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New Method for Analyzing Tyramine by Spectrofluorimetry: Application to Fish

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Abstract

A new method for direct spectrofluorimetric analysis of tyramine in various fish samples was established. Low limits of detection (LOD) between 0.53 ng/mL and 1.40 ng/mL and limits of quantification (LOQ) between 1.76 ng/mL and 4.68 ng/mL were obtained. Also, low values of relative standard deviations (RSD) between 0.30 % and 0.58 % were obtained; confirming good reproducibility of our measurements. The application of this method to our samples allowed the detection of tyramine with satisfactory recovery rates between 93.68 % and 106.60 %. Interference studies with different biogenic amines have shown that the determination of tyramine can be done without major problems in the presence of spermine, spermidine, and histamine. However, special attention should be paid when tyramine is determined in the presence of agmatine, cadaverine or putrescine. In all cases, the determination of tyramine is more influenced by the presence of tryptamine or dopamine in the sample.

Keywords: biogenic amine, tyramine, analysis, fish, interference, rate, fluorescence

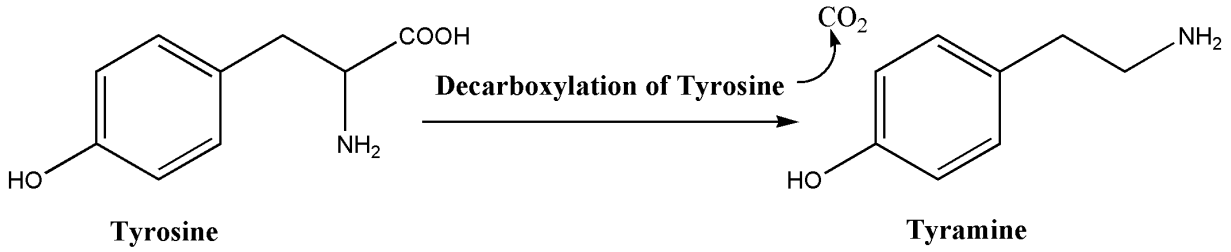
1. Introduction

Most food intoxications are caused by biogenic amines (BAs). The latter, also called bioactive amines, are basic nitrogen compounds of natural origin, biologically active and thermostable with relatively low molecular weight (Dasa, Bejo, & Abdo, 2022., Visciano, Schirone, & Paparella, 2020). They are ubiquitous in humans, animals, plants and bacteria. In general, BAs are derived from normal metabolic processes in living cells. Thus, they are obtained by bacterial decarboxylation of amino acids in food (Ekici & Omer, 2019., Stadnik & Dolatowski, 2010). This decarboxylation reaction occurs by removal of α-carboxyl group from precursor amino acid substrates to form the corresponding biogenic amine (Pessione, & Cirrincione, 2016., Ruiz-Capillas & Herrero, 2019). Thus, the accumulation of BAs in foods therefore requires the presence of microorganisms that produce specific amino acid decarboxylase enzymes.

BAs in low concentration confirm crucial functions in the human body. In fact, most of them are neurotransmitters (Ladero, Calles-Enríquez, Fernández, & Alvarez, 2010). Indeed, they play essential roles in the control of body temperature, stomach pH, gastric acid secretions, metabolic activity, nervous system (brain) activity, blood pressure and immune responses in the gastrointestinal tract (Ladero et al., 2010., EFSA, 2011). On the other hand, high levels of BAs in food products are considered a public health hazard (Ekici & Omer, 2020., Wójcik, Łukasiewicz, & Puppel, 2020) because they cause many neurodegenerative diseases and gastrointestinal cancers (Novella-Rodríguez, Veciana-Nogués, Roig-Sagués, Trujillo-Mesa, & Vidal-Carou, 2002). In fact, at a certain level their presence in food can cause adverse effects such as headaches, respiratory problems, heart palpitations, hyper or hypotension, hives, flushing, mouth burns, abdominal pain, cramps, diarrhea, vomiting, heart failure, cerebral hemorrhage, gastric problems and kidney poisoning (Ruiz-Capillas & Herrero 2019., Kalac & Krausová, 2005., Attaran, 2002). Therefore, their determination in a food sample is of great importance for health (Biji, Ravishankar, Venkateswarlu, Mohan, & Gopal, 2016).

In the current state of research, only the dosage of histamine in food has been regulated. However, many other ABs such as cadaverine, agmatine, tyramine, tryptamine are very toxic from a certain level in the food. Among these, tyramine,

which is the subject of this work, is the second type of amine involved in food poisoning (Novella-Rodríguez et al., 2002). The formation of tyramine (Scheme 1) is mainly due to the microbial decarboxylation of tyrosine, the main precursor.



Scheme 1. Scheme of tyramine formation from tyrosine

Marcobal et al., (2006) studied the impact of five physicochemical parameters (temperature, incubation time, environmental pH, added tyrosine concentration and pyridoxal-5-phosphate supplementation) on cell growth and tyramine production. They identified tyrosine concentration and incubation time as major contributors to tyramine production.

Storage conditions of different types of foods have a significant impact on the variation of tyramine levels, at least for fish and fish products. Indeed, while fresh fish contain very small amounts of tyramine (≤ 3 mg/kg), unfavorable storage conditions, however, result in rapid accumulation of tyramine. Some examples have shown that storage of Indian mackerel at room temperature (25°C - 29°C) resulted in an increase in tyramine concentration up to 200 mg/kg after 20 hours of storage (Chong et al., 2014., Da Silva, Pinho, Ferreira, Plestilová, & Gibbs, 2002).

Ingestion of certain amounts of tyramine can cause severe headaches and lead to cerebral hemorrhage or heart failure (Doeun, Davaatseren, & Chung, 2017). Tyramine indirectly causes an increase in blood pressure by inducing the release of noradrenaline from the nervous system. It can also cause lacrimation, salivation, increased heart rate and blood glucose concentration (Schirone, Visciano, Conte, & Paparella, 2022). In addition to this, the level of tyramine in protein-rich foods is an indicator of food quality. Indeed, studies have shown that the change in tyramine level in red meat could objectively reflect the spoilage process (Vinci & Antonelli, 2002., Galgano, Favati, Bonadio, Lorusso, & Romano, 2009). Therefore, it is necessary to propose regulation of tyramine content in foods not only because of its toxicological effects but also because of its role as a freshness index for protein-rich foods (Omer, Mohammed, Ameen, Abas, & Ekici, 2021).

For example, the European Food Safety Authority ([EFSA], 2011) has indicated that the consumption of 600 mg/person/meal of tyramine has no adverse effects on the health of people without monoamine oxidase inhibitor (MAOI) medication control. However, the safe limit is 50 mg for people taking drugs containing third-generation MAOIs, and this is only 6 mg for patients on conventional MAOIs (EFSA, 2011). It has been reported that normal tyramine intake is 100-800 mg/kg, whereas levels above 1080 mg/kg are considered toxic (Dasa et al., 2022., Karovičová & Kohajdová, 2005).

These low limits, therefore, require researchers to use very reliable and accurate measuring devices. Various methods have been developed for tyramine analysis, such as high-performance liquid chromatography (HPLC) (Saaid, Saad, Hashim, Ali, & Saleh, 2009), gas chromatography (GC) (Almeida, Fernandes, & Cunha, 2012), ion chromatography (Saccani, Tanzi, Pastore, Cavalli, & Rey, 2005), thin layer chromatography (TLC) (Lapa-Guimarães & Pickova, 2004), capillary electrophoresis (An et al., 2015), and electrochemical sensors (Calvo-Pérez, Domínguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2013). Although these methods can offer good selectivity and detection limits, they are often expensive and time-consuming techniques with complex pretreatment steps. In addition, the use of these devices often requires trained personnel.

