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Cadaverine level and pH: indicator of spoilage in the flesh of fish (carp, mackerel and jaw) storage at different temperatures

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ABSTRACT

Cadaverine (CAD) is an indicator of spoilage in fish. Its level was determined by spectrofluorimetric method in the flesh of selected species of fish stored at -10, 4, 22 and 32°C. Average recovery rates obtained ranged from 87.9 to 101.4% for all species. It was found that CAD was present in all fish species used and its level was essentially constant during storage at -10°C for 32 days. At 4°C, the variation in CAD level was characterized by a stable phase during the first two weeks followed by a descending phase. Over a period of three of storage, CAD levels tripled at temperatures of 22°C and 32°C. Variations in CAD levels were also accompanied by a significant variation in the pH value. Slight pH variations were also observed when fish was stored at -10°C for 32 days and for 18 days at 4°C. Beyond these times, pH values increased significantly. The pH of the fish flesh increased sharply during storage at 22°C and 32°C. The Pearson treatment correlation study indicated a strong positive correlation (0.81 and 0.93) between CAD and pH for all samples stored at 22°C and 32°C, respectively.

INTRODUCTION

The Ivorian economy is for the most part based on agriculture with remarkable performance in crop production (cocoa, coffee, pineapple, bananas, cashew nuts, cotton, sugar, food crops, *etc*.). However, Cote d'Ivoire has chronic deficits in animal and fish production. Fish is the main source of animal protein for the Ivorian consumer. Annual per capita fish consumption is estimated at 16 kg while the Ivorian fishing industry produces only a third of the fish consumed. To make up this deficit, Côte d'Ivoire imports about three hundred and sixty thousand tonnes per year (COIHAFAT, 2014).

The improvement of the sanitary quality of food products and particularly fish products is a major concern of the Ivorian government and consumer associations that are increasingly demanding quality because fish is highly perishable foodstuff. The contamination of fishing waters and poor hygiene conditions increase the risks of contamination of fish by microorganisms making them unsuitable and even dangerous for consumption. Thus, to ensure the freshness and quality of the fish, several

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techniques of analysis, conservation and processing are used. Among the quality assessment methods is the determination and quantification of biogenic amines. This has been of interest to many researchers because of the advantage of rapid analysis compared to microbiological methods.

Biogenic amines are degradation products of bacteria during spoilage. The production of biogenic amines is associated with certain groups of microorganisms with a positive amino acid decarboxylase enzyme. Their optimal activities occur at pH values between 4.0 and 5.5. These microorganisms tend to become part of the food through contamination before and during food consumption (Dapkevicius et al., 2000). Biogenic amines are also known as sources of nitrogen and precursors for the synthesis of hormones, alkaloids, nucleic acids and proteins (Bouchereau et al., 2000; Jansen et al., 2003). They can also influence metabolic processes in the body such as regulation of body temperature, synaptic transmission, allergic reactions, cell division, proliferation of malignant cells, control of blood pressure, blood pressure and cell growth (Jansen et al., 2003; Bashan et al., 2004; Önal, 2007). However, the absorption of a certain amount of biogenic amines can cause hot flashes, itching, dizziness, headaches, nausea, heart palpitations, changes in blood pressure, gastrointestinal distress, oedema and skin rashes. Histamine is the causative agent of scombrotoxin. Scombroid fish such as tauna, mackerel, bonito and saury contain high levels of free histidine. Scombrotoxin occurs in most cases after consumption these fish. In addition, the toxic effects of scombrotoxin can be potentiated by other biogenic amines such as putrescine and cadaverine (CAD); inhibiting the effects of regulatory enzymes such as monoamine oxidase and diamine oxidase (Nguyen et al., 2007). In severe cases, scombrotoxin can lead to death (Berry 2007; Önal, 2007; Standarová et al., 2008).

For these reasons, the content of biogenic amines in fish and fish products has been the subject of several studies (Tine and Douabale, 2008; Simat and Dalgaard, 2011). The level of free amino acids present in fish is a function of the degree of freshness of the fish, temperature and storage time due to proteolysis by endogenous and exogenous proteases (Carelli et al., 2007). Moreover, in unfermented foods, the presence of biogenic amines is indicative of undesirable microbial activity (Anli and Bayram, 2009).

Studies have shown that CAD can be used as an indicator of the degree of microbial spoilage in a large number of fish (Ruiz-Capillas and Moral, 2001). Free lysine, a precursor of CAD, is present in many species of fish (Antoine et al., 2004). The absence of CAD in a sample of fish therefore reflects the good quality of the fish (Li et al., 2012). In addition, because of its particular smell, CAD can be used as an indicator of the organoleptic quality of fish (Chytiri et al., 2004; Ehsani and Jasour, 2012). Similar to CAD levels, pH is also an indicator of the degree of freshness and deterioration of fish (Liu et al., 2010; Farid et al., 2014).

The present work aims to contribute to improving the hygienic quality of fish. In this work, a method of quantifying CAD has been developed by Traoré et al. (2017), along with a correlation with pH variation at different temperatures, (-10, 4, 22 and 32°C). Three species of fish were involved in this study: carp, jaw and mackerel. The object of this study is to ascertain the relationship between CAD levels, temperature and pH variation, using spectrofluorescence technique. This technique has been used because it is very sensitive and selective. Biogenic amines are always produced as a mixture and this technique uses the specific and unique wavelengths of excitation and emission of CAD.

MATERIALS AND METHODS

Materials

Products and solvents used

All chemicals and solvents used were of analytical grade. CAD dichlorohydrate (99%), orthophthalaldehyde (OPA, 97%, m/m), methanol (99%) and hydrochloric acid (37%, m/m) were sourced from Sigma-Aldrich (Taufkirchen, Germany). They were used without further purification. Distilled water was used for the preparation of the working solutions.

