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Research Paper

Detection of the indicatory micro-organisms of the contamination in the smoked fish and cool fish sold in the city of Kisangani (case of Oreochromis niloticus)

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Summary
Fish are very perishable food commodities, having a relatively elevated change speed after the fishing but their process of transformation and merchandising remains even artisanal and often affect their microbiological quality, capable to cause some illnesses. This survey aims to value the microbiological quality of fish smoked and sold expenses in the city of Kisangani, while identifying the responsible bacterial species of the family of Entérobactériacées of the infectious illnesses at the human beings.
The microbiological analyses carrying on the numbering of the total germs have been made according to the conventional norms. The rate of contamination by the total germs reached an average of: for the cool fish coming from the township of Kabondo 6,8. 10^{10} UFC/g, followed those of the common Makiso with 78 UFC/g. For the smoked fish, the most important abundances are recorded in the township of the Tshopo 4,8.10^{10} UFC/g, consistent of the township of Mangobo 4,68.10^{10} UFC/g, of the township of Kabondo 4,38.10^{10}UFC/g, of the township of Kisangani 3,41.10^{1} UFC/g, of the township of Lubunga 4,03.10^{9} UFC/g finally the township of Makiso 3,4.10^{9}UFC/g.
Are the concentrations of entérobactéries especially abundant among the cool fish of the township of Kabondo, reaching 4,3.10^{11} UFC/gs, followed of those of the common Makiso with 68 UFC/gs. For the smoked fish, the most important levels are observed in the township of Lubunga 5,84.$10^{1\,0}$ UFC/g, consistent of the common Mangobo 4,94.$10^{1\,0}$ UFC/g, of the common Makiso 4,53.$10^{1\,0}$ UFC/g, of the common Tshopo 4,38.$10^{1\,0}$ UFC/g, of the common Kisangani 4,62.10^{2}UFC/g finally the common Kabondo 4,22.10^{8} UFC/g.

Key words: *Let's pitch smoked, microorganismes, contamination.*

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I. Introduction

The food insecurity in the world, and more especially in the countries in development, is not only bound to a weak production of the food commodities but also to their bad distribution through the community. This situation worsens again by the problem that the conservation of these products poses. Thus, the food insecurity generates a nutritional effect that entails some serious repercussions, sometimes irreversible, on the physical, social and economic development of the communities concerned (FAO, 2003).

According to the last statistics of the world production of the freshwater fish (FAO, 2003), the Tilapias occupy the third rank after the cyprinidés and the salmonidés, with a rate of yearly growth of 13,9% (1970-2000) and a production of 1,256 millions of tons in 2002 of which 82% of the total production are represented by the species Oreochromis niloticus.

From then on, the healthiness of the fishing products constitutes a major preoccupation for the health of the population. Fish is unfortunately a very perishable food. His/her/its deterioration begins since the fishing and evolve very quickly under the climatic and environmental conditions of the region of the tropics. Fish alters himself/itself in less than 12 hours (Brigitte and al, 2005). To palliate to this problem, some techniques of

conservation so much artisanal that industrial (manuring, salting,) are put in place in the goal to increase his/her/its commercial life span and also to slow down the process of his/her/its deterioration.

The question of conservation of fish is always of actuality. Indeed, the main potential sources of contamination of this food are soil, water, air, the plants, the animals, the man, the equipment, the ingredients and of the packing materials (Lambert, 1989). This sector poses enormous nutritional, sanitary and environmental problems that are however likely to compromise the food security of the populations (Diouff, 1992).

In RDC, fish occupies a place of choice in the human food because of his/her/its yearly permanent availability. Every inhabitant consumes 5,7 Kg on average of it and he is very often presented in the country under salty shape, smoke and expenses (Anonymous, 2005).

In intensive aquaculture, the station food represents a part important of the cost of the production of fish. The interest economic of this type of raising is therefore very dependent of the availability and the cost of food (Tacon, 1996). So, the reduction of the loads bound to the food, and therefore the mastery of the cost of production of the raising fish, is one of the priorities in aquaculture (Watanabe, 2002).

In the region of Kisangani, Oreochromis niloticus commonly named "tilapia" is fish the more clear soup under shape expenses, salty and smoke. His/her/its production essentially takes place in the soft waters of the ponds piscicoles and therefore under controlled conditions. Being appreciated well of the consumers, his/her/its production does increase only and the techniques of his/her/its conservation diversify. Indeed, his/her/its consumption gets used mainly at the cool state and currently his/her/its shape smoke takes the size in this middle. This reality especially attracted our scientific curiosity and to the look of that that precedes, we asked the following questions:

Oreachromis fish cool niloticus and smoked traitors in the city of Kisangani are they carriers of the germs responsible for the infectious illnesses?

