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## Research Article

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# INFLUENCE OF STEAMING ON THE PROXIMATE AND MICROBIAL QUALITY OF FROZEN MUSCLE OF *CYPRINUS CARPIO* (LINN.)

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(Received: January 30, 2015; Accepted: March 24, 2015)

### ABSTRACT

An experiment was carried out to study the effect of freezing on the proximate and microbial quality of raw and steamed muscle of *Cyprinus carpio* stored for a period of 21 days. The proximate composition (protein, lipid, moisture and ash) and microbiological analysis was carried out at an interval of 7 days. The total percental decrease was 8.84%, 24.37, 2.71%, 35.15% in raw samples and 6.50%, 21.96%, 2.12%, 20.48% in steamed samples respectively. A significant increase in protein & lipid content and decrease in moisture content was observed during steaming. Results also revealed that the protein, lipid, moisture and ash content decreased during the entire storage period in both the samples. However, the microbial load was found to be significantly increased ( $p \leq 0.05$ ) during the entire storage period. Results showed that the microbial count of steamed samples was significantly lower than those in control samples ( $p < 0.05$ ).

**Keywords:** Freezing, steaming, proximate, microbial, *Cyprinus carpio*.

### INTRODUCTION

Fish is known to be a source of protein rich in essential amino-acids (lysine, methionine, cysteine, threonine and tryptophan). Fish muscle also contains micro and macro-elements and fat-soluble vitamins (Larsen et al., 2007). 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 (FAO, 2008). Besides, it has high content of polyunsaturated fatty acids (PUFA's) which contributes to essential fatty acid requirement. Omega-3 fatty acid decrease triglycerides, lowers blood pressure, reduce blood clotting, boost immunity and improve arthritis symptoms, keep skin youthful and supple, reduce the risk of coronary heart disease and atherosclerosis, as well as preventing certain form of cancer and in children may improve learning ability. Moreover, Fish is also a good

source of minerals, like calcium, phosphorus, iron and sodium that are vital to our health. It also provides several other nutrients such as Vitamin A, E and K. Fishes also contain Vitamin B6 and B12, which help in producing RBC's, antibodies and maintain central Nervous System. Furthermore, many fishes are rich in antioxidants like Selenium and Coenzyme Q-10 that help to fight disease and promote health and longevity. It also provides a good combination of essential amino acids, well suited to human nutritional requirement along with the essential vitamins and minerals.

Though, fish is highly nutritious, it is one of the exceptionally perishable foods and cannot be kept for long time for human consumption. This is due to its short shelf life. Generally spoilage tends to vary according to species, feeding habits, seasonality, gender, age and possibly geographical location. Shelf-life extension can be achieved by various preservation

methods, i.e., refrigeration, freezing, salting, brining (wet salting), icing, smoking, glazing, drying, frying, steaming etc. Refrigeration and freezing help in preserving fish by lowering temperature. At low temperature, micro-organisms become inactive, enzymatic activity also slows down, thus biochemical activities decreases. Consequently, the fish remain free from spoilage for longer duration.

Keeping this in mind, the present work has been designed to generate information on the changes in proximate and microbiological composition of raw and steamed muscle of a fish (*Cyprinus carpio*) available in the local market of Jammu, stored in freezer ( $-12\pm 20^{\circ}\text{C}$ ) so as to determine their quality change during storage period of 21 days. This study has immense importance to satisfy consumer's query relating to and how long fish muscle can be stored without any deterioration in domestic refrigerator for the betterment of the public health.

## MATERIALS AND METHODS

### Sample collection

Fresh samples of *Cyprinus carpio* were purchased from local market of Jammu city. They were immediately brought to the lab in polythene bags along with crushed ice.

### Sample processing

The viscera of fish were removed and the fish was washed with large amount of water. The fish was cut into pieces and

- To prepare raw sample (control), these pieces were washed and immediately wrapped in aluminium foil, kept in air tight plastic container and stored at  $-12\pm 2^{\circ}\text{C}$  (frozen storage).