In the present work, we are interested in the analysis of tyramine in aquatic products (fish) by spectrofluorimetry. In order to improve the fluorescence signal, the effect of different media on the emission spectra was evaluated. Also, parameters such as solvent effects, pH, temperature, and stability conditions were optimized. After obtaining very satisfactory analytical performances in various media, an application was performed in the quantitative analysis of tyramine in fish. Very good results were obtained by this new method. To further validate this method, an interference study of foreign species such as biogenic amines and salts likely to be present in food was done.

2. Experimental Survey

2.1 Products and Solvents Used

Tyramine (97 %, w/w), sodium hydroxide (98 %, w/w), hydrochloric acid (37 %, w/w) were purchased from Sigma Aldrich and used without further purification. The interference studies were done on the one hand with some biogenic amines (dopamine (DOP), tryptamine (TRYP), cadaverine (CAD), spermine (SPM), spermidine (SPD), agmatine (AGM), putrescine (PUT) and histamine (HIS) and on the other hand with salts (NaCl, Na_2CO_3, KI, Na_2PO_4, KCl). As solvents,

distilled water, methanol (MeOH), acetonitrile (ACN), and ethanol (EtOH) were used. All these chemicals and solvents were of analytical quality and supplied by Sigma-Aldrich.

2.2 Instrumentation

Fluorescence spectra were determined using a Perkin Elmer spectrofluorometer, model LS-45 interfaced with a computer equipped with FL-Winlab software. For weighing, a Sartorius precision 0.1 mg balance was used. A micropipette of 100 µL to 1000 µL, flasks and beakers of varying volumes from 5 mL to 100 mL, a Consort C6010 brand pH meter, and an SL16R Thermo scientific centrifuge were used. The use of OriginPro 8.5 and Chemdraw Ultra 8.0 software were required for data processing and molecule sketching, respectively. For fluorescence measurements, a quartz cell with five polished sides was used; the slit and the voltage of the apparatus were fixed at 10 nm and 600 volts respectively.

2.3 Proceedings

2.3.1 Preparation of the Solutions

A stock solution of tyramine of concentration 10^{-2} M was prepared in a 50 mL flask in an aqueous medium. From the stock solution, we proceeded to dilutions to have working solutions of desired concentrations. The solutions were protected from light with an aluminum foil and stored in a refrigerator.

2.3.2 Sampling

Fresh sardines (Sardinella aurita) were purchased at a local fish market in Soumbedioune (a small fishing port west of Dakar). The product purchased was intact, with a fresh smell and reddish gills. The fish were kept in ice to maintain their internal temperature as low as possible before and after purchase. However, to avoid the risk of changes in biogenic amine levels, all purchased samples were kept in a freezer. Similarly, canned fish (sardines in vegetable oil) and albacore tuna purchased from a local supermarket were kept in a freezer after opening.

2.3.3 Preparation of the Extract

To recover tyramine from fish, the solid phase extraction (SPE) procedure was applied from a certain frozen mass subjected to grinding. Then 10 g of grind was mixed with 100 mL of HCl (0.4 M)/pure methanol (20/80, v/v) solvent. The whole mixture was homogenized for 10 min using a magnetic stirrer. After shaking, the homogenate was centrifuged at 4000 rpm for 5 minutes at room temperature. The collected supernatant was decanted, filtered through Wittman filter paper, and stored in the refrigerator until used for the assay.

3. Results and Discussion

3.1 Fluorescence Spectra of Tyramine

Tyramine naturally fluoresces in different media. Well-resolved fluorescence spectra of tyramine were obtained in water, ethanol, methanol, acetonitrile, water-methanol (20/80, v/v) and water-acetonitrile (10/90, v/v) mixtures (Figure 1). No noticeable shift in wavelength maxima in both excitation and emission was observed when switching from one solvent to another. However, the signal intensity varied with the medium. In general, two peaks were observed in excitation varying between 217 nm and 222 nm for the first peak and between 273 nm and 278 nm for the second. On the other hand, the emission was characterized by a single peak between 306 nm and 308 nm depending on the solvent.

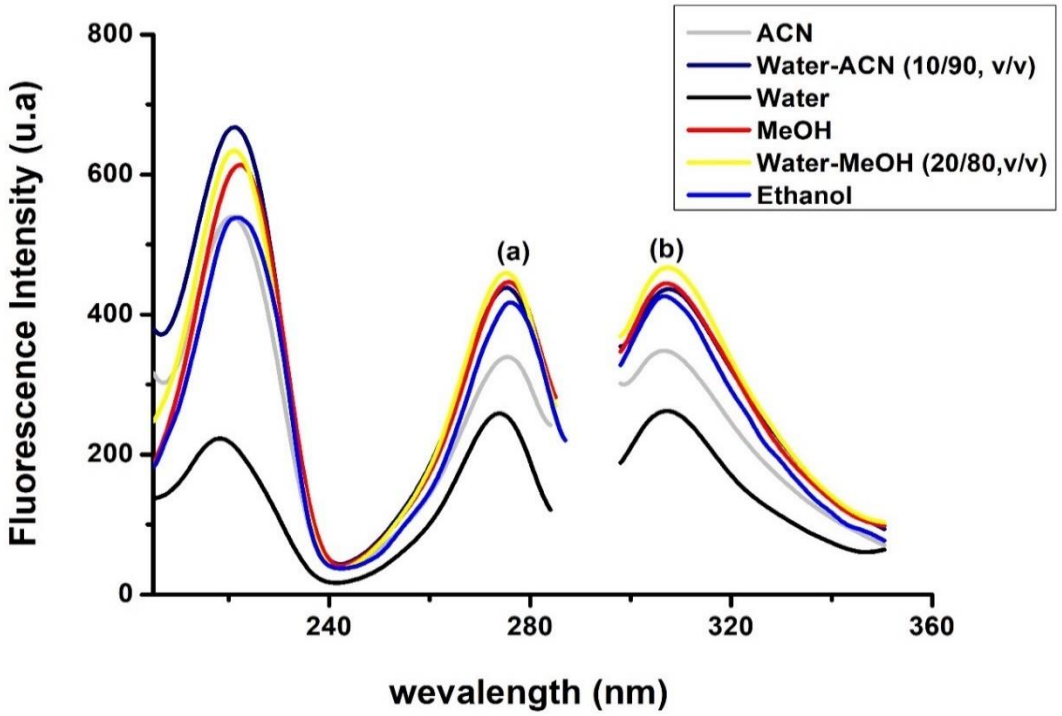


Figure 1. Excitation (a) and emission (b) spectra of tyramine ($6x10^{-7}$ M) in various solvents: water, methanol, acetonitrile, ethanol, water-methanol (20/80, v/v) and water-acetonitrile (10/90, v/v) mixtures

3.2 Optimization of the Analytical Parameters

To improve the sensitivity of the method, the effects of pH, temperature, percentage (v/v) of methanol in the water-methanol mixture as well as the stability of the tyramine fluorescence signal were determined.

