Int.J.Curr.Microbiol.App.Sci (2026) *15*(7): 106-112

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 7 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1507.011

Implementation of Hazards Analysis Critical Control Point (HACCP) On Fish Marketing Before and After Freezer Storage in Khartoum State

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Keywords

Microbial contamination, marketing, *Clarias lazera, Tetraodon fahaka,* storage

Article Info

Received:
05 May 2026
Accepted:
26 June 2026
Available Online:
10 July 2026

A B S T R A C T

Fishes are a sensitive food which had a rapid rate of spoilage if they were not handled properly. The action of spoilage begins immediately after rigor mortis. When fish is caught, the subsequent steps of handling play great role on keeping quality and safety of fish. Two hundred of swab samples were collected, 100sample from Central market and 100samples from Al-Mawrda market, each 100 samples divided into, 50 samples type 1 (*Clarias*), 50 samples type 2 (*Tetradon*), which were taken from five critical control points, fish skin, fish flesh, hands of employees, knives and tables of cutting. Then preserved in freezer at-18Co for (0, 14, 28days). Total bacterial count (TBC) analyses were performed using accredited methods, which showed no significant difference between the two markets, but the storage period was significantly affected at $p<0.015$ on the two typesof fish that (TBC) decreased with the increase of storage period and showed the higher rate of contamination before storage on (0) day for the two types of fish at all points of risk factors followed with that after 14 days and finally the lowest one after 28 days. In this study, TBC for all samples ranged between 8.17 to 8.21Log10 CFU/cm2 which was not within the acceptable food safety limits.

Introduction

Fish are the most than numerous of the vertebrates, with at least 20.000 known kinds, and more than half (58%) found in the marine environment and most common in the warm and temperate waters of the continental shelves (8000 kinds) In the cold polar water, about 1, 100 species are found.In the oceanic pelagic environment well away from the effect of land, there are only 225 species, Surprisingly, in the deeper mesopelagic zone of the pelagic environment (between 100 and 1000m depth) the number of species increases there are 1000 species of so-called mid-water fish (Webber and Thurman, 1984).

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Karrar, 2007).

Clarias lazera is a mud catfish belongs to the family *Clariidae.* This specie has maintained its aquaculture quality because of its high growth rate, large size, good flesh quality, tolerance to poor water quality even at larval stages, acceptance of cheap feeds and ability to withstand high stocking densities, disease resistance and good taste (Hongendoorn, 1981).

The family *Tetradontidae,* also popularly Known as *Pufferfish*, is the most specious group of the order *Tetraodntiformes* and is widespread across many tropical and subtropical regions of the world, it includes species that are typically marine, with occasional entering and occurring in brackish and fresh waters (Nelson, 2006).

Fish begins to spoil as soon as it is caught, perhaps even before it is taken out of the water. Therefore, the key to delivering high quality product is close attention to small details throughout the entire process of preparation, catching, landing, handling, storage, and transport. Fish that becomes spoiled or putrid is obviously unusable (Gopakumar, 2000). Austin, (2002) illustrated that the muscles of health fish were sterile and microorganism count in skin, gills and gastrointestinal tract of fin fish vary depending on the area of catch, however, the skin typically contained 104 cfu/cm2, the gills contained 106cfu/g and the digestive tract contained to 108 cfu/g. The limit of the total viable count (TVC) not exceeding than 106 cell/gram in whole raw fish and 104 cell /gram in raw fish fillets as reported by the Egyptian Standard Specification (2008). The evaluation of freshness quality of fish is becoming popular in modern research and development to ensure food safety (Dalgaard *et al.,* 2006; Cheng, 2015). Avariety of the indices such as sensory attributes, microbial contamination and chemical changes have been used for the assessment of fish quality during harvesting, distribution and storage (Alam, 2007; Oehlenschlager, 2013; Cheng *et al.,* 2015).

