Effect Of Microbes On Proximate Contents Of Stored Smoke-Dried African Cat Fish (Clarias Gariepinus) Sold In Ibadan Markets

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Abstract: Fish is the commonest source of protein for many homes all over the globe. Smoking as one of the methods for preserving fish is often adopted in most localities in Nigeria hence they are liable to spoilage soon after harvest. Five smoke-dried Clarias gariepinus each were purchase from one vendor in the surveyed four markets in Ibadan city for microbiological and proximate analyses for 60 days. General increase in microbial counts along storage period was observed. However, samples from market D had the highest microbial counts (2.64×10^4 – 4.50×10^4 Cfu/g) followed by fish samples from market B with load range of between (2.41×10^4 - 4.36×10^4 Cfu/g) and least count in samples from market A with counts of that range from 2.16×10^4 – 2.91×10^4 Cfu/g). Species of Staphylococcus aureus and Escherichia coli were isolated from all purchased fish samples while Salmonella species was only isolated from fish samples obtained from markets C and D. Proximate composition of the fish samples featured increase in moisture content, while other determined proximate parameters were in decrease values alongside days of storage. These resulted in the significant reasons to deduce reduction in the amount of nutrients of the fish samples. Proper processing and appropriate methods of storage is necessary to avoid microbial interactions that will sustain fish nutrients and extend shelf life after harvest.

Index Terms: Analysis, Smoke-dried, Fish, Market, Commercial, Stored

1. INTRODUCTION

Both the poor and rich in developing and developed countries of the world feed on fish diet or supplement on daily basis. In many regions where carbohydrate based foods is the food of choice for many due to poverty, fish is the common source of protein as they are readily available, affordable in markets and trapped in many surrounding streams and rivers.The various processing possibilities of fish are cooking, fermenting, drying, brining and frying among others. These processing forms of fish have preference in terms of taste or aroma, flavour, colour and texture for individual's choice; and also to extend its shelf life; hence fish is a perishable food that is easily degraded by microorganisms and enzymes soon after harvest to cause spoilage.In Nigeria, cultured and wild fish are available for sale to consumers in form of frozen, sun dried or fresh form (Akinwumi and Adegbehingbe, 2015) Particularly, where electricity supply is unreliable, at best about 70-80% of domestic catfish is smoke-dried as this is the most affordable and practicable method of preservation (Akinyemi et al., 2012). In Nigeria, smoked fish could be liable to contamination with microorganisms from hawking vessels and market environments before reaching the consumers because many hawkers or fish vendors usually display the fish in unprotected manners from microbial contamination.In many instances, left over fish are re-dried on fire by fish vendors to extend the shelf life. These re-smoked fish however are then mixed up with freshly purchased dried fish for consumers. The heat and smoke associated with the smoke-drying process has been reported to cause denaturation of protein content of fish which in other words alter the nutritional value.

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This method of vendors mixing old and newly smoked fishes for purchase by consumers with no regard for differing storage period is worrisome on the safety and quality of the smoke-dried fish offered for sale in the open markets (Ikutegbe and Sikoki, 2014) The criteria for consumer's choice for buying smoke-dried fish are the physical qualities such as firmness, wholesomeness, flavour and attraction. These characteristics however left out the microbiological quality that could stand an important aspect for evaluating quality and safety of the fish products. Following the wider concept of food quality, different attributes cover different dimensions from healthy and production method to environmental and social orientation (Moser et al., 2011). Since such characteristics cannot be verified, credence attributes require standards or communication to be communicated and to ensure consumers (Meixner and Haas, 2016; Sheldon, 2017)The aims of this study, therefore is to evaluate the microbiological and proximate composition of smoke-dried African catfish (Clarias gariepinus) sold in markets in Ibadan city.

2 MATERIALS AND METHODS

2.1 Collection of fish samples

Clarias gariepinus is a valued fish product in Nigeria for its cultivability in homes and wide spread in many streams and rivers; and its high demand for pepper soup delicacy, desirable taste and it's wholesome in soups and stews. Five smoke-dried C. gariepinus were purchase from one vendor each in four different markets in Ibadan city. They were packaged separately in sterile cellophane bags and represented with identity A, B, C and D. This gives a total of twenty smoke-dried fish samples for the experiment.The purchased fish samples were transported to the laboratory for microbiological analyses and proximate composition. From the day of purchase representing day 0, fish samples from each market were maintained at ambient temperature of 27±2 ^{o}C and analyzed for sixty days.