Biological material

The fish used were *Cyprinus carpio* (carp), *Arius sp*. (Jaw) and *Cybium tritor* (mackerel). The choice of fish species (carp, jaw and mackerel) is based on their abundance, their availability in the markets throughout the year and above all on the appreciation of consumers. These fish are eaten in all the coastal countries of West Africa, in particular in the Ivory Coast.

Instrumentation

- A Perkin-Elmer Model LS-55 spectrofluorometer connected to an HP Model LE1901w computer was used to record electronic fluorescence spectra.
- A VELP ARE Scientifica magnetic stirrer was used to for

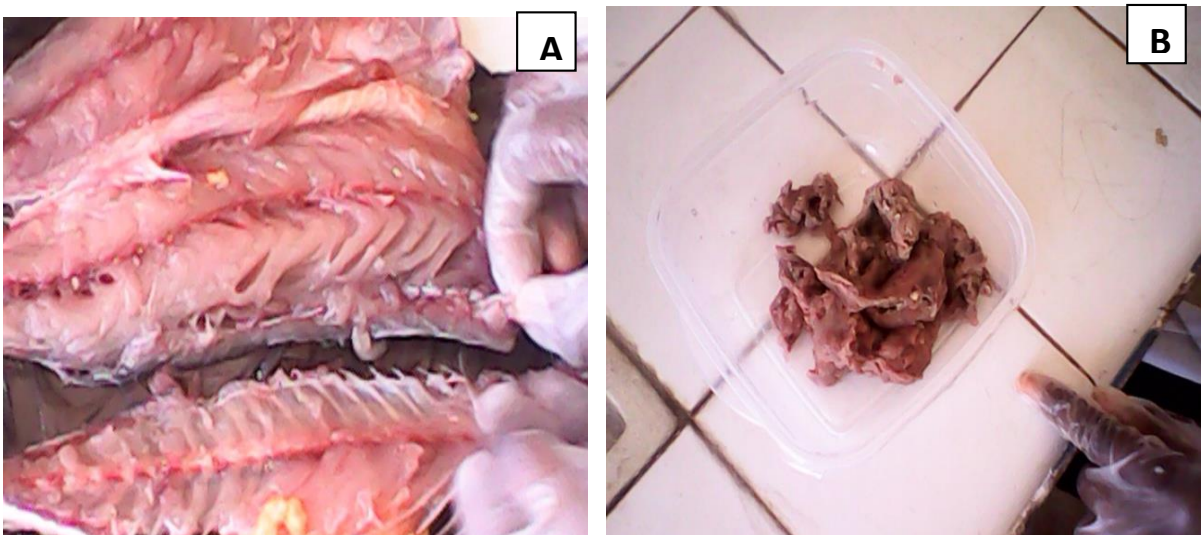

Figure 1. Photographs of fish filets (A) and blended filet in a plastic container (B).

stirring and heating. A Consort C6010 pH metre was used to adjust the pH of the samples studied and the prepared buffer solutions. The pH metre was previously calibrated with two buffer solutions (pH 4 and pH 7).

A grinder was useful to homogenize fish samples. The products were weighed using a Sartorius analytic balance with 0.1 mg accuracy. A quartz cell with five polished faces was used to record the fluorescence spectra. Pipettes, Eppendorf micropipettes ranging from 5 to 1000 µL and two Hewlett syringes of 10 and 50 µL were also used for sampling.

Methods

Preparation of solutions

Cadaverine (CAD) and orthophthalaldehyde (OPA) stock solutions were prepared in distilled water at a concentration of 10^{-2}M taking into account their purity. Since OPA is sparingly soluble in water, it was first dissolved in a small volume (approximately 1 mL) of methanol heated slightly for a few seconds and then made up to the desired volume (10 mL) with water.

All stock solutions prepared were placed in glass bottles and protected from light with aluminum foil. They were then stored in a refrigerator at 4 to 6°C. Solutions of different concentrations were prepared from stock solutions by serial dilution.

Sampling and transport of fish samples

The fish were purchased directly from sellers. The fish were bought early in the morning before cadaveric rigidity. They were wrapped individually in sterile bags, then placed in a cooler containing ice and transported immediately to the laboratory for analysis. The length and weight of the fish bought were estimated to be approximately 30 cm and 100 g, respectively. A total of six fishes were purchased: two fishes per species.

For each fish, the following parameters were recorded: date, weight, species and size. The fish was washed with tap water, scaled (if necessary), its gut removed. Washing was repeated and then the fish was cut into fillets; special care was taken to avoid bones. Fillets of the same species were homogenized using a Moulinex blender on the same day of sampling (day D1) (Figure 1). Trays were used to store the fillets; according to storage temperatures: -10, 4, 22 and 32°C. These temperatures were chosen because the fish were generally stored at -10°C for the freezers and 4°C for the refrigerator. In the covered markets, the temperatures of the fish on the vendors' stalls were around 22 and 32°C on the uncovered markets. Thus, depending on the storage temperature, the fluorescence measurements were made at different time intervals. For samples stored at -10°C, measurements were performed every 72 h. For samples stored at 4°C, measurements were performed every 48 h. Finally, for samples stored at 22 and 32°C, measurements were taken twice a day; in the morning and in the afternoon.

Extraction of the cadaverine

CAD was extracted from flesh of fish according to the method recommended by Nicole et al. (2011). The method uses a methanol-water mixture acidified with 0.4 M HCl (75:25, v/v) as extraction solvent. For the extraction, 10 g of the blended flesh was mixed with 65 mL of the solvent HCl/methanol (25:75, v/v). The resulting mixture was centrifuged at 4000 rpm for 5 minutes. To remove traces of methanol, the supernatant (extract) was collected and heated in a water bath at 60°C for 15 min. After cooling to room temperature, it was filtered using Wattman filter paper and stored in a refrigerator at 5 to 6°C.