3.2.1 Effect of pH on Tyramine Fluorescence Intensity

The fluorescence signal of organic compounds in aqueous media was very sensitive to the acidity or basicity of the medium (Coly & Aaron, 2009). Protonation or deprotonation of functional groups profoundly modifies many fluorophores sometimes affecting the shape of excitation or emission spectra.

For this purpose, a concentration of tyramine $1.2x10^{-6}$ M was prepared an aqueous solution; then the pH value was varied from 1 to 12 using two stock solutions HCl and NaOH of concentration 1 M respectively. The excitation and emission spectra were recorded for each pH value. The experiment showed that there was no observed change in the shape of the spectra. The plot of the fluorescence intensity I_f versus pH shows a relatively constant and higher value between pH 1 and pH 7; beyond this there was a decrease (Figure 2).

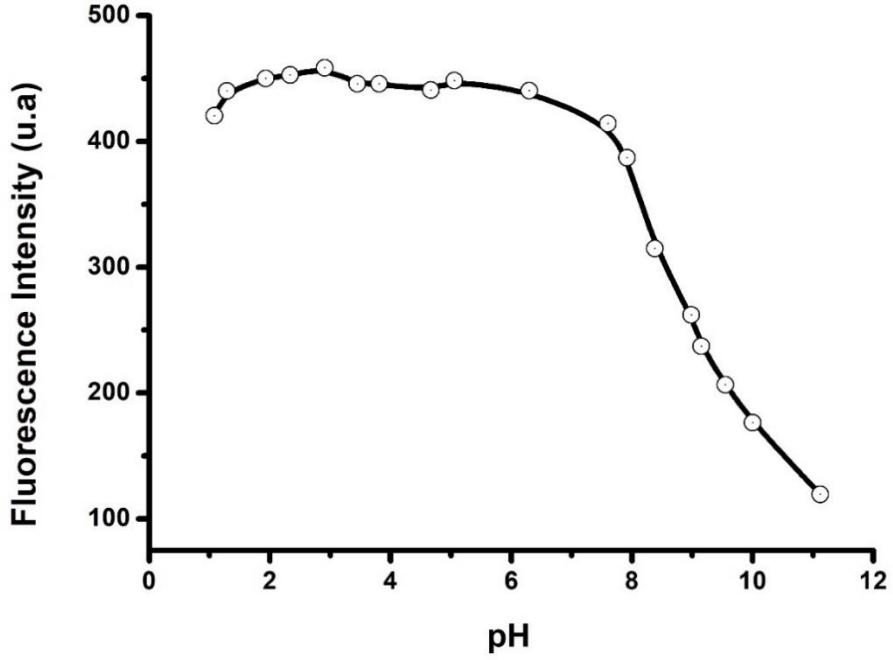


Figure 2. Effect of pH on the fluorescence intensity of tyramine ($1.2x10^{-6}$ M) in aqueous solution

3.2.2 Effect of Temperature on Tyramine Fluorescence

From tyramine solution ($1.2x10^{-6}$ M) the fluorescence excitation and emission curves were recorded between 25 °C and 80 °C. The result was that the increase in temperature did not affect either the excitation and emission wavelength maxima or the shape of the spectra. However, the fluorescence intensity decreased as the temperature increased (Figure 3). This fluorescence inhibition of tyramine in water can be explained by a temperature-dependent increase in the number of collisions with solvent molecules. The increase in collisions thus leads to a decrease in quantum yield, and fluorescence lifetime, in favour of non-radiative processes (Valeur, 2004).

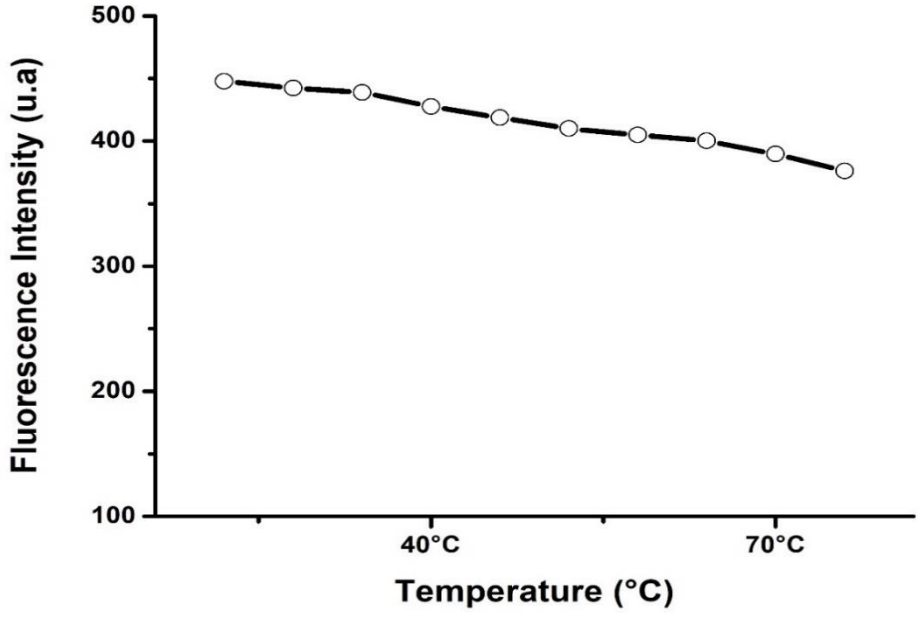


Figure 3. Temperature effect on the fluorescence intensity of tyramine ($1.2x10^{-6}$ M)

3.2.3 Effect of the Percentage of Methanol on the Fluorescence Intensity of Tyramine in Water at pH 4

To extract biogenic amines in food matrices, Custodio, Tavares and Gloria (2007) studied the efficiency of different extractors. In the context of selective extraction of amines these authors showed that aromatic amines (tyramine, phenylethylamine) and indolamines (tryptamine, serotonin) were best recovered with organic solvents (methanol and ethanol). In our case, methanol was used as extraction solvent and water-methanol mixture for analysis. Therefore, it was considered useful to study the effect of methanol level in this binary mixture on the fluorescence intensity of tyramine. For this purpose, a fixed concentration of tyramine $1.2x10^{-6}$ M was used for each analysis with varying levels of methanol in the mixture. No change in the shape of the emission spectrum was observed; however, the fluorescence intensity (I_f) curve as a function of methanol rate increased up to the 80 % value and then decreased immediately thereafter (Figure 4). In this work, 80 % level was used in the analysis of tyramine contained in various samples.