- To prepare steamed samples, 500ml of water was poured in to the bottom of the pot and heated to the boiling point, then these pieces were put on a middle layer of the pot (not in contact with water). After that, the muscle was cooled down and wrapped in aluminium foil, kept in air tight plastic bags and stored at  $-12\pm 2^{\circ}\text{C}$  (frozen storage).

Analytical procedures for biochemical and microbiological changes were done on 0, 7th, 14th and 21st day of storage in both the samples.

### Analyses

The proximate composition (ash and moisture) of the fish samples were evaluated using the standard AOAC procedure (AOAC, 1995). The protein content was determined using the Lowry et al. (1951). Fat content was

determined using Folch et al. (1957). The microbiological profile was determined according to APHA method (1984). Data were expressed as mean  $\pm$  SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

## RESULTS AND DISCUSSION

### Chemical Analysis

The proximate composition of raw and steamed muscle of *Cyprinus carpio* during storage period of 21 days has been shown in the table no. 1- 4.

**Protein Content:** Results shown in table 1 and 2 revealed that the total protein content of raw and steamed muscle showed a decreasing trend with increase in storage period. Initially, the protein content of steamed sample was found to be higher i.e.  $18.76\pm 0.05\%$  than that of raw sample i.e.  $16.85\pm 0.03\%$ . After that, it showed a decreasing trend. The total percent decrease was found to be 8.84% in raw samples and 6.50% in steamed samples on 21st day of storage. These results are in accordance with those of Hakimeh et al., (2010), Koubaa et al., (2012) and Zhang et al., (2013). They stated that the increase in protein content was due to the loss of water during steaming process. The low protein content in raw samples was perhaps mainly due to the increased microbial growth and higher water activity. In support with the present studies Keyvan et al., (2008) in Caspian white fish (*Rutilus frisii kutum*), El-Deen and El-Shamrey (2010) in Gahsh (*Lethrinus elongatus*) and Aberoumand (2013) 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 nitrogen substances formed by bacterial decomposition that escaped from tissue during frozen storage.

**Lipid Content:** In present investigation, a decreasing trend was observed in Total lipid content of both raw and steamed samples for a period of 21 days. Perusals of table 1 and 2 revealed that initially the lipid content of steamed muscle was found to be higher i.e.  $3.05\pm 0.01\%$  than that of raw sample i.e.  $2.01\pm 0.04\%$ . After that, it showed a decreasing trend. The total percent decrease was found to be 24.37% in raw samples and 21.96% in steamed samples on 21st day of storage. According to Sehgal et al. (2008) the total lipid content in raw fish mince was 0.34% which increased to 0.4% in steamed fish mince pakoras and to 3.36% in cooked

**Table1.** Proximate composition (wet weight basis) of raw fish muscle of (*Cyprinus carpio*) stored in freezer at  $-12\pm 2^{\circ}\text{C}$  during 21 days of storage

| Days              | 0 day                    | 7 <sup>th</sup> day      | 14 <sup>th</sup> day     | 21 <sup>st</sup> day     |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Total Protein (%) | 16.85 <sup>a</sup> ±0.03 | 16.03 <sup>b</sup> ±0.07 | 15.74 <sup>c</sup> ±0.02 | 15.36 <sup>d</sup> ±0.05 |
| Total Lipid (%)   | 2.01 <sup>a</sup> ±0.04  | 1.83 <sup>b</sup> ±0.02  | 1.65 <sup>c</sup> ±0.01  | 1.52 <sup>d</sup> ±0.03  |
| Moisture (%)      | 83.65 <sup>a</sup> ±0.03 | 83.01 <sup>b</sup> ±0.04 | 82.42 <sup>c</sup> ±0.02 | 81.38 <sup>d</sup> ±0.01 |
| Ash (%)           | 2.19 <sup>a</sup> ±0.02  | 1.94 <sup>b</sup> ±0.06  | 1.76 <sup>c</sup> ±0.03  | 1.42 <sup>d</sup> ±0.07  |

