

NEUROCHEMICAL NEXUS: AGMATINE'S CRUCIAL CONTRIBUTION IN BIOGENIC AMINES PHYSIOLOGY AND TOXICITY

Fatoumata Ndiaye Camara

Faculté des Sciences et Techniques, Université Cheikh Anta Diop, BP 5005 Dakar- Sénégal.

Abstract

Biogenic amines, nitrogen compounds derived from amino acids and protein-rich foods, are present in animals and some plants. At low concentrations, they serve vital physiological roles as neurotransmitters in vertebrates, while high levels can lead to toxicity with varying symptoms depending on the specific biogenic amine. Polyamines like histamine, cadaverine, putrescine, and tyramine can be found in various foods and dairy products, their concentrations influenced by factors such as maturation, storage duration, pH, temperature, and salt content. Monitoring and understanding biogenic amine levels in different contexts, from cancer diagnosis to food safety, is critical. This abstract provides insights into the multifaceted nature of biogenic amines and their importance in both health and food quality.

Keywords: Biogenic amines, Polyamines, Toxicity, Cancer diagnosis, Food safety

1. Introduction

Biogenic amines are nitrogen compounds found in animals and in some plants. They usually result from the enzymatic degradation of amino acids or of protein-rich foods (Ladero, Calles-Enríquez, Fernández, and Alvarez, 2010; Lonvaud-Funel, 2001). At low doses, biogenic amines have important physiological functions as neurotransmitters in vertebrates. Similarly, to facilitate the diagnosis of tumors and the monitoring of the treatment of cancer, measurements of polyamine levels in tumor tissue, blood and urine are increasingly used (Hougaard and Larsson 1982). At high concentrations, however, biogenic amines become toxic (Brink, Damink, Joosten, and Huis in't Veld, 1990), and symptoms vary according to the various biogenic amines. For instance, histamine can cause headaches, redness and hypotension, while tyramine causes salivation and lacrimation (Shalaby, 1996). In addition, the presence of cadaverine, putrescine and tyramine in foods may have a significant synergistic effect with an increase in histamine levels, thus causing acute toxicity (Santos 1996; Shalaby 1996). Biogenic amines are also found in dairy products. Their concentration depends, however, on several parameters: maturation, duration of storage, pH, temperature and salt (NaCl) content (Linares et al., 2012).

In most cases, agmatine (Figure 1) is synthesized in living organisms under the action of an enzyme called arginine decarboxylase (ADC). Its presence in foods such as meat, fish and cheese is a chemical indicator of hygienic quality (Yamanaka, Shiomi, and Kikuchi, 1987). In addition, agmatine performs a wide range of activities related to the nervous system functions, including interactions with

membranereceptors such as nicotine, N-methyl-D-aspartate (NMDA), 2-adrenergic and intracellular imidazoline (Gilad and Gilad, 2000; Li, Regunathan, and Reis, 1995; Reis and Regunathan, 2000). Indeed, agmatine acts as a potential neurotransmitter in the brain (Halaris and Plietz, 2007).

It is also a regulator of polyamine levels (Isome et al., 2007) and a precursor of putrescine under the action of bacteria (Alberto, Arena and Nadra, 2007; Landette, Arena, Pardo, De Nadra, and Ferrer, 2008). The presence of agmatine in the human body is of great interest. In fact, agmatine reduces the accumulation of collagen in diabetic patients (Marx, Trittenwein, Aufrich, Hoeger, and Lubec, 1995), plays a protective role against depression in the mouse (Mohseni et al., 20017; Neis et al., 2015), regulates the growth of epithelial cells during the healing of wounds (Gilad and Gilad, 2000) and, lastly, it increases muscle growth while improving physical condition. It is also used in the treatment of autism spectrum disorders in rats (Kim et al., 2017). For these reasons, agmatine is used as a dietary supplement (Gilad and Gilad, 2014). Keynan, Mirovsky, Dekel, Gilad, and Gilad. (2010) have shown, however, that the administration of a significant dose of agmatine can cause diarrhea and nausea. Yet, Gilad and Gilad (2014) observed no adverse reaction after having administered a dose of 2.67 g of agmatine sulphate to 2 patients six times a day for 5 years. Consequently, this experience shows that the toxicity threshold of agmatine is not well defined at present. In general, analysis methods used to determine the rate of agmatine are: high performance liquid chromatography (HPLC), gas chromatography (GC), electrochemical method, and enzymatic method (Chen, Turecki, and Mamer, 2010; Custodio, Tavares, and Gloria, 2007; Hajós, Sass-Kiss, Szerdahelyi, and Bardocz, 2000). These different methods usually require rather heavy equipment, highly qualified staff, and a time-consuming implementation. Among them, fluorimetric detection HPLC is the most widely used. Agmatine is not fluorescent. Its structure, however, contains primary and secondary amines. This is why various types of markers can be used to form fluorescent complexes with agmatin. To be mentioned among these markers are: Orthophthalaldehyde (OPA) (Figure 1), benzoyl chloride (ClB), 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBDF), 2,3-naphthalenedialdehyde (NDA), diethyl ethoxymethylenemalonate (DEEMM) and succinimidylferrocenyl propionate (PSF) (Dalluge, McCurtain, Gilbertsen, Kalstabakken, and Williams, 2015; Fairbanks et al., 2000; Loret, Deloyer, and Dandrifosse, 2005; Nishikawa, Tabata, and Kitani, 2012; Özdestan and Üren, 2010; Wang, Ye, Zhu, Wu, and Duan., 2014). In most cases, complex formation reactions between markers and agmatine are slow. Therefore, fluctuations obtained with HPLC make it difficult to obtain accurate results.

