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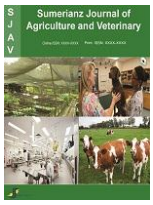

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Spoilage Organisms in Smoked Atlantic Cod (*Gadus morhua*) from Different Sources

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Abstract

This study was designed to establish the microbiological safety and suitability for human consumption of the cold-smoked Atlantic cod, *Gadus morhua* retailed in Oju-Ore, Sango, Iyana-Iyesi and Oja-Oba markets in Ota, Ogun State, Nigeria. The fish samples were randomly purchased from the four markets and analysed for Total Viable Count (TVC), Total Coliform Count (TCC), Staphylococcal count and Fungi count using Nutrient agar, Plate count agar (PCA), MacConkey agar, Mannitol salt agar (MSA) and Potato Dextrose Agar. Samples were stored in wire baskets and examined for the presence of bacteria and fungi on day 1, 4, 8, 12 and 16. The highest mean values were TVC, 5.7 X 10^6 CFU/g; TCC, 6.4 X 10^4 CFU/g and Staphy. Count, 7.9 X 10^3 CFU/g in the smoked Cod from Oja-Oba market, on day 16 of storage at ambient conditions. There were no fungal counts in all the smoked *Gadus morhua* samples from the four markets on day 1. However, from the 4th day, there were traces of fungi from 0.4 X 10^2 CFU/g in samples from Oju-Ore to 4.6 X 10^2 CFU/g on day 12 in samples from Oja-Oba market. By the 16th day, moulds have completely covered the smoked fish and it was too numerous to count (TNC). Also, through biochemical characterization, some bacterial isolates from the smoked fish were identified. Generally, smoked Cod from Oju-Ore had the best quality and least mean values of the microbial parameters, followed by that of Sango, Iyana-Iyesi and the highest values were the smoked fish from Oja-Oba markets. It was, however, recommended that the application of the Hazard Analysis Critical Control Point (HACCP) principles be introduced to the local fish processors, putting their literacy level into consideration for them to be able to understand and imbibe the concept of the practice.

Keywords: Atlantic cod; Markets; Microbiological safety; HACCP.

1. Introduction

Fish has been accounting for 40% of the protein intake in Nigeria for some time, reaching 6.5 to 7.5 kg per capital consumption [1]. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis [2, 3]. The fat of fish is an excellent source of vitamins A and D [4]. Meanwhile, meats and fish have been reported as one of the most common smoked foods [5]; the reason being that fish is a highly perishable commodity. In this modern world, there are several smoking methods such as the cold, hot, liquid and electrostatic.

Smoking is the most popular method of fish processing in Nigeria [6]. Fish is either eaten fresh, preserved or processed. Da Silva [7], stated that one third of the cured fish were smoked and about 20% of the smoked fish went into international markets. Smoke-drying methods used in Nigeria requires low capital investment and it is carried out in fishermen camps and fish processing chambers in traditional smoking kilns of clay, cement blocks, drums or iron sheets. Traditional fish smoking products dominate the Nigerian markets but this method is faced with a lot of challenges and characterised by uncontrolled burning, poor distribution of heat, low volume capacity and contamination with sand and ash from the wood [8]. Atlantic cod (*Gadus morhua*) is one of the many types of fish imported into Nigeria. It is a popular table fish marketed widely, primarily for human consumption. The liver of Atlantic cod is also processed to produce cod liver oil which is used as a vitamin supplement. Cod is a type of fish commonly processed and widely acceptable in the south-western part of Nigeria as cold-smoked products which are processed at a temperature not exceeding 30°C [9].

The safety of a fish for consumption in terms of the microbial load and the presence or absence of food-borne pathogens is one of the factors considered in assessing the quality of the fish. Fish quality is definitely the most important factor which stimulate consumers' demand; as a product of low quality will have a respectively low market value. Fish products which does not meet a required standard of quality, is often rejected and the producer will suffer economic loss [3]. Smoked fish and shellfish products have been reported as a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* spp., and *Clostridium botulinum* [10]. Also, Martin [11] stated that bacteria (*Staphylococcus aureus*), yeasts (*Saccharomyces cerevisiae*) and moulds (*Penicillium* and *Aspergillus*) were the commonest microorganisms associated with smoked fish.

