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Determination of Tryptamine Level by Spectrofluorimetric Method: Optimization and Application to Canned Fish

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Abstract

In this article, we propose developing a reliable and sensitive method for determining tryptamine (TRYP) levels in canned fish samples using spectrofluorimetric analysis. The analytical performance obtained was satisfactory, with detection limits (LOD) ranging from 0.09 ng/mL to 1.16 ng/mL and quantification limits (LOQ) from 0.30 ng/mL to 3.87 ng/mL. Additionally, low values of relative standard deviation (RSD) between 0.56% and 5.56% were achieved, demonstrating a good reproducibility of our measurements. The application of this method to our samples allowed for the detection of tryptamine with satisfactory recovery rates between 94.35% and 107.14%. Finally, the potential interference from biogenic amines, which may be present in fish, was studied. Our results showed that the spectrofluorimetric method is simple, rapid and sensitive enough for routine analysis, requiring neither expensive equipment nor tedious chemical pre-treatments.

Keywords

Tryptamine, Analysis, Fish, Levels, Fluorescence

1. Introduction

Biogenic amines are low molecular weight organic bases. They can be produced by the metabolism of plants, animals and microorganisms [1] [2]. These amines

are also formed by the transamination of aldehydes and ketones, hydrolysis of nitrogenous compounds, thermal decomposition, or by the decarboxylation of amino acids [2] [3]. The decarboxylation of amino acids is the primary pathway for the formation of biogenic amines and involves the removal of the α-carboxyl group from the amino acid structure, forming the corresponding amine [4]. This reaction can occur via two biochemical pathways: through the action of endogenous decarboxylase enzymes, *i.e.*, enzymes naturally present in foods, or through exogenous decarboxylase enzymes produced by microorganisms [5] [6]. The concentration and formation of different types of amines are directly related to the nature of the food and the type of microorganism present [7]-[9]. Biogenic amines are present in low concentrations or not detected in fresh foods; however, animal-derived foods such as fish, meat, eggs, cheese and fermented foods, can be present in high concentrations capable of inducing chemical intoxication [6]. The accumulation of biogenic amines in food depends on the availability of free amino acids and the presence of microorganisms with decarboxylase activity on amino acids [10]. In addition to the availability of precursor amino acids, the formation of biogenic amines depends on both intrinsic and extrinsic parameters of the food, such as temperature and pH, oxygen tension, availability of carbon sources, presence of vitamins, coenzymes, concentration of free amino acids and fermentable carbohydrates [11]-[14]. Thus, the main factors that can influence the biosynthesis of these compounds are storage conditions, good manufacturing practices [15] [16], the amount of microorganisms with decarboxylase activity [5] [17], the quality of raw materials and the availability of free amino acids [18]-[20].

The concentration level of these biogenic amines (BAs) can be used as an indicator of food spoilage [21]-[25]. For public health protection, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [22], as well as the European Food Safety Authority (EFSA) [23], have established a maximum acceptable concentration of 50 mg/kg for histamine (HIS) in food. Although HIS is the only BA with a defined threshold level [21], FAO/WHO and EFSA highlight other BAs such as tryptamine (TRYP), 2-phenylethylamine (PHE), putrescine (PUT), and cadaverine (CAD), as they may have a significant synergistic effect when combined with increasing histamine levels, thus causing acute toxicity [22] [23]. It is therefore crucial to monitor their presence in food due to their potential health impacts, especially since these amines are heat-stable [22] [23] [26].

Tryptamine (TRYP), a biogenic amine, is a low-molecular-weight nitrogenous organic compound [27], derived from the enzymatic decarboxylation of free tryptophan and proteins [28]. It is present in a variety of foods and beverages such as fish, cheese, salami, sausages, wine, beer, certain fruits and vegetables [29]. Once formed, it is difficult to destroy through pasteurization or cooking. Tryptamine plays an important role in humans as it can act as a neuromodulator or neurotransmitter [29]. However, consuming foods containing high concentrations of tryptamine can be harmful to human health, causing symptoms such as nausea, palpitations, headaches and increased blood pressure [2] [9] [30]. Although high

concentrations of tryptamine can cause these health effects, its threshold amount is not regulated.