In this study, a method for spectrofluorimetric analysis of agmatine is optimized. After determining the stoichiometry of the OPA-AGM complex, stirring and temperature effects on the fluorescence intensities of the complex were optimized. Such optimization allowed us to achieve quite satisfactory analytical performances. This method was then applied to the determination of the rate of agmatine in shrimps, fishery products intended for consumption and export.

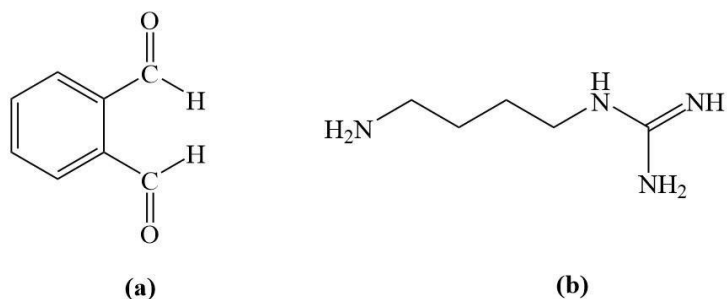


Figure 1: Molecular structures of orthophthalaldehyde (a) and agmatine (b) 2. Experimental

2.1 Material

Fluorimetric analyses were performed using a Varian Cary Eclipse spectrofluorometer by setting the voltage to 650 volts and the slot to 5 nm. To determine fluorescence spectra, a five-sided polished quartz cell (1 cm optical path, 3.5 mL inside volume) was used. Weighings were carried out using a Sartorius AG Gottingen precision scale (Type BA 110S-OF1), with an accuracy of 0.1 mg. Pipettes, micropipettes, flasks and beakers were used to prepare the solutions. The use of a scientific SL16R centrifuge was necessary for the extraction and separation of the solid and solvent phases. Also necessary was the use of a Consort C6010 pH-meter. The different software packages used were: WinUV for recording fluorescence spectra, OriginPro 8.5 for data processing, and Chemdraw Ultra 8.0 for the representation of molecules.

2.2 Products and solvents

The products used were: agmatine sulfate (97%), orthophthalaldehyde (97%), cadaverine dihydrochloride (99%), dopamine (100%), histamine dihydrochloride (99%), putrescine dihydrochloride (98), serotonin hydrochloride (99%), spermidine trihydrochloride (99%), tryptamine (98%), tyramine (99%), sodium hydroxide, chloridic acid (37%), trichloroacetate acid (99%), NaCl, Na₃PO₄, KI, CaCl₂, and FeCl₂. Also used were some solvents such as demineralized water, methanol (MeOH), acetonitrile (ACN) and N, N-dimethylformaldehyde (DMF). All reagents were of analytical quality and purchased from Sigma-Aldrich.

2.3 Methods

2.3.1 Preparation of solutions

Fresh stock solutions of agmatine and OPA (10⁻² M) were prepared in 25 mL-flasks in aqueous medium. Daughter solutions at desired concentrations were prepared from the stock solutions. All solutions were protected from light with aluminium foil and stored in a refrigerator.

2.3.2 Preparation of the extract

500g of common prawn (*Palaemon serratus*) and 500 g of giant prawn (*Panaeus monodon*) purchased at La Halle de Dunkerque, a fish shop, and already-eviscerated and dried prawn (*Palaemon serratus*) from Senegal, were used to determine the quantity of agmatine in these different species. The solid phase extraction (SPE) process was used to determine agmatine in these shrimp species (Ozyurt, Kuley, Ozkutuk, and Ozogul, 2009). After evisceration and crushing of the shrimps, 2 g thereof were homogenized with a magnetic stirrer for 10 minutes in 10 mL of 6% TCA. The mixture was then

centrifuged at 5000 rpm for 20 minutes at 4°C and filtered with Whatman filter paper. Lastly, this filtrate was protected by aluminium foil and stored in a refrigerator at 278 K until analysis. For the analysis of the extract, 10 µl of the filtrate and 50 µl of OPA (10⁻² M) were mixed in a 5 mL vial. Then, this mixture was supplemented with an NaOH solution to obtain a pH equal to 13.

2.3.3 Calculation of the mass rate of agmatine in shrimp

The shrimp extracts were analyzed by diluting 10 µl of the extract in 5 mL of demineralized water. Co concentrations (ng/mL) of agmatine in this solution were determined from the standard addition lines. Since we know Co, we can deduce the mass (m) of pure agmatine contained in the 2 g (mt) of crushed shrimp. Thus, for a mass (mt) of shrimp, the mass rate (τ) can be (τ) written: $\tau (\%) = \frac{m}{m_t} \times 100$.

In this relation, m and mt are expressed in grams (g). This relation may still be written: $\tau (\%) = \frac{510^{-4}}{m_t} C_0$ (Equation 1)

$$4 \quad m_t = 2 \text{ g} \Rightarrow \tau (\%) = \frac{510^{-4}}{2} C_0$$

In our case: with Co expressed in ng/mL

Thus, the mass of pure agmatine consumed per kg of shrimp (m) can be written:

$$m_j = 10 \cdot \tau (\%) \quad (\text{Equation 2})$$

3. Results and discussion

3.1 Optimization of analytical parameters

3.1.1 3D fluorescence spectrum of the OPA-AGM complex

To determine excitation (λex) and emission (λem) wavelengths of the complex, we mixed two equimolar solutions of OPA and AGM (4.10⁻⁶ M) in alkaline medium (pH 13). In fact, the work Nedeljko, Turel, and Lobnika (2015) has shown a high fluorescence intensity of the complex in basic medium. For this reason, experiments were performed in basic pH 13 medium. Scanning in the UV-visible wavelength range allowed us to obtain the 3D fluorescence spectrum of the OPA-AGM complex (Figure 2). This figure shows two excitation wavelength maxima at 230 nm and 333 nm, and a single emission band whose maximum is around 473 nm (Figure 2).