"What are the families of the bacterial and fungal species responsible for the present infectious illnesses on these fish?

Do "the bacterial and fungal species responsible for the infectious illnesses done carry by these fish present them a real sanitary danger for the consumers?

The major objective pursued by this survey is to value the microbiological quality of fish smoked and sold expenses in the city of Kisangani, to count and to identify the bacterial species of the family of present Entérobactériaceae in fish smoked Oreochromis niloticus and expenses.

II. Materiel and methods

Site of sampling

Work has been done in the city of Kisangani, County seat of the Province of the Tshopo, situated to the Northeast of the RD Congo. His/her/its geographical coordinates are 0°31' North latitude, 25°11' longitude is and 396-410 m of altitude (Ebwa 2024).

II.2 Materiel

- **Biologic Material**

Fish smoked O. niloticus and sold expenses in the city of Kisangani.

- **Non biologic Materials**

The microbiological analyses done to the Provincial Laboratory of the Public Health of Kisangani have been facilitated while using the following materials :

- Surroundings of culture and reactive: Nourishing soup, Gélose of Mac Conkey, Middle of Kligler Hajna, middle of Chapman, citrate of Simmons, Voges Proskaue, etc.
- Matériels of sterilization: Pasteur oven, Autoclave, Beak bunsen, etc.
- Other materials: clamps, portoir to tube to test, match, shackle of turntable, etc.
- Various devices: Incubator, Hood to flux laminaire, Double boiler, balance of precision, etc.
- Glassware: boxes of Kneaded, Tubes to test, Pipettes, Erlenmeyers, etc.

II.3. Methods

a) Sampling and microbial preparation

❖ **Places of withdrawal**

The samples of poisons to analyze in this work have been appropriated in different markets being in the city of Kisangani notably: Isomela (Lubunga), Balese (Mangobo), social Home (Kabondo), Limanga Fond of advance (Kisangani), Fifteenth avenue (Tshopo) and central Market (Makiso).

❖ **Actual withdrawal and conditioning of the samples**

The sampling has been done the before-noon between 10h30 and 11h30. Every fish bought by the seller has been put in a sterile jar in order to minimize the most possible the accidental contaminations of the sample thus collected.

❖ **Preparation of the culture surroundings.**

The quality of the final product often depends on the technique used to put back the surroundings some solution, for it it is necessary to follow the main described below stages:

Stage n°1: Weighed

One starts with weighing the quantity of wanted powder, one or several constituent in the case of middle composed of several ingredients, by means of a calibrated analytic balance.

The precision doesn't influence too much on the preparation of the culture surroundings except for weighed it of some chemical body.

Stage n°2: Dissolution

The substances to add will be made in the order indicated of the formula of preparation of the culture surroundings in the case where the middle is constituted of several ingredients.

Stage n°3: Adjustment of the pH

It is the most sensitive phase of the whole preparation while being indispensable, because the pH of water purified can vary according to the conditions of obtaining and storage.

The verification of the pH and the adjustment so necessary to the value indicated on the technical card while respecting the interval, corresponds to the concentration in ions hydrogen of the middle that it is necessary to respect in order to preserve the life of the bacteria to study.

The measure of the pH, must be done therefore as correctly as possible.

Stage n°4: Sterilization

Except contrary indication, the sterilization is done the most often to the autoclave to 121°C during 15 minutes.

Stage n°5: Adjustment of the pH after sterilization

Same operation mentioned however in the stage n° 3 the solutions of adjustment of NaOH and HCls must be sterile and the operation takes place in conditions of total asepsis owing a Beak bunsen

Stage n°6: Distribution of the culture surroundings

The surroundings of culture are the most often distribute either in tubes, either in them limp of kneaded, either in small bottles syrups according to the need and it is after sterilization.

Retorted the small bottles once are labeled the labels must contain; name of the middle, used code, date of expiration and conservation limit so necessary and to keep them in a cool place safe from dusts in the fridge has a temperature between 2 in 8 °C.

After the stage of sterilization the middle must be manipulated in aseptic conditions in order to protect it against the outside contaminations.

❖ **Setting in culture and conservation of the microbial species**

On every sample, a withdrawal (smoked 1g fish) has been done with the help of a sterile swab that has been dived in 10 ml of nourishing soup (préculture) and kept in the refrigerator to 4°C in order to slow down the microbial multiplication. Once the actual conditions of setting in culture on gélose will be filled, the préculture will be activated to 37°C to act as solution-mother to different dilutions.

