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Assessing the Microbiological Quality and Conditions of Sales of *Cyprinus carpio*, *Arius* sp. and Cybium tritor: Three Fish Species Mostly Consumed in Côte D'ivoire

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Abstract: The microbiological quality and the conditions of sale of the three species of fish, *Cyprinus carpio, Arius* sp. and *Cybium tritor*, highly enjoyed and consumed in Côte d'Ivoire were assessed. Forty eight fishes of each species were analyzed. Total aerobic count, fecal coliforms, *Staphylococcus aureus*, anaerobic sulfite-reducers, *Vibrio* and *Salmonella* were found in fish sold at both unloading area and on stalls in the market. The monitoring of the distribution chain and the determination of temperature of fish storage in the market showed that fishes are liable to very poor hygienic conditions (temperature, cleanness…) during handling, transportation, storage and selling. The level of contamination regarding the total aerobic count ranged from $2.2.10^4$-$4.5.10^5$ CFU g^{-1}; for fecal coliforms, it randed from 10-2.10^2 CFU g^{-1}, for *S. aureus*, from 10^2-$1.2.10^2$ CFU g^{-1} and for anaerobic sulfite-reducers it ranged from 1-3 CFU g^{-1}. *Salmonella* and *Vibrio* were not detected in the fishes analyzed. *C. carpio* was the most contaminated fish by total aerobic count ($1.2.10^5$ CFU g^{-1} found in fish bought at the unloading area and $4.1.10^5$ CFU g^{-1} in fish bought to the retailer on fish market) and fecal coliforms (21 and 2.10^2 CFU g^{-1}). Levels of *S. aureus* and anaerobic sulfite-reducers were similar in all species. For the 3 species, the fish bought at the fish market show higher percentage of unacceptable microbiological quality (17%) compared to those bought at the unloading place (4%). Ninty four percent of *C. tritor* are of acceptable microbiological quality whereas percentages of *C. carpio* and *Arius* sp. with acceptable microbiological quality were, respectively 89 and 88%. *C. carpio* and *Arius* sp. show the highest percentages of fishes with unacceptable microbiological quality, (respectively 11 and 12%), the lowest percentage obtained for *C. tritor* is 6%. The bacteriological quality of fishes studied is not dangerous for consumers and will not be a public health risk if the fish are properly cooked before consumption.

Key words: Fish, microbiological quality, distribution chain

INTRODUCTION

Pollution of aquatic environments by domestic and industrial activities, strongly contributes to the contamination of seafood (Kosmala, 1998). The artisanal fisheries are endemic to post harvest losses. In most cases, during fishing or catching, the bacterial infection set in the flesh of the fish is the result of injuries caused by traps and nets. The spoilage of fish can also be caused by catching methods resulting in wounds on their skin (Aldrin, 1965). Open wounds and stress out of struggling accelerate the deterioration of fish, especially if they are not stored in a chilling or cold room (Bolorundino *et al*., 2005; Huss, 1995). Deteriorations can be source of human infection because fish are highly implicated in food poisoning. Lipp and Rose (1997) indicated that from 1983-1992, in the United States of America, fish and other seafood held the third place of the food categories responsible for food infections.

In some West African countries, fish has become the essential commodity and the main animal protein because the existing climatic conditions are an obstacle to cattle farming; consequently, this situation has resulted in the improvement of fish consumption. Côte d'Ivoire is both a coastal and forest country and the populations of this country are consumers of halieutics products and great fish consumers as well in all forms (fresh, smoked, salted, fermented, dried...). In this country, like in most of the African ones, after catching, fish are transported, stored and sold on markets which represent the major site

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supplying retailers and consumers. Unfortunately, the adequate cold storage or packaging is not observed from harvesting to selling. It is therefore predictable that microbial contamination of fish occurs resulting in risk for consumers. That being the case, it is significant to assess the hygienic quality of fish sold in the above described conditions.

The aim of this study was to evaluate the hygienic quality of 3 species of fish most consumed and widespread in Côte d'Ivoire: *Cyprinus carpio* (carp), *Arius* sp. (machoiron) and *Cybium tritor*, also called *Scomberomorus tritor* (mackerel). *Cyprinus carpio* is a resistant fish especially living in rivers and lakes, but capable to adapt to various aquatic environments and easy to breeding (Kottelat, 1997; Koehn, 2004) *Arius* sp. are river fish and sea fish while *Cybium tritor* is a marine species which extends to cold water (Aldrin *et al*., 1972; Colette and Nauen, 1983).