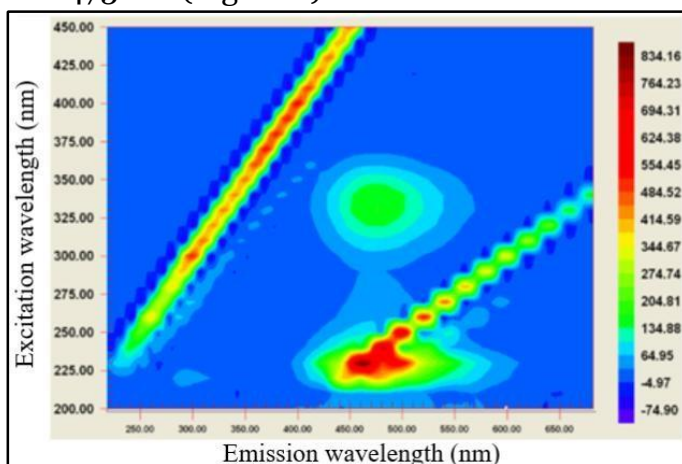


Figure 2: 3D Fluorescence spectra of OPA-AGM complex in pH 13 water

$$[\text{OPA-AGM}] = 4 \cdot 10^{-6} \text{ M}$$

3.1.2 Stoichiometry of OPA-AGM complex

In order to study the stoichiometry of the derivation reaction between OPA and agmatine, the limiting reagent method (successive additions) was applied. This method consists in fixing agmatine concentration at $8 \cdot 10^{-6} \text{ M}$ and changing OPA concentration, between 10^{-6} and 10^{-4} M . The variation of fluorescence intensity of the complex versus the OPA concentrations gives two intersecting straight lines. The first straight line with steeper slope corresponds to the formation of a complex (Figure 3). At the end of the reaction, any addition of OPA corresponds to the other line, with exaltation of the fluorescence signal. The stoichiometry of the complex is determined from the intersection point of the two lines. At this intersection point, the number of OPA moles poured was exactly equal to the number of initial AGM moles. Thus, the stoichiometry of the complex between OPA and AGM is of a 1:1 type.

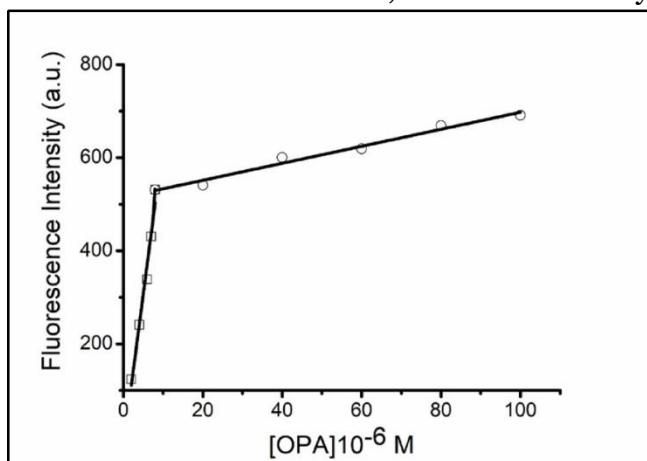


Figure 3: Variation of the fluorescence intensity of the complex according to OPA concentration $[\text{AGM}] = 8 \cdot 10^{-6} \text{ M}$, $\lambda_{\text{ex}} = 333 \text{ nm}$, $\lambda_{\text{em}} = 473 \text{ nm}$

3.1.3 Effect of stirring on the fluorescence intensity of the complex

The formation of the OPA-agmatine complex is a slow process. Thus, the effect of stirring on the formation kinetics of this complex was studied. Figure 4 shows that the stirring time plays a fairly important role on the signal of the complex. Indeed, there is a clear exaltation of the fluorescence signal under the effect of stirring. For the rest of the work, 20 minutes were chosen as optimal time for stirring before any measurement.

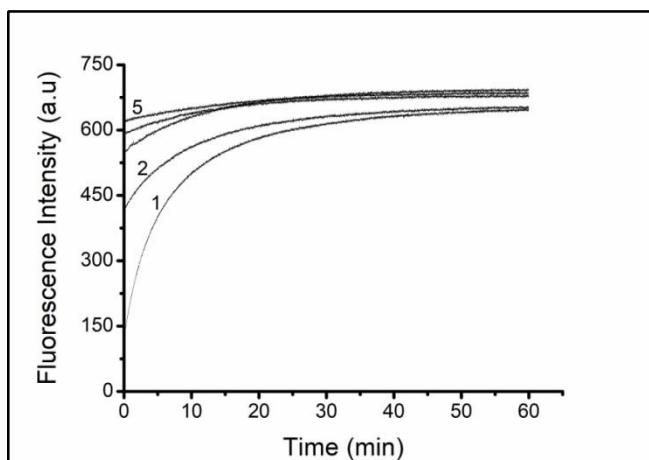


Figure 4: Formation kinetics of OPA-AGM complex in water:

