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Effect of smoking on the microbiological quality of horse mackerel (*Trachurus trachurus*) processed and marketed by artisanal fishermen from Vilankulos, Mozambique

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Abstract Smoking is one of the fish conservation techniques used by some artisanal fishing communities. Horse mackerel is a fish widely traded and consumed in Mozambique. This study aimed to evaluate the effect of smoking on the microbiological quality of horse mackerel sold in district Desse in Vilankulos. Twelve samples were collected and analyzed for following microbiological parameters (total viable microorganisms, total coliforms, thermotolerant coliforms, *Escherichia coli*, *Salmonella spp.*, *Staphylococcos aureus, Vibrio cholerae* and *Vibrio parahaemolyticus*). The total viable microorganisms were quantified by the NMKL method and the total coliforms, fecal coliforms, and *Escherichia coli* were quantified by the MPN method. Statistical analysis were performed using the SPSS program, and data were submitted to the normality test using the Shapiro-Wilk test at a significance level of 5%. The results showed the existence of significant differences in the amount of aerobic microorganisms between frozen horse mackerel (3.59 ± 0.87 CFU/g) and smoked horse mackerel (2.39 ± 0.88 CFU/g), total coliforms (0.22 ± 0.53 CFU/g), and (0.06 ± 0.19 CFU/g), respectively, and thermotolerant coliforms (0.12 ± 0.25 CFU/g) and (0.06 ± 0.19 CFU/g), respectively. Smoked horse mackerel had less contamination compared to the frozen fish. There was no significant growth of other microorganisms analyzed.

Keywords: microbiological quality, fish conservation, contamination

1. Introduction

Trachurus trachurus is a pelagic species of the Carangidae family, which is present in several seas, mainly in the Atlantic and the Indian Ocean and has become a commercial species of relevant importance in Africa and Europe. *Trachurus trachurus* (horse mackerel) is a semi-fat species-rich in proteins and omega-3 unsaturated fatty acids. This species has adequate nutritional components for a healthy diet (FAO 2015). The Vilankulos district located in the southern region of Mozambique, in the coastal zone, has great potential in fisheries resources (PES 2015). The extraction of these resources is done by artisanal fishermen, where at times they manage to capture considerably larger quantities, reaching or exceeding 5 tons daily (PES 2015). The commercialization of fishery products from artisanal catches is carried out almost exclusively on the domestic market. Fish is usually sold fresh, and only a small part (less than 3%) is sold dry, salted, or in brine (FAO 2015).

Preservation of fish through smoking had its origin linked to the beginnings of civilization, and reached industrial characteristics, with the consequent evolution of the techniques used and the improvement of equipment, reaching current quality standards, as it causes changes in sensory attributes such as odor, taste, color, and texture (Souza 2007). The technology for fish processing can provide consumers innovative products, with quality and affordable price with a certificate and that fulfill the characteristics of being convenient, easy to prepare, hygienically correct, prioritizing raw material quality (Oetterer 2009). Successful preparation of smoked products depends on the application of smoke and the combination of physical and chemical factors. It is necessary to strictly control each of the stages of smoking, taking into account that the composition of the smoke is composed of numerous substances such as phenols, aldehydes, alcohols, organic acids, among others, where some of these are harmful to human health if consumed in larger quantities. Among the compounds mentioned, phenols and aldehydes are the ones that give the characteristic aroma to smoked products (Kubitza 2000).

In the Vilankulos district, horse mackerel is one of the most captured species every year compared to other fishery resources and is important among the population in terms of food diet. Due to the capture in absolutely larger volumes by artisanal fishermen, lacking adequate refrigeration, the quality of smoked fish existing in the markets is questioned, being

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purchased by many consumers at an affordable price, as they constitute a viable alternative to the diet. Thus, this study aimed to analyze the effect of smoking on the microbiological quality of horse mackerel (*Trachurus trachurus*) compared to the quality of the frozen product.

2. Materials and Methods

2.1. Processing of samples

In the study, 12 samples of smoked *Trachurus trachurus* and an equal number of frozen *Trachurus trachurus* were used, randomly selected from four artisanal fishermen and three replicates were made. To avoid unwanted sensory changes such as gills and fins dry and faded, covered with sticky slime, cloudy eyes and sunken into the head, unpleasant, sharp smell of trimethylamine, the samples were placed in transparent plastic bags, placed in isothermal boxes, and transported to the Fish Inspection Laboratory (LIP), where they were frozen at a temperature of -4°C. In the laboratory, the samples (Figure 1 and 2) were placed in sterile stomacher bags, properly identified and transported to the microbiological analysis section.