--Mean±SD with different superscripts in a row differs significantly (P<0.05)

**Table2.** Proximate composition (wet weight basis) of steamed fish muscle of (*Cyprinus carpio*) stored in freezer at  $-12\pm 2^{\circ}\text{C}$  during 21 days of storage.

| Days             | 0 day                    | 7 <sup>th</sup> day      | 14 <sup>th</sup> day     | 21 <sup>st</sup> day     |
|------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Total Protein(%) | 18.76 <sup>a</sup> ±0.05 | 18.18 <sup>b</sup> ±0.08 | 17.85 <sup>c</sup> ±0.2  | 17.54 <sup>d</sup> ±0.03 |
| Total Lipid (%)  | 3.05 <sup>a</sup> ±0.01  | 2.87 <sup>b</sup> ±0.04  | 2.59 <sup>c</sup> ±0.07  | 2.38 <sup>d</sup> ±0.15  |
| Moisture (%)     | 81.54 <sup>a</sup> ±0.03 | 81.26 <sup>b</sup> ±0.05 | 80.64 <sup>c</sup> ±0.01 | 79.81 <sup>d</sup> ±0.06 |
| Ash (%)          | 2.05 <sup>a</sup> ±0.02  | 1.95 <sup>b</sup> ±0.04  | 1.79 <sup>c</sup> ±0.09  | 1.63 <sup>d</sup> ±0.1   |

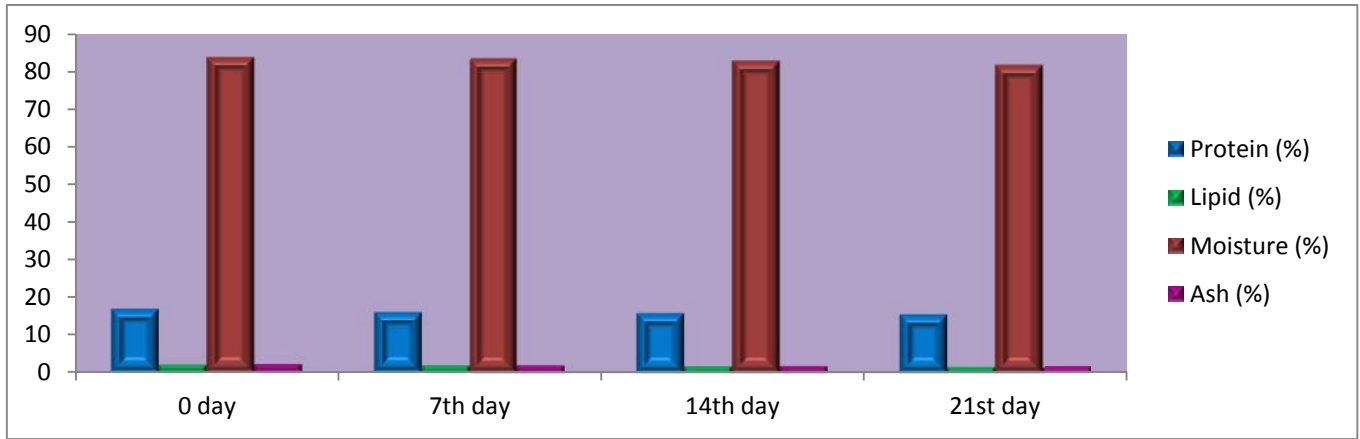
--Mean±SD with different superscripts in a row differs significantly (P<0.05)

**Table 3.** Percental decrease in proximate composition of raw muscle of *Cyprinus carpio* during frozen storage at  $-12\pm 2^{\circ}\text{C}$  from 0 day to 21<sup>st</sup> day.