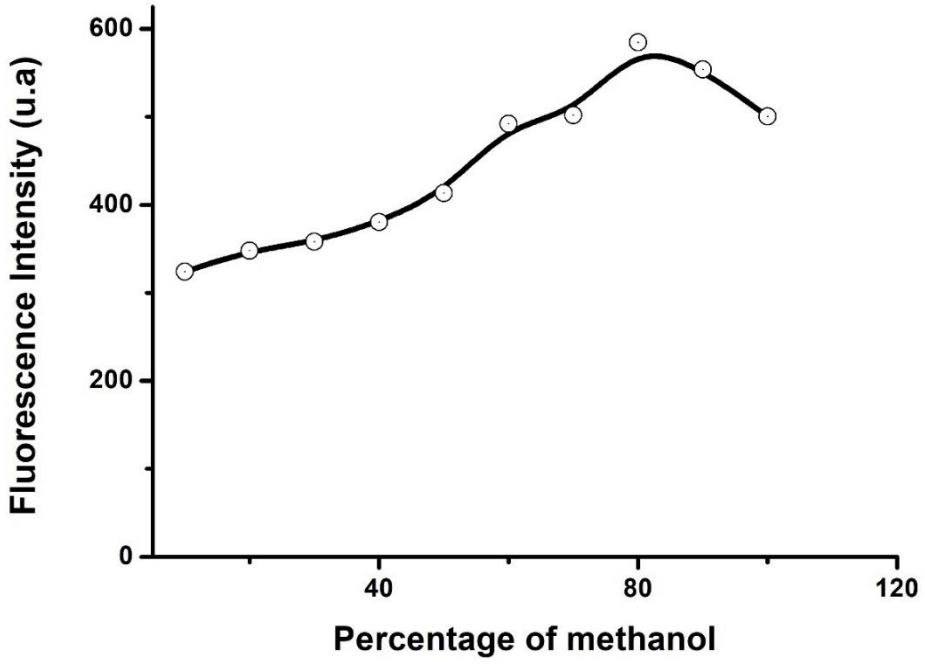


Figure 4. Effect of the percentage of methanol on the fluorescence intensity of tyramine ($1.2x10^{-6}$ M)

3.2.4 Study of the Fluorescence Signal Stability

To do this, the evolution of the fluorescence signal as a function of time for a given concentration of tyramine was followed after fixing the excitation and emission wavelength maxima (Figure 5). The results of this study showed that the fluorescence of tyramine was relatively stable in all the solvents studied.

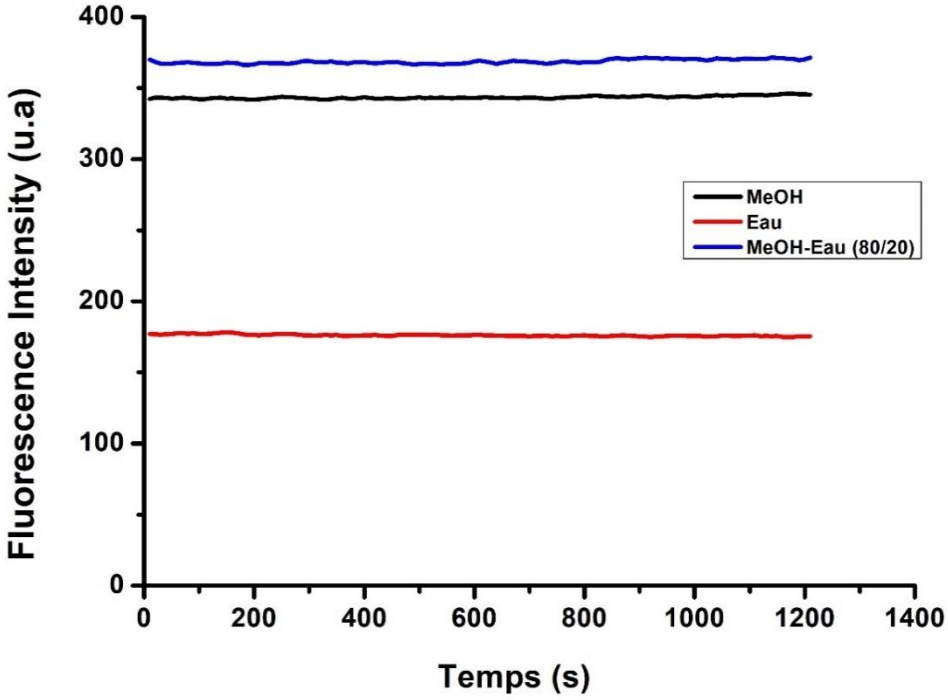


Figure 5. Study of the evolution of the fluorescence signal of tyramine (10^{-6} M) as a function of time in aqueous and organic solution

3.3 Analytical Performance of the Spectrofluorimetric Method

The analytical evaluation by direct fluorescence allowed us to determine the analytical performances in different media studied under optimal conditions. Linear calibration lines were obtained in all the solvents used with correlation coefficients between 0.9990 and 0.9999 very close to unity, which indicates the precision of our measurements (figure 6). In pure water the slope was relatively lower, in the water-methanol mixture the highest slope was obtained.

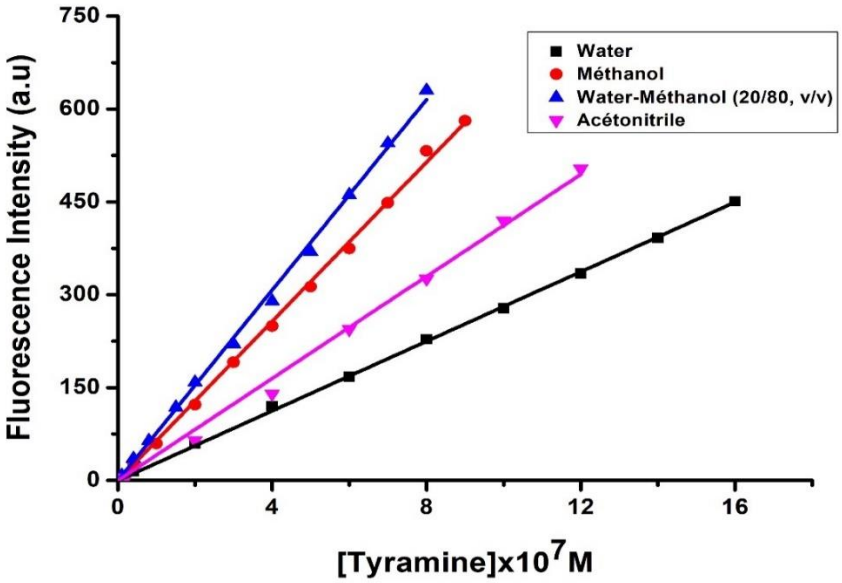


Figure 6. Calibration lines in different solvents

From the calibration curves the analytical parameters: limits of detection (LOD), limits of quantification (LOQ), and relative standard deviations (RSD) were determined (Table 1).

Table 1. Analytical parameters in aqueous (pH 4) and organic media

Solvants	λ_{ex}/λ_m[a](nm)	I_f[b] Standardized	r^2[c]	DL[d] (ng/mL)	LOD[e] (ng/mL)	LOQ[f] (ng/mL)	RSD[g]
Water (pH 4)	217; 273/308	1	0.9999	5.49-219.49	1.40	4.68	0.58
Methanol	222; 276/308	1.7	0.9997	1.37-123.46	0.56	1.87	0.46
Water-Methanol (20/80, v/v)	222; 276/307	1.8	0.9995	1.37-109.74	0.53	1.76	0.30
Acetonitrile	222; 276/306	1.3	0.9990	2.74-274.36	0.92	3.06	0.43

Notes: [a]Excitation (λ_{ex}) and emission (λ_{em}) wavelengths, [b]Fluorescence intensity normalized to the minimum value; [c]Linear correlation coefficients, [d]Domain of Linearity, [e]Detection limit defined as the analyte concentration giving a signal-to-noise ratio (S/N) equal to 3 (IUPAC criterion), [f]Quantification limit defined as the analyte concentration giving a signal-to-noise ratio (S/N) equal to 10 (IUPAC criterion), [g]Relative Standard Deviation (n = 6).

