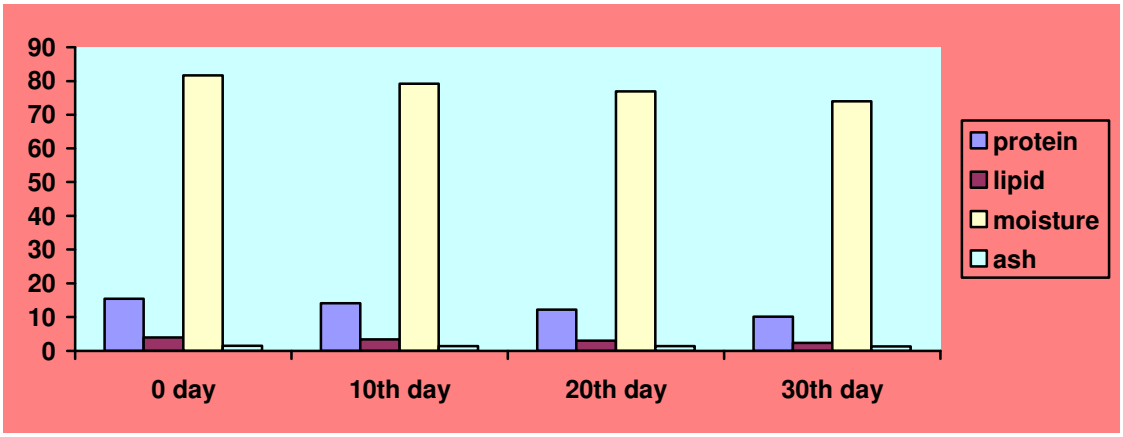

Figure-1
Change in proximate composition of Wallago attu during frozen storage

Moisture: The total moisture content of the fish sample decreased from 81.66±0.03% on day 0 to 74±0.05 % on day 30th. Total percent decrease was 3.01%, 5.78% and 9.3% on 10th, 20th and 30th day of frozen storage table-2. These results are favoured by the findings of Le-blanc and Le-blanc[15], Arannilewa *et al*[13], El-deen and El-shamrey[11] and Aberoumand[12] who proposed that decrease in moisture is due to condensation of water during frozen storage.

Ash: In the present studies table-1, ash content showed a decrease with an increase in frozen storage period. Total percental decrease was 4.72% on day 10th and it increased upto 10.13% on day 30th of frozen storage. These results are in agreement with Emire *et al*[16] in Tilapia (*Oreochromis niloticus*) and Aberoumand[12] in Iranian fishes and Gandotra *et al*[17] in *Labeo rohita.*

Biochemical Composition: Thiobarbituric acid (TBA): The TBA value is an index which measures the malondialdehyde (MDA) content and is a widely used method for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen[18]. The present study showed a progressive increase in TBA value (secondary oxidation product) with increase in storage period under frozen conditions. The values rose from 0.16±0.2 on day 0 to 4.48±0.05 on 30th day of frozen storage period. These results are in consistence with the studies of] Bao *et al*[19] in Arctic charr (*Salvelinus alpinus*), Orak and Kayisoglu[20] various fish species and Jezek and Buchtova[21] in Common carp (*Cyprinus carpio L.*) and Silver carp (*Hypophthalmichthys molitrix V.*). The increase in TBA is attributed to the fact that freezing and thawing accelerates the accumulation of secondary oxidative products released due to the destruction of cell membrane by crystals of ice and release of pro-oxidants, especially haem-iron[22].