Measurement of fluorescence intensity

The spectrofluorometer was switched on for about 30 min before the start of the measurements in order to have a stable source of radiation. The fluorescence spectra were acquired using the FL-Winlab software. The repetitive assay method was used for the measurement of fluorescence intensities. Thus, after each measurement, the content of the measuring cell was poured away, the cell was rinsed before a second reading was performed at room temperature under the same conditions (same scanning speed, same sensitivity and same slit).

Measuring of the pH of tissue of fish

The pH measurements were carried out according to the procedure described by Li et al. (2013). It consists of adding 90 mL distilled water to 10 g of ground fish flesh. After 30 min of stirring, the resulting mixture obtained was filtered using Whattman filter paper and the pH of the filtrate is measured with/using a pH meter.

Determination of the recovery rate

The CAD recovery rate (%R) was determined according to the following relationship below.

$$\%R = \frac{Ct}{Ca+Co} \times 100$$

Ct: Concentration of CAD found; Ca: concentration added; C_0: Blank concentration (13.5 ng mL^{-1}). The rate of recovery made it possible to evaluate the interference effects for each concentration within the domain of linearity of the calibration curve. Thus, to the extent of its applicability, in agreement with the validation of analytical methods, this rate must be between 80 and 120%.

Determination of the mass concentration of extracted cadaverine

The standard addition method was used to determine the concentration of CAD extracted from the fish. From the CAD concentrations expressed in µg/ml. Since we know C_0, we can deduce the mass (m) of pure CAD contained in the 10 g (mt) of crushed fish. The mass (mt) in rates per gram of flesh was determined according to the formulae below.

$$T(\%) = \frac{m}{mt} \times 100$$

Data processing

The fluorescence spectra acquired using the FL-Winlab software were processed by the use of Microcal Origin version 8.5 software. The calculation of the correlations between the levels of CAD and pH were done using SPSS Statistics 22 software according to Pearson treatment.

RESULTS AND DISCUSSION

Results

Confirmation of the presence of cadaverine in extract

The excitation and emission spectra of the OPA-CAD complex from the standard and the extract showed zero spectral difference in shape and wavelength. This confirmed that the sample extract contained CAD (Figure 2).

Determination of cadaverine recovery rate

Standard calibration and addition lines for each fish species studied were established and used to determine the level of CAD in each fish species. The slopes of the calibration and standard addition lines were very close to unity. The lines had good parallelism indicating a weak

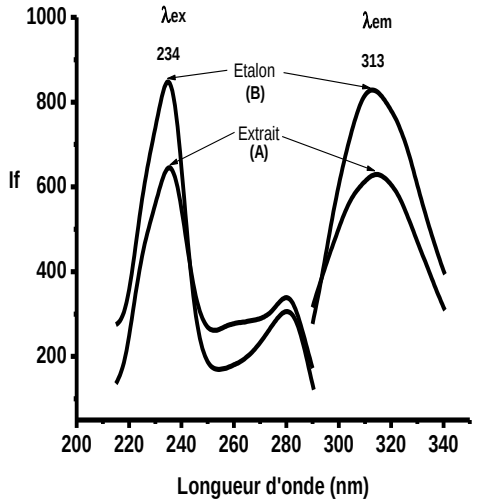

Figure 2. Comparison of the excitation and emission spectra of the OPA-CAD complex: extract (A) and standard (B).

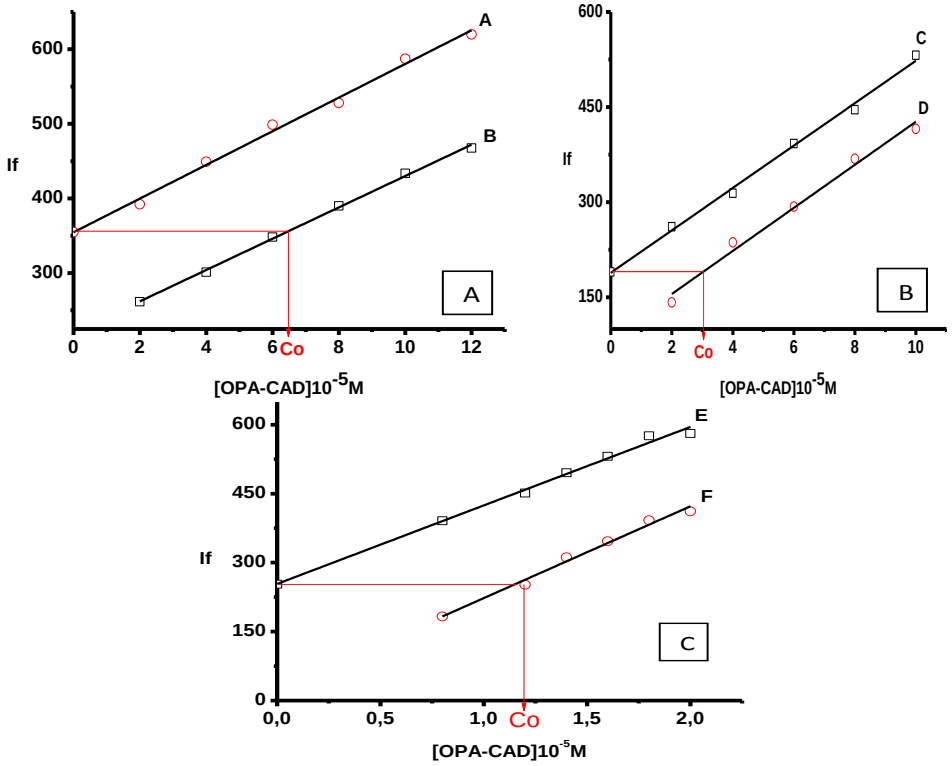

Figure 3. Standard calibration and addition curves for CAD in acetate buffer pH 4.62: A) Mackerel, B) Jaw, and C) carp.

matrix effect. However, the parallelism obtained in the case of carp fish was less appreciable than the first two (Figure 3). The characteristics of the calibration and standard addition lines are summarized in Table 1. The recovery rates obtained were between 82.4 and 98% for mackerel, between 99.1 and 101.4% for jaw fish, and

Table 1. Characteristics of the calibration and standard addition lines.