2.1 Preparation of samples for microbiological analysis

The fish samples from each market on every analysis were soaked with sterile distilled water in a warring blender cup for

20 minutes before blending. This was necessary because all parts of the fish must be soft enough to expose every tissue for smooth blending. 1 ml aliquot of samples was obtained and serially diluted to 107. From each dilution, 1 ml was obtained with sterile pipette and pour plated with plate count agar, nutritional agar, man Rogosa chapman agar for bacterial cultivation, violent Red Bile agar and malt extract agar for fungal cultivation in triplicates. For aerobic bacterial cultivation plates were incubated at 37 ^{o}C for 24-48 hrs. Anaerobically, plates were inserted in an anaerobic jar for 24-48 hrs, while fungal plates were incubated at 27±2 ^{o}C for 72 hrs. Bacterial counts were reported in colony forming unit per 1 gram of fish (Cfu/g) and fungal counts in spore per 1 gram of fish sample (spore/g).The isolated species were identified based on their cultural, morphological, physiological and biochemical characteristics with the criteria of Holt et al. (1994). The fungal species were identified with the criteria of Barnett et al. (2000),

2.3 Proximate analysis

The proximate composition of the smoke-dried fish samples were determined using the methods described by Association of official Analytical Chemists, (2000).

2.4 Statistical analysis

The experiment was carried out by using Randomized Design (RD). Experimental trials were conducted in triplicates. The results were expressed as mean ± standard deviation (SD) and were subjected to one way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pair using mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($P < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Microbiological analysis

The results of microbiological analysis on the smoke-dried fish samples are presented in Table 1. This study becomes necessary because of the inadequacy in smoking of fish adopted by many local fish vendors in Ibadan city of Nigeria. This inadequate smoking of fish has resulted in consumers not deriving quality from dried fish due to microbial spoilage. Elevated temperature is not used for smoking of fish and so, would only partially burn the external fish surfaces thereby leaving the internal parts for microbial activities. For this reason, fish sizes are only reduced with a little loss in weight which would make consumers buy them for high price. Such fish when purchased and not consumed immediately will degraded from inside which may not necessarily reflect on the fish surface for easy detection because of the tick leather-like skin and thick flesh of cat fish. This could be misleading from the trust consumers' demands from vendors hence they are twisted to buy a product according to the attributes that it does not worth (Eugenio et al., 2013).

TABLE 1

Microbial counts in fish samples

	Fish samples			
	A	B	C	D
Total Bacterial Counts (Cfu/g)				
Day 0	2.16×10^4	2.41×10^4	2.28×10^4	2.64×10^4
Day 15	2.23×10^4	2.53×10^4	2.64×10^4	2.93×10^4
Day 30	2.55×10^4	3.24×10^4	3.25×10^4	3.34×10^4
Day 45	2.72×10^4	3.81×10^4	3.76×10^4	4.18×10^4
Day 60	2.91×10^4	4.36×10^4	4.29×10^4	4.50×10^4
Total fungal count (Spore/g)				
Day 0	16×10^2	32×10^2	44×10^2	42×10^2
Day 15	23×10^3	41×10^2	58×10^2	54×10^2
Day 30	48×10^2	67×10^2	76×10^2	66×10^2
Day 45	62×10^2	75×10^2	94×10^2	82×10^2
Day 60	84×10^2	91×10^2	1.13×10^3	1.04×10^3
Total Coliform count (Cfu/g)				
Day 0	40×10^2	26×10^2	31×10^2	55×10^2
Day 15	62×10^2	51×10^2	49×10^2	84×10^3
Day 30	76×10^2	84×10^3	63×10^2	1.23×10^3
Day 45	84×10^2	1.05×10^3	84×10^2	1.65×10^3
Day 60	98×10^2	1.28×10^3	1.10×10^3	1.81×10^3
Total E. coli count (Cfu/g)				
Day 0	15×10^2	9×10^2	13×10^2	22×10^2
Day 15	34×10^3	15×10^2	26×10^2	41×10^2
Day 30	51×102	34×10^2	47×10^2	63×10^2
Day 45	78×10^2	58×10^2	63×10^2	84×10^2
Day 60	94×10^2	77×10^2	86×10^2	1.26×10^2
Total S. aureus count (Cfu/g)				
Day 0	97×10^2	1.22×10^3	1.29×10^2	53×10^2
Day 15	1.14×10^3	1.74×10^3	1.78×10^3	85×10^2
Day 30	1.38×10^3	2.18×10^3	2.43×10^3	1.13×10^3
Day 45	1.64×10^3	2.63×10^3	2.45×10^3	1.58×10^3
Day 60	1.93×10^3	3.16×10^3	3.44×10^3	2.65×10^3
Total Salmonella count (Cfu/g)				
Day 0	-	-	24×10^2	15×10^2
Day 15	-	-	43×10^2	31×10^2
Day 30	-	-	8×10^2	54×10^2
Day 45	-	-	95×10^2	73×10^2
Day 60	-	-	1.47×10^3	1.10×10^3
Total Clostridium count (Cfu/g)				
Day 0	-	-	-	-
Day 15	-	-	-	-
Day 30	-	-	-	-
Day 45	-	-	-	-
Day 60	-	-	-	-