(1) without stirring, (2) 5 minutes, (5) 20 minutes stirring, $[OPA - AGM] = 8 \cdot 10^{-6} M$ $\lambda_{ex} = 333 nm$, $\lambda_{em} = 473 nm$

3.1.4 Effect of temperature on the kinetics of OPA-AGM complex formation

Figure 5 shows that the formation kinetics of the complex is stable for all temperatures after stirring about 20 minutes. The intensity of fluorescence decreases slightly, however, as the temperature increases. This is due either to an increase in the non-radiative transition as a function of temperature (Basavaraja, Inamdar, and Kumar, 2017), or to a deformation of chromophores at high temperatures, which can destabilize the structure of the complex (Vernotte and Moya, 1973).

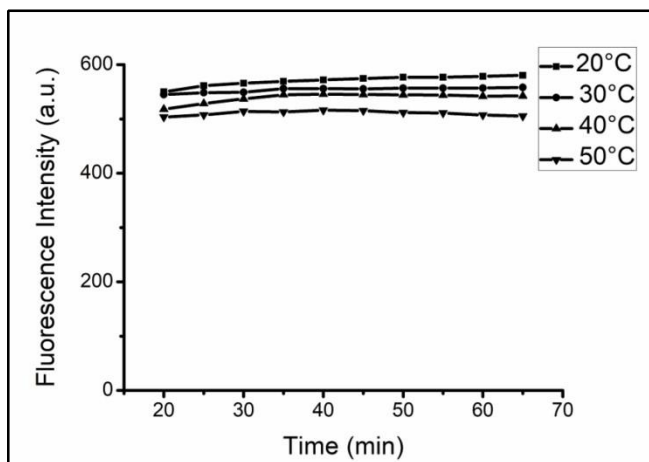


Figure 5: Effect of temperature on the kinetics of OPA-AGM complex formation ($8 \cdot 10^{-6} M$)

$[OPA - AGM] = 8 \cdot 10^{-6} M$ $\lambda_{ex} = 333 nm$, $\lambda_{em} = 473 nm$

3.2 Analytical performances

To assess the interest of the method proposed, analytical performances were determined under optimal conditions in water and various organic solvents. Linear correlations were noted in all solvents, with coefficients ranging between 0.9991 and 0.9992. These correlation coefficients close to unity indicate the good accuracy of our measurements. The relative standard deviation (RSD), ranging between 0.1

and 1.5 indicates the good replicability of this method. The detection limit (DL), between 0.36 and 2.52 ng/mL, and quantification limit (QL); between 1.62 and 8.40 ng/mL, obtained with this method are among the lowest experimental values found in literature (Nedeljko, Turel, and Lobnika, 2015; Gómez-Alonso, Hermosin-Gutiérrez, and Garcia-Romero, 2007; Triki, Jimenez-Colmenero, Herrero, and Ruiz-Capillas, 2012; Smit, Du Toi, Stander, and Du Toi, 2013). All results are grouped in Table 1.

These values are lower in aprotic organic solvents (DFM and ACN) than in methanol and water. This difference may be due to a possible dehydration of the OPA in polar environment (Isogai, Isumaki, and Eguchi, 2012).

Thus, these experimental data show that this method is highly advisable for analysis of the matrix containing agmatine, even at trace levels.

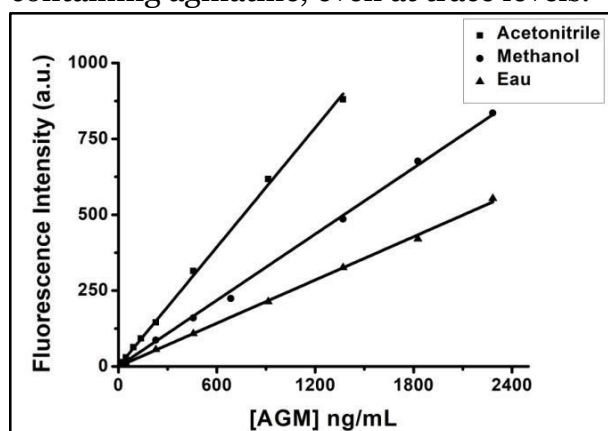


Figure 6: Calibration curve in acetonitrile, water, and methanol
($\lambda_{ex} = 333 \text{ nm}$, $\lambda_{em} = 473 \text{ nm}$)

Table 2: Analytical parameters in aqueous and organic solvents

Solvents	$\lambda_{ex}/\lambda_{em}^a$ (nm)	r^2^b	DL ^c (ng/mL)	QL ^d (ng/mL)	RSD ^e (%)
Water (pH 13)	333/473	0.9992	2.52	8.40	0.1
		0.9991	0.49	1.62	1.5
	342/470	0.9992	0.36	1.20	1.4
	336/463	0.9992	0.21	0.71	0.5

Methanol 333/465

DFM

Acetonitrile

A Excitation (λ_{ex}) and emission wavelengths (λ_{em}), b Correlation coefficient, c Detection Limit, d Quantification Limit, e Relative standard deviation

3.3 Application on shrimp

3.3.1 Detection of agmatine in shrimp

Agmatine was detected in shrimp extracts by comparing excitation and emission spectra of the standard solution with those of the extracts (dried and fresh prawn, and giant prawn) under the same conditions (pH 13). Figure 7 shows a quasi-superimposition of both the excitation and the emission spectra. This substantiates the presence of agmatine in shrimp.

