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Efficiency of the ice-salt mixture in the conservation of trevallies (*Caranx hippos*) caught at the artisanal fishing port of Cotonou (Southern Benin)

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Abstract

Fish is a food consumed by many animal species, including humans. In addition to providing high quality protein, fish is a source of many essential micronutrients, including several vitamins (D, A and B), minerals (calcium, iodine, zinc, iron and selenium) and omega polyunsaturated fatty acids. 3 Unfortunately, it is a highly perishable product because of its high water content. This study aimed to improve the conservation process of the *Caranx hippos*. For that, the effectiveness of the ice-salt mix on fish conservation was assessed. The samples were stored in ice-salt mixture and characterized on sensory and microbiological levels. Results from the sensory evaluation showed that all samples were in good fresh condition and that the ice-salt mixture was more appreciated on several characteristics in relation to ice only. Microbiological analyses showed that samples have a low microbial load marked by the absence of total coliforms, staphylococci, salmonella, and anaerobic sulfuto-reducing bacteria agents. There were fewer than 10 CFU of yeast and mold per gram of samples. Nevertheless, samples were contaminated by the total flora (1.7×10^5 CFU/g) for sample conserved which a mixture of ice and salt and $2.3.10^5$ CFU/g for the sample preserved with ice only). This method needs to be optimized in view of its extension.

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Introduction

Just over 100 million tonnes of fish are consumed worldwide each year and provide 2.5 billion people with at least 20% of their average animal protein intake per capita (Godfray et al., 2010). In Benin, fisheries play an important role in socio-economic development, contributing about 3% of GDP (FAO, 2006). It is the most important source of animal protein in the diet of the population (Béné and Heck, 2005). However, fish are very perishable food items, with a relatively high rate of spoilage after fishing (Ghaly et al., 2010). The conservation of fish in tropical countries with relatively high temperatures is difficult because of its composition, the lack of adequate conservation infrastructure and the climatic environmental conditions that favor its and degradation within a few hours (Ayernor et al., 2005). Like other countries in the West African sub-region, post-harvest losses in Benin are estimated at around 20%, despite the efforts of more than 4,000 women each year to limit these losses by ensuring conservation of fresh fish by various traditional techniques (Anihouvi et al., 2006). About three quarters of the national fish production is consumed fresh, the rest being either smoked, dried, salted or fried before being distributed in domestic markets of the country. Fish should be quickly processed or kept in a chilled or frozen environment after fishing (Degnon et al., 2013). At the Artisanal Fishing Port of Cotonou (POPAC), the fishmongers keep the fish caught in ice to be able to sell them fresh. This preservation system acts as an artisanal freezer and as long as the fish is at a temperature of o°C or less, it retains all its qualities intact and the minerals are the preserved. Nevertheless fishmongers unanimously recognize that the more the conservation in the hard ice, the more the fish loses its flavor and its quality gradually deteriorates. Another drawback of this practice is the continuous supply of ice cream by the fishmongers. Indeed, ice is manufactured and stored in electrical cold production devices that struggle to operate continuously due to untimely power cuts in the country (Benin). This is why this research tends to explore new avenues for reducing the amount of ice used in conservation while at the same time preserving the microbiological and nutritional quality of fish. One of these is to combine salt with ice for fish conservation. The effectiveness of this alternative method has not yet been the subject of published study. It is to compensate for this insufficiency that we have undertaken the present work whose general objective is to evaluate the effectiveness of the conservation by the ice-salt mixture on the *Caranx hippos* fish.

Materials and methods

Materials

The material used in this study were *Caranx hippos* species, ice flakes and salt, sampling equipment, and laboratory reagents.

Experimental device

At a fisherman, six (06) Caranx hippo fish on one lot were collected. Three fishermen were collected in total. From a total of 18 fish, two lots of nine (09) were formed. Preservation was done in used freezers with water evacuation. For the first batch, 5kg of ice was poured into the bottom of the freezer, then we spread a given amount of salt and then we introduced the samples of Caranx hippos that we covered again with 5kg of ice on which we have shed a given amount of salt again. A total of 10 kg of ice and 69.24 g of salt were used. The storage under these conditions was held for five (5) days with renewal of the mixture when it decreased. For the second batch (control) the fish were kept only under ice with the same technique. Sample collection for microbiological and sensory analyses takes place 5 days after storage.