Free fatty acids (FFA) : The values for Free Fatty Acids (FFA) were 0.88±0.02 on day 0 and it rose to 2.86±0.2, 4.88±0.33 and 6.93±0.05 on 10th, 20th and 30th day of frozen storage respectively. These results are in accordance with Keyvan *et al*[10] in Caspian Sea white fish (*Rutilus frisi kutum*), Jezek and Buchtova[23] in Silver carp and Gandotra *et al*[24] in *Mystus*. These findings are devoted to the release of lipase from liposome during frozen storage which degrades the fats, thus increasing the free fatty acids[25].

pH: The pH values also showed an increasing trend with increase in frozen period. The pH values ranged from 5.9 on day 0 to 7.11 on 30th day. These results are in line with Arannilewa *et al* (2005)[13] in frozen Tilapia (*Sarotherodun galiaenus*), Jezek and Buchtova (2011)[23] in Common carp (*Cyprinus carpio L.*) and Silver carp (*Hypophthalmichthys molitrix* V.) and Erkan and Ozden (2007)[26] in Gutted sardines (*Sardina pilchardus)* who attributed this increase to the production of basic components induced by bacterial growth.

Microbial quality+: The quality of fish meat is largely dependent on its microbial contamination. Inquistive study of table- 4 shows an increasing trend for TPC, CC and PC during the frozen storage period. Initially the values for TPC were 2.18±0.02 log cfu/g and increased to 6.87±0.1 lof cfu/g at the end of storage, thus crossing the permissible limits of 6 lof cfu/g on 20th day of storage.

Table-3
Change in bio-chemical composition of *Wallago attu* during frozen storage

Days	0 day	10th day	20th day	30th day
TBA(mg MA/kg)	0.16 [a] ±0.2	1.89[b]±0.01	3.39 [c] ±0.4	4.48[d]±0.05
FFA (%)	0.88 [a] ±0.02	2.86 [b] ±0.2	4.88 [c] ±0.33	6.93 [d] ±0.05
pH	5.9[a]	6.4 [b]	6.9[c]	7.11 [d]

Mean ±SD with different superscripts in a row varies significantly (P<0-05)

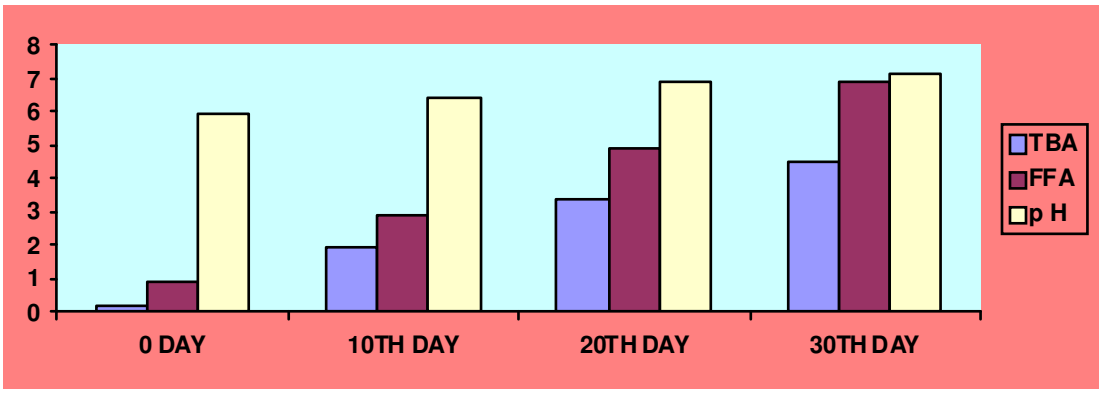

Figure-2
Change in bio-chemical composition of *Wallago attu* during frozen storage

Table-4
Changes in Total Plate Count (TPC), Coliform Count (CC) and Psychrophillic Count(PC) of fish muscle of *Wallago attu* under frozen storage

Days of storage	0 day	10th day	20th day	30th day
TPC log cfu/g	2.18^{a} ±0.02	4.4^{b}±0.12	6.1^{c}±0.04	6.87^{d}±0.1
CC log cfu/g	2.02^{a}±0.04	3.1^{b}±0.05	3.82^{c}±0.06	5.25_{d}±0.2
PC log cfu/g	2.43^{a}±0.03	3.48^{b}±0.11	4.7^{c}±0.05	5.99^{d}±0.02