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This study is to assess the smoking procedures and post-mortem qualities of cold- smoked Atlantic cod, *Gadus morhua* from the selected traditional fish trading centres and verify the microbial safety of the fish retailed in four major markets: Oju-Ore, Sango, Iyana-Iyesi and Oja-Oba markets in Ota, Ogun State, Nigeria.

2. Materials and Methods

Employing 20% sampling intensity on the retailers, a stratified random sampling technique was used to collect smoked samples *Gadus morhua* from Oju-Ore, Sango, Iyana-Iyesi and Oja-Oba markets in Ota. Fifteen (15) whole samples of smoked Cod were purchased from three retailers randomly picked from each of the four market; making a total of 60 pieces. Fish samples were collected from the selected retailers aseptically wrapping them in aluminum foil and taken to the Microbiology Laboratory of Bells University of Technology, Ota. Samples were examined for the presence and occurrence of pathogenic bacteria and fungi, every 4 days until the 16th day. The selected samples of the study were coded as follows:

M = Market =M1 (Oju-Ore), M2 (Sango), M3 (Iyana-Iyesi) and M4 (Oja-Ota) and

R = Retailer = R1, R2 and R3.

Therefore, M1R1 = Oju-Ore market, retailer 1

2.1. Sample Handling

The smoked fish samples were taken from the different retailers in the four markets inside sterile polythene bags. After the initial analyses on day 1, the remaining samples were stored in big different wire baskets, according to the four markets. The wire baskets were lined with muslin cloth to allow for aeration but prevented rat and insects from getting to the fish.

2.2. Sample Processing

All samples were collected in triplicates and treated within 3 hours of collection. Whole smoked Cod were taken from each of the four baskets on day 1, 4, 8, 12 and 16 for analyses. The samples were grinded using a blender until semi-fine samples were obtained. Thereafter, five (5g) were taken for each trial of analysis and the remaining part of smoked fish carcasses were disposed off.

2.3. Microbiological Analyses

Cultivation and enumeration of bacteria, Total Viable Count (TVC), Total Coliform Count (TCC), Staphylococcal count and Fungi count in the selected samples of cold-smoked *G. morhua* were determined using Nutrient agar, Plate count agar (PCA), MacConkey agar, Mannitol salt agar (MSA) and Potato Dextrose Agar. These were prepared according to standard guidelines and analysed using the microbiological methods described by Fawole and Osho [12].

Other tests carried out include: isolation, characterisation and identification of bacteria in the fish samples and gram staining [13]; catalase test and coagulase test Cruickshank, *et al.* [14] and Cheesebrough [15]; citrate utilisation test Marshall [16]; indole test Cheesebrough [15]; motility test Ogbonna and Igbenijie [17]; hydrogen sulphide production test Cheesebrough [15] and Kligler iron agar test

3. Data Analysis

The mean values of the microbial parameters obtained from the analyses of the different samples of cold-smoked *Gadus morhua* were presented in Tables 1 and 2.

4. Results

In this study, evaluation of microbes present in cold-smoked *Gadus morhua* were carried out to determine proliferation of the target food borne pathogens in terms of Total Viable Count (TVC), Total Coliform Count (TCC), Staphylococcal count and Fungi count; the result is presented in Table 1.

The least mean value of the TVC was 4.1 X 10^6 CFU/g in smoked sample from Oju-Ore market on day 1 while the highest was 5.7 X 10^6 CFU/g from Cod from Oja-Oba on day 16 of storage at ambient conditions.

On the Total Coliform Count (TCC) of the smoked *Gadus morhua*, the least mean value was 4.3 X 10^4 CFU/g from samples in Oju-Ore market on day 1 while the highest on day 16 was 6.4 X 10^4 CFU/g in the one from Oja-Oba market.

For the Staphylococcal count, the mean value increased progressively from 2.3 X 10^3 CFU/g in smoked fish sample on day 1 and Oju-Ore market to 7.9 X 10^3 CFU/g in Oja-Oba market on the 16th day.

There were no fungal counts in all the smoked *Gadus morhua* samples from the four markets: Oju-Ore, Sango, Iyana-Iyesi and Oja-Oba on day 1. However, from the 4th day, there were traces of fungi from 0.4 X 10^2 CFU/g from Oju-Ore samples to 4.6 X 10^2 CFU/g on day 12 in samples from Oja-Oba market. By the 16th day, moulds have completely covered the smoked fish and it was too numerous to count (TNC).