The freezing process partially or completely hinders disruptive microbiological or enzymatic reactions, nevertheless, it cannot repair damage already caused. The fish should be frozen after handling for a better quality. The great advantage of the freezing process is the ability to achieve stability without damaging the initial quality (Amin *et al.,* 2023)

The HACCP system was introduced in 1971 during the National food protection conference. Sea food processors must keep in mind that HACCP does not replace GMPs or guard against fraudulent practices (Russell *et al.,* 2004) marketing in Sudan, mostly carried out in an environment exposed to direct sun heat and dust. The market is just a piece of landscape fenced with rabbit wire where inadequate fish filleting plat forms have been built. Fish marketing is largely managed by private individuals. Similar observations were reported in Tanzania by (Rupamoarthy, 1982).

Materials and Methods

Area of survey

The survey was conducted in AL-Mwrada and central fish markets to assess the effect of personal practices on microbial contamination of fish before and after freezer storage.

Sampling

A total of 200 swab samples were taken randomly from Al-Mwrada and central fish market for *Clarias lazera* and *Tetradon fahaka* fish species. 100 swab samples were taken from each fish market (50 samples for *Clarias* lazera.) and (50 samples for *Tetradon fahaka)* from 5 critical control points, fish skin, fish flesh, hands of employees, knives and tables, then preserved in freezer at-18C°. Random samples were taken for microbial analysis after (0, 14, 28) days respectively.

Statistical analysis

The data was collected as means of triplicate s± Standard Deviation (SD). For statistical analysis, the differences among the fish markets, fish species and risk factors data were assessed using the SPSS 11.5 at P <0.05 degree of significance. The difference among the storage durations had been determined by analysis of variance (ANOVA).

Results and Discussion

Total Bacterial Count: Bacteriological analysis showed the effect of market, (source) and storage period on the total bacterial count (TBC), for the two types of fish at different risk factors in each Central and Al-Mwrada market. Table (1) showed the TBC range (8.17-8.24 $\log_{10}$ cfu/cm2), there was no significant difference between the two markets for skin flesh, knives, hands and tables at $p<0.05$. The two types of species, type 1 (*Claries*), type 2 *(Tetradon)* were significantly different at skin and flesh $p<0.01$, but there was no significant difference at hands,

knives and tables $p<0.05$. On the other hand, the storage period as in table (1) affected significantly $p<0.01$ on TBC at (0-14-28) days for all risk factors. Table (2) showed, the Interaction effects among fish types and storage period for the total bacterial count at different risk factors, that the higher rate of contamination was recorded before storage (0) day for the two types of fish at all points of risk factors followed by that after 14 days and finally the lowest one after 28 days at $p<0.01$.

Researchers studied interactions between fish and bacteria (Olafsen, 2001, Austin 2002), because fishes are found in water, they are affected by the bacterial load and bacteria species present in water and sediments (Olafsen, 2001). Some researchers reported higher bacterial loads on/in fishes present in polluted waters (Zmyslowska *et al.,* 2001 and Ali *et al.,* 2015). It is difficult to differentiate between the indigenous microflora and transient microflora on/in fish, which could be present in the water film around fish (Austin *et al.,* 2002), moreover extraneous bacteria are capable of surviving in fish (Del Rio Rodriguez., 1997). This study clarified that, the total bacterial count for fresh fish was ranged between 8.17-8.21 $x10^5$.log_{10}cfu/cm^2 which disagree with the limit reported by SSMO (Sudanese Standards and Metrology Organization, SDs 357) which was 5 x 10^5. cfu/g for fresh fish product. According to (ICMF 1986) and (Atiken *et al.,* 1982) who say that, any fish that have more than $x10^6$ bacteria count in one gram is not suitable for human consumption, but since gut and gill are always being removed and discarded, there is a tendency for safety.

The results also did not agree with the study of (Kapute *et al.,* 2013) Who foundthat, the total bacterial count reach 2.1 x 10 log_{10} cfu/cm^2 and (Hisham, 2017) who reported the total count of bacteria 2.50 log_{10} cfu/cm^2, that may be due to good handling in their studies. However, (Okoro 2010) found the range of bacteria count in different fish $8.7x10^5$ cfu/g in Mullet Nigerian Marine fish which agree with the presentresults.