The fish canning industry is an important part of the Senegalese agri-food industry. To ensure the safety of canned fish, we deemed it necessary to check the tryptamine levels in cans sold in local markets.

Various methods have been developed to quantify tryptamine. Among these methods, one can mention: high-performance liquid chromatography (HPLC) coupled with a UV-visible detector or a fluorescence detector, which is the most commonly used [30]-[32], ion chromatography (IC) [33], micellar chromatography [34], capillary zone electrophoresis [35], gas chromatography coupled with mass spectrometry (GC-MS) [36], thin-layer chromatography (TLC) [37], electrochemical methods [29] [38] and immunoassays [39]. Although these methods can offer good selectivity and detection limits, they are often expensive, have long analyses, require complex sample preparation and demand qualified personnel. Therefore, the development of an appropriate analytical technique to detect tryptamine remains a significant challenge.

In this article, we developed a direct fluorescence method in aqueous and organic media for the determination of tryptamine. To achieve this, we studied the fluorescence spectral properties of tryptamine in various organic solvents and aqueous environments, including pH, temperature, signal stability, and the analytical performance of the method. A detailed study of interference effects with other biogenic amines that could be present in the food matrix was conducted before any applications.

2. Experimental Study

2.1. Products and Solvents Used

The following products were used: tryptamine (98%, w/w), sodium hydroxide (98%, w/w), hydrochloric acid (37%, w/w), glacial trichloroacetic acid (99%, w/w), and glacial acetic acid (99%, w/w). We also used other biogenic amines such as dopamine (DOP), tyramine (TYR), cadaverine (CAD), spermine (SPM), spermidine (SPD), agmatine (AGM), putrescine (PUT), and histamine (HIS) to study the interferences with tryptamine (TRYP). As solvents, we used distilled water, methanol (MeOH), acetonitrile (ACN) and ethanol (EtOH). All these products and solvents were of analytical grade and were provided by Sigma-Aldrich.

2.2. Instrumentation

Fluorescence spectra were recorded using a Perkin Elmer LS-45 spectrofluorometer interfaced with a computer equipped with FL-Winlab software. For weighing, we used a Sartorius precision balance with an accuracy of 0.1 mg. A 100 to 1000 µL micropipette, flasks and beakers with varying volumes from 5 to 100 mL, a Consort C6010 pH meter, and a Thermo Scientific SL16R centrifuge were used. The software programs OriginPro 8.5 and ChemDraw Ultra 8.0 were helpful for data processing and molecular schematic drawings, respectively. During all exper-

iments, a quartz cuvette with five polished faces was chosen, and for the spectrofluorometer, we set the slit to 10 nm and the voltage to 600 volts.

2.3. Experimental Procedures

2.3.1. Preparation of the Solutions

A stock solution of tryptamine with a concentration of 10^{-2} M was prepared in a 50 mL flask using methanol. From this stock solution, dilutions were made to obtain desired concentrations for the working solutions. The solutions were protected from light with aluminum foil and stored in a refrigerator.

2.3.2. Samples

Canned fish (sardines in vegetable oil) and white tuna in water (albacore), purchased from a local supermarket, were stored in a freezer after opening. However, to prevent the risk of biogenic amine levels evolving, all samples purchased were kept in a freezer.

2.3.3. Preparation of the Extract

We applied the solid-phase extraction (SPE) procedure to recover tryptamine from the canned fish. After grinding, 10 g of the ground material was mixed with 80% pure methanol and 20% distilled water in a 100 mL volumetric flask. The mixture was homogenized for 10 minutes using a magnetic stirrer. The homogenate was then centrifuged at 4000 rpm for 5 minutes at room temperature. The supernatant was decanted, filtered and stored in the refrigerator until use.