Figure 1 Frozen horse mackerel

Figure 2 Smoked horse mackerel

Culture media were prepared according to instructions on the label of the media (LIP 2011). The following culture media were prepared: Plate Count Agar (PCA-Oxoid), Lauryl Sulfate Broth (LSB-Merck), Bright Green Bile Broth (BGB-Merck), *Escherichia Coli* Broth (ECB-Merck), Tryptone (Merck KGaA), Baird-Parker Agar Base, Xylose Lysine Deoxycholate (XLD-Biolab), Thiosulfate Citrate Salt Sucrose (TCBS-Merck KGaA), Rappaport-Vassiliadis Soy Peptone Broth (RVS-Oxoid) (LIP 2011).

2.2. Microbiological analysis

The analysis and identification of microorganisms were carried out based on the methodologies in use at the Maputo Fish Inspection Laboratory. The NMKL (Nordic Food Analysis Committee) method was used to quantify the total viable microorganisms by plate seeding, NMKL (method nr. 86 2013). The reading consisted of counting the colony found on the PCA plates. The expression of the results was done in CFU/g. The other methods used were for the determination of total coliforms, fecal and *Escherichia coli*, confirmation of thermotolerant coliforms, NMKL (method nr. 96 2009). Confirmation of *Staphylococcus aureus*, NMKL (2003). Detection of bacteria of the genus *Salmonella* (method nr. 71 1999), *Vibrio cholerae,* and *Vibrio parahaemolyticus* NMLK (method nr. 156, 1997).

2.3. Statistical analysis

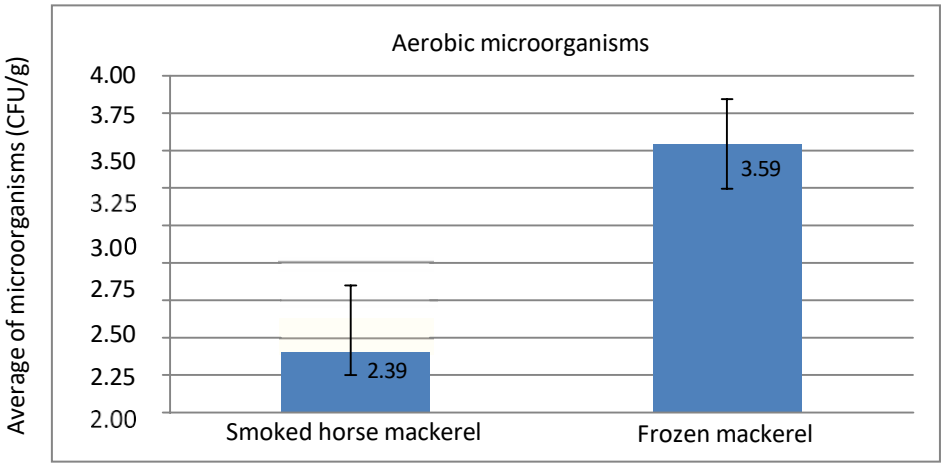
The data collected were organized in Excel spreadsheets and were submitted to statistical analysis in the SPSS version 21 program using non-parametric tests. The graphical representation was performed using the GraphPad Prism version 7 software. Data were submitted to a normality test using the Shapiro-Wilk test at a probability level of 0.05.

3. Results and discussion

3.1 Quantification of Aerobic Microorganisms

Statistically significant differences were observed (Mann-Whitney U test = 14.50; $P<0.05$) between frozen horse mackerel and smoked mackerel. The mean values of the quantification of aerobic microorganisms, as observed in Graph 1, it was found that the frozen horse mackerel had a higher number of microorganisms (3.59 ± 0.87 CFU/g) than smoked horse mackerel (2.39 ± 0.88 CFU/g).