This table shows that the LODs ranged from 0.53 ng/mL to 1.40 ng/mL, and the LOQs ranged from 1.76 ng/mL to 4.68 ng/mL depending on the solvent. These low LOD values showed that direct fluorescence can be applied to the determination of tyramine in different samples. The relative standard deviations (RSD) obtained between 0.30 % and 0.58 % depending on the solvent, showed the good reproducibility of our measurements. It should be noted that the different values obtained in this work were lower than those found in the literature (Baranowska & Płonka, 2015., Jia, Ryu, Kwon, & Lee, 2013., Sirocchi, Caprioli, Ricciutelli, Vittori, & Sagratini, 2014). These results also show that the water-methanol mixture (20/80, v/v) is the appropriate medium for the quantitative analysis of tyramine by fluorimetric.

3.4 Analytical Application

3.4.1 Qualitative Study: Detection of Tyramine in Fish

The respective comparison of the excitation and emission spectra of the standard and the extract showed with some exceptions a zero spectral difference in shape and wavelength (Figure 7). This confirms that the extract in the various samples did contain tyramine. Taking into account these results, we can therefore affirm that tyramine was indeed present in these different extracts (sardine and albacore tuna). For both extracts, the band obtained between 350 nm and 450 nm corresponded to the emission of tryptamine.

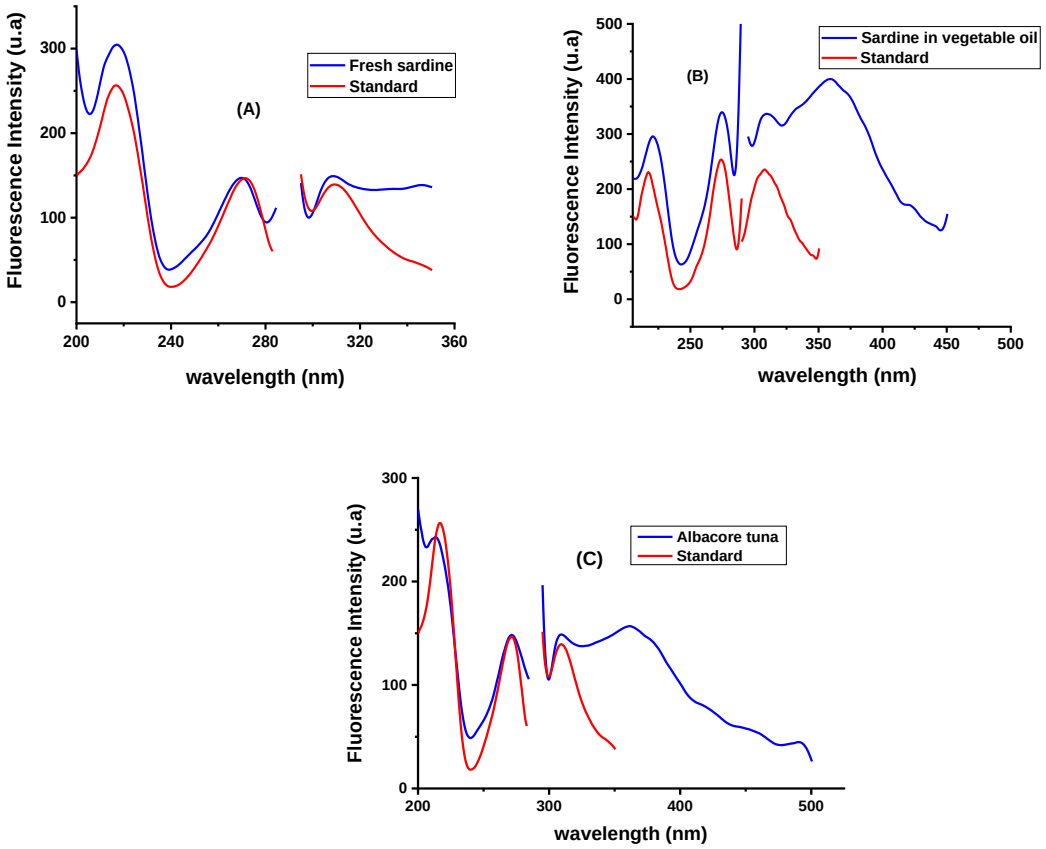


Figure 7. Comparison of the excitation and emission spectra of the standard with those of the different extracts: (A) fresh sardine, (B) sardine in vegetable oil and (C) albacore tuna

3.4.2 Quantitative Study on the Determination of Tyramine in Fish Extracts

We evaluated the applicability of the proposed method to the quantitative analysis of fish samples. The existence of parallelism between the calibration line and the standard addition line shows the non-involvement of the matrix effect on the determination of tyramine level. From these curves the percentage recovery (% R) was determined according to the following relation:

$$\%R = \frac{C_t}{C_0 + C_a} \times 100 \quad (1)$$

In this formulation, C_t represents the concentration of tyramine found using the calibration line, C_a is the added concentration, and C_0 is the blank concentration.

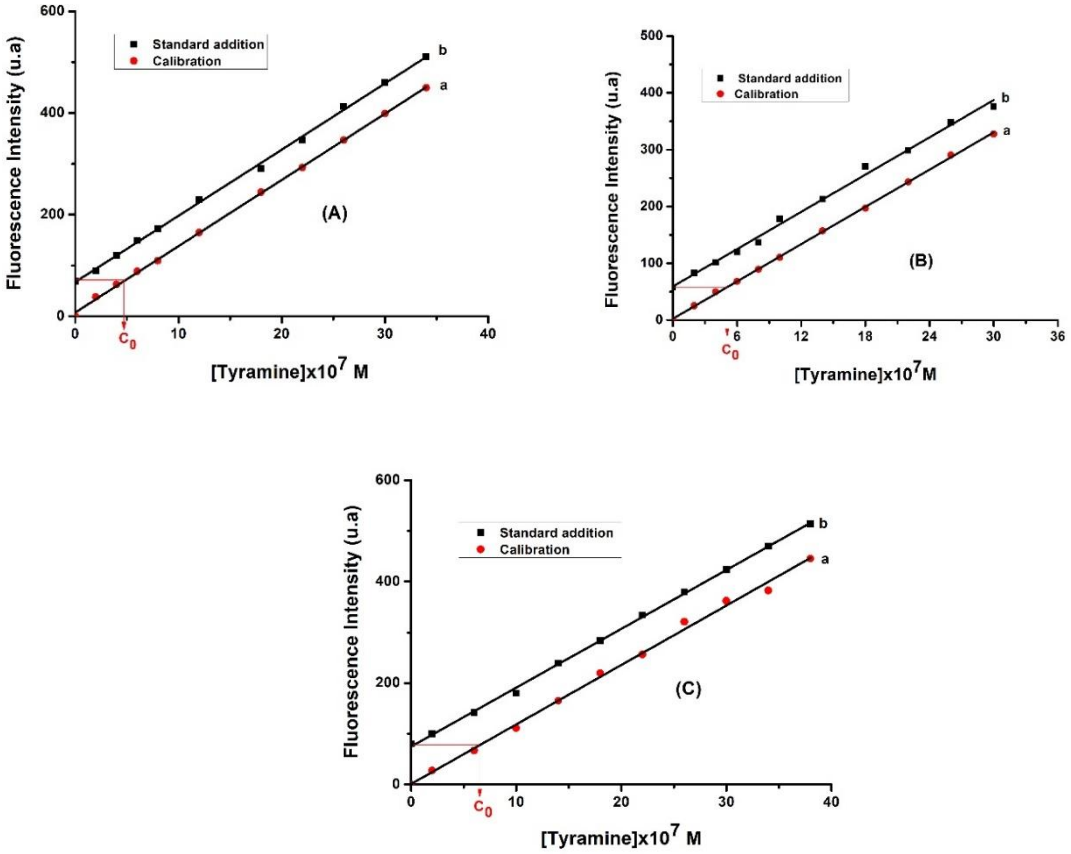


Figure 8. Calibration (a) and standard addition (b) curves of tyramine in various extracts: (A) fresh sardine, (B) sardine in vegetable oil and (C) albacore tuna