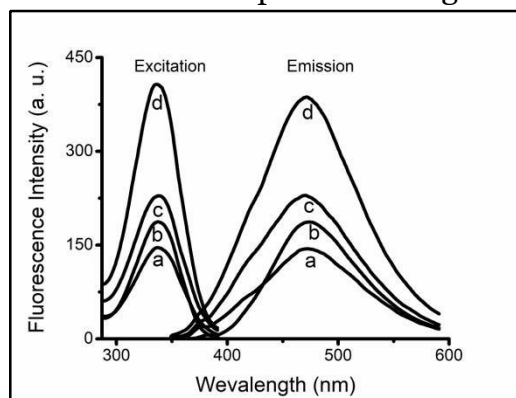


Figure 7: Fluorescence spectra of the OPA-AGM complex: (a) fresh prawn; (b) standard solution; (c) giant prawn; (d) dried prawn

3.3.2 Quantitative analysis of agmatine in shrimp

To determine the amount of agmatine in these shrimp samples, standard addition curves were established in all three cases (dried and fresh prawns, and giant prawns). All these curves are parallel to the calibration curve of agmatine (Figure 8). This close parallelism shows that the matrix effect is quite insignificant in all our measurements. From these curves, the recovery percentage (R%) was determined according to the following relationship (Traoré et al., 2017):

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

In this relationship, C_t represents the concentration of agmatine found, C_a the added concentration and C_0 the blank concentration.

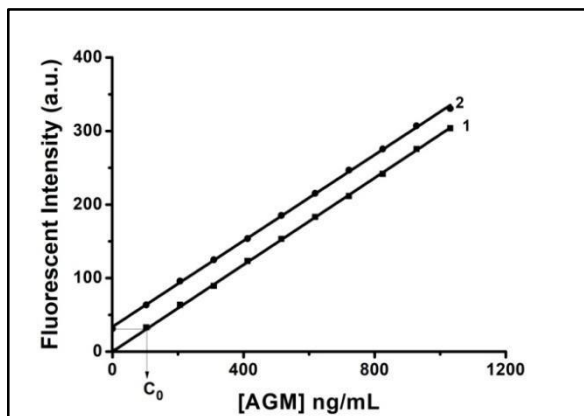


Figure 8: Calibration curves of agmatine in water (1) and standard addition corresponding to the fresh prawn (2) ($\lambda_{ex}=333$ nm, $\lambda_{em}=473$ nm).

In all samples, satisfactory recovery percentages, between 96.3 and 103.4%, were found (Table 2). These values close to 100% show the efficiency of the extraction method. Similarly, the very low relative standard deviations (RSD), ranging between 0.2 and 0.5%, show a high potential for replicability of measurements. The values found are thus consistent with international standards for the validation of analytical methods.

From standard addition curves, the mass concentration (C_0) of agmatine contained in each species of shrimp was determined. Determination of C_0 allowed us to calculate the mass percentage of agmatine contained in the different samples of shrimp based on Equation 1. Using equation 2, calculations show that for a kilogram (kg) of shrimp consumed, a quantity of 0.26 g of agmatine is taken in by fresh prawn, 0.95 g by dried prawn, and 0.41 g by fresh prawn. These results show that the quantity of agmatine found in these shrimps is lower than that used by Keynan, Mirovsky, Dekel, Gilad, and Gilad(2010) in testing the secondary effects of agmatine in the human body. Accordingly, when they are well preserved, these shrimp species may be consumed on a large scale without fear of the secondary effects of agmatine.

Table 2: Evaluation of recovery values in shrimp by solid-phase extraction procedure (SPE)

Type of sample	Added (Ca) (ng/mL)	Found (Ct) (ng/mL)	Recovery (R%)	Interval Recovery (%)	RSD (%)
Fresh prawn	0	104.0	-	99.8-103.4	0.2
	103.1	206.2	99.6		
	206.2	320.9	103.4		
	309.3	424.7	102.7		
	412.4	515.5	99.8		
	515.5	618.5	99.8		
	618.6	732.9	101.4		

Dried prawn	0	363.3	-		
	103.1	472.5	101.3		
	206.2	558.8	98.1	96.3	
	309.3	647.8	98.4	98.2	96.3-101.3
	412.4	763.6	99.6		0.5
	515.5	862.8			
	618.6	978.5			
Raw giant prawn	0	168.2	-		
	103.1	275.0	101.4		
	206.1	379.9	98.0	98.4	
	309.3	571.7	97.8	97.8-101.4	0.5
	412.4	671.0	100.2		
	515.5	796.6	99.9		
	618.6	889.2			

3.4 Interference of added alien species

3.4.1 Biogenic amines

Several studies have shown the presence of biogenic amines in shrimp (cadaverine, histamine, putrescine, spermidine, and tyramine) (R. A. Benner, Staruszkiewicz, and Otwell, 2004; López-Caballero, Gonçalves, and Nunes, 2002; Saaid et al., 2009; Salazar, Smith, and Harris, 2000; Thaw, Aung, Myint, and Bisswanger, 2004). Their simultaneous presence in the matrix of shrimp may thus cause interference effects during the analysis of agmatine by spectrofluorimetric method. This is why their effect on the determination of agmatine was studied. Since most biogenic amines yield fluorescent complexes with OPA, a large quantity of this marker (10^{-4} M) was used, whereas that of agmatine was set at 4.10^{-6} M. Concentrations of each potential interfering amine ranged between 4.10^{-7} M and 10^{-5} M. The effect of variable concentrations of each potential interfering species on the fluorescence signal of the OPAAGM complex was tested. From this signal change, the tolerance limit for each amine added was determined. This tolerance limit of alien interfering species was defined as the concentration limit of these species for which the percentage of signal change of the complex did not exceed $\pm 5\%$. For each concentration of interfering species, we calculated the percentage of signal change using the following expression:

$$\Delta F (\%) = [(F_0 - F)/F_0] \times 100.$$

In this expression, $\Delta F (\%)$ represents the percentage of the signal change of the complex; F_0 and F indicate the fluorescence signal of the complex in the absence and in the presence of interfering species, respectively.