Since there are no previous national studies regarding the hygienic quality of those fishes, the present work will monitor the distribution system of these three species, identify possible critical points of contamination and determine factors influencing contamination. This will improve recommendations and policy formulations in order to reduce post-harvest fish losses and to prevent public health disease as well. More over, the objective of this work is to study the microbiological quality of these fishes sold in markets by the determination of the level of micro-organisms such as Total Aerobic Count (TAC), fecal coliforms, Anaerobic Sulfite-Reducers (ASR), *Staphylococcus aureus* and pathogenic bacteria like *Salmonella* and *Vibrio*.

MATERIALS AND METHODS

The study was carried out on 144 fresh fish bought in the market of *Adjamé* (North of Abidjan, Côte d'Ivoire) including 48 *C. carpio*, 48 *Arius* sp. and 48 *C. tritor*. 12 space periods were defined, with 4 fish of each type per period. For each period, 2 fish pertaining to each species of 20 cm in size and weighting between 65-70 g were provided both from the unloading site and from the stalls in the market.

Monitoring of fish distribution chain: A survey was carried out with the fishermen and the other actors of fishery in order to clearly identity the critical points of contamination in fresh fish distribution chain. A similar survey was also carried out in the market of "*Adjamé*" (Abidjan), in order to describe the selling conditions of fish, insisting on the hygienic conditions (cleaning of stall, disinfection…).

Measurement of fish storage temperature: A thermometer (SILDER DRAND) was used to check the storage temperature of fishes preserved in refrigerators in the market.

Sampling: Samples were supplied during two days in the week, Monday at the unloading site and Tuesday at the salesmen's stalls. Each sample placed into a sterile plastic bag, identified, stored in an ice box and conveyed to laboratory.

Preparation of fish for analyses: Once in the laboratory, 25 g of flesh from each sample were added to 225 mL of sterile buffer peptone water contained in a plastic stomacher bag and mixed together. Decimal dilutions from this solution were then carried out in buffered peptone water.

Research and enumeration of micro-organisms and determination of microbiological quality of fishes: The following count was carried out according to the AFNOR methods (1996): Total aerobic count, fecal coliforms, *Staphylococcus aureus*, ASR, *Salmonella* and *Vibrio*. Samples of each suitable dilution were plated in duplicate in an appropriate media for total aerobic count, fecal coliforms, *S. aureus* and ASR. Suspected colonies of *S. aureus* were submitted to staphylocoagulase test. The steps required for *Salmonella* and *Vibrio* detection (pre-enrichment, enrichment, isolation and identification) were carried out for these 2 genera. Results of bacterial count were used to determine the microbiological quality of the fishes according to the AFNOR criteria for fresh fish (AFNOR, 1996).

RESULTS AND DISCUSSION

Fish distribution chain and fish storage temperature: Monitoring the fish distribution chain showed that more than 80% of the quantity of fish sold in Abidjan is supplied by the unloading site and the remaining 20% is supplied by fishermen working at the lagoon. The cautious follow-up of the distribution chain of fish shows that from harvest to the retailer at the market, fish goes through several intermediaries before selling to the market. The channel of sales is the following one: wholesalers, retailers and consumers. Fish is delivered without adequate chilling method. In fact, during transportation to the markets, fish are not preserved at low temperature. As a matter of fact, micro-organisms are likely to proliferate in the products, strongly contributing to fish spoilage.

At the market, fish is stored in inadequate cold room. Retailers stored the fish in an old open refrigerator containing ice and are due to be sold within 10 days. The average temperature of fish storage is 8±2.5°C. This storage temperature is not sufficient to stabilize or reduce microbial growth in fish and can induce deterioration of this food. According to Nixon (1971) the relative rate of deterioration of fish is directly correlated with the temperature of storage. More ever, several authors came to conclusion that spoilage rate of many fish species increases when these products are subjected to temperatures above 0°C (Olley and Quarmby, 1981; Cann *et al*., 1983).

For selling, fish is displayed to the surrounding temperature during the day on improperly cleaned and not disinfected stalls, this can enhance development of micro-organisms such as the pathogenic bacteria. In order to make more profit, retailers used cunning to bring client buy the damaged fish: To this end, fishes are either washed to eliminate sticky secretion, scaled, or the head and the tail are tightly joined together to maintain fishes in a rigid position so that the beginning of deterioration is cover up or hidden. Removing the soiled gills and faded fish innards is also the means of eliminating degradation evidences of the products. The poor storage and hygienic conditions in the market and the selling practices of retailers (selling damaged products) rapidly lead to fish deterioration resulting in the risk for consumers to be poisoned. A defective hygiene presents a risk of contamination of fish and consumers are exposed to food borne diseases especially if theses products are undercooked or poorly processed. In addition to the possible risk of micro-organism infection, there is a risk of intoxication due to toxic compounds such as histamine. Histamine is a thermostable biogenic amine frequently found in seafood; it is produced by the decomposition of histidine related to the presence of bacteria in food and to storage temperature of these products (Stratten and Taylor, 1991; Foo, 1975; Emborg and Dalgaard, 2006). Intoxication by histamine is known to be the first cause of food toxi-infection associated with the consumption of fish and other seafood (Lipp and Rose, 1997).