Also, colonial morphology, microscopy and biochemical characterisation of the bacterial isolates from smoked Cod from the different retailers and markets are shown in Table 2. *Klebsiella pneumonia, Enterobacter* spp, *Staphylococcus* spp, *Salmonella* spp, *Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli* were identified.

5. Discussion

Generally, smoked Cod from Oju-Ore had the best quality and least the least mean values of the microbial parameters-Total Viable, Total Coliform, Staphylococcal and Fungi counts, followed by that of Sango, Iyana-Iyesi and the highest values were the smoked fish from Oja-Oba markets.

The TVC of smoked samples were the highest throughout the period of storage and the fish sample were completely covered by mould after the 12th day of storage. The results obtained were similar to those reported by Kester, *et al.* [18] where the highest TVC in smoked *Gadus morhua* was 5.7 x 10^6 CFU/g and the least was 4.11x 10^6 CFU/g. Also, this result is comparable with that of the microbiological and proximate assessment of cold-smoked *Gadus morhua* in Traditional Fish Centres in Ota Metropolis as reported by Daramola, *et al.* [19].

Significant increase in coliform population occurred in all the smoked Cod samples during the 16-day storage period. The result was similar to that reported by Da Silva [7] where the coliform in the control sample showed 2.6x10^6 CFU/g. This finding is of concern as a result of the associated public health implications. For example, generally, hot smoked fish are consumed in the tropics with little or no further processing or further cooking; thus, they fall into the high-risk category of foods [20]. Hence there is a need for the use of appropriate percentage of choice antimicrobial agent as preservative.

Also, the population of the fungi increased from the 4th day in all the smoked fish samples during storage to when it became too numerous to count. This not a surprise as the fish sample were usually cold-smoked and still retain a high level of moisture which is favourable to high microbial growth. This is so as samples were collected and stored during the raining season with corresponding high humidity. This agreed with Gibson, *et al.* [21] findings that atmosphere composition had a great impact on mould growth with humidity being the most important variable. In addition, Eyo [3] stated that smoked fish samples may have a relatively high water activity level which is a prerequisite for microbial growth. Also, this is in agreement with the findings of Abolagba and Iyeru [22] who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a very high microbial load.

Furthermore, the higher mean microbial loads associated with smoked fish samples from Oja-Oba and Iyana-Iyesi markets relative to the lower loads of the ones from Oju-Ore and Sango markets can be attributed to the heavy traffic of humans and vehicles in the vicinity of the former than the latter markets. According to Bankole, *et al.* [23], food processors may be sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage. It was also observed that smoked fish samples were displayed on woven mats close to the ground without cover and as a result of which the samples were prone to microbial contamination from the air, dust and soil. Likewise, the unhygienic environment and handling of the smoked fish products coupled with other sources of contaminants, such as unclean hands, unsanitary containers, equipment, unclean water and sewage and contamination between raw and processed product is of great concern [24].

Meanwhile, considering the bacterial isolates from the smoked Cod, processed seafood products are usually considered to present a low risk for infections attributed to *Salmonella spp* [25]. Also, the presence of *Clostridium* and *Salmonella* is usually at zero tolerance level [24]. The presence of pathogens such as *Salmonella* is considered a case 10 hazard, and cooking would reduce the degree of hazard posed by the pathogens. The heat treatment during preparation might have destroyed or inactivated the pathogens [26]. Hence, re-heating of products from the markets before consumption might be necessary.

Notwithstanding the high mean values of the microbial parameters recorded in the smoked fish samples from the four markets, they are considered as good products since the counts were either below or within the recommended standards of International Commission on Microbiological Specification for Foods-ICMSF [20].

6. Conclusion

The microbial loads of all the samples of cold-smoked *Gadus morhua* obtained from the four study markets in Ota still qualified the entire fishery product for safe consumption and of no serious harmful consequence to the health of the consuming public.

References

[1] Olatunde, A. A., 1989. "Approaches to the studies of fisheries biology in Nigerian inland waters. Ayeni and olatunde (eds.) " In *Proc. Nat. Conf. of Two Decades of Research on Lake Kainji.* pp. 538-541.

[2] Abolagba, O. J. and Melle, O. O., 2008. "Chemical composition and keeping qualities of a scaly fish Tilapia (Oreochromis niloticus) smoked with two energy sources." *African J. Gen. Agric.*, vol. 4, pp. 113-117.