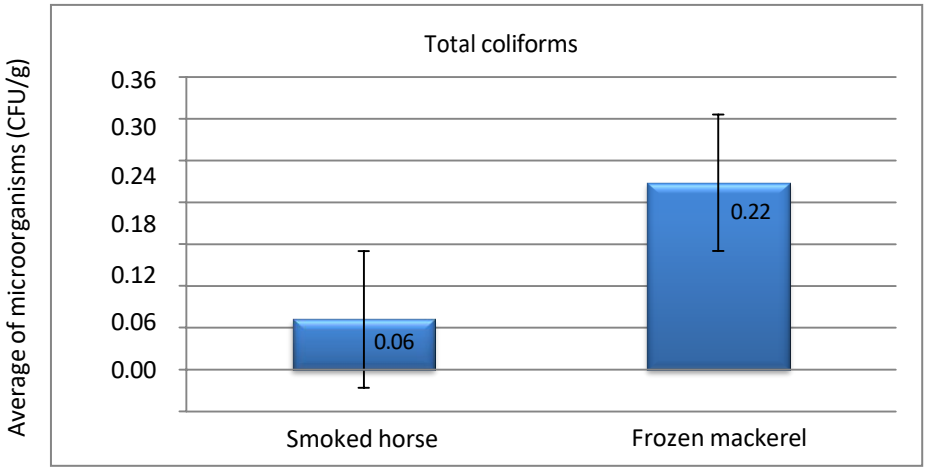
The low number of aerobic microorganisms in the smoked horse mackerel can be explained by the submission of the horse mackerel to heat during the smoking process. Regarding the frozen product, the high number of microorganisms is attributed to the change in temperature during the transport and storage of fish, taking into account that in the Desse market, most sellers contacted did not have an adequate system for the conservation of fish at low temperatures. Despite the presence of aerobic microorganisms in frozen and smoked horse mackerel, these results are within limits established by Mozambican legislation (MIPES 2001), which fixes the range from $5x10^5$ to 10^7 CFU/g in 2 of the 5 sub-samples analyzed.



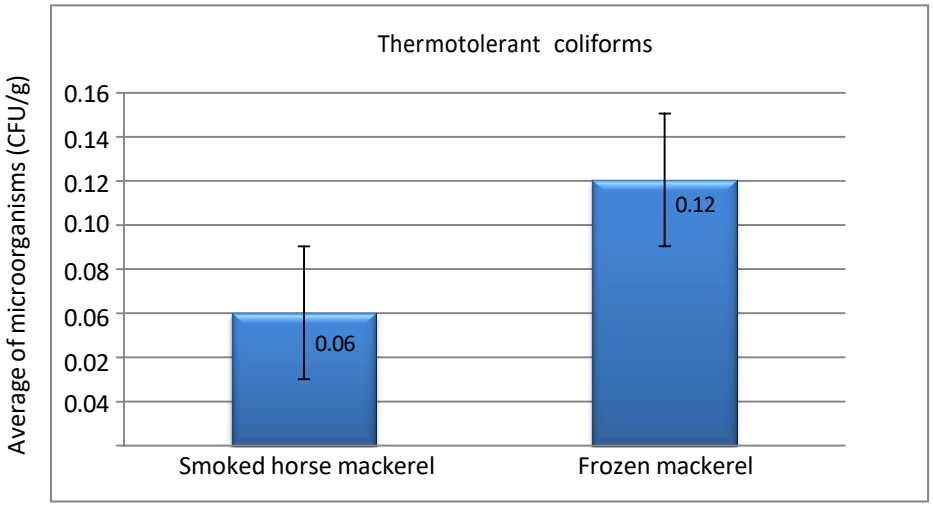
Graph 1 Comparison of aerobic microorganisms between smoked and frozen horse mackerel (mean ± standard deviation).

3.2 Quantification of total and thermotolerant coliforms

Frozen and smoked horse mackerel showed a statistically significant difference in the quantification of total and thermotolerant coliforms (Mann-Whitney U test = 40.50; P ≤ 0.05). In graphs 2 and 3, it can be seen that frozen horse mackerel had a higher number of total (0.22 ± 0.53 CFU/g) than smoked horse mackerel (0.06 ± 0.19 CFU/g). The same was observed in relation to the thermotolerant coliforms, frozen horse mackerel (0.12 ± 0.25 CFU/g), and smoked horse mackerel (0.06 ± 0.19 CFU/g).



Graph 2 Comparison of total coliforms between smoked and frozen horse mackerel (mean ± standard deviation).



Graph 3 Comparison of thermotolerant coliforms between smoked and frozen horse mackerel (mean ± standard deviation).