3. Results and Discussion

3.1. Fluorescence Spectra of Tryptamine

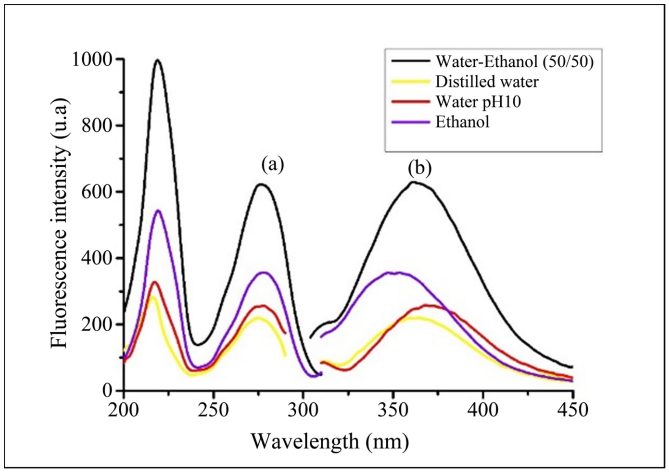

Figure 1. Excitation spectrum (a) and emission spectrum (b) of tryptamine (10^{-7} M) in distilled water, water at pH 10, ethanol, and in a 50/50 (v/v) ethanol-water mixture.

Tryptamine is a biogenic amine that exhibits natural fluorescence. We examined the excitation and emission spectra of a 10^{-7} M tryptamine solution in both aqueous and organic media (**Figure 1**). The resulting curves were well-resolved. The

excitation spectra were characterized by two peaks, one at 216 nm and the other at 278 nm, while the emission spectrum was characterized by a single peak, with a maximum between 352 nm and 371 nm. Figure 1 shows that when transitioning from an aqueous medium to ethanol, there was a hypsochromic shift in the emission wavelength, accompanied by an increase in fluorescence intensity. This can be explained by the fact that tryptamine was highly soluble in ethanol.

3.2. Optimisation of Analytical Parameters

3.2.1. Effect of pH on Tryptamine Fluorescence Intensity

Using a 1 M HCl solution and a 1 M NaOH solution, we adjusted the desired pH value between 1 and 13 for a 10^{-7} M aqueous tryptamine solution. We recorded the excitation and emission spectra for each pH value (Figure 2(A)). This figure shows that pH variation does not affect the shape of the emission spectrum. However, there was a bathochromic shift of the band when transitioning from acidic to basic conditions. This is not surprising, as it has already been proven that pH is a factor that strongly affects the fluorescence signal of organic compounds in aqueous media [40] [41]. Additionally, protonation or deprotonation of functional groups profoundly modifies the fluorophores, sometimes altering the shape of excitation or emission spectra and the fluorescence intensity.

Regarding fluorescence intensity (Figure 2(B)), in acidic conditions, there is an increase in fluorescence intensity up to pH 4, followed by a slight decrease in intensity until pH 7. In basic conditions, an increase in intensity was observed up to pH 10. Beyond this pH value, a sharp decrease in fluorescence intensity was noted, reaching pH 13. At pH 10, the intensity is higher compared to other pH values on the curve (Figure 2(B)). Therefore, pH 10 was chosen as the optimal pH for the subsequent analytical work in aqueous media.

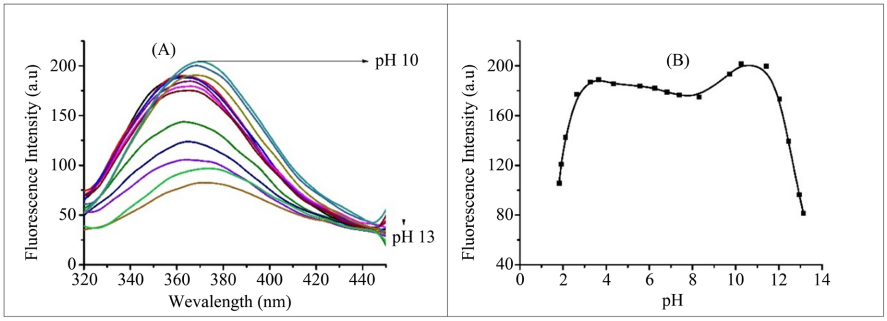

Figure 2. Effect of pH on the emission spectrum (A) and on the fluorescence intensity (B) of tryptamine (10^{-7} M) in aqueous solution.