Sample	Type of Curve	Slope	R^2
Mackerel	Etalonnage	$2.259x10^6$	0.999
	Addition standard	$2.099x10^6$	0.996
Jaw	Etalonnage	$3.396x10^6$	0.998
	Addition standard	$3.344x10^6$	0.993
Carp	Etalonnage	$1.716x10^7$	0.995
	Addition standard	$1.997x10^7$	0.99

finally for carp between 87.9% and 89.5%. The best recovery rate was obtained with jaw fish, followed by mackerel and carp fish. The relative standard deviation (RSD) values obtained were 5.7, 1.07 and 9.4% for mackerel, jaw fish and carp, respectively; all were below 10%. These low RSD values indicated the good reproducibility of our measurements (Table 2).

Evolution of the CAD (CAD) level in the fish at different storage temperatures

At low temperatures (-10 and 4°C)

The curves in Figure 4 show the evolution of CAD levels in the filets of fish samples as a function of time (in days). For samples, kept at -10°C, the evolution curves of CAD levels can be split into two phases (Figure 4A): the first phase (from the 1st to the 5th day of storage) is marked by a significant increase in CAD content. The second phase (from the 5th to the 32nd day of storage) is characterized by a small increase in CAD content. For samples stored at 4°C, the evolution curves of CAD content show three phases (Figure 4B): the first phase (from the 1st to the 7th day of storage), during which a significant increase in CAD content was observed. The second phase (from the 7th to the 16th day of storage) is characterized by a slight increase in the CAD content. Finally, the third phase (from the 16th to the 32nd day of storage), the content of CAD gradually decreases.

Variation of cadaverine content with time at 22 and 32°C

The curves in Figure 5 show a rapid increase in CAD levels in the filets of fish samples with time. By the 3rd day of storage, the CAD content had almost tripled for each fish species.

Variation in the pH of the flesh of fish stored at -10 and 4°C

For samples stored at -10°C, all pH variation curves were essentially constant. The average pH values were 5.7; 6.1 and 7 for mackerel, carp and jaw fish samples, respectively (Figure 6A). For samples stored at 4°C, the pH variation curves could be split into three phases (Figure 6B). First, the 1st phase (1st to 18th day of storage) was characterized by a plateau. During this phase, the pH values were almost the same as when stored at -10°C. Then, the second phase (from the 18th to the 23rd day of storage) was marked by a sharp increase in pH. The pH values increased from 5.7 to 8.4; from 6.1 to 8.4 and from 7 to 8.4 respectively for the mackerel, carp and jaw fish samples. Finally, in the third phase (from day 23 to day 32 of storage), plateaus were observed after the sharp increases observed in phase 2. The pH values were all above than 8.

Variation in the pH of the flesh of fish stored at 22 and 32°C

The variation curves showed a rapid increase in pH. The pH exceeded 8 in only 3 days for all three types of fish species (Figure 7). In general, a small decrease in pH was observed the day after the beginning of the storage at all temperatures and for all fish species.

Correlation between cadaverine level and pH of fish flesh

All the correlation results according to Pearson's treatment are reported in Table 3. The pH values and CAD levels were highly correlated for samples stored at -10°C. The correlation was positive for carp with r = 0.95 but negative with r = - 0.90 and r = - 0.74 for jaw fish and

