INFLUENCE OF ANTHROPOGENIC ACTIVITIES ON THE HYGIENIC QUALITY AND BIOCHEMICAL COMPOSITION OF SACCOSTREACUCULLATA, SOLD IN THE CITY OF MAHAJANGA

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SUMMARY

The coastal environment of Mahajanga is subject to multiple sources of contamination of human or animal origin. Also, the oyster samples from three collection points Port Schneider (PS) and the village of Katsepy (KP) could present a danger to the health of consumers. Our main objective is to better understand the physico-chemical properties of oysters sold in the city of Mahajanga as well as its microbiological quality. To do this, analyzes of the contents of proteins, lipids, crude ash and carbohydrates as well as the counts of Total Mesophilic Flora (FTM), Coliforms, Staphylococci, Sulfuto-Reducing Anaerobes (ASR) germs were carried out. The results of experimentation showed that the oysters proved to be rich in protein with 9.14±0.03g/100g M.S and poor in lipids, with 2.63±0.56 g/100g M.S. They contain 322.08 ±2.98 mg/100g of calcium, 89.64±5.44mg/100g M.S of potassium, 89.64±5.44mg/100g M.S of magnesium and 6.15±0.27mg/100g M.S of iron. In microbiology, hygiene indicator germs such as FTM, CF, Escherichia coli, ASR germs have been observed in oysters. The concentrations of MTF according to the collection zones are respectively on average 7.2.105 ± 12.7, 1.7. 105 ± 29.5 and 5.0.105 ± 88.5 for sites PS1, PS2, KP. As for the levels of faecal coliforms, they are all below the reference criteria except for the results obtained from the KP site (42.5 ± 2.5 cfu/g). E.coli colony count results from all sites meet required standards. Concerning the ASR germs, the samples collected at the Schneider bridge, their level of contamination amounts to 39.5 ± 9.19 cfu/g against 10 cfu/g for the reference criteria.The levels of the other germs sought, namely SP, Vibriosp. , Salmonella sp in all the sites all meet the required standards, except for the presence of the Vibrio sp. in oysters from Katsepy. This research deserves to be deepened by determining the contents

of essential amino acids and fatty acids as well as vitamins. The eco-toxicological study is of great interest in the continuation of this work to reveal the possible presence of heavy metals and organic contaminants. Prior purification of oysters by chlorination before sale would improve the hygienic quality of the product.

Keywords: Oysters, Mahajanga, Physico-chemical parameters, Microbiological quality, Nutrients, Food safety

Introduction: The oyster is a sentinel species that directly reflects qualitative and/or quantitative changes in the quality of the ecosystem. The coastal environment is subject to multiple sources of contamination of human or animal origin: urban wastewater, rainwater runoff on agricultural land, wildlife. By filtering the water, the shellfish concentrate the microorganisms present. In our case, the Fisheries Health Authority does not intervene for the preliminary checks of the products for sale. As for consumers, they have the dietary habit of eating them raw and untreated. We also hypothesize that the oysters sold in the markets of the city of Mahajanga could be contaminated and thus make them unhealthy for consumption. Urbanization and climate change affect their nutritional value as well as their health quality. Thus, the purpose of our study is to assess the state of physico-chemical and microbiological safety of oysters sold in the city of Mahajanga in order to ensure consumer health.

Research methodology: Oyster samples were taken in a simple random way in the city of Mahajanga. The type of descriptive analytical study was adopted. The samples come from three Port Schneider collection points (PS 1 Bazar be saleswoman; PS 2 Seaside saleswoman), from the village of Katsepy (KP Bazar be saleswoman). The samples were placed in insulated STOMACHER bags.

Biochemical analyses: The following analytical methods were used to determine the various physico-chemical and biochemical parameters.

For the determination of the water content, three different samples of oyster tissue were taken, A test portion of 5 g each was placed in a previously tared capsule. The analysis was carried out in n=3 for each sample. The capsules were then placed in the oven at 103°C ± 2°C for a minimum of 4 hours. They were then left to cool in a desiccator for 1 hour before weighing them. The percentage of moisture is obtained by the following relationship

H%=((C2-C1))/((C2-C0))×100

H%: Humidity or water content for 100g of samples;

C2: Mass in grams of the capsule fitted with the test portion before steaming;

C1: Mass in grams of the capsule fitted with the test portion after steaming;

C0: Mass in grams of the empty capsule.