3.2.2. Effect of Temperature on Tryptamine Fluorescence

For each temperature value set between 25˚C and 80˚C, we recorded the excitation and emission fluorescence curves of a 10^{-7} M aqueous tryptamine solution at regular intervals of 5˚C or 10˚C. The results showed that increasing the temperature does not affect either the maxima of the excitation and emission wavelengths or the shape of the spectra (Figure 3(A)). However, we observed that fluorescence

intensity decreases as the temperature increases (**Figure 3(B)**). We can concluded that tryptamine fluorescence in water was inhibited at higher temperatures. This can be explained by an increase in thermal agitation and a higher number of collisions between solvent molecules, which may lead to a decrease in fluorescence quantum yield and fluorescence lifetime, resulting from a greater efficiency of non-radiative processes [42].

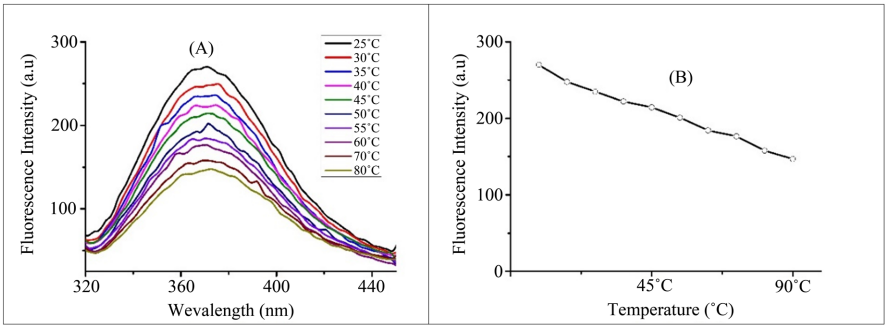

Figure 3. Effect of temperature on the fluorescence intensity of tryptamine (10^{-7} M).

3.2.3. Study of Fluorescence Signal Stability

To gain more insight into the stability of tryptamine in aqueous media, we studied the kinetics by monitoring the evolution of the fluorescence signal over time at the analytical excitation (277 nm) and emission (366 nm) wavelengths (**Figure 4**). The results of this study showed that tryptamine is relatively stable at the optimal pH.

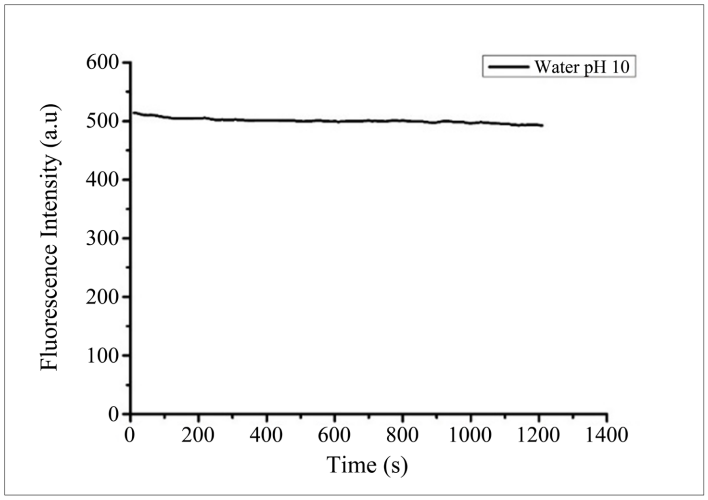

Figure 4. Study of the evolution of the fluorescence signal of tryptamine (2×10^{-7} M) over time at pH 10.

3.3. Analytical Performance of the Spectrofluorimetric Method

To assess the effectiveness of the proposed method, the analytical performance was determined under optimal conditions in different solvents, namely water (pH 10), acetonitrile, methanol, and ethyl acetate. Linear correlations were observed in all solvents, with correlation coefficients (r^2) ranging from 0.9977 to 0.9996.

These correlation coefficients, which are close to unity, indicate the high precision of our measurements (Figure 5).