[3] Eyo, A. A., 2001. *Textbook on fish processing technology in the tropics*. Nigeria: Published by National Institute for Freshwater Fisheries Research, New Bussa. University of Ilorin Press. p. 403.

[4] Norman, N. and Joseph, H. H., 1996. *Investigation of heat and mass transfer in hot smoking of small fish*. 5th ed. Nikitin, B.M. ed. Food Science, pp. 115-119.

[5] McGee, H., 2004. *Wood smoke and charred Wood. On food and cooking*. Scribner, pp. 448-450.

[6] Bako, W. S., 2005. "The role of women in fish processing: Handling and marketing in Kainji Lake basin." In *Proceedings of 19th annual conference of the Fisheries Society of Nigeria (FISON), Nov.29-Dec.3, 2004, Ilorin, Nigeria.*

[7] Da Silva, L. V. A., 2002. *Hazard analysis critical control point (HACCP), microbial safety, and shelf life of smoked blue catfish (Ictalurus furcatus)*. Master Thesis, Louisiana State University, p. 100.

[8] Balogun, A. M., 1992. "Fish handling and quality control." In *Aquaculture development in Africa, training and references manuals for aquaculture extentionists. Cowx I.G., ed, Commonwealth Secretariat, London.* pp. 290-318.
[9] Doe, P. E., 1998. *Fish drying and smoking production and quality*. Lancaster, Pennsylvania: Technomic Publishing Co., Inc.
[10] Heinitz, M. L. and Johnson, J. M., 1998. "The incidence of Listeria sp., Salmonella sp. and Clostridium botulinum in smoked fish and shellfish." *J. Food Prot.*, vol. 61, pp. 318-323.
[11] Martin, A. M., 1994. *Fisheries processing biochemical applications*. London: Published by Chapman and Hall.
[12] Fawole, M. O. and Osho, B. A., 1995. *Laboratory manual of microbiology*. Shalom Prints Ibadan Nigeria, pp. 25–30.
[13] Obire, O., Nwaubeta, O., and Adue, S. B. N., 2002. "Microbial community of a waste- dump site." *J. of Appl. Sci. and Environ. Mgt.*, vol. 6, pp. 78-83.
[14] Cruickshank, R., Marmion, B. P., and Swain, R. H. A., 1975. *Medical microbiolog*. 12th ed. New York: Churchill Livingstone. pp. 32-36.
[15] Cheesebrough, M., 2000. *Biochemical tests to identify bacteria. In: District laboratory practice in tropical countries*. part 2: low price ed. United Kingdom: Cambridge University Press. pp. 35-38.
[16] Marshall, E., 2010. "MCB Course." Available: www.marshall.edu/library/mcbsciences/default.asp
[17] Ogbonna, D. N. and Igbenijie, M., 2006. "Characteristics of Microorganisms associated with waste collection sites in Port-harcourt city, Nigeria." *Nig. J. of Microbiol*, vol. 20, pp. 1427-1434.
[18] Kester, C. T., Adelekan, A. O., and Ajose, D. J., 2012. "Assessment of the microbial hazardous status of cold- smoked Atlantic Cod, Gadus morhua, retailed in some selected markets in Ota, Ogun State, Nigeria." *African Journal of Microbiology Research*, vol. 6, pp. 3970-3975.
[19] Daramola, J. A., Kester, C. T., Osofero, S. A., and Ojiakor, C. C., 2014. "Microbiological and proximate assessments of cold-smoked Gadus morhua in traditional fish smoking centres in Ota metropolis." *The Pacific Journal of Science and Technology*, vol. 15, pp. 297-305. Available: https://www.researchgate.net/publication/323078983_Microbiological_and_Proximate_Assessments_of_Cold-smoked_Gadus_morhua_in_Traditional_Fish_Smoking_Centers_in_Ota_Metropolis
[20] International Commission on Microbiological Specification for Foods-ICMSF, 1986. *Microorganisms in foods 2, sampling for microbiological analysis. Principles and specifications*. 2nd ed. Oxford: Blackwell Science.
[21] Gibson, A. M., Baranyi, J., Pitt, M. J., Eyles., M. J., and Roberts, T. A., 1994. "Predicting fungal growth: The effect of water activity on Aspergillus flavus and related species." *Int. J. Food Microbial.*, vol. 23, pp. 419-431.
[22] Abolagba, O. J. and Iyeru, O. A., 1998. "Study of insect pests infecting traditionally processed fish sold in Benin city metropolis, Nigeria." *Nig. J. Applied Sci.*, vol. 16, pp. 25-29.
[23] Bankole, M. O., Oladimeji, D. S., and Omemu, A. M., 2005. "Microorganisms associated with the palms of food vendors in Abeokuta metropolis." In *The book of abstracts of the 29th annual conf. by Nigerian society for microbiology (NSM), University of Agriculture, Abeokuta.* p. 17.
[24] Food and Drug Administration-FDA, 2001. "Department of health and human services. FDA and EPA safety levels in regulations and guidance." In *fish and fisheries products hazards and controls guidance: Third Edition. Appendix 5.* p. 258.
[25] Varnam, A. H., 1991. *Foodborne pathogens: an illustrated text*. London: Mosby-Year Book, Inc.
[26] Fernandez, C. F., Flick, J. G., Silva, J. L., and McCaskey, T. A., 1997a. "Comparison of quality in aquacultured fresh catfish fillets II. Phatogens E. coli O157: H, Campylobacter, Vibrio, Plesiomonas and Klebsiella." *J. Food Prot.*, vol. 60, pp. 1182-1188.