Sensory inspection

The evaluations were the mucus color, eyes appearance, the color, body rigidity, gills quality, adhesion of the scales to the skin, anus appearance, and the smell of fish. The sensory evaluation consisted in assigning notes to these various parameters in order to appreciate the freshness of the products. Thus, for each studied parameter, the scores varying between 1 and 9, ranging from satisfactory conditions to degradation of the product (unsatisfactory) were attributed. The sensory evaluation panel contained 10 fishermen, 10 fishmongers and 10 clients. Table 1 presents the sensory evaluation grid in relation to the freshness state of the fish.

Microbiological analysis

After five (05) days of storage, samples were evaluated by searching using standard methods, microbiological parameters of quality. Thus, the total mesophilic aerobic flora was enumerated by inoculation on the Plate Count Agar medium (PCA) and incubation at 30°C for 24-48 h (NF Vo8-051), whereas the total and thermotolerant coliforms were searched on the Violet Red Bile Lactose medium (VRBL) with incubation at 30 and 44 °C respectively for 24 h (NF Vo8-050). Positive coagulasse staphylococci was tested on Baird Parker medium with incubation at 37°C for 24-48 (NF EN ISO 6888-1 / A1), while yeasts and molds on Sabouraud medium with chloramphenicol were incubated at 25 °C for 3 to 5 days (NF Vo8-059). The Anaerobic Sulpho-Reducing Bacteria were investigated on Tryptone Sulfite Neomycin (TSN) agar with incubation at 46 ° C for 20H (NF ISO 15213). Finally Salmonella search was performed by pre-enrichment of the stock solution at 37 ° C for 19 h. Enrichment was made on Rappaport Vassiliadis (RV), Müller Kaüffmann (KM) media and isolation on Xylose-Lysine-Decarboxylate (XLD) and Hektoen (ISO 6579) media.

Statistical analyzes

Data from three independent replicate trial were subjected to statistical analysis using Minitab 16. Differences between means were tested by ANOVA test. Sensory analysis has been subject to a Principal Component Analysis (PCA) in this same software.

Results

Sensory characteristics of preserved fish

The analysis of the eigen values and eigen vectors of the correlation matrix generated by the principal component analysis showed that the first principal component explains 100% (Table 2) of the information relating to the preserved fish samples.

Table 1. Recognition characteristics of a freshfish and an alteredfish (Huss, 1988).

Freshfish	Spoiledfish				
Light, pleasant, reminiscent of seaweed	Unpleasant, acrid, acidic, ammoniacal				
for sea fish, or aquatic weeds for water	putrid				
fish douce					
Brilliant with metallic luster and	Matte, without glare or glare				
iridescent reflections, no blood around					
the head and along the column					
Rigid body, arched, consistent and at	Flaccid body, soft, soft consistency,				
the same time elastic	finger pressure leaving marks				
Wet fish, transparent mucus, no visible	Present and slimy				
secretions					
Strongly adherent, brilliant	Easily detached when lifted				
Tight, well adherent	Wrinkled, discolored, easily tears				
Clear, bright, bright, shiny, convex,	Term, glassy, opaque, concave, sagging				
transparent, occupying the entire orbital	in orbit				
cavity					
Adhere, without spot of blood	Slightly raised, with red-brown spots				
Wet, bright, pink or blood red	Dry, grayish				
Not inflated, sagging, tense or torn	Flabby, deformed, often swollen, with				
	blot, dark blue, greenish or black				
Hermetically closed	Gaping, often prominent				
	Light, pleasant, reminiscent of seaweed for sea fish, or aquatic weeds for water fish douce Brilliant with metallic luster and iridescent reflections, no blood around the head and along the column Rigid body, arched, consistent and at the same time elastic Wet fish, transparent mucus, no visible secretions Strongly adherent, brilliant Tight, well adherent Clear, bright, bright, shiny, convex, transparent, occupying the entire orbital cavity Adhere, without spot of blood Wet, bright, pink or blood red Not inflated, sagging, tense or torn				

It emerges that the odor, the rigidity of the body, the secretions, the scales, skin, flesh, and overall rating were positively correlated while the gills, abdomen, and anus are negatively correlated (Table 3). From the comparative exploitation of figure 1A and 1B, it

appears that samples stored fish with ice were better appreciated for the attributes of their abdomen, gills and anus. On the other hand, those preserved by a mixture of ice-salt have the best characteristics with regard to the eyes, the skin, the odor, the body rigidity, the smell, the pulpit, the scales, the secretions and were best preserved (overall rating) according to evaluators.