From the calibration and standard addition lines, the concentration C_0 expressed in ng/mL was determined respectively. Taking into account the dilution effect, the knowledge of C_0 allowed us to determine the mass levels of tyramine in milligrams per kilogram of fish (mg/kg) for each sample studied. For these three types of samples, the content in the mass rate of tyramine obtained were: 79.5 mg/kg for fresh sardine, 298.0 mg/kg for sardine in vegetable, oil and 139.7 mg/kg for canned albacore tuna in water. These found values are acceptable for consumption because they are lower or within the range of the tolerance limit of tyramine set between 100 mg/kg and 800 mg/kg (Paulsen, Grossgut, Bauer, & Rauscher-Gabernig, 2012., Food and Agriculture Organisation [FAO], 2013). The high value of tyramine obtained in sardines preserved in oil compared to fresh sardines would be due in part to the time taken before and during preservation.

Moreover, the mass of tyramine per kg of tuna obtained was higher than that in fresh sardine. This was consistent with the study by El-Ghareeb, Elhelaly, Abdallah, El-Sherbiny and Darwish (2021), which showed that for the same mass, tuna had a higher tyramine content than sardine.

For each of the three samples to be assayed, the relative standard deviations (RSD), the percentages (% R) and the intervals (IR) of recovery were determined (Table 2).

Table 2. Evaluation of recovery percentages in fish by the solid phase extraction (SPE) procedure

Sample type	C_{aj}[a] (ng/mL)	C_t[b] (ng/mL)	% R[c]	%RI[d]	% RSD[e]
Sardines (*Sardinella aurita*)	0	63.65	-	96.10-102.15	0.36
	27.43	87.53	96.10		
	54.87	119.21	100.58		
	82.31	149.10	102.15		
	109.74	173.89	100.29		
	164.62	231.41	101.38		
	246.92	298.91	96.25		
	301.80	358.46	98.09		
	356.67	425.00	101.11		
	411.54	474.58	99.87		
	466.41	527.25	99.47		
Sardine in vegetable oil	0	89.44	-	93.38-99.15	1.27
	27.44	114.34	97.83		
	82.21	164.88	96.06		
	137.18	211.61	93.38		
	192.05	279.10	99.15		
	246.92	331.65	98.60		
	301.80	389.30	99.50		
	356.67	441.84	99.04		
	411.54	494.51	98.71		
	466.41	549.06	98.78		
	521.28	601.62	98.51		
White tuna canned in water	0	69.93	-	94.61-106.60	0.43
	27.44	99.41	102.09		
	54.87	125.35	100.44		
	82.31	151.30	99.38		
	109.74	169.99	94.61		
	137.18	218.25	105.38		
	192.05	265.50	101.34		
	246.92	337.76	106.60		
	301.80	372.68	100.26		
	356.67	434.27	102.50		
	411.54	470.03	97.62		

Notes: [a]: Added concentration, [b]: Found concentration, [c]: Percent recovery, [d]: Recovery interval, [e]: Relative standard deviation

In this table, the relative standard deviations between 0.36 % and 1.27 % showed the very good reproducibility of the measurements. Similarly, the percentages of recovery between 96.10 % and 102.15 % for fresh sardines, 93.38 % and 99.15 % for sardines in vegetable oil and 94.61 % and 106.60 % for canned albacore tuna in water, showed the good efficiency of our method.

3.5 Interference Study with Biogenic Amines and Salts

3.5.1 Interference with Biogenic Amines

Many studies have shown the presence of biogenic amines (cadaverine, histamine, putrescine, spermidine, tryptamine, and agmatine) in fish to a greater or lesser extent (Zhang et al. 2019., Huang et al. 2016., Alizadeh, Kamalabadi, & Mohammadi, 2017., Bueno-Solano, López-Cervantes, Sánchez-Machado, & Campas-Baypoli, 2012). Their simultaneous presence in fish can thus cause interference effects in the analysis of tyramine in spectrofluorimetry. This interference depends on the content and the excitation and emission range of the interferent. Thus, for a tyramine concentration fixed at $2x10^{-7}$ M (0.0274 µg/mL), the interference effects with another amine were evaluated by gradually increasing the concentrations of the interfering biogenic amine from 10^{-7} M to $4.5x10^{-7}$ M (Figure 9).

In general, in our case, the addition of interfering species did not change the appearance of the excitation and emission spectra of tyramine in a significant way. Furthermore, no shift in the wavelength maxima was observed. However, a more or less important variation of the fluorescence intensity of tyramine was noted as soon as the first concentrations of the interfering species were added. An increase in intensity was noted for dopamine, tryptamine, cadaverine, putrescine and histamine. On the other hand, a slight decrease in intensity was noted after addition of spermine, spermidine and agmatine. Figure 9 shows that biogenic amines such as spermidine (SPD), histamine (HIS) and spermine (SPM) hardly interfered with the tyramine assay. Similarly, this figure shows that the interference of putrescine and agmatine on the determination of a sample containing tyramine is only noticed if their respective concentrations exceed at least that of tyramine; for cadaverine much higher concentrations were required; close to twice that of tyramine, to observe an interference phenomenon. On the other hand, very low concentrations of dopamine and tryptamine, less than a quarter of that of tyramine in the sample, were sufficient to interfere with the assay.

To further confirm these statements, quantitatively the tolerance limits and mass levels for each added amine were determined (Table 4).