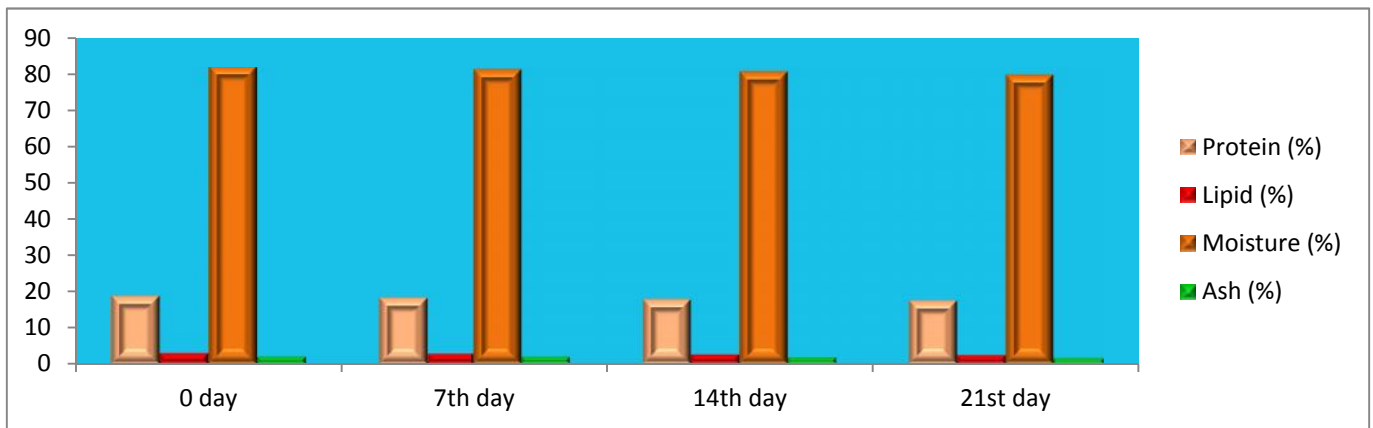
| Days | Protein (%) | Lipid (%) | Moisture (%) | Ash (%) |
|------|-------------|-----------|--------------|---------|
| 0-7  | 4.86        | 8.95      | 0.76         | 11.41   |
| 0-14 | 6.58        | 17.91     | 1.47         | 19.63   |
| 0-21 | 8.84        | 24.37     | 2.71         | 35.15   |

**Table 4.** Percental decrease in proximate composition of steamed muscle of *Cyprinus carpio* during frozen storage at  $-12\pm 2^{\circ}\text{C}$  from 0 day to 21<sup>st</sup> day.

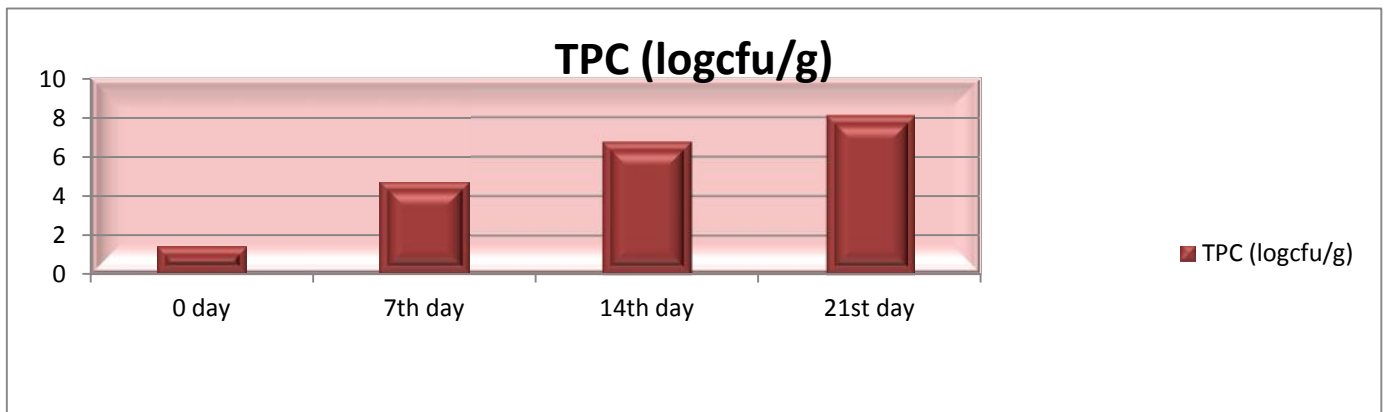
| Days | Protein (%) | Lipid (%) | Moisture (%) | Ash (%) |
|------|-------------|-----------|--------------|---------|
| 0-7  | 3.09        | 5.90      | 0.34         | 4.87    |
| 0-14 | 4.85        | 15.08     | 1.10         | 12.68   |
| 0-21 | 6.50        | 21.96     | 2.12         | 20.48   |