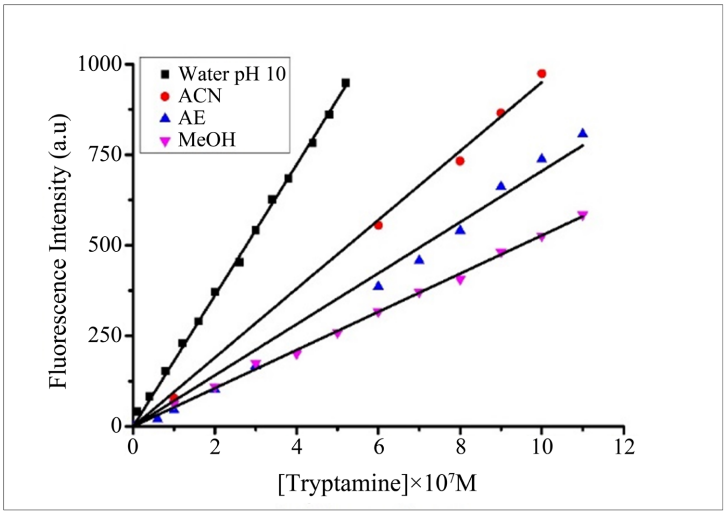

Figure 5. Calibration curves in water at pH 10, acetonitrile, ethyl acetate and methanol.

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

Table 1. Analytical parameters in aqueous (pH 7) and organic media.

Solvants	λ_{ex}/λ_m[a] (nm)	r^2[b]	DL[c] (ng/mL)	LOD[d] (ng/mL)	LOQ[e] (ng/mL)	RSD[f]
Water (pH 10)	216; 277/366	0.9995	1.602 - 83.31	0.09	0.30	1.17
Acetonitrile	217; 277/347	0.9996	16.02 - 160.22	0.41	1.36	5.20
Methanol	219; 276/347	0.9997	16.02 - 176.24	1.16	3.87	0.56
Ethyl Acetate	276/333	0.9977	9.61 - 176.24	0.62	2.06	5.56

[a]Excitation (λ_{ex}) and emission (λ_{em}) wavelengths, [b]Linear correlation coefficients, [c]Linearity range, [d]LOD = Limit of detection, defined as the analyte concentration giving a signal-to-noise ratio (S/N) equal to 3 (IUPAC criterion), [e]LOQ = Limit of Quantification, defined as the analyte concentration giving a signal-to-noise (S/N) ratio of 10 (IUPAC criterion), [f]Relative Standard Deviation (n = 6).

From the obtained linear calibration curves, we determined the analytical parameters (Table 1). The relative standard deviations (RSD) ranged from 0.56% to 5.56%, depending on the solvent. These values demonstrate the good reproducibility of our measurements. The limits of detection (LOD) and limits of quantification (LOQ) vary from 0.09 ng/mL to 1.16 ng/mL and from 0.30 ng/mL to 3.87 ng/mL, respectively. These low limits highlight the high sensitivity of the method. These values indicate that this method can be applied to the analysis of trace amounts of tryptamine in food products.

3.4. Method Validation

The direct fluorimetric method we developed has detection limits lower than those reported by authors using various methods, such as ultrasonic-assisted dispersive liquid-liquid microextraction (UA-DLLME) combined with high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) (LOD = 3.2 ng/mL) [43], capillary electrophoresis with UV detection (500 ng/mL) [44], LPME-HPLC with UV detection (LOD = 20 ng/mL) [45], DLLME-SFO-LC with UV detection (LOD = 5 ng/mL) [46] and DLLME-GC with mass spectrometry (LOD = 1.7 ng/mL) [36]. Therefore, the LOD and LOQ values obtained in this study are very low, suggesting that direct fluorescence can be considered an appropriate and sensitive method for determining tryptamine in canned fish.

3.5. Analytical Application

3.5.1. Detection of Tryptamine in Fish

We recorded the excitation and emission spectra of tryptamine in the standard solution and in the canned sardines in vegetable oil and canned white tuna samples (**Figure 6**). These spectra showed no spectral difference in terms of shape or wavelength. Therefore, we can conclude that not only was tryptamine present in the fish, but methanol proved to be more effective in extracting tryptamine from fish than trichloroacetic acid.