Microbiological assay: Bacterial counts are described in Table 1 and 2. The bacteriological analysis showed that total aerobic count, fecal coliforms, *S. aureus* and anaerobic sulfite-reducers are found in fish. All the fishes bought both at the unloading place and from retailers on stalls are contaminated by these micro-organisms but the levels of these micro-organisms are higher in fish bought on stalls than in fish directly bought at unloading places. The level of total aerobic count contained in fish

Table 1: Bacterial count of fishes bought on unloading in market

	Fish species		
Germs (CFU g^{-1})	*Cyprius carpio*	*Arius* sp.	*Cybium tritor*
Total aerobic count	$1.2.\ 10^5 \pm 3.10^4$	$2.2.\ 10^4 \pm 7.8.10^3$	$3.9.\ 10^4 \pm 3.5.10^3$
Fecal coliforms	16±9	10±1	21±10
S. aureus	$10^2 \pm 7$	$10^2 \pm 5$	$10^2 \pm 5$
ASR	1	1	1
Salmonella	-	-	-
Vibrio	-	-	-

- : not detected

Table 2: Bacterial count of fishes bought on stalls in market

	Fish species		
Germs (CFU g^{-1})	*Cyprius carpio*	*Arius* sp.	*Cybium tritor*
Total aerobic count	$4.1.10^5 \pm 8.3.10^4$	$3.\ 10^5 \pm 8.1.10^4$	$1.9.\ 10^5 \pm 2.4.10^4$
Fecal coliforms	$1.2.10^2 \pm 54$	$2.10^2 \pm 95$	46±9
S. aureus	$1.2.10^2 \pm 60$	$10^2 \pm 10$	$10^2 \pm 15$
ASR	1	5±2	2±1
Salmonella	-	-	-
Vibrio	-	-	-

- : not detected

bought at the unloading place is $1.2.10^5$ CFU g^{-1} for *C carpio*, $2.2.10^4$ for *Arius sp.* and $3.9.\ 10^4$ for *C tritor*. The level of the same aerobic count contained in the fish purchased from retailers is $4.1.10^5$, $3.\ 10^5$ and $1.9.\ 10^5$ CFU g^{-1}, respectively for *C. carpio*, *Arius* sp. and for *C tritor*. Concerning the fecal coliforms, the level for carps is 21 CFU g^{-1} for those bought from the unloading place while it reaches 2.10^2 CFU g^{-1} for those bought on the stalls. As regarded to *Arius* sp. the number of fecal coliforms is 10 and $1.2.10^2$ CFU g^{-1}, respectively for fish bought at the unloading place and for those bought from stalls. For *C. tritor*, the level of these bacteria ranges from 16 CFU g^{-1} (unloading place) to 46 CFU g^{-1} (stalls in the market). The number of *S. aureus* (10^2-$1.2.10^2$ CFU g^{-1}) and ASR (1-3 CFU g^{-1}) is similar in all the species. Total aerobic count, fecal coliforms, *S. aureus* and anaerobic sulfite-reducers are often found in fresh fish confirming that sea and lagoon pollution constitutes a risk for seafood and for humans (Fernandes *et al*., 1997; Morales *et al*., 2004; Herrera *et al*., 2006; Andreji *et al*., 2006).

Vibrio and *Salmonella* were not detected in all the fishes analyzed (Table 1 and 2). Several works on bacteriological study of fresh fish (Herrera *et al*., 2006; Pullela *et al*., 1998; Koffi *et al*., 2006) by other authors had presented similar results. The absence of these pathogenic bacteria is a good indicator of the microbiological quality of fishes. It can be justified by the fact that in water, the presence of other bacteria predators is responsible for the low resistance of the pathogenic bacteria (Carmouze and Caumette, 1985).