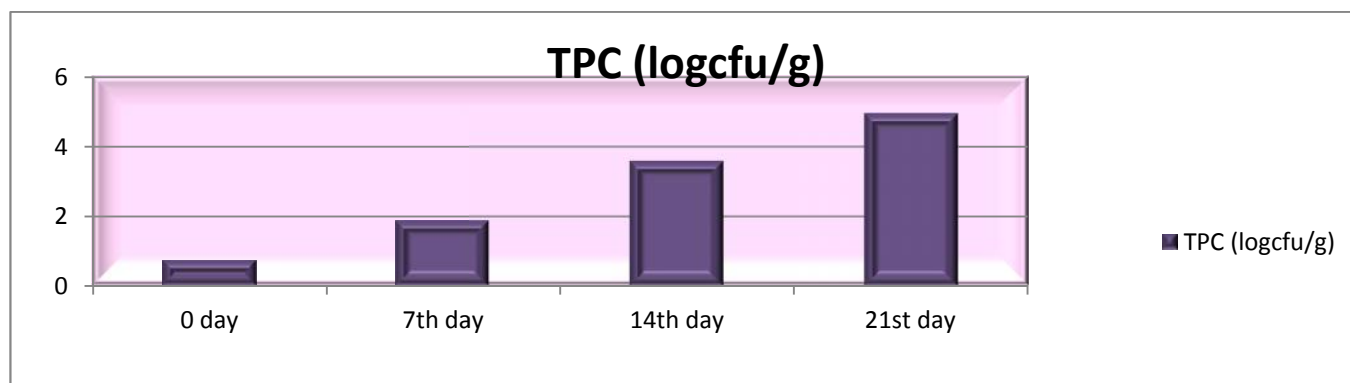
**Figure 1:** Change in Proximate composition (wet weight basis) of raw muscle (*Cyprinus carpio*) subjected to frozen storage for 21 days.



**Figure 2:** Change in Proximate composition (wet weight basis) of steamed muscle (*Cyprinus carpio*) subjected to frozen storage for 21 days.



**Fig 3:** Change in Total Plate Count of raw muscle of *Cyprinus carpio* stored at 12±2°C for up to 21 days.



**Fig 4:** Change in Total Plate Count of steamed muscle of *Cyprinus carpio* stored at  $-12\pm 2^{\circ}\text{C}$  for up to 21 days.

fish mince pakoras. The increase in fat content in steamed samples was due to the loss of moisture (Koubaa et al., 2012). In support of present findings ozogul et al.(2011) in Common sole (*Solea solea*) found significant increase in peroxide value(PV) and decrease in lipid content during 24 days of storage. The decrease in lipid content was associated to the fat hydrolysis. Similarly Siddique et al. (2011) found that total lipid content decreased during frozen storage of three species of *Puntius*.

**Moisture Content:** Results shown in table 1 and 2 revealed that the moisture content decreased significantly from  $83.65 \pm 0.03\%$  to  $81.38 \pm 0.01\%$  in raw and  $81.54 \pm 0.03\%$  to  $79.81 \pm 0.06\%$  in steamed muscle on 21st day of storage at  $-12\pm 2^{\circ}\text{C}$ . The total percent decrease in moisture content was found to be 0.76%, 1.47%, 2.71% in raw and 0.34%, 1.10%, 2.12% in steamed samples on 7th, 14th and 21st day respectively during frozen storage of 21 days. In support of present findings Bhat et al., 2010 also observed decreasing trend (15.77% decreases) in 7 days of storage in moisture content of *Chevon Harrisia* with storage time at  $4\pm 10^{\circ}\text{C}$ . They associated this loss of moisture on refrigerated storage with the surface loss of moisture by refrigeration and due to the poor moisture barrier offered by the packaging material. The decrease in moisture content was attributed to the sublimation of ice in frozen storage and drip loss during thawing process (Benjakul et al. 2005). Similarly, Akter et al., (2012), Gandotra et al., (2012), Aberoumand (2013), Gandotra et al., (2013) and Mahmoud Sharaf (2013) also observed decreasing trend in moisture content of fish muscle under frozen storage conditions.