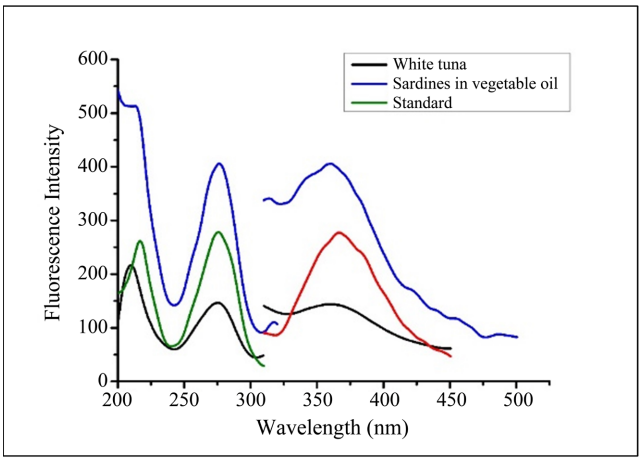

Figure 6. Comparison of excitation and emission spectra of the standard with different extracts: sardines in vegetable oil and canned white tuna.

3.5.2. Quantitative Analysis of Tryptamine in Fish

To determine the amount of tryptamine in canned fish, the standard addition curve was established. This curve was nearly parallel to the tryptamine calibration curve (**Figure 7**). This good parallelism indicated that the matrix effect was very negligible in all our measurements. From this curve, the recovery rate of tryptamine (%R) was determined using the following equation:

$$\%\mathbf{R} = \frac{\mathbf{C_t}}{\mathbf{C_0} + \mathbf{C_a}} \times 100$$

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.

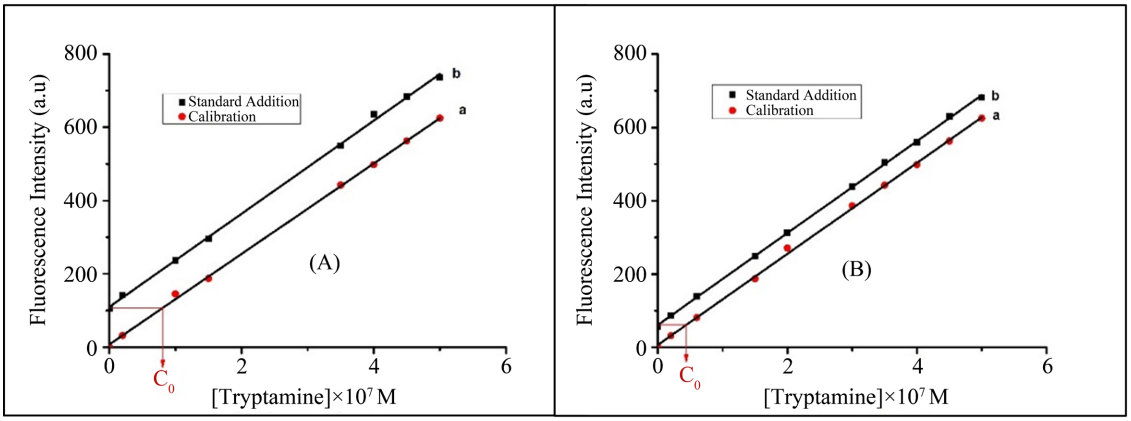

Figure 7. Determination of C_0 from the calibration curves (a) and standard addition (b) of tryptamine in the extract: (A) sardines in vegetable oil and (B) canned white tuna.

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 in vegetable oil	0	12.90	-	94.35 - 107.14	0.8
	3.20	17.25	107.14		
	16.02	30.11	104.11		
	24.03	37.76	102.25		
	56.08	70.63	102.39		
	64.09	81.94	106.43		
	72.10	88.02	94.35		
	80.11	94.80	101.92		
White tuna in water	0	6.98	-	100.46 - 104.04	1.42
	3.20	10.29	101.08		
	9.60	17.25	104.04		
	24.03	31.34	101.06		
	32.04	39.67	101.66		
	48.07	56.02	101.76		
	56.08	64.36	102.06		
	64.09	71.67	100.84		
	72.10	80.55	101.86		
	80.11	87.49	100.46		

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

In all the samples, satisfactory recovery percentages, ranging from 94.35% to 107.14%, were found (**Table 2**). These values, close to 100%, demonstrated the effectiveness of the extraction method. Similarly, the very low relative standard deviations (RSD), ranging from 0.8% to 1.42%, show the good reproducibility of the measurements. The values obtained were therefore in accordance with international standards for analytical method validation [47].