Total of 60 days were used for the microbiological analysis whereby microbial counts was carried out at intervals of 15 days. General increase in microbial counts along storage period was observed during the study. However, samples from market D had the highest bacterial count where count of 2.64×10^4 in day 0 increased to 4.60×10^4 Cfu/g in day 60 of storage. This was followed by fish samples procured from market B with bacterial load of 2.41×10^4 Cfu/g in day 0 and increased to 4.36×10^4 Cfu/g in day 60 of storage. The fish samples from market A had the least bacterial count of 2.16×10^4 Cfu/g in day 0 and 2.91×10^4 Cfu/g in day 60 of storage. Total fungal count was also higher in fish samples from market D with count of 42×10^2 Spore/g in day 0 which increased to 1.04×10^3 Spore/g in day 60 of storage line. This was followed by fish samples for market C where count of

44×10^2 Spore/g in day 0 increased to 1.13×10^3 Spore/g in day 60. Least fungal count of 16×102 Spore/g in day 0 and 84×10^3 Spore/g in day 60 was recorded from market A. Akwuobu et al. (2019) have reported similar fungal counts from smoke-dried fish from a neighboring state in Nigeria. The result obtained from the microbiological analysis of C. gariepinus from four different markets within Ibadan city showed variations in microbial counts. However, significant differences occurred in the microbial load of fish samples in the surveyed markets from day 0 of purchase to day 60 of storage period. This increase in microbial load on the length of storage may not approve their acceptability by consumers in terms of safety, low nutritional values and alteration in the sweet flavour desired in quality fish. This observation has made us deduce one of the reasons, why retailers re-smoke left over fish from their daily sales, in order to reduce microbial load thus salvaging the nutrients and other fish qualities for consumers. Though smoking and re-smoking have been reported to have effect on the nutritional values (Morris et al., 2004); this preservative method extends fish shelf life. However, effect of re-smoking fish could cause loss in gain as the size of the fish would be reduced to make buyer negotiate for low price. Some researchers (Kumolu-Johnson et al., 2010; Ikutegbe and Sikoki, 2014) have reported increase in microbial load during storage of which similar result is reported in this study. Total coliform count was higher in fish samples from market D where a count of 55×10^3 Cfu/g in day 0 increased to 1.81×10^3 in day 60 of storage time. This was followed by fish samples from market A with count of 40×10^2 Cfu/g in day 0 and 98×10^2 in day 60. Least count of 31×10^2 Cfu/g was recorded in day 0 and 1.10×10^3 in day 60 of storage time from market C fish samples. Market D fish sample were however, populated with high E. coli count of 22×10^2 Cfu/g in day 0 which increased to 1.26×10^3 Cfu/g in day 60 of storage line. This was followed by fish samples from market A with count of 15×10^2 Cfu/g in day 0 and 94×10^2 Cfu/g in day 60. Least count of 9×10^2 Cfu/g in day 0 which increased to 77×102 in day 60 was recorded for samples from market B. Staphylococcus aureus count was more in market C fish samples which increased from 1.29×10^3 Cfu/gin day 0 to 3.44×10^3 Cfu/g in day 60. This was followed by fish samples from market B where count of 1.22×10^3 Cfu/g increased from day 0 to day 60 with 3.16×103 and least count of 53×10^2 Cfu/g in day 0 to 2.65×10^3 in day 60 in fish samples from market D. Salmonella species were more in fish samples from market C with 24×10^2 in day 0 and increased to 1.47×10^3 Cfu/g in day 60. Following this are fish samples from market D where a count of 15×10^2 for day 0 increased to 1.1×10^3 Cfu/g in day 60 of storage. However, fish from markets A and B were void of Salmonella counts. Clostridium species was however, not recorded for fish from the four markets surveyed for this study. From the microbial load perspective, coliform group of bacteria such as E. coli and the presence of afflatoxin producing fungus (Aspergillus flavus) were reported in increasing number during the storage period. The presence of these microorganisms in the fish could put consumers at risk for their pathogenic nature known to man. Dutta et al. (2018) have reported both on total coliform and faecal coliform from smoked fish during storage which is in correlation with the report in this study.The variations of microbial counts and types from the fish samples could result from human handling, environment of storage, improper drying process and from culture ponds or tanks.