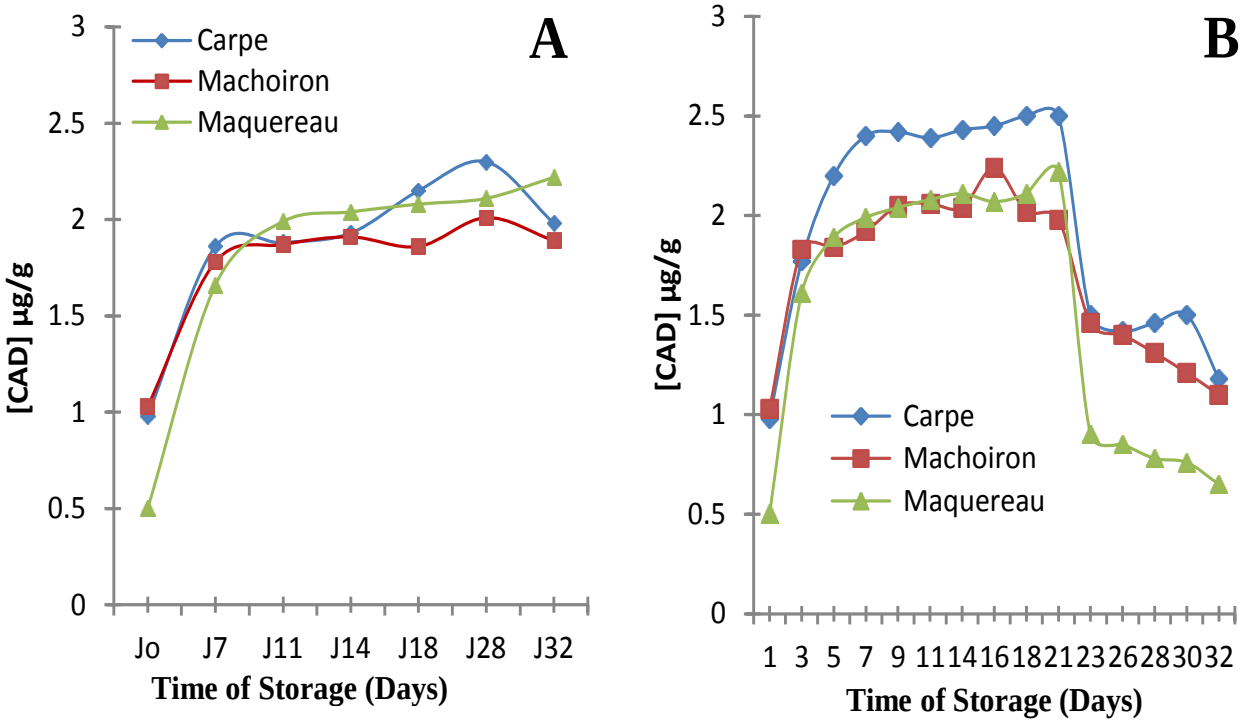

Figure 4. Evolution of CAD content in fish filets as a function of storage time: A at (-10°C); B at (4°C). CAD: Cadaverine.

Table 2. Statistical results of standard addition method: relative standard deviation, recovery and recovery rates.

Sample	Standard addition method			Recovery rate	DSR (%)
	Ca (µg/ml)	Ct (µg/ml)	R (%)		
Mackerel	0	13.5	-	**82.4 % - 98 %**	**5.7**
	3.5	14.0	82.4		
	7.0	19.1	93.2		
	10.5	23.5	98.0		
	14.0	25.4	92.4		
	17.5	30.3	97.7		
	21.0	32.6	94.5		
Jaw	0	5.14	-	**99.1 % - 101.4 %**	**1.07**
	3.5	8.6	99.5		
	7.0	12.07	99.4		
	10.5	15.5	99.1		
	14.01	19.0	99.2		
	17.01	22.47	101.4		
Carp	0	2.22	-	**87.9 % - 89.5 %**	**9.4**
	1.40	3.24	89.5		
	2.10	3.84	87.9		
	2.45	4.13	89.2		
	2.8	4.44	88.4		
	3.15	4.74	88.4		
	3.5	5.04	88.1		

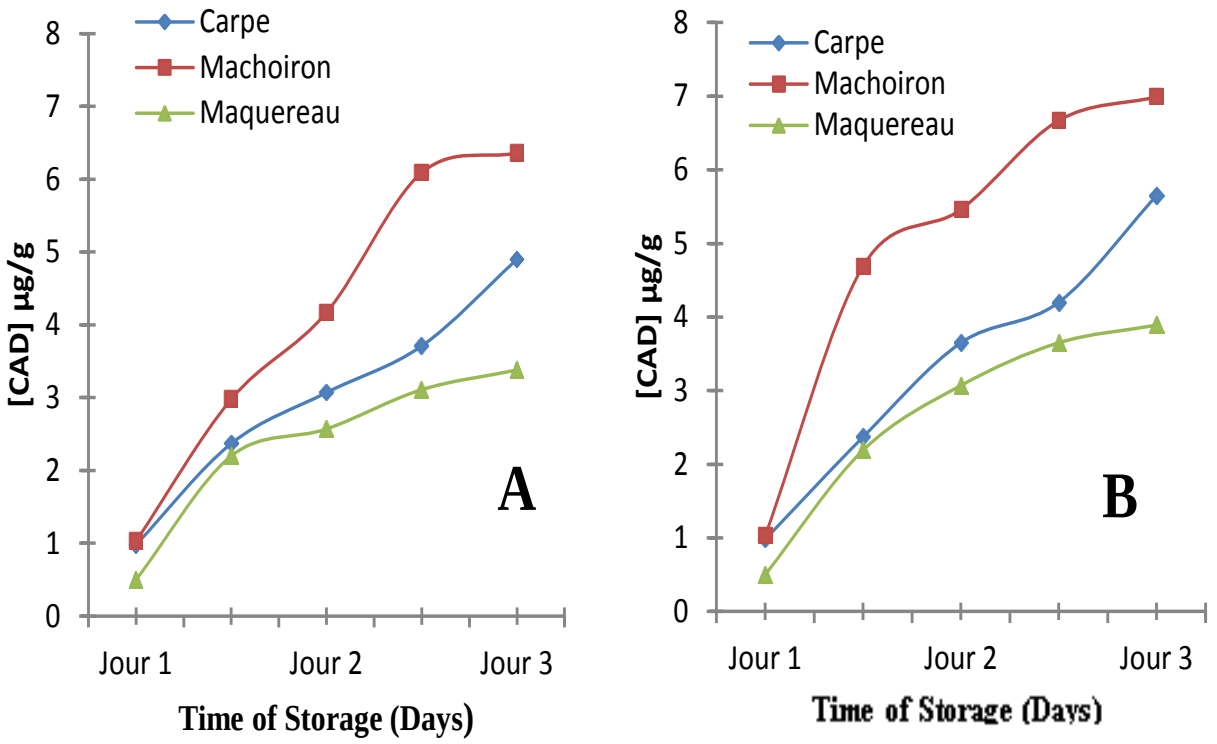

Figure 5. Evolution of CAD levels in fish filets as a function of storage time: A at (22°C); B at (32°C).