B. Detection and numbering microbial

II.3.2.Numbering of the microorganisms

The numbering of the microorganisms made itself thanks to the decimal dilution technique. To arrive there, the named solution-mother "otherwise solution 100" permitted the preparation of the other dilutions that has been sowed on the surroundings of culture to search for the germs.

a) Decimal Dilutions

The solutions besides to more dilute of the microorganisms will be prepared from the solution-mother. To arrive there, 1ml of the solution-mother (10^0) is appropriated aseptically and introduces in another tube to test containing 9 ml of water distilled sterile. One gets a solution 10^{-1}. Of this tube, one appropriates 1 ml that is introduced in another tube to test containing 9 ml of water distilled, what gives a solution 10^{-2}, again. The operation continues until the obtaining of the solution 10^{-10} used for the numbering of the total germs (Sanders, 2012).

b) Numbering of the entérobactéries

The numbering of the entérobactéries is made in the middle of Mac Conkey.

Principle

The middle of Mac Conkey is in general a selective environment of the entérobactéries. He/it permits to eliminate the secondary flora of the products polymicrobiens thanks to the action of two inhibitors: the purple crystal (inhibitory of flora to gram-positive) and the biliary salts (selection of the entérobactéries). The use of this middle is recommended to isolate and to count the entérobactéries in waters, milk, the food matters and urines. He/it can also be used for the research of salmonella, of shigella and E. coli in the fecal matters (Benhafed, 2020).

Operative fashion

A quantity (1ml) takes in every dilutions (10°, 10-1, 10-2, …10-10) is sowed in them limp of kneaded containing the middle Mac Conkey prepared according to manufacturer's norm. The incubation to 37°C during 24 hours gives some colonies red fattening pond that is the entérobactéries.

C. Identification of the microorganisms

1°) Identification of the entérobactéries

Some tests have been applied on the colonies that pushed in the middle of Mac Conkey among which the Test of Kligler Hajna, The test of uréase, Test of indole, Test of use of citrate, Test of mobility, ONPG, Vogue Pronscar, Red of Methyl,. A key of identification allowed to determine the species found in the colonies.

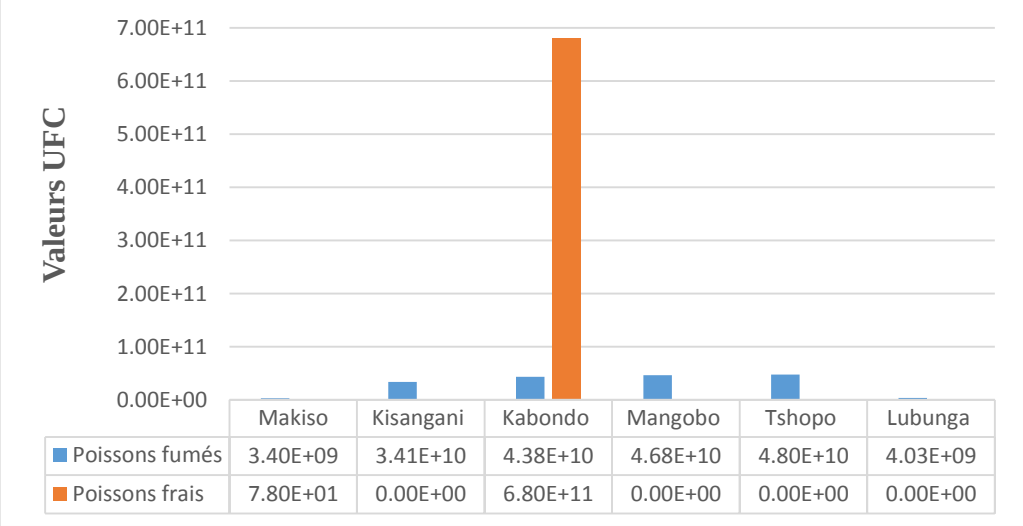
II.4. statistical Analysis

The results have been analyzed according to the device in split contact whose main factor is the type of fish (expenses and smoked) and the secondary factor is the type of the markets (different townships). The recorded data have been treated and have been analyzed with the help of the software Statistix 8.0 according to the model ANOVA 2. The LSD test has been used to the doorstep of p=0,05 for the comparison of the different averages.