From the standard addition curves, the mass concentration (C_0) of tryptamine in each fish sample was determined. The determination of C_0 allowed us to calculate the mass percentage of tryptamine in the different canned fish samples. Taking dilution effects into account, knowing the concentration C_0 enables us to determine the mass rates of tryptamine in milligrams per kilogram of fish (mg/kg) for each sample studied. For both types of samples, the mass rates of tryptamine were obtained as follows: 86.0 mg/kg for canned sardines in vegetable oil and 39.88 mg/kg for canned white tuna. These values show that for every kilogram of sardines in vegetable oil consumed, 86.0 mg of pure tryptamine was ingested. Other researchers have conducted work on the determination of tryptamine in fish using HPLC. For example, Bilgin *et al.* [48] detected tryptamine in about 42.85% of all their study samples, with values ranging from ND to 190.61 mg/Kg. Furthermore, Zhai *et al.* [49] reported that tryptamine was detected in the range of 10.12 to 20.15 mg/kg in fish samples. High levels of tryptamine may be linked to the use of low-quality raw fish. Köse *et al.* [50] reported that tryptamine was detected in marinated anchovies at 55.8 mg/kg. In their study, tryptamine levels were generally higher than 20 mg/kg, especially in marinated anchovies. Some technological processes, such as marination, fermentation and salting, can increase the possibility of biogenic amine formation. A low pH of 4.0 to 5.5, which can be reached in salted anchovies, is favorable to increased activity of amino acid decarboxylase [51].

3.6. Study of Tryptamine Interference with Other Biogenic Amines

In this study, we focused on biogenic amines with primary amine groups such as histamine (HIS), spermidine (SPD), cadaverine (CAD), putrescine (PUT), dopamine (DOPA), tyramine (TYR), and agmatine (AGM). These amines are present in many foods (plant products and fish) [52] [53] and could interfere with tryptamine within our studied wavelength range. We studied the interference effects of these biogenic amines, which are likely to be present in food along with the biogenic amine of interest. A fixed concentration of 10^{-7} M (0.0160 µg/mL) of tryptamine was used in the study (**Figure 8**). To evaluate the interference effects, we progressively added concentrations of biogenic amines that may interfere with tryptamine. The influence of dopamine (DOPA), cadaverine (CAD), spermine (SPM), spermidine (SPD), agmatine (AGM), putrescine (PUT), histamine (HIS) and tyramine (TYR) on the spectra and fluorescence intensities of tryptamine was examined.

The addition of these amines did not alter the shape of the spectra or shift the maximum emission wavelength. However, a decrease in fluorescence signal was

observed for dopamine (DOPA), cadaverine (CAD), spermine (SPM), spermidine (SPD), putrescine (PUT), histamine (HIS), and tyramine (TYR), whereas agmatine (AGM) led to an increase in fluorescence intensity at the added concentrations. We summarized the tolerance limit (LT) values and the mass percentage of each interfering species in Table 3.

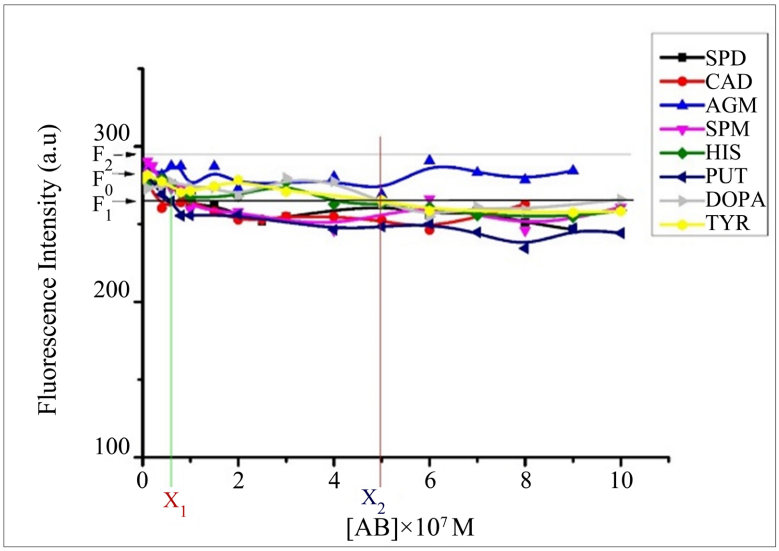

Figure 8. Effect of biogenic amines on tryptamine fluorescence intensity.