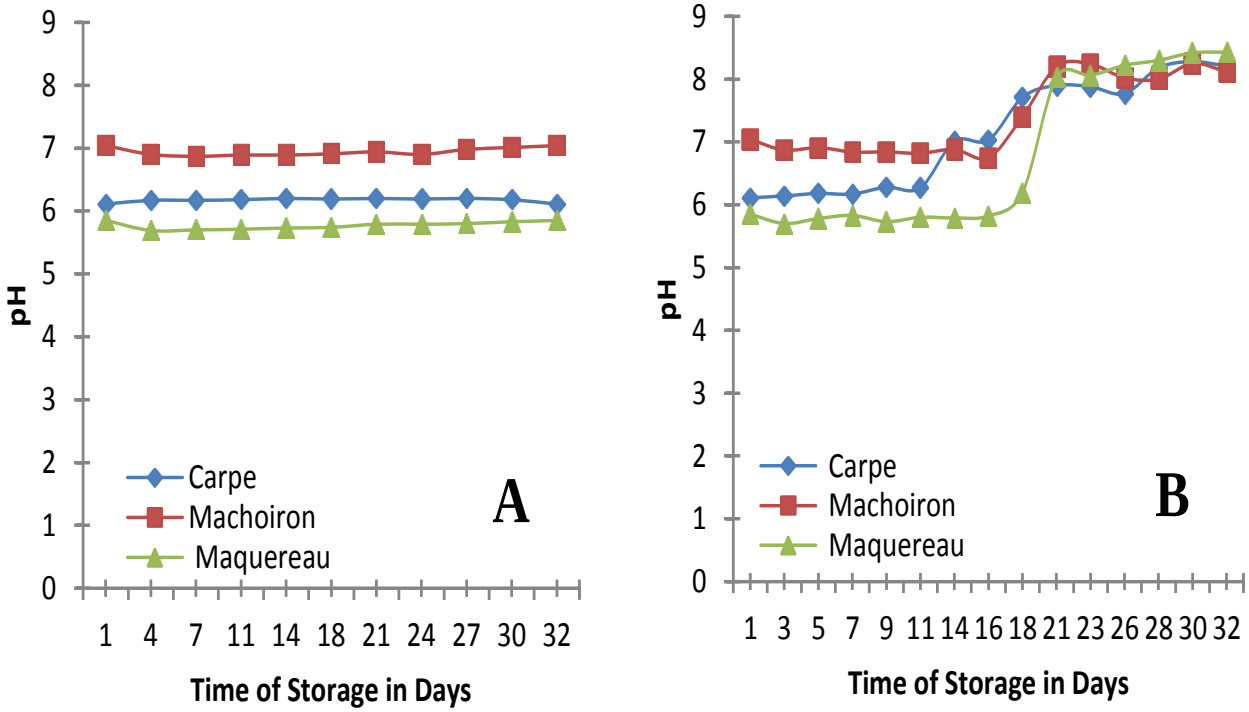

Figure 6. Evolution of pH of fish flesh as a function of storage time: A at (-10°C); B at (4°C).

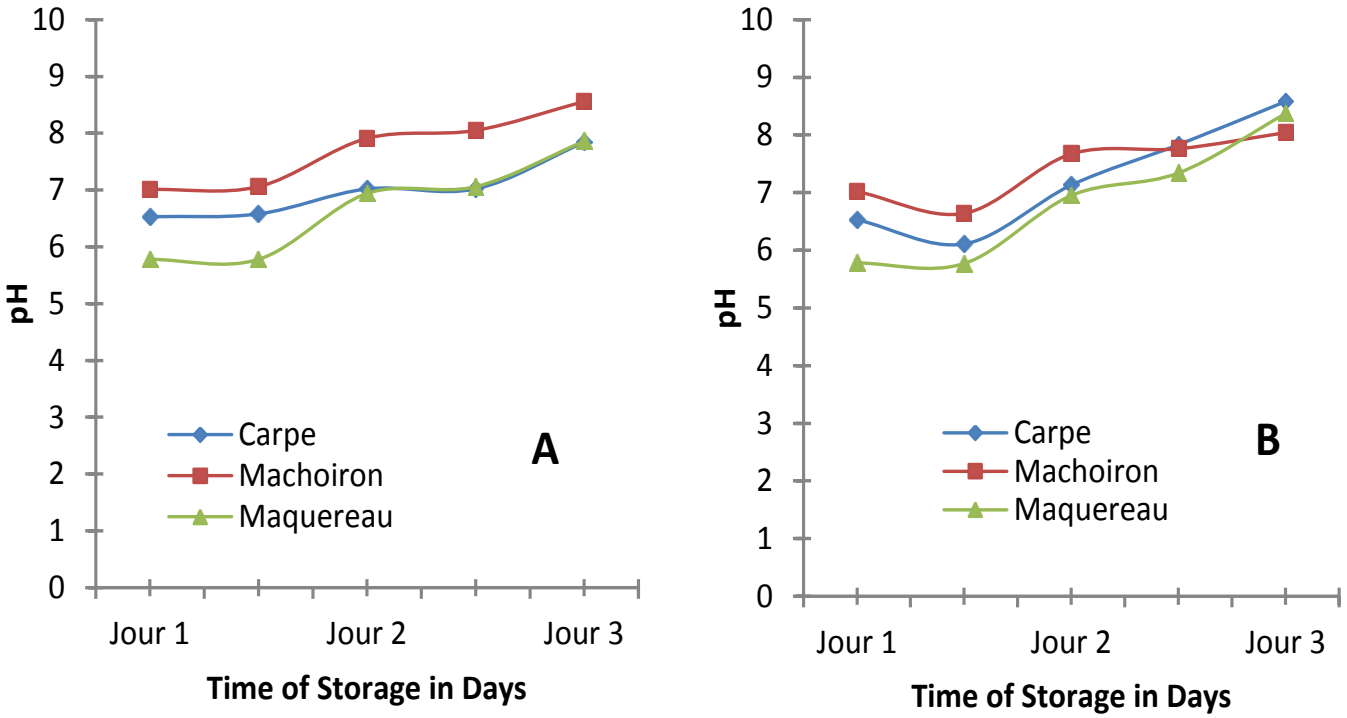

Figure 7. Evolution of pH of fish filets as a function of storage time: A at (22ºC), B at (32ºC).