3.2 Proximate composition

The fish samples' proximate values are presented in Figures 1-6. Apart from values of the fishes in moisture evaluation where increase was recorded, other proximate parameters determined decreased with days of storage. The moisture content recorded for fish samples purchased from market A was the least among the surveyed markets. The moisture content recorded for fish purchased from markets B and C were of no significant difference in value between days 0 to 60of storage line. However, highest moisture content was recorded for fish samples purchased from market D. The proximate value of this for day 0 was 9.15±0.21% and increased to 10.63±0.08% in day 60 of storage (Fig. 1).

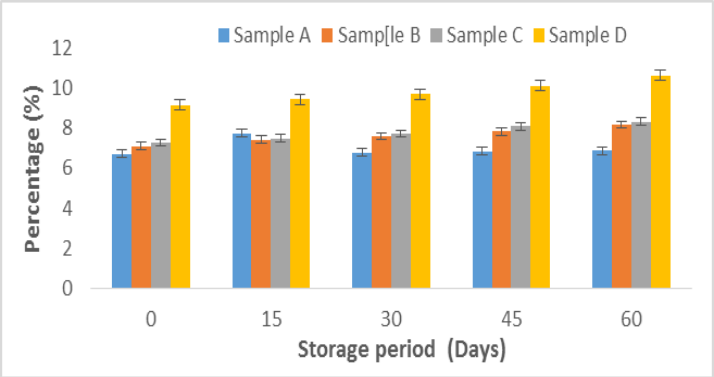

Figure 1: *Moisture content of fish sample (%)*

The moisture content observed from the fish samples dictated the tune of microbial load; where samples with high moisture content had higher microbial load especially bacteria species. There were statistically significant differences ($P< 0.05$) among the fish samples alongside day 0 of purchase to day 60 of storage time. This was pertinent because the fish were stored in cool dry place that encouraged increase in microbial growth and the non-re-drying of the fish for the period of storage as well encouraged the increase encountered in moisture content alongside storage time of the samples. The geometrical increase in moisture of fish samples with storage period observed connotes with the earlier reports by Ikutegbe and Sikoki, (2014). The bases of smoke drying fish is to achieve weight loss to minimal level for discouragement of microbial growth thus extending the shelf life. However, storage of smoke-dried fish in room temperature enhances moisture hence it is not an appropriate condition for fish storage. Meanwhile, only the fish samples from market D could not meet up with the standard of moisture content in fish for adequate storage potential. The smokes from wood when drying fish is an aerosol produced by pyrolysis of wood during burning at high temperature and reduce oxygen. The preservative effect of smoke is by the component of compounds such as furans, esters, lactones, carbonyls, phenols, acids and alcohols. Outside preservation of fish by these compounds in smoke, it also add acceptable flavour to fish for consumers. Martin et al. (2010); Varlet et al. (2010) have reported on the use of liquid smoke in food systems for flavour and safety of foods.Crude fibre values recorded from the surveyed fish samples decreased along days of storage. Highest crude fibre of 0.92±0.26% was recorded from fish samples purchased from market C. This was followed by fish samples from market A, where value of 0.76±0.28% and

decreased to 0.13±0.19% in day 60 of storage, fish samples from market Meanwhile, least crude fibre was recorded for fish samples obtained from market B where value of 0.73±0.15% for day 0 decreased to 0.09±0.11%on day 60 of storage (Fig 2).