**Ash Content:** The ash content of fish sample decreased from  $2.19\pm 0.02\%$  to  $1.42\pm 0.07\%$  in raw and  $2.05\pm 0.02\%$

to  $1.63\pm 0.1\%$  in steamed sample on 21st day of storage. The total percent decrease was 20.48% and 35.15% in steamed and raw samples respectively. In favour of present findings Okeyo et al., (2009) in Nile Perch and Emire et al., (2009) in Nile Tilapia fish (*Oreochromis niloticus*) found a decrease in total ash content during its frozen storage. The decrease in ash content was associated to the drip loss during thawing process. Similar results were obtained by Gandotra et al., (2012), Gandotra et al., (2013) and Mahmoud Sharaf (2013).

#### Microbial Analysis:

For the determination of microbial quality of fish before and frozen storage, Total Plate Count (TPC) was analysed.

#### Total Plate Count:

Results shown in fig. 3 and 4 revealed that the Total Plate Count increased with increase in storage period in both the samples. In raw samples, Total Plate Count was found to increase from  $1.38\pm 0.01$  log cfu/g to  $8.10\pm 0.03$  log cfu/g and in steamed samples from  $0.72\pm 0.01$  log cfu/g to  $4.96 \pm 0.04$  log cfu/g. In present studies, it has been found that the TPC in steamed samples was within the permissible limit i.e.6 log cfu/g (ICMSF, 1986) up to 21s day and in raw samples, it crossed the permissible limit after 7th day. TPC for steamed fish muscle shows comparatively slow increment as compared to raw muscle which is because of the significant water loss during steaming and frying process. These findings get a strong support from findings of Subramanian (2007) who attributed a significant affect of cooking process on decreased bacterial load of cuttle fish from  $2.6\times 10^7$  CFU/g in raw crab to  $6.57\times 10^6$  CFU/g in cooked crab and  $2.4\times 10^7$  CFU/g in raw cuttlefish to  $9.7\times 10^6$  CFU/g in cooked cuttlefish. Similar findings were

reported by Marshall et al. (2006) who showed a slight bacteria count (i.e., only >5 CFU/ml) in fish muscle after high pressure treatment stored at 4°C and negligible counts at 3 day of storage period but recorded 10 CFU/ml on 6th day of storage. They further advocated these variations probably occurs due to plating contamination and the observed load of bacteria during storage i.e., on 3rd day onwards, may be resulted due to cross contamination of the sample during transfer to the package for storage and handling during experimentation process. Similar findings were also encountered during present investigations. Obemeata et al (2011) showed a significant increase in bacterial count when Tilapia was subjected to frozen storage at -18°C than at 4°C. They stated that freezing of fish at -18°C created unfavourable environmental conditions for the growth and the survival of the micro-organisms.