Dosage of raw ash: 5 g of samples are placed in a porcelain crucible weighed beforehand. The whole is left in a muffle furnace set at 550°C ± 5°C until white or light gray ashes, devoid of carbonaceous particles, are obtained for 6 hours. Subsequently, it is removed to be placed in a desiccator for 1 hour before being reweighed. The analysis was performed in duplicate for each sample. The raw ash content is obtained by the following formula:

CB%=(Pf-Pi)/(P.e)×100

Pf: mass in grams of the crucible with the ashes (after incineration);

Pi: mass in grams of the empty crucible;

P.e: mass in grams of the test sample.

Assay of the total lipid content: The method used is an extraction with hexane using the Soxhlet apparatus. Approximately 5 g of sample were introduced into an extraction cartridge. The cartridge was placed in a Soxhlet extractor and the extraction of the lipids by hexane lasted 6 hours. The hexane contained in the flask was then removed by evaporation using a rotavapor. The flask containing the residual lipids was placed for 1 hour in the oven at 103°C, then 30 minutes in the desiccator before weighing it. The percentage of total lipids was calculated from the following formula:

MG%=(B1-B0)/(P.e) □ 100

Fat%: Fat content in grams per hundred grams of raw material;

B1: Weight of the flask containing the dry residue after desiccation;

B0: Weight of the empty balloon before extraction.

Assay of crude proteins: The total nitrogen content is determined by the Kjeldahl method (Ould El Hadj M.D et al. 2001). Organic matter is destroyed by oxidation, under the combined effect of sulfuric acid, catalysts and optionally substances intended to raise the boiling point of the mixture. In these conditions; nitrogen is transformed into ammonium

salt. The ammonia is released by treatment of the ammonium salt and by soda. It is then collected in a solution of boric acid (40 g/l). In the presence of concentrated and hot sulfuric acid, the carbon, oxygen, hydrogen and nitrogen of the organic compounds are found under form of C02, H20 and NH3. The sulfuric acid being in excess, we have:

2 NH3 + H2S04 2 NH4+ + SO42-

Total nitrogen is therefore obtained in the mineral form NH4+ (ammonium ion).

During mineralization, sulfuric acid is partially decomposed and reduced to S02 and S03 which form irritating and toxic white fumes. After mineralization, nitrogen is found in the mineralizate in the form of NH4+. total is an acid-base ratio. The ammonium ions of the mineralized product being in an excess of sulfuric acid, they cannot be dosed directly. At first we will therefore move the ammonium ions from the mineralized in the form of NH3 (ammonia), then it will be necessary to recover the ammonia alone to be able to dose it using a titrated solution of sulfuric acid (0.1N). As the nitrogen content is variable (depending on the amino acids present and their proportions), a different conversion factor should be used for each type of protein. The total nitrogen content (N%) is given by Godon's formula, Loisel, 1991:

$$N\% = (V \times T \times 0.014)/m \times 100$$

V: Volume in ml of H2SO4 at 0.1N;

T: normality of H2SO4 (0.1N);

m: mass in grams of the test sample.

The crude protein content (CP%) in g/100g of fresh material is obtained by the following formula:

$$PB\% = N\% \times 6.25$$

Determination of the carbohydrate content by difference: The carbohydrate content (G%) in the samples is determined by the difference between 100% and the sum of the protein (Pr), lipid (L), crude ash (C) and water (H) (RDE Legislation 1970).

Carbohydrates = 100% – (MG + Pr + CB + H)%

The determination of the different mineral elements is done by the atomic absorption spectrophotometer method from the ash obtained.

Microbiological analyses: Spoilage germs, Total Mesophilic Flora (FTM) were counted on Plate Count Agar medium at 30°C (AFSSA 2008). As for the indicator germs of faecal or personal contamination, Termotolerant Coliform (CF), Violet Red Bile Lactose deep agar (NF V 08-060 March 1996) and Escherichia Coli (EC) on Tryptone Bile X-β-D- were used. Glucuronide (NF V 08-053 November 2002) Staphylococci (SP) on Baird Parker medium NF-V08-057-1 (November 1994 - IC: 08-057-1). For telluric germs: Sulfur-reducing anaerobic (ASR) on Tryptone Sulfite medium with Cycloserine at 37°C was used (XP V 08-061 October 1996). The assessment of microbial loads is interpreted according to a three-class plan at the exception of Vibrio and Salmonella. Results below the reference criteria are satisfactory, acceptable in the event of a tie and unsatisfactory when they are significantly higher.

Results and discussion

The study of chemical composition and bacterial contamination is of great interest. In recent years, the marine ecosystem has suffered the effect of climate change as well as various urban effluents and industrial discharges that may affect these parameters. .