Microbiological characteristics of conserved fish

The results from analyzes carried out on the various samples of *Caranx hippos* have been recorded in Table 4. From the analysis of this table, there was an absence of total coliforms, staphylococci, salmonella and sulphite-reducing bacteria. Nevertheless, it was noted that the number of total bacteria was 1.7.10⁵

CFU/g for the ice and salt mixture and $2.3.10^{5}$ UFC / g for ice only. At the level of presumed coliforms, there was a colony number equal to $6.7.10^{2}$ UFC/g for ice only whereas at the level of the mixture of ice and salt, this number is less than 10ufc / g. From the same table we note that the number of yeast and mold counted in the control samples was all less than 10.

From this it can be deduced that ice and salt inhibits the proliferation of total bacteria and presumed coliforms.

Table 2. Analysis of the correlation matrix eigenvalues.

Eigenvalues	11.000	0.000	0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
Proportions	1.000	0.000	0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
Cumulated	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Discussion

A fish, when it is removed from the sea, dies, then putrefies after a more or less long time according to the external conditions. As for other animal or plant substances this decomposition is caused by two categories of active agents: diastases and microorganisms. The cold tempers these processes and thus, allows preserving the state of freshness of the fish (Fikiin and Fikiin, 2000).

Table 3. Analysis of the correlation matrix eigen vectors.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Odor	0.302	0.302	-0.147	-0.233	-0.017	-0.377	0.240	0.027	-0.180	0.769	0.008
Body rigidity	0.302	-0.150	0.362	0.772	-0.134	-0.051	0.164	0.274	-0.060	0.065	-0.170
secretions	0.302	0.484	-0.081	0.043	-0.392	0.277	0.441	-0.488	-0.002	-0.055	-0.026
Scales	0.302	0.346	0.346	-0.010	-0.065	0.041	-0.096	0.566	0.565	0.014	0.096
Skin	0.302	-0.419	-0.414	-0.042	-0.327	0.381	-0.177	0.163	-0.448	-0.139	0.173
Eyes	0.302	-0.564	-0.159	0.029	0.053	-0.240	0.111	-0.402	0.528	-0.223	0.046
Gills	-0.302	-0.188	0.175	-0.315	-0.586	-0.218	0.431	0.335	0.081	-0.214	-0.054
Abdomen	-0.302	0.101	-0.186	0.336	-0.526	-0.347	-0.445	-0.237	0.066	0.175	0.257
Anus	-0.302	0.058	-0.058	0.335	0.298	-0.201	0.492	0.089	-0.197	-0.173	0.237
Flesh	0.302	0.141	0.381	-0.110	0.074	-0.242	-0.000	0.040	-0.149	-0.223	0.771
Global	0.302	0.229	-0.139	-0.094	-0.006	-0.555	-0.204	0.007	-0.304	-0.420	-0.458
appreciation											

PC : Principal Component.

The results from the sensory characteristics evaluation showed that those fishes conserved with ice were better appreciated for the parameters abdomen, gills and anus. On the other hand, those preserved by a mixture of ice-salt have the best characteristics with regard to the eyes, the skin, the odor, the bodyrigidity, the smell, the pulpit, the scales, the secretions and are the best retained (overall assessment) by evaluators. So, ice-salt mixture allowed better conservation of the fish. This could be explained by the fact that salt melts ice (which is water in solid form) but at the same time as the ice melts, the temperature of the ice-salt mixture drops sharply (Ababouch *et al*, 1989).