III. RESULTS

III.1. Numbering of the microorganisms

1.1. Total germs (UFC/g)



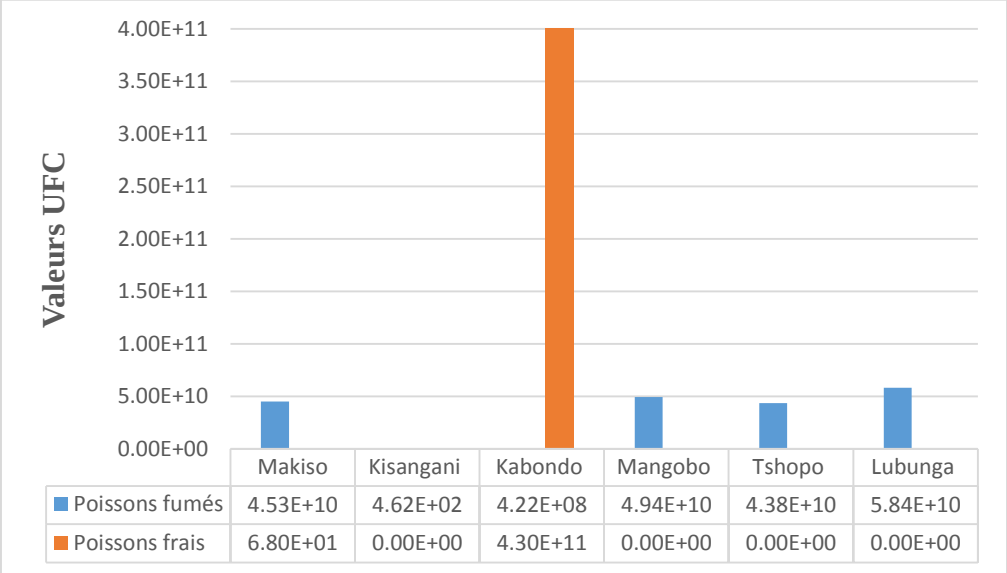
	Makiso	Kisangani	Kabondo	Mangobo	Tshopo	Lubunga
Poissons fumés	3.40E+09	3.41E+10	4.38E+10	4.68E+10	4.80E+10	4.03E+09
Poissons frais	7.80E+01	0.00E+00	6.80E+11	0.00E+00	0.00E+00	0.00E+00

Face 1. Total Germs

This face shows that the levels of total germs are especially raised among the cool fish coming from the township of Kabondo, reaching $6{,}8.10^{11}$ UFC/g, followed of those of the common Makiso with 78 UFC/gs.

For the smoked fish, the most important abundances are recorded in the township of Tshopo (4,8.10^{10}UFC/g), consistent of Mangobo (4,68.10^{10}UFC/g), Kabondo (4,38.10^{10} UFC/g), Kisangani (3,41.10^{10}UFC/g), Lubunga (4,03.10^{9}UFC/g), and finally Makiso (3,4.10^{9}UFC/g).
The statistical analysis achieved with the help of an ANOVA to two factors reveals meaningful differences, so much between the types of fish that between the different townships (LSD=3.49).

1.2. Enterobacteries



Face 2. Enterobacteries

This face reveals that the concentrations of entérobactéries are especially to cramming among the cool fish of the township of common Kabondo, reaching 4,3.10^{11} UFC/g, followed of those of the common Makiso with 68 UFC/g.
For the smoked fish, the most important levels are observed in the township of common Lubunga (5,84.10^{10} UFC/g), consistent of the common Mangobo (4,94.10^{10} UFC/g), the common Makiso (4,53.10^{10}UFC/g), common Tshopo (4,38.10^{10} UFC/g), common Kisangani (4,62.10^{2} UFC/g), and finally common Kabondo (4,22.10^{8} UFC/g).
The statistical analysis done with an ANOVA to two factors gave a p been worth lower to 0,05, indicating that the abundance of the entérobactéries varies meaningfully according to the type of fish and their township of origin (LSD=4.18).

A. FISH SMOKES.

III.2. relative Results to the identification of the microorganismes.

This picture presents the relative results to the identification of the entérobactéries in the different townships of the city of Kisangani.

Table 1: Identification of the entérobactéries.

Communes	Milieu Kligler				Citrate	Indole	Urée	Mobilité	Espèce
	Glucose	Lactose	Gaz	H_2S					
MAKISO	+ +/-	- +/-	- +	+ -	- -	+ +	+ -	+ +	*Proteus vulgaris* *Escherichia coli*
KISANGANI	+ +	- +	- -	+ -	- +	+ -	+ -	+ -	*Proteus mirabilis* *Citrobacter boseri*

KABONDO	+ +	+ -	- +	+ +	+/- -	- +/-	+ -	- +	*Klebsiella oxytoca* *Citrobacter freundii*
MANGOBO	+ +	- -	- +	+ +	+ +	- -	+ -	+ +	*Proteus mirabilis* *serratia*
TSHOPO	+	+/-	-	-	+	-	-	+	*Enterobacter*
LUBUNGA	+ +	+ +	+ -	- -	+ +	- -	- +	+ -	*Enterobacter cloaceae* *Clebsiella*