All our results are grouped in Table 3. Thus, these results show that the presence of one of these amines in a sample causes more or less important interferences in the determination of agmatine.

Among all these amines, histamine (HIST), and cadaverine (CAD) are the most interfering factors in the dosage of agmatine. This high interference of histamine and agmatine was predictable because each of them forms a very fluorescent complex with OPA in alkaline medium (Douabalé, Dione, Coly, and Tine, 2003). In addition in such medium, there is considerable overlap between the excitation and

noted for NaCl, with a tolerance limit of 0.0073 µg/mL. Yet, NaCl is used on a large scale in the preservation of shrimp (Einarsson, and Lauzon, 1995; Gonçalves and Ribeiro, 2009). It is therefore important to take into account the presence of NaCl in the analysis of agmatine, at least for preserved shrimp. That is why it is recommended to soak the shrimp samples in demineralized water before any extraction when measurements are to be made by spectrofluorimetry.

Table 4: Tolerance limit of different salts with agmatine

Alien species	Tested concentration range (µg/mL)	Tolerance limit (µg/mL)	
Salts a			
FeCl ₂ (Fe ²⁺ , 2Cl ⁻)	0.0027-7.762	5.058	
MgSO ₄ (Mg ²⁺ , SO ₄ ²⁻)	0.0024 ^{12.42} -7.872	0.405	∞
NaCl (Na ⁺ , Cl ⁻)	0.00058 ^{2.668}	0.0073	
Na ₃ PO ₄ (3Na ⁺ , PO ₄ ³⁻)	0.0038 ^{7.636}		∞
KI (K ⁺ , I ⁻)	0.0 ^{16-7.636}		∞

a Concentration set = 1.83 µg/mL for agmatine; ∞ non-interfering

These results of interference show that some biogenic amines and some salts interfere in the dosage of agmatine. For the samples processed, however, no significant interference has been noted, since the standard calibration straight lines are completely parallel to the calibration line. This is confirmed by the recovery rates obtained, between 96.3 and 103.4%, in all three types of samples. In fact, these samples have not been altered. Therefore, the levels of cadaverine and histamine were not too high to influence the results of the analysis.

4. Conclusion

In this study, a simple, sensitive, accurate and inexpensive spectrofluorimetry-based method for the determination of agmatine was optimized. The low limits of detection and quantification found indicate the high level of sensitivity and accuracy of this method. Similarly, the small relative standard deviations found show the easy replicability of measurements. This method allowed to obtain very satisfactory recovery percentages for the analysis of agmatine in shrimp. The study of the interference effects also shows that some biogenic amines and some salts may interfere with agmatine. For our study, however, no significant interference effect has been observed in the determination of the rate of agmatine in the three samples of shrimp studied. Actually, a close parallelism was obtained between the straight lines of standard addition and the calibration curve. This parallelism points to the absence of interference effects. Accordingly, these results show the effectiveness of this new method of analysis. Therefore, this method could be proposed for the analysis of agmatine in food products.

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References

- Alberto, M. R., Arena M. E., and Nadra, M. C. M. (2007). Putrescine production from agmatine by *Lactobacillus hilgardii*: effect of phenolic compounds. *Journal of Food Control*, 18, 898-903.
- Basavaraja, J., Inamdar, S. R., and Kumar, H. M. S. (2017). Effect of quencher and temperature on fluorescence intensity of laser dyes: DETC and C504T. *Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy*, 170, 124-130.
- Brink, B. T., Damink, C., Joosten, J. M. L. J., and Huis in’t Veld J. H. J. (1990). Occurrence and formation of biologically active amines in foods. *Journal of Food Microbiology*, 11, 73-84.
- Chen, G. G., Turecki, G., and Mamer, O. A. (2010). A novel liquid-liquid extraction and stable isotope dilution NCIGC-MS method for quantitation of agmatine in postmortem brain cortex. *Journal of Mass Spectrometry*, 45, 560-565.
- Custodio, F. C., Tavares, E., and Gloria, M. B. A. (2007). Extraction of bioactive amines from grated parmesan cheese using acid, alkaline and organic solvents. *Journal of Food composition and analysis*, 20, 280-288.
- Dalluge, J. J., McCurtain, J. L., Gilbertsen, A. J., Kalstabakken, K. A., and Williams, B. J. (2015). Determination of agmatine using isotope dilution UPLC-tandem mass spectrometry: application to the characterization of the arginine decarboxylase pathway in *Pseudomonas aeruginosa*. *Analytical and Bioanalytical Chemistry*, 407(18), 5513-5519. Doi: 10.1007/s00216-015-8724-0.
- Dayal, J. S., Ponniah, A. G., Khan, H. I., Babu, E. P. M., Ambasankar, K., and Vasagam, K. P. K. (2013). Shrimps - a nutritional perspective. *Current Science*, 104(11), 1487-1491.
- De Carvalho, M. A., Andrade, P. F., and Corbi, F. C. A. (2013). A simple method to synthesize fluorescent modified gold nanoparticles using tryptamine as the reducing and capping agent. *Synthetic Metals*, 185, 61-65.
- Douabalé, S. E., Dione, M., Coly, A., and Tine, A. (2003). Contributions to the determination of histamine rate by measuring out the histamine-orthophthalaldehyde complex in the absorption and fluorescence. *Journal of Talanta*, 60, 581-590.