Comparing between the risk points in the two markets, Centeral Market and Al-Mwrada market, reported highest TBC in flesh (8.25 $x10^5$. cfu/cm^2), the lowest (8.17 $x10^5$. cfu/g,) in hands as in Al-Mwrada Market however, there was no significant difference among all points, which did not agree with (Alam *et al.,* 2014) who found TBC range in two retail fish markets between 5.08 to 6.63log_{10} CFU/g and in super shops 3.74 to 5.81 log_{10} CFU/g, (Islam *et al.,* 2021) found the total plate counts (TPC) in all five fish species from the retail markets 'and super shops in Dhaka city for the fishes collected from the retail markets, is higher than the samples from the super shops. Also, a significant difference ($p<0.05$) in TPC in three species between retail markets and super shops. (Mhango *et al.,* 2010; Hossain and Barman, 2016) found, high microbial contamination of the fishes in retail markets that agreed with the results in this research and it may be due to no or delay in icing, rough handling, compactness, lack of sanitation, contaminated water, contaminated ice, soiled surfaces and boxes. Upon Comparing the two types in the two markets, there was no significant difference among knives, hands and tables, but there was significant difference between flesh and skin, however type 1 reported the highest TBC.

Arafat (2013) found bacterial load in fish skin between 8 x 10^3 log_{10}cfu\g to 9 x 10^4 log_{10}cfu\g in Ed Dueim fish market that agreed with the results in this study. The freezing process partially or completely hinders disruptive microbiological or enzymatic reactions, nevertheless, it cannot repair damage already caused. The fish should be frozen after handling for a better quality. The great advantage of the freezing process is the ability to achieve stability without damaging the initial quality. This study clarified that, type (1) reported highest TBC at (0) day 8.53+0.10 in hands while the lowest TBC 7.88+0.37 after (24) days on knives, however the highest TBC in type (2) in (0) day 8.57+0.06 on flesh but lowest TBC7.74+0.39 after (24) days in hand, The decrease of TBC with the increase of the storage period might be due to the impact of frozen storage on the bacteria. These results are relatively agreed to those obtained by (Nanto *et al.,* 1993), the spoilage in fish can be reduced by maintain low temperature and good handling during unloading fish catch,

The rate of spoilage occurs varies with species of fish, handling method, hygienic condition and chilling storage method, (Mahmoudzadeh, *et al.,* 2010) reported that burgers from P. *elevatus* and *S.undosquamis* had low number of microorganisms at the end of storage period (-18 ○), and (El-Sherif *et al.,* 2011) found that the total bacterial count (TBC) of raw Mullet fish during storage period at -18° were 2.01, 4.32, 3.05 and 2.65log_{10} cfu /g sample after 0, 60, 120 and 180 days of storage, respectively, while TBC of raw Tilapia fish were 2.35, 5.75, 4.15 and 2.95 log_{10} cfu/g sample after the same periods of frozen storage the first and simples method to both preserve and process fish is to keep it cool.

Table.1 Effect of Storage period on Total Bacterial Count for Clarias lazera and Tetradon fahaka fish in Central and Al-Mwrada fish markets at different risk factors.

Main effect	Total bacterial count (log10 cfu/cm2) per risk factor				
	Skin	Flesh	Knives	Hands	Table
Overall	8.21	8.24	8.20	8.19	8.22
SEM+	0.017	0.013	0.017	0.018	0.016
Fish source (markets)					
Central	8.23	8.24	8.23	8.21	8.23
Al-Mwrada	8.19	8.25	8.18	8.17	8.22
Standard error of mean	0.024	0.019	0.023	0.026	0.023
Significant	NS	NS	NS	NS	NS
Fish species					
Type 1	8.24	8.27	8.19	8.22	8.23
Type 2	8.17	8.22	8.21	8.16	8.22
Standard error of mean	0.024	0.019	0.023	0.026	0.023
Significant	*	*	N.S	N.S	N.S
Storage period/day					
0	8.35^{a}	8.55^{a}	8.52^{a}	8.55^{a}	8.51^{a}
14	8.17^{b}	8.20^{b}	8.17^{b}	8.17^{b}	8.18^{b}
28	7.92^{c}	7.97^{c}	7.87^{c}	7.85^{c}	7.98^{c}
Standard error of mean	0.024	0.019	0.026	0.026	0.023
Significant	**	**	**	**	**