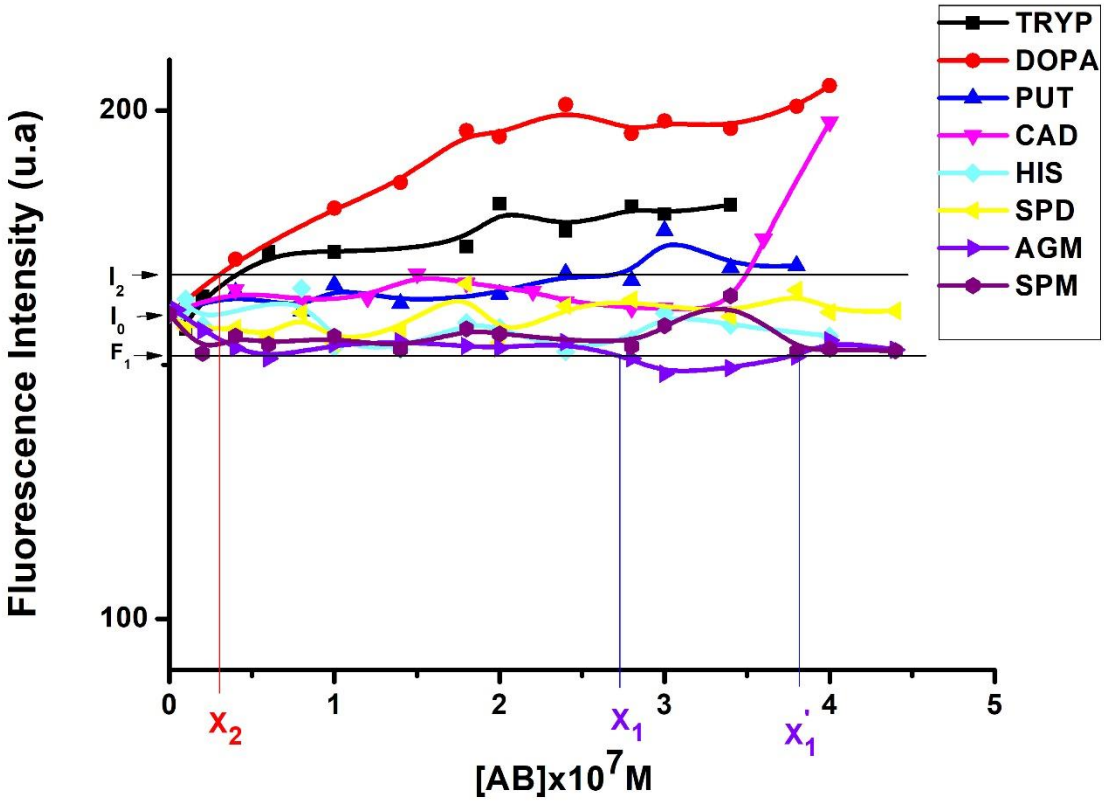


Figure 9. Effect of biogenic amines on tyramine fluorescence intensity

In our case, the tolerance limit (TL) is defined as the limiting concentration of the interferent for which the percentage change in the tyramine fluorescence signal does not exceed ± 5 %. This can be written as:

$$|\Delta I(\%)| = \frac{I_0 - I}{I_0} \times 100 = 5\ \% \quad (2)$$

In this expression, ΔI (%) represents the percent signal change of tyramine; I_0 and I indicate the fluorescence signal of tyramine in the absence and presence of the interfering species, respectively. For our case the value of I_0 is equal to 109.11 relative unit of fluorescence intensity.

From this expression, we can deduce the limit values that correspond to this precision. We can therefore have two limit values $I_1$and I_2.

If $|\Delta I(\%)| \geq 0$ we write $\frac{I_0 - I_1}{I_0} \times 100 = +5\ \%$ we will have :

$$I_1 = \frac{95 \times I_0}{100} = 103.65 \quad (3)$$

If $|\Delta I(\%)| \leq 0$ we write $\frac{I_0 - I_2}{I_0} \times 100 = -5\ \%$ we will have :

$$I_2 = \frac{105 \times I_0}{100} = 114.57 \quad (4)$$

Thus, the concentrations that do not interfere with tyramine emission will correspond to intensities between I_1 and I_2. Outside this interval the accuracy of the method exceeds ± 5%; the measurement therefore becomes less accurate.

From the intersection of the curve I = f[AB] with the lines $y = I_1$ or $y = I_2$ one can determine the tolerance limits (TL) at X_1or X_2 respectively. If there is no intersection, the amine does not interfere with the tyramine determination.

Table 3 groups the concentration ranges tested the different tolerance limit values, and the corresponding mass ratios at the tolerance limits of each interfering species with respect to tyramine.

Table 3. Tolerance limits and corresponding mass rates according to the range of biogenic amines tested

Biogenic amines	Concentration ranges tested (µg/mL)	Tolerance limit (LT) X_i (µg/mL)	Mass rates (%)
Tryptamine	0.001602-0.054474	0.00693	25.29
Dopamine	0.00758-0.09482	0.00603	22.01
Putrescine	0.0064428-0.0612066	0.04316	157.52
Cadaverine	0.007004-0.07004	0.06119	223.32
Histamine	0.0014725-0.073628	∞	∞
Spermidine	0.0020234-0.109263	∞	∞
Agmatine	0.000913-0.1004	0.062[a](X_1); 0.0866[b](X_1')	226.27[a]; 316.06[b]
Spermine	0.00405-0.08903	∞	∞

Notes: Fixed concentrations $= [TYR]_0 = 0.0274$ µg/mL ; ∞ : not interfering; Mass ratio $= \frac{[AB]}{[TYR]_0} \times 100$; **a**) upper limit; **b**) lower limit

Taking into account the tolerance limits or mass levels in relation to tyramine, this table 3 shows that, dopamine and tryptamine interfere much more in the determination of tyramine followed by putrescine then cadaverine and finally agmatine. There is no interference in the determination of tyramine when the sample also contains histamine, spermidine or spermine.

It is noted that dopamine and tryptamine exalt the fluorescence of tyramine. However, the very low tolerance limits or mass rates observed in the case of these two amines compared to tyramine can be explained by the existence of overlap of their excitation or emission spectra compared to those of tyramine (Figure 10).

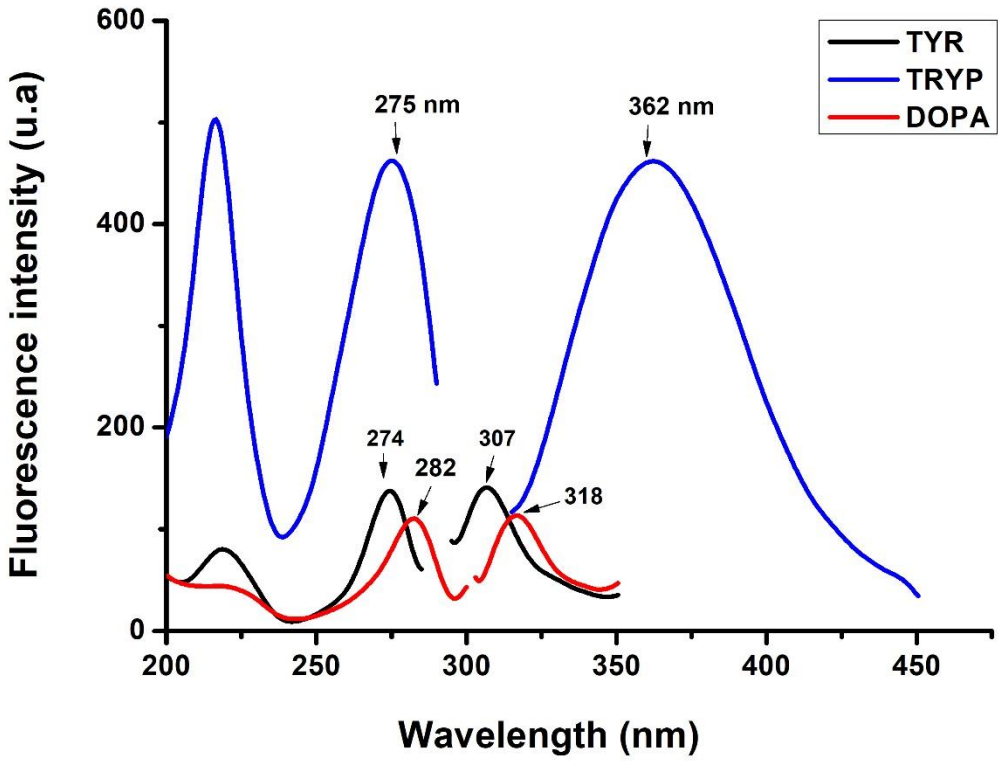


Figure 10. Excitation and emission spectra in water of tyramine, tryptamine and dopamine (pH 4 at 25 °C)

Nevertheless, it is possible to determine tyramine with an accuracy of ± 5 % in a sample containing tryptamine whose mass does not exceed 25% of that of tyramine. Similarly, to determine tyramine with ± 5 % accuracy in a sample containing dopamine, its mass must not exceed 22 % of that of tyramine. In a sample containing putrescine or cadaverine the determination of tyramine can be done with the same accuracy for a much higher level of tyramine (157 % for putrescine and 223 % for cadaverine). The determination of tyramine in a sample containing agmatine can be done with the same accuracy if its mass ratio to tyramine is less than 226 % or more than 316 %. Indeed, between 226 % and 316 % the precision will be higher ± 5 % (figure 9).