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

$$\left|\Delta F(\%)\right| = \frac{F_0 - F}{F_0} \times 100 = 5\%$$

In this expression, ΔF (%) represents the percentage variation in the tyramine signal; F_0 and F represent the fluorescence signal of tyramine in the absence and presence of interfering species, respectively. In our case, the value of F_0 is equal to 279.21 relative fluorescence intensity units.

$$\left|\Delta F(\%)\right| = \frac{F_0 - F}{F_0} \times 100 = 5\%$$

From this expression, we can deduce the limit values corresponding to this precision. Therefore, we can have two limit values, F_1 and F_2.

If $\left|\Delta F(\%)\right| \geq 0$, we write $\frac{F_0 - F_1}{F_0} \times 100 = +5\%$ we will have:

$$F_1 = \frac{95 \times F_0}{100} = 265.25.$$

If $\left|\Delta F(\%)\right| \leq 0$, we write $\frac{F_0 - F_2}{F_0} \times 100 = -5\%$ we will have:

$$F_2 = \frac{105 \times F_0}{100} = 293.17.$$

Thus, concentrations that do not interfere with the emission of tryptamine will correspond to intensities between F_1 and F_2. Outside of this range, the precision of the method exceeds ± 5%; the measurement thus becomes less precise.

From the intersection of the curve F = f [AB] with the lines y = F_1 or y = F_2, we can determine the tolerance limits (TL) at X_1 or X_2, respectively. If there is no intersection, the amine does not interfere with the tryptamine assay. Table 3 summarizes the concentration ranges tested, the different tolerance limit values, and the corresponding mass rates at the tolerance limits for each interfering species relative to tryptamine.

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) (µg/mL)	Mass rates (%)
Tyramine	0.0013718 - 0.13718	0.0686	428.75
Dopamine	0.001896 - 0.1896	0.09486	592.875
Histamine	0.00184 - 0.18407	0.0737	460.625
Cadavérine	0.001751 - 0.1400	0.00633	39.56
Putrescine	0.00161 - 0.16107	0.009659	60.37
spermidine	0.002546 - 0.22916	0.0244	152.5
Agmatine	0.00228 - 0.2054	∞	∞
Spermine	0.002023 - 0.2023	0.0194	121.25

Fixed concentration = $[TRYP]_0$ = 0.0160 µg/mL; ∞ : non-interfering; Mass rates = $\frac{[AB]}{[TRYP]_0} \times 100$.

On the one hand, Table 3 shows that dopamine, histamine, and tyramine are the amines that interfere the least with the analysis of tryptamine. No interference was noted in the quantification of tryptamine when the sample also contained agmatine.

On the other hand, the table reveals that cadaverine and putrescine interfere much more with the determination of tryptamine, followed by spermine and spermidine. Nevertheless, it is possible to measure tryptamine with an accuracy of ±5% in a sample containing cadaverine, as long as the mass of cadaverine does not exceed 40% of that of tryptamine. Similarly, for tryptamine quantification with an accuracy of ±5% in a sample containing putrescine, the mass of putrescine must not exceed 60% of that of tryptamine. In a sample containing spermine or spermidine, tryptamine can be quantified with the same precision even at much higher concentrations (121% for spermine and 153% for spermidine). Tyramine interferes at a mass percentage of 429%, histamine at 461%, and dopamine at 593%.

4. Conclusions

In this study, we developed a spectrofluorimetric method for determining tryptamine in fish products. The low detection and quantification limits found, in the ng/mL range, indicated the high sensitivity and precision of this method. Similarly, the low values of relative standard deviations (RSD) demonstrated the good reproducibility of the measurements. The method provided very satisfactory recovery percentages (94.35 to 107.14%) for the analysis of tryptamine in fish. The study of interference effects also showed that certain biogenic amines may interfere with tryptamine. In our case, no significant interference effect was observed in determining the tryptamine content in the two types of samples studied. Indeed, a close parallelism was obtained between the standard addition lines and the calibration lines. This parallelism indicated the absence of marked interference effects. These results thus demonstrated the effectiveness of this new analytical method.

Therefore, this simple, sensitive, precise and cost-effective method could be proposed for the analysis of tryptamine in food products.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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