For this table and subsequent tables:
N=30 fish sample/species
**=Significant difference at p<0.01, *=significant difference at p<0.05-N. S=No significant difference.
Means with different superscript letters in the same column significantly different at p<0.05.
Type1=*Clarias lazera.*
Type2=*Tetradon fahaka*

Table.2 Total Bacterial Count of Fish Species at Different risk Factors

Factors		Total bacteria count (log10 cfu/cm2) per risk factor				
Fish species	**Storage period (days)**	**Skin**	**Flesh**	**Knife**	**Hand**	**Table**
Type 1	0	8.51+0.11a	8.53+0.07a	8.53+0.09a	8.53+0.10a	8.52+0.09a
	14	8.18+0.11b	8.20+0.08b	8.17+0.11b	8.16+0.10b	8.18+0.11b
	24	8.04 + 0.10*c*	8.07+0.012c	7.88+0.37c	7.97+0.23c	8.00+0.31c
Type 2	0	8.55+0.08a	8.57+0.06a	8.52+0.10a	8.57+0.07a	8.50+0.23a
	18	8.17+0.011b	8.21+0.013b	8.21+0.10b	8.18+0.10b	8.19+0.11b
	24	7.80+0.039d	7.87+0.028d	7.92+0.22c	7.74+0.39d	7.96+0.08c
Significant		**	**	**	**	**

Cooled fish kept longer than uncooled fish, although both will spoil in a matter of hours (Tawari and Abowei, 2011). Arannilewa *et al.,* (2005) found total bacterial count increase 3.0 x 103-5.6 x 104 -6.5x104-6.7x104-4.0x 106-7.5 x 106 log_{10}cfu\g in (0) day to 60 days respectively the increasing total coliform counts of fish is best before frozen storage and that quality of frozen fish is better achieved in the first ten days of storage.

(Eman 2021) found that, the total bacterial count was 4.10, 2.11 and 2.71 (cfux10^3/g) /at zero, 6 and 12months for frozen Catfish fillets, respectively. In addition, there were significant differences ($p<0.05$) between 6and 12 months of frozen storage also as reported by (Alam *et al.,* 2005; Mahmoud, 2004). Thatcher and Clark, (1973) earlier reported that, the kind and number of microorganisms found on frozen fish is dependent on the source of the fish, additional contamination introduced in the fishing boat, freezing temperature during storage, the severity of freezing process with respect to lethality to microorganisms and contamination by handlers and market sellers. For this, it, therefore, calls for great attention to handle fish in more hygienic ways to keep up to the microbiological standard of the use of fishing equipment which often serves as a route of contamination of frozen fish and storage environment. Brooks *et al.,* (2004) however, concluded that one of the sources of infection is contaminated food.

Foodstuffs safety are generally ensured by a preventive approach, which could be achieved by the implementation of the practice of good hygiene and application of the procedures based on hazard analysis and critical control point (HACCP) principles. Popovic *et al.,* (2010), have therefore reported that microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. Some of the isolated microbes might not be directly associated with the fish but it cleared that microorganisms localized in frozen fish and it is a message that fish must be properly processed either by cooking, frying or drying for safe consumption.

In conclusion, Fish preservation and processing is a very important aspect for fisheries. Poor hygiene practices during fish marketing make fish highly hazardous food. Freezing technology is a good method for keeping quality and safety of fish.

Acknowledgement

The authors have great appreciation to all the staff of the college of Veterinary Medicine.

Author Contributions

D. H. Enas: Investigation, formal analysis, writing—original draft. M. M. Maha: Validation, methodology, writing—reviewing. A. A. Mohamed:—Formal analysis, writing—review and editing. A. S. Ali: Investigation, writing—reviewing. Ramzy A. Yousif: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Enas DifAlla Hashim Mohmmed1, Maha Mubarak Mohammed, Mohamed A. A., Ali A. S. and Ramzy A. Yousif. 2026. Implementation of Hazards Analysis Critical Control Point (HACCP) On Fish Marketing Before and After Freezer Storage in Khartoum State. *Int.J.Curr.Microbiol.App.Sci.* 15(7): 106-112.
doi: https://doi.org/10.20546/ijcmas.2026.1507.011