3.5.2 Interference with Salts

The interference of NaCl, KCl, Na_3PO_4, Na_2CO_3, KI on tyramine fluorescence was studied (Figure 11). These salts are all present in the food matrix (Albarracín, Sánchez, Grau, & Barat, 2011). This figure shows that except for NaCl and Na_2CO_3 the other salts have little influence on the fluorescence intensity of tyramine.

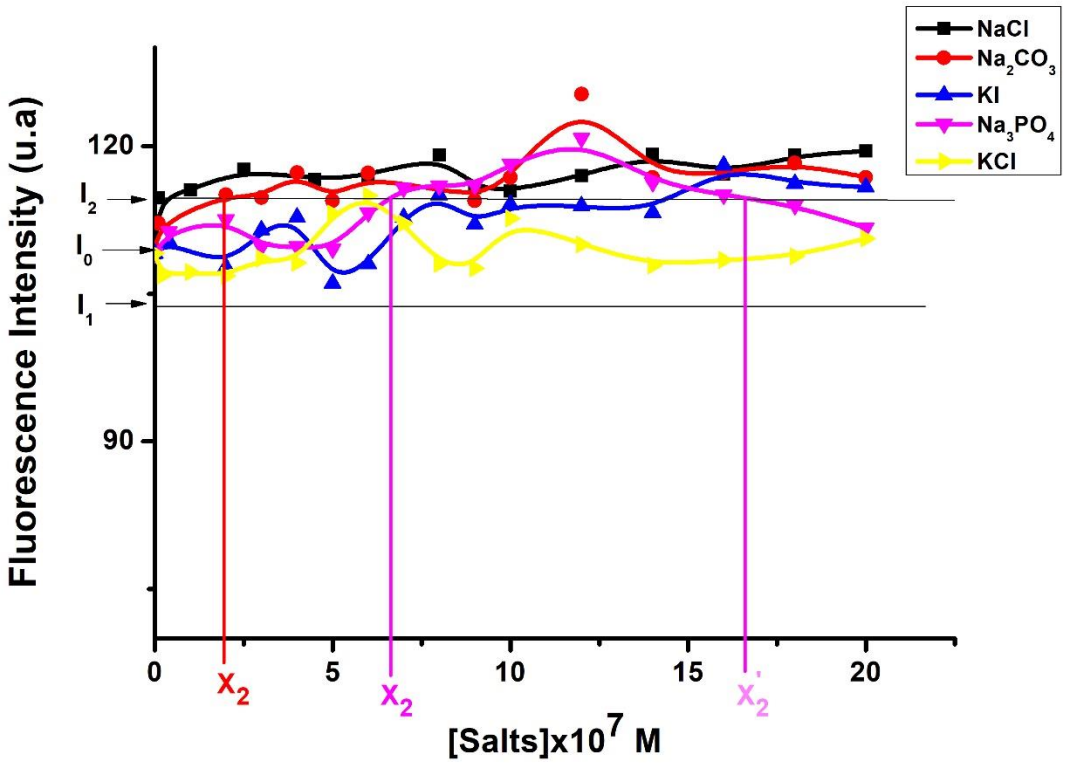


Figure 11. Effect of salts on tyramine fluorescence intensity

From this figure, it was possible to determine for each salt its tolerance limit and its mass rate with respect to tyramine. The tolerance limits and the mass rates obtained were calculated in the same way as before (interference with biogenic amines). All the results are grouped in table 4.

Table 4. Tolerance limits and corresponding mass rates according to the range of salts test

Salts	Tested Concentrations Ranges (µg/mL)	Tolerance Limits (µg/mL)	Mass rates
Na^+,Cl^-	0.000584-0.11688	0.002417	3.52
K^+, I^-	0.00664-0.332	0.2374	346.11
$2Na^+$, CO_3^{2-}	0.0010598-0.21196	0.0195	28.43
$3Na^+$, PO_4^{3-}	0.006557-0.3278	$0.1076^a(X_2)$; $0.2758^b(X'_2)$	156.87^a; 402.09^b
K^+, Cl^-	0.001491-0.1491	∞	∞

Notes: Fixed concentrations = $[TYR]_0$= 0,06859 µg/mL; ∞ non-interfering; Mass ratio= $\frac{[sel]}{[TYR]_0} \times 100$; **a**) upper limit; **b)** lower limit

No interference was noted with KCl. In contrast, strong interference was noted with NaCl, with a tolerance limit of 0.00242 µg/mL corresponding to a mass level of 3.52 % (Table 4). Thus, even at trace levels, sodium chloride interferes significantly with tyramine fluorescence at least for the 5 % accuracy. Therefore, it is important to perform a thorough washing of the food matrix containing NaCl before any measurement. Indeed, in most cases fish are preserved by NaCl. The other salts interfere in a more or less important way. Na_2CO_3 interferes with a mass rate of 28.43 %, KI with a mass rate of 346.11 % and Na_3PO_4 with a mass rate between 157 % and 402 %.

Interference studies show that the presence of certain biogenic amines and salts in a food sample may interfere to a greater or lesser extent during the determination of tyramine. In contrast, no significant interference was found during the assays performed on our treated fish samples. Indeed, the standard addition and calibration lines are totally parallel; this confirms the absence of a matrix effect during the assay. These samples were therefore not altered.

4. Conclusion

In this study, a simple, sensitive, accurate and inexpensive spectrofluorimetric based method for the determination of tyramine was optimized. The low detection and quantification limits found indicate the good sensitivity and accuracy of this method. Similarly, the low relative standard deviation values showed the good reproducibility of the measurements. This method provided very satisfactory recovery percentages for the analysis of tyramine in fish. The study of interference effects also showed that some biogenic amines and salts can interfere with tyramine. For our case, no significant interference effect was observed in the determination of tyramine for both types of samples studied. Indeed, a close parallelism was obtained between the standard addition lines and the calibration lines. This parallelism indicated the unmarked absence of interference effects. However, our study showed that it is very important to wash dried or NaCl preserved fish before any assay. These results showed the effectiveness of this new method of analysis. Thus, this method could be proposed for the analysis of tyramine in food products.

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Authors contributions

Dr. Khémesse Kital, Dr. Moussa Mbaye and Prof. Alphonse Tine were responsible for study design. Papa A. Ndione and Latyr Ndione were responsible for data collection. Prof. Serigne Omar Sarr and Prof. Djibril Fall were responsible for supervision. Papa A. Ndione, Dr. Moussa Mbaye, Prof. Lamine Cissé, Prof. Atanasse Coly, and Prof. Alphonse Tine drafted the manuscript. Papa A. Ndione, Dr. Moussa Mbaye, Prof. François Delattre and Prof. Mame D. Gaye-Sèye revised it. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal’s policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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