Table 3. Correlation matrix (r) between CAD level and pH of fish filets stored at -10, 4, 22 and 32°C.

Storage temperature	Storage period (Days)	Species of fish		
		Carp	Jaw	Mackerel
-10ºC	1-32	0.95	- 0.90	- 0.74
4ºC	1-32	- 0.39	- 0.69	0.72
4ºC	1-18	0.45	-0.88	0.67
4ºC	18-32	- 0.53	- 0.19	- 0.89
22ºC	1-3	0.93	0.92	0.82
32ºC	1-3	0.89	0.81	0.86

mackerel, respectively. For samples kept at 4°C, first, we established a correlation between pH and Pearson's CAD level during 32 days of storage. The results showed a weak correlation on the part of the carp. This weak correlation could be explained by the existence of a high level of CAD *in vivo*. Thus, in this case the pH is very little influenced by the variation of CAD. Then, we calculated the correlation coefficients between the pH and the CAD level during the storage phase from the 1st to the 18th day characterized by substantially constant values. There is a very good correlation between CAD level and flesh pH for jawbone and mackerel. As before, we did not find a good correlation in the case of carp. In fact, for carp, if the CAD level remains constant during this period, the pH of the flesh begins to increase significantly from the 11th day which is not the case for the other two species of fish. Between the 18th and the 32nd day of storage, the correlation between the CAD level and the pH is low for the jawbone and average for the carp. However, we have a good correlation for mackerel (Table 3). These weak correlations could be explained by the progressive loss of freshness of the flesh which is more important for the jawbone, followed by that of carp and finally of mackerel. This phenomenon would explain why the jawbone is more consumed smoked than fresh. Likewise at this temperature, experience shows that mackerel flesh is more resistant to weathering.

For fish stored at 22 and 32°C, the CAD level and the

pH are significantly correlated with each other. Indeed, we obtain in all cases correlation coefficients between 0.81 and 0.93, close to unity. These coefficients being all positive. These parameters pH and CAD level evolve in the same direction. Values of r close to unity show that there is a good relationship between the pH and the level of CAD. However, the correlations obtained are generally better at the storage temperature of 22°C. Finally, CAD level and pH were significantly correlated for fish kept at 22 and 32°C with r values between 0.81 and 0.93.

DISCUSSION

The parallelism observed between the calibration curve and the standard addition line shows that the involvement of disturbing phenomena was not very significant and that the matrix effect was negligible (Khonté et al., 2015). The results of the recovery rates were in agreement with the degree of parallelism obtained for each fish species. The recovery rate of between 87.9 and 89.2% obtained in the case of carp could therefore be explained by the less appreciable degree of parallelism of its calibration curve and standard addition lines compared to those of jaw and mackerel. However, these recovery rates were all consistent with international standards for analytical methods which stipulate that the recovery rate should be between 80 and 120% (Diaw et al., 2014). Moreover, authors have shown that extraction carried out with methanol-acidified water mixture allows a better recovery of CAD; this is independent of the fat content, processing conditions and sample size of the fish. Moreover, these researchers have shown that a single extraction was sufficient with this type of mixture (Nicole et al., 2011).

The study was conducted for 32 days for samples stored at -10°C as opposed to 4°C. On the first day of analysis, the CAD levels detected were low and approximately the same for all three samples at both -10 and 4°C. CAD detected from day 1 of storage may be endogenous. This result confirms several previous works. Indeed, researchers have shown that free lysine (CAD precursor) is produced in many fish species (Ruiz-Capillas and Moral, 2001; Antoine et al., 2004). It is present in the form of metabolites and is involved in many biological processes such as cell growth, cell division, allergies etc. (Wallace, 2009). Results show that CAD levels vary from one fish species to another. Similar results have also been observed by several fish researchers. They have shown that a difference in biogenic amine formation can exist within the same fish species. In fact, the formation of biogenic amines can be influenced by both internal factors (availability and quantity of free amino acids, pH, ionic strength, etc.) and external factors (hygiene and handling practices) (Ruiz-Capillas and Jiménez-Colmenero, 2010).

The increase in the quantity of CAD observed between the 1st and 5th day of storage at -10°C and between the 1st and 7th day of storage at 4°C could be explained by a continuity of its production by microorganisms not yet totally inhibited under the effect of these temperatures. In reality, the fishes were caught in tropical waters with temperatures above 20°C, which suggests that the original floras of these fish are mesophilic.

During the second phase, characterized by a plateau between the 5th and 32nd day for storage at -10°C and between the 7th and 16th day for storage at 4°C, the CAD content slowly increased. This slight increase in CAD content could be explained by a halt in the production of amines by microorganisms due to their inhibition. Indeed, the storage temperatures applied (-10 and 4°C) are able to stop microbial growth and multiplication. However, even in the case of inhibition of the microbial flora, the enzyme activity can still continue, which resulted in a continuous production of even small amounts of amines. The results agree with some researchers who say that CAD is the only biogenic amine that increases significantly during storage at low temperatures (Rossi et al., 2002; Pons-Sánchez et al., 2006). In addition, the possible presence of psychotolerant bacteria (*Photobacterium phosphoreum* and *Psychrotolerans morganella*) in fish may actively contribute to amine accumulation at high levels, even during storage below 5°C (Dalgaard et al., 2006; Rezaei et al., 2007).

Carp had higher CAD content than the other two species. This is probably due to the higher amounts of lysine in carp (Dromigny, 2011). For samples stored at 4°C, the progressive decrease in CAD content from the 16th day of storage can be explained by its degradation. Indeed, microorganisms such as *Brevibacterium linen*, *Staphylococcus xylosus* are able to degrade primary biogenic amines by deamination to produce aldehydes, hydrogen peroxide and ammonia (Leuschner et al., 1998).