#### REFERENCES

1. 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.
2. Akter, T., Begum, R., Abu Ahmed, A.T., Khaleque, M.A. and Begum, M., Comparative studies on some undesirable quality changes during freezing preservation of Tilapia (*Oreochromis niloticus*, Hamilton-Buchanan, 1822) and Bele fish (*Glossogobius giuris*, Linnaeus, 1766) at laboratory condition. *Trends In Life Sciences*, 1(2): 20-23.
3. AOAC., *Official Methods of Analysis*. 16th Edn., Association of Official Analytical Chemists, Washington DC., USA.
4. APHA., *Compendium of method of microbiological examination of foods*. 2nd Edn., American Public Health Association, Washington DC.
5. Benjakul, S., Visessanguan, W., Thongkaew, C. and Tanaka, M., Effect of frozen storage on chemical and gel forming properties of fish commonly used for surimi production in Thailand. *Food hydrocolloids*, 19:197-207.
6. Bhat, Z.F., Pathak, V., Bukhari, S.A.A., Ahmad, S.R. and Bhat, H. , Quality changes in Chevon Harrisa (Meat based product) during refrigerated storage. *Intr. J. Meat Sci.*
7. El-Deen, G. and El-Shamery, M.R., Studies on contamination and quality of fresh fish meats during storage. *Egypt. Acad. J. Biolog. Sci.*, 2(2): 65-74.
8. 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.
9. FAO. Report of the FAO/SWIOFC Regional Workshop on Safety at Sea for Small-scale Fisheries in the South West Indian Ocean. Moroni, Union of the Comoros: Pp 76.
10. Folch, J., Less, M. and Sloane, G.W.S. (1957). A Simple Method For The Isolation And Purification Of Total Lipids From Animal Tissues. *J. Biol. Chem.* 226, 497-509.
11. 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 (Ham-Buch). *IOSR Journal of Pharmacy and Biological Sciences*, 2 (1): 13-17.
12. Gandotra, R., Gupta, V., Koul, M. and Gupta, S., Quality changes in the muscles of Wallago attu during frozen storage (-12±2°C) conditions. *Res.J.Animal, Veterinary and Fishery Sci.* 1(5): 16-20.
13. Hakimeh, J.A., Akram, A.A., Bahareh, S. and Alireza, S.M., Physicochemical and sensory properties of silver carp (*Hypophthalmichthys molitrix*) fillets as affected by cooking methods. *International Food Research Journal*, 17: 921-926.
14. International Commission on Microbiological Specifications for Foods (ICMSF), Sampling plans for fish and shellfish, In: *Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Scientific Applications*, 2(2) University of Toronto Press, Toronto, Canada: 181-196.
15. 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 frisii kutum*). *J. Fish. Aquatic Sci.*, 3: 404-409.
16. Koubaa, A., Mihoubi, N.B., Abdelmouleh, A. and Bouain, A., Comparison of the effects of four cooking methods on fatty acid profiles and nutritional composition of Red Mullet (*Mullus barbatus*) muscle. *Food Sci. Biotechnol.*, 21(5): 1243-1250.
17. Larsen, R., Stormo, S.K., Dragnes, B.T. and Elvevoll, E.O., Losses of taurine, creatine, glycine and alanine from cod (*Gadus morhua* L) fillets during processing. *J Food Compos. Anal.*, 20: 396-402.
18. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
19. Mahmoud Sharaf, M., Influence of domestic freezing on the biochemical composition and mineral contents of fish muscles. *Egypt. Acad. J. Biolog. Sci.*, 5 (1): 11-16.
20. Obemeata, O., Nnenna, F.P. and Christopher, N., "Microbiological assessment of stored Tilapia guineensis", *Afric. J.Food Sci.* 5(4):242-247.
21. Okeyo, G.O., Lokuruka, M.N.I. and Matofari, J.W., Nutritional composition and shelf life of the lake victoria Nile perch (*Lates Niloticus*) stored in ice. *African Journal of Food Agriculture and Nutrition and Development*, 9(3): 901-919.

22. 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.
23. Subramanian, T.A., Effect of processing on bacterial population of Cuttle fish and Crab and determination of bacterial spoilage and Rancidity developing on frozen storage. *Journal Of Food Processing And Preservation* ,31(1): 13 – 31.
24. Zhang, J., Wu, D., Liu, D., Fang, Z., Chen, J., Hu, Y. and Ye. X., Effect of cooking styles on the lipid oxidation and fatty acid composition of Grass carp (*Ctenopharyngodon idellus*) fillet. *Journal of Food Biochemistry*, 37(2): 212-219.