The high contamination observed in frozen horse mackerel may be related to the handling of fish, the conservation method and the lack of knowledge about the application of good handling practices. In addition to the factors mentioned, there were also cases of exposure of fish in places with some level of contamination (places close to garbage containers, existence of flies). Bastos (2010) evaluated the processing and conservation of fish in Brazil and obtained greater contamination with total and thermotolerant coliforms in frozen horse mackerel compared to smoked horse mackerel, factors related to the lack of knowledge of Good Management Practices by sellers in the Municipality of Ceará. Similar results were also observed by Huss (1997), who detected greater contamination by total and thermotolerant coliforms in frozen horse mackerel when compared to smoked mackerel. The reasons that led to these differences may be related to the hygienic-sanitary quality and the inadequate conditions for handling and processing the fish. Table 1 shows non-growth of *Coliforms 45*°C *, Escherichia coli*, *Salmonella spp.*, *Staphylococcos aureus, Vibrio cholerae* and *Vibrio parahaemolyticus.*

Table 1 Microbiological analysis of frozen and smoked horse mackerel.

	Coliforms 45°C	*E. coli*	*S. aureus*	*Salmonella* 25g	*V. cholerae*	*V. Parahaemolyticus*
Smoked horse mackerel	10^2 MPN/g	<10 CFU/g	5×10^3 CFU/g	Absent	Absent	Absent
Frozen mackerel	10^2 MPN/g	<10 CFU/g	5×10^3 CFU/g	Absent	Absent	Absent

(*) All analyzes were performed in triplicate.

In both groups of samples, *E. coli* growths were not identified. Similar results were obtained by Almeida (2013) during the evaluation of the quality of frozen and smoked fish in the Northeast region of Pernambuco, not having identified the presence of *E. coli*. On the contrary, Nunes (2011) obtained values that ranged from 40 to 930 MPN/g. Therefore, results above those foreseen in the legislation; are caused by poor and precarious hygiene conditions during the processing and marketing of fish. There was no growth of *Staphylococcus aureus* in both frozen and smoked horse mackerel samples. The results were within the standard established by the Mozambican legislation (Imprensa Nacional de Moçambique 2011), which establishes, as an unacceptable limit for consumption, values above 10^3 to 10^4 CFU/g in 3 of the 5 sub-samples analyzed.

There was no evidence of the growth of typical *Salmonella* colonies in all samples. Some factors such as handling, transport, and sale, if practiced incorrectly, can cause the fish to have the risk of contamination and consequently endanger the health of the consumer (Santos Castro 2011). The results of analysis of fish sold in supermarkets and local markets carried out by Arumugaswamy et al (1995) detected 25% positive samples for *Salmonella spp*.

According to ANVISA (2011), the presence of this bacteria in food indicates the inadequacy of the product for consumption, constituting a serious health problem. Bacteria of the genus *Salmonella* are not part of the fish's natural microflora and their presence can be justified by inadequate handling in the stages of the production chain or contact with contaminated water, through sewage effluent discharges, fishing basins, representing a way of transmission of these bacteria (Lira and Pereira 2001). No growth of typical *Vibrio cholerae* colonies (yellowish colonies) nor typical *Vibrio parahaemolyticus* colonies (greenish colonies) was detected in all samples analyzed. These results are in accordance with the maximum admissible values established by the Mozambican legislation, Imprensa Nacional de Moçambique (2011), which establishes as standard for product rejection a total above 10^2 to 10^3 bacteria in 1 of 5 analyzed subsamples. The Brazilian legislation through the National Health Surveillance Agency - ANVISA (2001), also fixes as maximum tolerance for *V. parahaemolyticus* 10^3 MPN/g. The results were within the established and acceptable limits for human consumption.

4. Conclusions

Hot smoking was shown to have a positive effect on the microbiological quality of horse mackerel, providing a safe product for potential consumers. Due to the deficient refrigeration conditions of the horse mackerel right after the capture, there was a slight increase in the number of microorganisms. However, it is essential to reinforce the hygiene measures and storage conditions of the horse mackerel in markets and other places of sale, as well as in the periphery, so as to avoid some vehicles from contamination. Frozen horse mackerel had a higher level of contamination compared to smoked horse mackerel. However, it's not a danger to consumers, as the subsequent steps, before its preparation, will contribute to reducing the likelihood of the risk.

Conflict of Interest

The authors declare that they have no conflict of interest.

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