From an organoleptic point of view, storage at -10°C has been shown to be more effective. Sample quality was okay for 32 days at -10°C and 18 days at 4°C. Thus, temperature is an important factor in the behavior of microorganisms and thus on the shelf life of fish (Rezaei et al., 2007; Hernández-Orte et al., 2008).

The rapid increase in CAD content during storage at 22 and 32°C could be explained by the rapid spoilage of the flesh. Indeed, these temperatures are favorable for the development of mesophilic bacteria and proteolysis. Thus, the lysine obtained from proteolysis was rapidly transformed into CAD from the lysine decarboxylase activity of microorganisms (Moini et al., 2012). Similar results were observed in barramundi and mackerel when they were kept under essentially the same conditions (Bakar et al., 2010). CAD levels of 2 to 20 times higher

were found in some fish species kept at 10°C compared to samples kept at 2°C (Hu et al., 2012). In addition, mackerel had the highest content and was therefore less well preserved than carp and mackerel at 22 and 32°C.

Organoleptically, signs of decomposition were visible and perceptible from the first day of storage at 22 and 32°C. The decomposition process was rapid and the flesh of the fish had changed color. By the 3rd day of storage, the flesh had a very unpleasant odor.

The pH values varied from fish to fish. This difference in pH could be explained by the diversity of the microbial flora, the rate of glycolysis and the buffering capacity which differ from one fish species to another (Assogba et al., 2018). Researchers have found pH values between 5.5 and 7.5 depending on the species, size, season of capture, water composition, geographical location, stress levels during catch and muscle type (Daskalova and Pavlov, 2015). Indeed, carp and mackerel are freshwater fish and the pH of this environment varies between 6.5 and 7.5. However, mackerel is a marine fish and the pH of the flesh is between 5.8 and 6 from day one (FAO, 1999). The decrease in the pH value observed on the day after the start of storage could be explained by the formation of lactic acid in the flesh of the fish. Indeed, the formation of lactic acid is the consequence of the anaerobic degradation of the glycogen present in the fish flesh. It occurs during the onset of rigor mortis. In fish (Channastriata) stored at 4°C, the pH decreased for 3 days before increasing until the 18th day of storage (Assogba et al., 2018).

The small variation in pH during storage at -10°C shows that the fish were well-preserved at this temperature. Indeed, at -10°C, the bacteria responsible for decomposition were inhibited. Moreover, small pH variations were also observed when the fishes were stored at 4°C for 18 days. Above this temperature, the pH values increased significantly. Thus, at 4°C, the fish was well-preserved for at least 18 days, which corroborates several previous studies. According to these studies, the first signs of spoilage were reported after 12 to 16 days when the fish were stored at 0°C (Rosset et al., 2002).

In addition, the pH of fish (*Oreochromisshiranus*) stored at room temperature of 27-30°C increased rapidly in 12 h while the pH value of *Channastriata* stored at 4°C increased slowly until the 18th day of storage (Assogba et al., 2018). The increase in pH therefore marks the beginning of spoilage and can be attributed to the overproduction of alkaline substances following the degradation of proteins by the action of endogenous microorganisms (Ruiz-Capillas and Moral, 2001).

The longer the storage time, the more pasty the fish flesh became after thawing. This suggests a gradual loss of water retention capacity and firmness. For this reason, it is advisable to take out of the freezer only the necessary amount of food required and to reduce the thawing time as much as possible.

The rapid increase in pH observed at 22 and 32°C was probably due to the gradual loss of freshness of the fish. Indeed, the temperatures were favorable for the growth and multiplication of mesophilic bacteria, which led to the rapid decomposition of the samples. The compounds resulting from the activity of these bacteria therefore contributed to the increase in the pH of the flesh. Thus, the increase in pH of the flesh during storage at 22 and 32°C can be attributed to the overproduction of basic compounds such as ammonia, trimethylamine and other biogenic amines by spoilage bacteria present in the fish (Kyrana et al., 1997). For many researchers, there is a close link between the evolution of pH and the quality of the fish. Any increase in the pH value after the capture of fish indicates a loss of freshness (N'Ganguem, 2007; Assogba et al., 2018). In general, a pH value of flesh above 7.5 indicates that the fish is inconsumable at least for most species, with the possible exception of Pacific salmon where the limit is 6.2 (Adolphe, 2006). In addition to temperature and storage time, other parameters such as glycogenolysis, fishing conditions and fish species may affect pH variation (Assogba et al., 2018).

As for the correlation between CAD and pH, it was weak for carp ($r = -0.39$) kept at 4°C. This low correlation could be explained by the existence of a high CAD level *in vivo*. Thus, in this case the pH could have very little influence on the variation of CAD. For fish kept at 22 and 32°C, all correlation coefficients were all positive and close to unity. The r values close to unity showed that there was a good relationship between pH and CAD levels. However, the correlations obtained were generally better at the storage temperature of 22°C.

Conclusion

The study showed that CAD was always present even at low levels in the three fish species studied. It remained substantially constant during storage at -10°C and this for at least 32 days. In contrast, at 4°C, the change in the CAD levels was characterized by a stable a phase during the first two weeks followed by a descending phase. During storage at 22 and 32°C, a significant increase in the CAD level was observed from the first day of storage.

As for the pH, its small variation at -10°C shows that the fishes were well preserved at this temperature. Moreover, small variations in pH were also observed when the fishes were kept at 4°C for 18 days. Beyond that, the pH values increased sharply. Thus at 4°C, these fishes were well-preserved well for at least 18 days. The pH of the fish filets increased strongly during storage at 22 and 32°C.

A good correlation was obtained between the pH and the level of CAD from the Pearson treatment. It was

much more significant when the fish were kept at 22 and 32°C.

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