Microbiological quality of fish: According to microbiological criteria for fresh fish, most of fish studied have an acceptable quality: 79% for fish bought at the

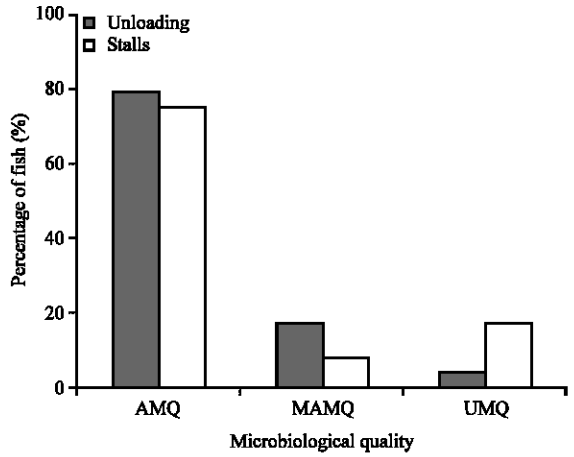

Fig. 1: Microbiological quality of fishes according to site of purchase. AMQ: Acceptable Microbiological Quality; MAMQ: Marginally Acceptable Microbiological Quality UMQ: Unacceptable Microbiological Quality

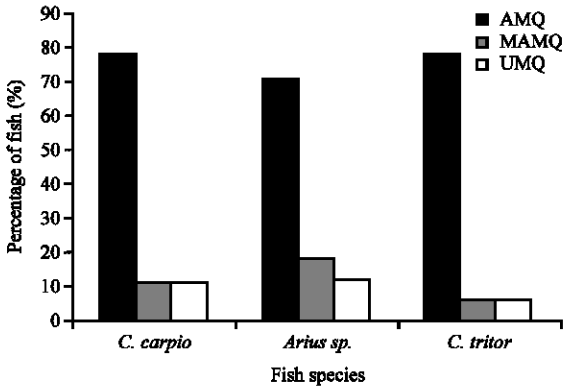

Fig. 2: Microbiological quality according to species of fish studied. AMQ : Acceptable Microbiological Quality; MAMQ: Marginally Acceptable Microbiological Quality Umq: Unacceptable Microbiological Quality

unloading place and 75% for those bought on the stalls in the market. However, the number of fish with unacceptable microbiological quality is related to the place of supply: 17% from stalls and 4% from unloading point (Fig. 1). These results correlate with those of the distribution chain monitoring indicating that fish storage and selling conditions are not done in compliance with hygienic conditions or requirements (high temperatures of conservation, improperly cleaned stalls and bad practices in selling damaged fish). These poor hygienic conditions correlate with the high presence of fecal coliforms (from 10-2.10^2 CFU g^{-1}) and *S. aureus* (10^2-1.2.10^2 CFU g^{-1}) in the analyzed samples (Table 1 and 2). Previous works have indicated that at storage temperatures above 0°C, species of Enterobacteriaceae family (coliforms and non-coliforms) and the Gram positive bacteria are often responsible for seafood deterioration (Gram *et al*., 1987; Gram *et al*., 1990; Liston, 1992). Such species can be opportunist pathogen and can cause food borne diseases and fish death (Balarin and Hatton, 1979; Roberts and Sommerville, 1982).

The three species studied showed different percentage of microbiological quality: Percentage of samples of *C carpio* and *C tritor* with an acceptable microbiological quality are more important (78%) than those of *Arius* sp. (70%). *C. carpio* and *Arius* sp. showed the highest percentages of samples with unacceptable quality (11 and 12%, respectively) the lowest percentage being obtained for mackerels *C. tritor* (6%) as shown in Fig. 2. These results correlate with those of bacteria count which indicated that *C. carpio* and *Arius* sp. contained the highest level of micro-organisms. *C. carpio* are scales fishes, consequently, their deterioration can be easily hidden. It the reason why the high rate of unacceptable quality is observed for this species. Hiding scale fish deterioration is known to be easier than hiding scale less fish (Murray and Burt, 1969). *Arius* sp. are scaleless fish, yet the percentage of unacceptable quality is high. This result could be explained by the fact that because they are more expensive fish and are likely to be hardly sold or bought. Consequently, this fish is stored for long time and is more deteriorated than others. The low percentage of *C tritor* with unacceptable quality (6%) is due to one the fact that it is inexpensive fish, easily bought and stored in a short period. It is also because it is a scaleless fish and as such any deterioration is easily and quickly detectable.

CONCLUSION

This study showed that the three species of fish analyzed (*C. carpio*, *Arius* sp and *C. tritor*) are not only handled, transported, stored and sold in poor hygienic conditions but also contaminated by various micro-organisms. The total aerobic count, fecal coliforms, *S. aureus* and ASR were found in all the fishes. But most of the fish had an acceptable microbiological quality. *C. carpio* and *Arius* sp. showed the highest percentage of fishes with unacceptable microbiological quality, while *C. tritor*, the lowest percentage. The difference between the various species can be related on the one hand, to the type of fish (scale fish or

scaleless fish) and on the other hand to the cost of fish, because expensive fish is stored for a long period before being sold and therefore are more likely to be deteriorated.

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