- Einarsson, H., and Lauzon, H. L. (1995) Biopreservation of Brined Shrimp (*Pandalus borealis*) by Bacteriocins from Lactic Acid Bacteria. *Applied and Environmental Microbiology*, 61 (2), 669-676.
- Fairbanks, C. A., Schreiber, K. L., Brewer, K. L., Yu, C-G., Stone, L. S., Kitto, K. F., Nguyen, H. O., Grocholski, B. M., Shoeman, D.W., Kehl, L. J., Regunathan, S., Reisi, D. J., Yezierski, R. P., and Wilcox, G. L. (2000). Agmatine reverses pain induced by inflammation, neuropathy, and spinal cord injury. *Journal of PNAS*, 97(18), 10584-10589.
- Gilad, G. M., and Gilad, V. H. (2014). Long-term (5 years), high daily dosage of dietary agmatine-evidence of safety: a case report. *Journal of Medicinal food*, 17, 1256-1259.
- Gilad, G. M., and Gilad, V. H. (2000). Agmatine, and polyaminoguanidine-bound heterocyclic compounds for neurtrauma and neurodegenerative diseases. *Journal of Neuroscience Letters*, 296, 97-100.
- Gómez-Alonso, S., Hermosin-Gutiérrez, I., and Garcia-Romero, E. (2007). Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. *Journal of Agricultural and Food Chemistry*, 55, 608-613.
- Gonçalves, A. A., and D. Ribeiro, J. L. (2009). Effects of phosphate treatment on quality of red shrimp (*Pleoticus muelleri*) processed with cryomechanical freezing. *Lebensmittel-Wissenschaft und Technologie*, 48(8), 1435-1438.
- Hajós, G., Sass-Kiss, A., Szerdahelyi, E., and Bardocz, S. (2000). Changes in biogenic amine content of tokaj grapes, wines, and aszu-wine. *Journal of Food Science*. 65, 1142-1144.
- Halaris, A., and Plietz, J. J. (2007). Agmatine Metabolic Pathway and Spectrum of Activity in Brain. *CNS Drugs*, 21, 885-900.
- Hougaard, D. M., and Larsson L. I. (1982). Polyamine cytochemistry. Use of a novel o-phthalaldehyde method for visualizing spermidine and spermine. Comparisons to the formaldehyde-fluorescamine method. *Journal of Histochemistry*, 76 (2), 247-259.
- Isogai, K., Isumaki, H., and Eguchi, D. (2012). Crystalline hydrate of ortho-phthalaldehyde, disinfecting agent and biocide containing same, and method for manufacturing ortho-phthalaldehyde. WO2012147865A1.
- Isome, M., Lortie, M. J., Murakami, Y., Parisi, E., Matsufuji, S., and Satriano J. (2007). The antiproliferative effects of agmatine correlate with the rate of cellular proliferation. *Journal of Am. Physiol Cell Physiol*, 293, 705-711.

- Keynan, O., Mirovsky, Y., Dekel, S., Gilad, V. H., and Gilad, G. M. (2010). Safety and efficacy of dietary agmatine sulfate in lumbar disc-associated radiculopathy. An open-label, dose-escalating study followed by a randomized, double-blind, placebo-controlled trial. *Pain Medicine*, 11, 356-368.
- Kim, J-W., Seung, H., Kim, K. C., Gonzales, E. L. T., Oh, H. A., Yang S. M., Ko, M. J., Han, S-H., Banerjee, S., and Shin, C. Y. (2017). Agmatine rescues autistic behaviors in the valproic acid-induced animal model of autism. *Journal of Neuropharmacology*, 113, 78-81.
- Ladero, V., Calles-Enríquez, M., Fernández, M., and Alvarez, M. A. (2010). Toxicological Effects of Dietary Biogenic Amines. *Current Nutrition & Food Sciences*, 6, 145-156.
- Landette, J. M., Arena, M. E., Pardo, I., De Nadra, M. C. M, and Ferrer, S. (2008). Comparative survey of putrescine production from agmatine deamination in different bacteria. *Journal of Food Microbiology*, 25, 882-887.
- Li, G., Regunathan, S., and Reis, D. J. (1995). Agmatine is synthesized by a mitochondrial arginine decarboxylase in rat brain. *Annals of the New York. Academy of Sciences*, 763, 325-329.
- Linares, D. M., Río, B. D., Ladero, V., Martínez, N., Fernández, M., Martín, M. C., and Álvarez, M. A. (2012). Factors influencing biogenic amines accumulation in dairy products. *Journal of Food Microbiology*, 3, 1-10.
- Lonvaud-Funel, A. (2001). Biogenic amines in wines: role of lactic acid bacteria. *Journal of Microbiology Letters*, 2001, 1999, 9-13.
- López-Caballero, M. E., Gonçalves, A., and Nunes, M. L. (2002). Effect of CO₂/O₂-containing modified atmospheres on packed deepwater pink shrimp (*Parapenaeus longirostris*), *Eur. Food Res. Technology*, 214, 192-197.
- Loret, S., Deloyer, P., and Dandrifosse, G. (2005). Levels of biogenic amines as a measure of the quality of the beer fermentation process: Data from Belgian samples. *Food Chemistry*, 89, 519-525.
- Marx, M., Trittenwein, G., Aufrich, C., Hoeger, H., and Lubec, B. (1995). Agmatine and Spermidine reduce collagen accumulation in kidneys of diabetic db/db mice. *Journal of Nephron*, 65, 155-158.
- Mohseni, G., Ostadhadi, S., Imran-Khan, M., Norouzi-Javidan, A., Zolfaghari, S., Haddadi, N-S., and Dehpour, A-R. (2017). Agmatine enhances the antidepressant-like effect of lithium in mouse forced swimming test through NMDA pathway. *Journal of Biomedicine and Pharmacotherapy*, 88, 931-938.