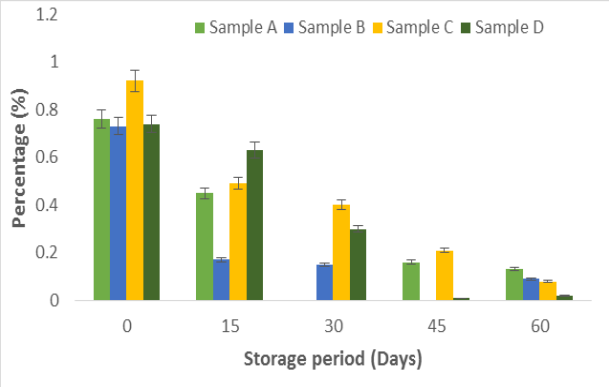

Figure 2: *Crude fibre of fish sample (%)*

The fibre values of the fish samples were dependent on the relative moisture contents where values were higher in fish samples with low moisture content and lower in fish with high moisture content. The former suggests quality storability of the fish hence the fibre which results from hardness of the fish tissue will not enable microorganism's easy access into the fish for degradation.The crude protein value of fish was in the trend of observed moisture content values of fish samples. In that trend, highest value of crude protein was recorded for fish samples purchased from market D, followed by samples from markets C, B and A, but with decrease in values from day 0 to day 60. There was decrease in the fish protein values along days of storage and this resulted in the observed significant differences in values of the fish from day of purchase to termination of experimental (day 60). However, fish of higher protein values in day 0 of purchase had reduced values at day 60 of storage. This could be due to microbial activities in the fish samples, thus the less value than the fish samples with low moisture content. Hence fish of higher moisture content contains high protein than the ones with low moisture content because of re-drying for protection against loss by vendors, it will be of necessity to purchase and consume freshly smoke-dried fish. of storage time irrespective of fish samples from the various markets (Fig. 3).

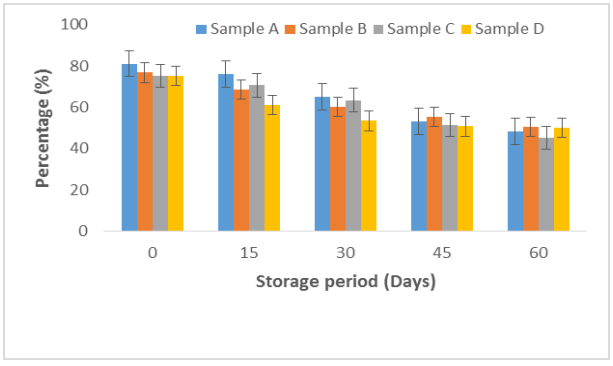

Figure 3: *Crude Protein contents of fish Samples*

The decline in protein values obtained during storage correlates with the findings of other studies (Fapohunda and Ogunkoya, 2006; Olayemi et al., 2011; Daramola et al., 2013). General decrease in ash content was recorded in the fish samples along line days of storage. However, samples obtained from market B had the highest ash content of 16.42±0.24%, followed by samples from market C (16.41±0.02%) and least in samples from market D (15.30±0.16%) (Fig. 4).

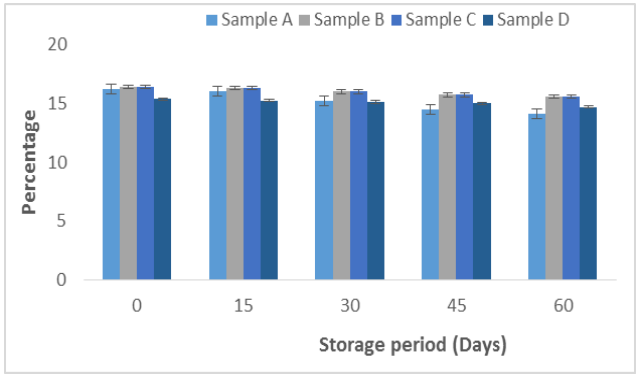

Figure 4: *Ash contents of fish samples*

Highest fat content of 9.28±0.02% was recorded in fish samples obtained from market A which decreased to 8.18±0.04 in day 60th of storage. Samples from market B had initial fat content of 11.34±0.11% but decreased to 10.31±0.20% in day 60 of storage. Initial fat content in fish samples obtained from market C was 14.48±0.44 which decreased to 13.17±0.04% and samples from market D fat content from 15.26±0.03% to 14.40±0.13 in day 60 of storage (Fig. 5). Meaningful ash content was recorded from the fish samples though with little variations that was not significantly different from one and another in the surveyed markets.