Similarly, CC and PC also showed an increasing trend with 5.25±0.2 lof cfu/g and 5.99±0.02 log cfu/g values for CC and PC respectively at the end of frozen storage period. Likewise, Arannilewa *et al*[13] found an increase in Coliform count with the increasing storage period in frozen Tilapia. Ozogul *et al*[27] also reported a significant statistical increase in total viable counts of whole gutted common sole (*Solea solea)* over the storage period of 24 days. Simiilarly, Ola and Oladipo[28] and Liu[29] found an increasing trend for psychrotrophs during storage period. This increase in microbial count is attributed to growth promoting effect of moisture during frozen storage[30].

Conclusion

The main objective of this study was to observe nutritional, biochemical and microbial changes in *Wallago attu* during frozen conditions. The freezing of fish at low temperature makes it less prone to spoilage by decreasing the bacterial activity. However, it was observed that there was a decrease in the nutritional parameters while an increase was observed in biochemical composition and microbial count during frozen storage. Therefore, it could be concluded that we should try to consume fish while it is fresh only. Since, all fishes are not available throughout the year; hence, freezing is best preferred when preservation of such fish species is of priority.

References

1. Nettleton J. A. Omega-3 Fatty Acids and Health, New York: Chapman & Hall, **(1995)**

2. AOAC, Official Methods of Analysis, 16th Edn., Association of Official Analytical Chemists, Washington DC., USA**, (1995)**

3. Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J., Protein measurement with phenol reagent, *J. Biol. Chem.,* **193,** 265-275 **(1951)**

4. Folch J., Less M. and Sloane G.W.S., A simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem*., **226,** 497–509 **(1957)**

5. Witte V.C., Krause G.F. and Bailey M.B., A new extraction method for determining 2-thiobarbituric acid analysis, *J. Food Sci.,* **35,** 582 **(1970)**

6. Koniecko E.K., In: Handbook for meat chemists. Avery Publishing group Inc., Wayne, New Jersey, USA, **(1979)**

7. Strange E.D., Benedict R.C., Smith J.L. and Swift C.E., Evaluation of rapid tests for monitoring alterations in meat quality during storage, *J. Food Prot*., **10,** 843-847 **(1977)**

8. Keller J.E., Kelly G.C. and Acton J.C., Effect of meat particle size and casing diameter on summer sausage properties during drying, *Journal of Milk Food Technol*, **37,** 101-106 **(1974)**

9. APHA, Compendium of method of microbiological examination of foods, 2nd Edn., American Public Health Association, Washington DC **(1984)**

10. Keyvan A., Moini S., Ghaemi N., Haghdoost A.A., Jalili S. and Pourkabir M., Effect of frozen storage time on the lipid deterioration and protein denaturation during Caspian sea white fish (*Rutilus frisi kutum*), *Journal of fisheries and aquatic sciences,* **3(6),** 404-409 **(2008)**

11. El-deen G. and El-shanrey M.N., Studies on contamination and quality of fresh fish meats during storage, *Egypt.Acad. J. biolog. Sci*., **2(2),** 65-74 **(2010)**

12. Aberoumand A., Impact of freezing on nutritional composition of some less known selected fresh fishes in Iran. *International food research journal,* **20(1),** 347-350 **(2013)**

13. Arannilewa S.T., Salawu S.O., Sorungbe A.A. and Ola-salawu B.B**.,** Effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (*Sarotherodun galiaenus), African journal of biotechnology***,* **4(8),** 852-855 **(2005)**

14. Siddique M.N., Hasan M.J., Reza M.Z., Islam M.R., Boduruzaman M., Forhadur, M. and Reza S**.,** Effect of freezing time on nutritional value of Jatpunti (*Puntius sophore), Sarpunti (P. sarana) and Thaisarpunti (P. gonionotus*), *Bangladesh Research Publications Journal,* **5(4),** 387-392 **(2011)**