- Nedeljko, P., Turel, M., and Lobnika, A. (2015). Fluorescence-based determination of agmatine dietary supplements. *Journal of Analytical Letters*, 48, 1619-1628.
- Neis, V. B., Moretti, M., Manosso L. M., Lopes, M., Leal, R. B., and Rodrigues, A. L. S. (2015). Agmatine enhances antidepressant potency of MK-801 and conventional antidepressant in mice. *Journal of Pharmacology Biochemistry and Behavior*, 13, 09-14.
- Nishikawa, H., Tabata, T., and Kitani, S. (2012). Simple Detection Method of Biogenic Amines in Decomposed Fish by Intramolecular Excimer Fluorescence. *Food and Nutrition Sciences*, 3, 1020-1026
- Özdestan, Ö., and Üren, A. (2010). Biogenic amine content of kefir: a fermented dairy product. *Eur Food Res Technol*, 231, 101-107.
- Ozyurt, G., Kuley, E., Ozkutuk, S., and Ozogul, F. (2009). Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry*, 114, 505-510.
- Padovan, G. J., Leme, I. A., Fassini, P. G., Junior, N. I., and Marchini, J. S. (2014). A new O-phthaldialdehyde (OPA) solution for fluorescence HPLC amine group detection without boric acid preparation. *Journal of Chromatography Separation Techniques*, 5(3), 1-6.
- R. A. Benner, J. R., Staruszkiewicz, W. F., and Otwell, W. S. (2004). Putrescine, Cadaverine, and Indole Production by Bacteria Isolated from Wild and Aquacultured Penaeid Shrimp Stored at 0, 12, 24, and 368 C. *Journal of Food Protection*, 67, 124-133.
- Reis, D. J., and Regunathan, S. (2000). Is agmatine a novel neurotransmitter in brain? *Trends Pharmacological Science*, 21, 187-193.
- Saaïd, M., Saad, B., Ali, A. S. M., Saleh, M. I., Basheer, C., and Lee, H. K. (2009). In situ derivatization hollow fibre liquid-phase microextraction for the determination of biogenic amines in food samples. *Journal of Chromatography A*, 1216, 5165-5170.
- Salazar, M. T., Smith, T. K., and Harris, A. (2000). High-Performance Liquid Chromatographic Method for Determination of Biogenic Amines in Feedstuffs, Complete Feeds and Animal Tissues. *Journal of Agricultural Food Chemistry*, 48, 1708-1712.
- Santos M. H. S. (1996). Biogenic amines: their importance in foods. *International journal of Food Microbiology*, 29, 213-231.
- Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Journal of Food Research International*, 29, 675-690.

- Smit, A. Y., Du Toi, W. J., Stander, M., and Du Toi, M. (2013). Evaluating the influence of maceration practices on biogenic amine formation in wine. *LWT-Food Science and Technology*, 53, 297-307.
- Thaw, M. M., Aung, O., Myint, A., and Bisswanger, H. (2004). Determination of Biogenic Amines in Different Shrimp Species for Export. *Journal of the Myanmar Academy of Arts and Science*, 2, 51-66.
- Traoré, M., Kital, K., Mbaye, M., Mbaye, O. M. A., Diop, C., Camara, M. K., Cissé, L., Seye, M. D. G., Coly, A., and Tine, A. (2017). New Spectrofluorimetric Method for Determining Cadaverine Following Derivation with Orthophthalaldehyde: Application in Fish Tissue. *International Journal of Chemistry*, 9, 10-18.
- Triki, M., Jimenez-Colmenero, F., Herrero, A.M., and Ruiz-Capillas, C. (2012). Optimisation of a chromatographic procedure for determination biogenic amine concentrations in meat and meat products employing a cationexchange column with a post-column system, *Journal of Food Chemistry*, 130, 1066-1073.
- Vernotte, C., and Moya, I. (1973). Action de la température sur la durée de vie de fluorescence et le rendement de fluorescence de la c-phycocyanine en solution. *Journal of Photochemistry and Photobiology*, 17, 245-254.
- Wang, Y-Q., Ye, D-Q., Zhu, B-Q., Wu, G-F., and Duan, C-Q. (2014). Rapid HPLC analysis of amino acids and biogenic amines in wines during fermentation and evaluation of matrix effect. *Food Chemistry*, 163, 6-15.
- Yamanaka, H., Shiomi, K., and Kikuchi, T. (1987). Agmatine as a Potential Index for Freshness of Common Squid (*Todarodes pacificus*). *Journal of Food Science*, 52, 936-938.