Biochemical composition

The table below summarizes the results of different biochemical parameters

Table 1: Biochemical composition of Saccostreacucullata (g/100g D.M.)

Biochemicalparameters	Quantities
Humidity	80,61±0,35
fat	2,63±0,56
rawash	3,11±0,01
Proteins	9,14±0,03
Carbohydrates	4,51± 0,0

By observing these results, the Mahajanga oysters revealed a certain biochemical similarity to those of Gujan-Mestras in macronutrients because they are rich in protein, with a content of 9.14%; carbohydrates with 4.51% and finally lipids with 2.63%.

Mineral salt content

The mineral salt contents of oyster tissue are shown in Table 2.

Table 2: Micronutrient content of Saccostreacucullata. (Content in mg/100g of D.M.)

Mineralelements	Quantities
Calcium	322,08±2,98
Magnesium	77,31±1,60
Potassium	89,64±5,44
Iron	6,15±0,27

This table tells us that the oysters harvested near the city of Mahajanga are rich in calcium followed by potassium, magnesium and finally iron. These results are similar to those obtained by (Jouzier E. 1998).

3- Microbial load for each sampling site (CFU/g).

The averages of the microbial loads of the various essential microbiological indicators of food safety between various sites are indicated in the table below.

Table 3: Comparison of microbial loads between various sites.

Samples	**MFT**	**CF**	**EC**	**ASR**	**SP**	*Vibriosp.*	*Salmonella sp.*
AveragePS1	**$7,2.10^5$±12,73**	**7,5±3,54**	3±1,41	39,5±9,19	<50	Absence	Absence
AveragePS2	**1,7. 10^5±29,5**	**<5**	**<1**	3±1,41	**<50**	Absence	**Absence**
Average KP	**$5,0.10^5$±88,5**	**42,5±2,5**	**9±1**	**9±1**	**<50**	Presence	**Absence**
Benchmarks	**1.10^5**	**1.10**	**1.10**	**1.10**	**1.10^2**	Absence in 25g	**Absence in 25 g**

MTF concentrations vary according to collection areas. They are respectively on average 7.2.105 ±12.7, 1.7. 105 ±29.5 and 5.0.105 ±88.5 for sites PS1, PS2, KP. The relatively high values in the PS1 and KP variants could be explained by the non-compliance with the storage conditions and/or the vendors' hygiene. The faecal coliform levels are all below reference criteria. However, the results obtained from the KP site (42.5±2.5 ufc/g) are higher than that of PS (7.5±3.54 ufc/g) the existence of these germs indicating faecal contamination informs us that the good hygiene practices are not always respected and/or this could be explained by contaminated urban waste water runoff. The results of the E.coli colony counts meet the required standards. By comparing between the sites, the samples taken from the Schneider bridge have a level of contamination in ASR germs rising to 39.5 ± 9.19 cfu/g against 10 cfu/g for the reference criteria. This increase is probably due to the accumulation of mud from the Betsiboka River around the Schneider bridge. The levels of the other germs sought, namely SP, Vibrio sp., Salmonella sp. in all the sites all meet the required standards, with the exception of the presence of the Vibrio sp germ in the oysters from Katsepy. During subsistence fishing for oysters, good hygiene practices are not always respected both at the level of the collectors and in the disgorging areas where some local residents even practice defecation in the open air.

Conclusion: The oyster samples collected on the three sites have a very interesting nutritional value thanks to its different constituents: carbohydrates, proteins, lipids and mineral salts. From a microbiological point of view, oysters, due to their ability to filter water, are true indicators of the presence of microorganisms in the marine environment. It appears from this study that the levels of FTM hygiene indicator germs are of unsatisfactory quality. Overall, the levels of total coliforms, staphylococci and ASRs from the three sites meet the required standards, not exceeding the acceptable limits except for the case of ASRs from Port Scheider 1. In addition, the loads of pathogenic germs (Vibriosp ., Salmonella sp.), are satisfactory for samples from Port Schneider. On the other hand, the batch from Katsepy is unsatisfactory, due to the presence of characteristic colonies of the Vibriosp germ, which is a very dangerous germ for human health. is necessary. At the end of this study, we will also retain that a preliminary purification of the oysters before the sale would improve the hygienic quality of the product. This research deserves to be deepened by

determining the contents of essential amino acids and fatty acids, vitamins. The eco-toxicological evaluation is of great interest in the continuation of this work to reveal the possible presence of heavy metals, organochlorine and organophosphorus compounds.

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