15. Le Blanc R.J. and Le Blanc E.L., Effect of superchilling with CO2 snow on the quality of commercially processed cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*) fillets, In Huss, H.H., (eds) Quality Assurance in the Fish Industry (115-124), Elsevier Science Publishers B.V. **(1992)**

16. Emire A.S. and Gebremariam M.M., Influence of frozen period on the proximate composition and microbiological quality of nile tilapia fish (*Oreochromis niloticus*), *Journal of Food Processing and Preservation,* **34,** 743-757 **(2009)**

17. Gandotra R., Koul M., Gupta S. and Sharma S., Change in proximate composition and microbial count by low temperature preservation in fish muscle of *Labeo rohita(Hambuch*), IOSR *Journal of pharmacy and biological sciences*, **2(1),** 13-17 **(2012)**

18. Sallam K.I., Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chem*., **2101(2),** 592–600 **(2007)**

19. Bao H.N.D., Araso S. and Iorarinsdottir K.A. Effects of dry ice and superchilling on quality and shelf life of Arctic charr (*Salvelinus alpinus*) fillets. *International journal of food engineering***, 3(3)/7,** 1-27 **(2007)**

20. Orak H.H. and Kayisoglu S., Quality changes in whole, gutted and filleted three fish species(*Gadus euxinus, Mugil cephalus, Engraulis encrasicholus*) at frozen storage period (-26ºC), *Acta Sci. Pol.,Technol.Aliment*., **7(3),** 15-28 **(2008)**

21. Jezek F. and Buchtova H., Shelf life of freeze thawed fillets of common carp (*Cyprinus carpio L.)* and silver carp (*Hypophthalmichthys molitrix V.*) packed under air, 20th Int. Symp. "Animal science days", Kranjska gora, Slovenia, Sept. 19th -21st, 2012 **(2012)**

22. Benjakul S. and Bauer F., Biochemical and physicochemical changes in cat fish (Silurus glanis) muscle as influenced by different freeze thaw cycles, *Food chemistry*, **72,** 207-217 **(2001)**

23. Jezek F. and Buchtova H., Monitoring of physicochemical changes in frozen fish muscle tissue. *Agriculturae conspectus scientificus*, **76(3),** 201-204 **(2011)**

24. Gandotra R., Sharm S., Koul M. and Gupta S., Effect of chilling and freezing on fish muscle), *IOSR Journal of pharmacy and biological sciences,* **2(5),** 5-9 **(2012)**

25. Rodriguez A., Losada V., Larrain M.A., Quitral V., Vinagre J. and Auborg S.P., Development of lipid changes related to quality loss during the frozen storage of farmed coho salmon (*Oncorhyncus kisutch*), *J. Amer oil chem. Soc.*, **84,** 727-734 **(2007)**

26. Erkan N. and Ozden O., Quality assessment of whole and gutted sardines (*Sardine pilchardus*) stored in ice, *International journal of food science and technology,* **43,** 1549-1559 **(2007)**

27. Ozogul Y., Boga E.B., Tokur B. and Ozogul F., Changes in biochemical, sensory and microbiological quality indices of common sole (*Solea solea*) from the mediterranean sea during ice storage, *Turkish Journal of Fisheries and Aquatic Science*, **11,** 243-251 **(2011)**

28. Ola J.B. and Oladipo A.E., Storage life of croaker (*Pseudotholitus senegalensis*) in ice and ambient temperatire, *Afr. J.Biomed.Res*., **7,** 13-17 **(2004)**

29. Liu S., Fan W., Zhong S., Ma C., Li P., Zhou K., Peng Z. and Zhu M., Quality evaluation of tray-packed tilapia fillets stored at 0°C based on sensory, microbiological, biochemical and physical attributes, *African Journal of Biotechnology,* **9(5),** 692-701 **(2010)**

30. Lawrie R.A., The storage and preservationof meat II. Moisture control, *In:Lawrie's Meat Science, 6th Edn.:* 191-194 **(1998)**