Table-1. Mean Microbial Loads of Smoked Cod from Different Markets

Total Viable Counts ($X10^6$ CFU/g)				
Days	**Oju-Ore Market (M1)**	**Sango Market (M2)**	**Iyana-Iyesi Market (M3)**	**Oja-Oba Market (M4)**
1	4.1	4.3	4.4	4.5
4	4.4	4.4	4.6	4.7
8	4.5	4.7	4.7	4.8
12	4.7	4.8	4.9	4.9
16	5.2	5.4	5.5	5.7
Total Coliform Count ($X10^4$ CFU/g)				
1	4.3	4.3	4.6	4.7
4	4.5	4.6	4.8	4.9
8	4.9	5.1	5.0	5.2
12	5.4	5.5	5.5	5.5
16	5.7	5.9	6.2	6.4
Staphylococcal Counts ($X10^3$ CFU/g)				
1	2.3	2.4	2.6	2.9
4	3.4	3.5	3.9	4.1

8	5.7	5.8	6.0	6.2
12	6.6	6.6	6.8	6.9
16	7.0	7.3	7.6	7.9
Fungal Count (X10^2 CFU/g)				
1	0.0	0.0	0.0	0.0
4	0.7	0.8	1.1	1.2
8	1.5	1.9	2.4	3.0
12	3.6	3.8	4.2	4.6
16	TNC	TNC	TNC	TNC

TNC = Too Numerous to Count

Table-2. Biochemical Characterisation of Bacterial Isolates From Smoked Cod (Retailers/Markets)

	Gram	**Catalase**	**Citrate**	**Motility**	**Indole**	**Sulphide**	**Kligler**	**Oxidase**	**Shape**	**Probable organism**
M1										
R1	-	+	+	-	-	-	-	-	Rod	*Klebsiella pneumoniae*
R2	-	-	+	+	-	-	+	-	Rod	*Enterobacter* spp
R3	+	+	-	+	-	-	+	-	cocci	*Staphylococcus* spp
M2										
R1	-	-	+	+	-	-	-	-	Rod	*Salmonella* spp
R2	-	+	+	+	-	-	+	+	Rod	*Pseudomonas aeruginosa*
R3	-	-	+	-	-	+	+	-	Rod	*Proteus mirabilis*
M3										
R1	+	+	-	+	-	-	-	-	Cocci	*Staphylococcus* spp
R2	-	-	+	-	-	+	+	-	Rod	*Proteus mirabilis*
R3	-	+	+	+	-	-	-	-	Rod	*Salmonella* spp
M4										
R1	-	+	+	+	-	-	-	-	Rod	*Salmonella* spp
R2	-	+	-	+	+	-	+	-	Rod	*E. coli*
R3	+	+	-	+	-	-	+	-	cocci	*Staphylococcus* spp

M = Market =M1 (Oju-Ore), M2 (Sango), M3 (Iyana-Iyesi) and M4 (Oja-Ota) and
R = Retailer = R1, R2 and R3.