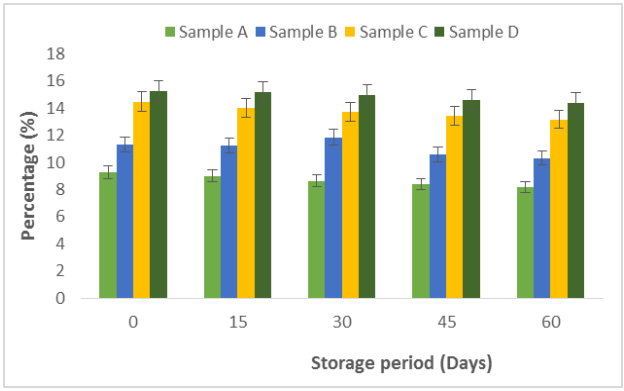

Figure 5: *Fat contents of fish samples*

The reasonable ash content of the fish samples indicates that the fish could be rich in mineral sources such as iron, potassium, zinc, calcium and magnesium even as reported by Andrew, (2000). Fish contains polyunsaturated fat (PUFA) needed for preventing or reducing premature heart diseases (Pigott and Tucker, 1987) and also susceptible to oxidation due to its unstable nature. While we reported decrease in fish fat content in storage line which is similar to the reports of other authors (Daramola et al., 2007; Ikutegbe and Sikoki, 2014; Ogbonnaya and Ibrahim, 2009) reported increase in

storage line. However, lipid oxidation results in deterioration in food quality for off flavour and off-odour (Hus in't Veld, 1996). Ikutegbe and Sikoki, 2014) have reported that the decline in fish fat on storage time could be due to the underlying biological characteristics of the species analyzed and its susceptibility to fat oxidation. In conformity, higher microbial load was recorded from the fish samples with high fat content than those with low fat content.Carbohydrate content of the fish was highest in samples from market A with 1.95±0.06% at 0 day but however decreased to 1.40±0.08 in day 60 of storage time. This was sequentially followed by samples from markets B, C and D (Fig. 6). Significant differences in carbohydrate values were also observed in the fish samples on storage line. This suggests utilization of the fat content by microorganisms for growth and multiplication

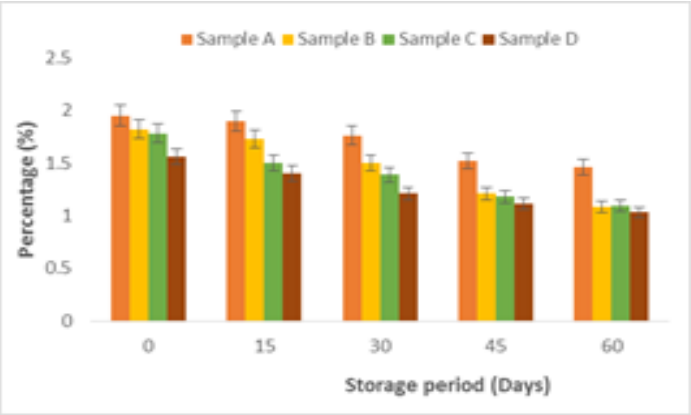

Figure 6: *Carbohydrate contents of fish samples*

4 CONCLUSIONS

Proper processing of fish and appropriate methods of storage have influence in fish preservation. These factors interferes with microbial interaction in fish for upkeep of the desired nutrients derived in fish. Wild fish and cultured fish are both processed for sale in markets without identity. However, the microbial status of fish environment has impact in fish microbial colonization to result in spoilage if not adequately processed. Low moisture content in fish gives longer shelf life though with reduction in nutrients than those with high moisture content but with shorter storage time hence liable to microbial contamination.

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