Parameters	Fish / ice	Fish / Salt ice
Total bacteria (CFU/g)	$2,3.10^{5}$	1,7.105
Total coliforms (CFU/g)	6,7.10 ²	$2,3.10^{5}$
Thermotolerant coliforms (CFU/g)	<1	<1
Positive coagulasse Staphylococci (UFC/g)	<1	<1
Sulphito-reducing bacteria (CFU/g)	<1	<1
Yeasts (Y) and molds (M) (CFU/g)	Y<10	L<10
	M<10	M<10
Salmonella in 25g	<1	<1

Table 4. Microbiological characteristics of fish sample.

Indeed, when salt is added to ice, the freezing temperature (solidification) of the water is lowered, by a few degrees to -10 °C, depending on the amount of salt. It is known that the solidification of water is

the passage from a liquid state (disordered water molecules) to a solid state (water molecules arranged side by side in an orderly fashion).

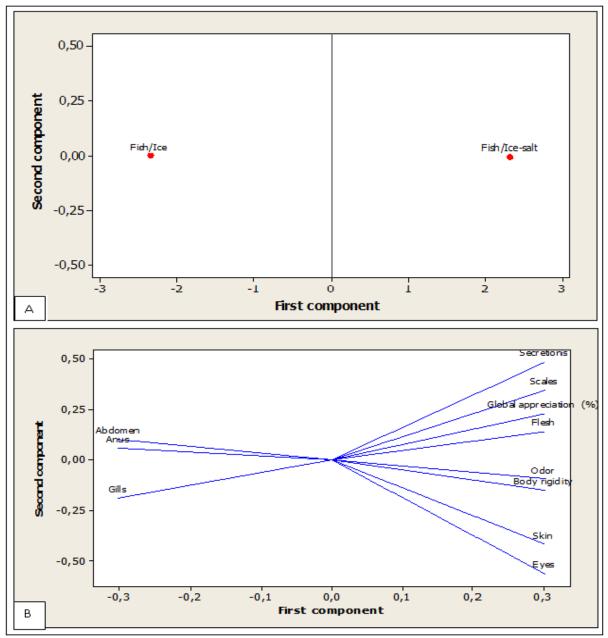


Fig. 1. Diagram of contributions (A) and scores (B) of sensory attributes of samples.

If the water is pure, the water molecules can be ordered at o °C. If the water contains salt, it will interfere with the storage of the water molecules: the constituents of the salt (the chloride and sodium ions) intervene between the water molecules, introducing disorder. For the water to solidify, it is necessary to compensate for this disorder thanks to a temperature lower than o ° C, because the low temperature favors the "storage" of the molecules to form the solid. This drop in temperature promotes the conservation of fish's sensory attributes by reducing enzymatic activities and the proliferation of microorganisms (Rahman, 2007). This would explain the fact that the level of microbiological contamination observed for samples stored in ice is higher than that of those preserved in the ice-salt mixture.

Moreover, the of microorganisms presence enumerated during this study can be variously appreciated. The contamination of the samples by the total flora showed that the latter are the subject of poor handling and poor hygienic conditions. Contamination flora often consists of Enterobacteriaceae, Bacillus, Pseudomonas, or other potentially pathogenic agents. Their presence beyond standards can mean a lack of hygiene in handling and poor storage conditions (Pothakos et al., 2012).

Total coliforms are of faecal origin (10-15%). These bacteria serve as indicators of faecal contamination because they come from the intestines and excrement of humans and warm-blooded animals. The presence of these pathogenic bacteria is very risky for the consumer'shealth (Chedad *et al.*, 2007).

For yeasts and molds their presence in small quantities could be explained by the low osmophile nature of the fish. They are germs responsible toproducts alterations, causing loss of organoleptic quality and food poisoning (due to the secretions of aflatoxin for example by molds). It is important not to neglect this contamination (Rahman, 2007).

Conclusion

This study evaluated the conservation efficiency of

Caranx hippos fish with a mixture of ice and salt. The results showed that the salt-ice mixture inhibits better the growth of the microbial flora that can alter the fish. Ice mixed with salt has melted much more slowly. Therefore, the frequency with which the fishmongers will need to renew the amount of ice to keep the cold in the insulated boxes will be reduced. The use of the ice-salt mixture could be therefore less expensive than keeping ice fish exclusively for fishmongers.

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