In relation to the method of isolation and identification of the entérobactéries, he/it is to note picture 2 that the pathogenic bacteria can develop itself/themselves easily in the smoked Oreochromis niloticus are: Proteus bulgaris and Escherichia colli in the common Makiso; Proteus mirabilis Citrobacter boseri in the common Kisangani; Klebsiella oxytoca, Citrobacter freundii in the township of Kabondo; Proteus mirabilis, serratia in the common Mangobo; Enterobacter in the common Tshopo; Enterobacter Clebsiella cloaceae in the common Lubunga.
The absence of the pathogenic germs as the Salmonella can be explained by a weak activity of the fish water smoked consecutive to the dehydration bound to the temperature raised at the time of manuring.

B. COOL FISH

III.3. relative Results to the identification of the microorganisms.

Table 2: Identification of the entérobactéries.

Communes	**Milieu Kliger**				**Citrate**	**Indole**	**Urée**	**Mobilité**	**Espèce**
	Glucose	**Lactose**	**Gaz**	**H_2S**					
MAKISO	+ +	+ -	- -	- +	+ +	- +	- -	- +	*Citrobacter koseri* *Proteus mirabilis*
KABONDO	+ + +	+ - -	- + -	- + +	+ - +	- - -	- - +/-	- + +	*Citrobacter koseri* *Citrobacter freundi* *Proteus mirabilis*

The pathogenic bacteria who are developed themselves in the smoked Oreochromis niloticus are the citrobacter koseri, the ptoteus mirabilis in the common Makiso; Citrobacter koseri, Citrobacter freundi, Proteus mirabilis in the common Kabondo.

IV. CONCLUSION AND DISCUTION

The major objective pursued by this survey was to value the microbiological quality of fish smoked traitors in the city of Kisangani, to count and to identify the bacterial and fungal cash of the families of the enterobacteria, Staphylococacéeses, and Saccharomycetacées responsible for the infectious illnesses.

With regard to the total germs we got the results following for the smoked fish: the common Makiso ($3{,}4.10^9$ UFC/g), the common Kisangani ($3.41.10^{10}$ UFC/g), the common Kabondo ($4.38.10^{10}$ UFC/g), the common Tshopo ($4.8.10^{10}$ UFC/g), the common Mangobo ($4.68.10^{10}$ UFC/g) and the common Lubunga ($4{,}03.10^9$ UFC/g), are superior to those gotten by Abotchi (2010) and Degnon and al. (2013). these raised a mean load of $7{,}69.10^5$ UFC/g respectively in fish smoked on the other hand of Sarda sarda and Trachurus spp in Togo and $2{,}4.10^6$ UFC/gs on the site 1 on the site 2, a load of $1{,}4.10^6$ UFC/gs in the samples of Trachurus trachurus at the time of the putting on sale in Benin, but as superior to the one of Atobla and al., (2022) that had an average of $6{,}61.10^6$ UFC/gs. this load would be due to the contamination of the products after manuring following the various manipulation or to the conditions of hygienes. The rate of contamination found in this work is on the other hand defer the results of Oulai and al., (2007) that got a mean load of $3{,}1.10^7$UFC by gram of product while working sur150 samples coming from the traditional manuring of the fish of lagoon of Ebrié in Coast of Ivory. The total

flora in a foodstuff reflects the general microbiological quality of the product and constitute this fact an indication of the sanitary quality (Anihouvi and al.2009). during the technological treatments, the numbering of the total flora permits to judge the impact of the various operations (Bornert, 2000). According to Djinou (2001), the total germs are germs "test of hygiene" because they give account of the general hygiene or an inefficient conservation. On the enterobacteria the found results are superior in relation to the results found by Wafaa L.2003 that is of 45.10^3 UFC/g and $7{,}2.10^2$ UFC/g by Catsaras (1980). But as superior to those gotten by Benkirate and al., (2015) that are of 5.10^2 UFC/g to the levels of the gills, 6.10^2 UFC/gs to the levels of mucuses, $0.2.10^2$ UFC/gs to the levels of the flesh: while refering to the norms of healthiness of fish (max 10UFC/g) (Jouve, 1996).
The present survey permitted to show that the smoked fish and sold expenses in the different markets of the city of Kisangani are contaminated by the enterobacteria, this contamination tightened due to the lack of hygiene but also to the exhibition of fish to the market at the time of the sale.

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