Fast and Direct Detection of Biogenic Amines in Fish by GC-IMS Technology

Cláudia Espalha, Jorge Fernandes, Mário Diniz and Valentina Vassilenko

***Abstract*—The presence of biogenic amines in food is of great concern to the food industry as, given the potential risk to human health, there is a growing demand from consumers and control authorities to reduce the allowed limits of biogenic amines in food and beverages.**

Several methodologies, such as differential culture media, specific enzyme methods and liquid chromatography, have been developed for the detection of biogenic amine-producing bacteria and to study some of their formation pathways. Along with the development of these techniques, the separation and quantification of biogenic amines have been studied and reported. Usually, their analysis and identification in foods is performed using chromatographic techniques.

For the first time, a gas chromatography-ion mobility spectrometer (GC-IMS) device was used to detect biogenic amines without any sample preparation. Histamine, putrescine, cadaverine, tyramine, tryptamine, spermine, spermidine and phenethylamine were analyzed and identification from specific patterns was obtained. The monitoring of non-volatile amines emission from fish tissues matrices was also accomplished and the presence of some biogenic amines was detected.

***Keywords*—Biogenic Amine; Detection; Fish Spoilage; Ion Mobility Spectrometry; Gas Chromatography.**

I. Introduction

Biogenic amines (BAs) are non-volatile amines characterized by the presence of at least one amino group in their molecular structure, which can be formed and degraded during the normal metabolism of microorganisms, animals and plants. Being mainly formed through the decarboxylation of their precursor amino acids, these amines are usually present in food either naturally or as products of microbial action during food processing or storage [1][2].

The consumption of food containing high amounts of some BAs can have toxicological effects leading to several health issues due to their toxicity, making it critical to monitor these compounds in foods. Histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), tryptamine (TRP), spermine (SPM), spermidine (SPD), and phenethylamine (PEA) are considered the most important biogenic amines occurring in foods, in terms of toxicology [3][4].

C. Espalha at LIBPhys – Laboratory for Instrumentation, Biomedical Engineering and Radiation Physics, Physics Department, NOVA School of Science and Technology, NOVA University of Lisbon, 2896-516 Caparica, Portugal (e-mail: c.espalha@campus.fct.unl.pt).

J. Fernandes and V. Vassilenko at NMT – Tecnologia, Inovação e Consultoria, S.A, Madan Parque, Caparica, Portugal, and LIBPhys – Laboratory for Instrumentation, Biomedical Engineering and Radiation Physics, Department of Physics, NOVA School of Science and Technology, NOVA University of Lisbon, 2896-516 Caparica, Portugal, (e-mail: j.manuel@campus.fct.unl.pt and vv@fct.unl.pt).

M. Diniz at UCIBIO, REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University of Lisbon, 2896-516 Caparica, Portugal (e-mail: mesd@fct.unl.pt).

The most serious foodborne intoxications caused by BAs are usually related with high concentrations of histamine. Several outbreaks of histamine poisoning have occurred after ingesting different food products characterized by containing high levels of histamine such as fish and fish products, dairy products, meat and meat products, and alcoholic beverages such as wine and beer.

Tyramine, tryptamine and phenethylamine are essentially vasoactive amines, causing peripheral vasoconstriction and an increase in the cardiac output. Although much less toxic than histamine and tyramine, some BAs such as putrescine, cadaverine, spermine and spermidine, can react with nitrite to form nitrosamines and produce carcinogenic compounds [5].

During decomposition of fishery products, especially during storage at elevated temperatures, various amounts of BAs are produced, depending on the fish species. Histamine, tyramine, putrescine and cadaverine are the most common BAs found in seafood associated with spoilage, in which histamine and tyramine are the most biologically active during spoilage. Usually the concentrations of BAs increase during spoilage, as a result of microbial concentrations and deterioration of sensory quality [6].

In the last decade innovative ion mobility spectrometry (IMS) applications have emerged and have been used in quality control of toxic contaminants, beverages and food products. Among these are the assessment of food freshness or the degree of spoilage of food products and the detection of pathogenic microorganisms or toxins [7].

IMS technology has the advantage of being easily coupled with separation techniques such as gas chromatograph (GC) or multi capillary column (MCC) for pre-separation of complex sample matrices, providing compound separation and increasing selectivity. IMS is an extremely sensitive technique that can analyze a multiplicity of compounds at low concentrations with detection limits typically in the low ppb-range or even ppt-range [8].

II. Principles of Ion Mobility Spectrometry

Being used for detection, identification and monitoring of trace levels of chemical compounds in different matrices, IMS is an analytical technique of ion separation based on the differences of ion mobilities in a drift tube with a defined electric field. This separation is based on the specific drift times, that ionized compounds take to travel a fixed distance under an electric field (E) [9]. When reaching the equilibrium, the ions move with a constant drift velocity (v_d) proportional to the electric field and in the same direction. Due to the relation between these two components it is possible to extract an independent component for each ion, ion mobility K (1) [10].

$$K = \frac{v_d}{E} = \frac{L}{t_d E} = \frac{L^2}{t_d U} \quad (1)$$

Drift velocity is expressed in cm^2s^{-1} and the electric field is in Vcm^{-1}, hence the ion mobility K is expressed in $cm^2V^{-1}s^{-1}$. L is the length of the drift tube, expressed in cm, t_d the drift time expressed in s and U the drift potential difference in V.

Ion mobility depends on the collision frequency, being susceptible to both pressure and temperature. Therefore, by normalizing to the standard pressure and temperature we get the reduced ion mobility K_0 (2) [10].

$$K_0 = K\left(\frac{P}{P_0}\right)\left(\frac{T_0}{T}\right) \quad (2)$$

P and T represent the values of pressure and temperature during the experiment and in standard conditions P_0=760 Torr and T_0=273.15 K [10].

The reactant ions yield a peak in the spectrum, known as the reactant ion peak (RIP). RIP is a constant feature in IMS spectra even in the absence of any sample. As the product ions are produced, the number of available reactant ions in the reaction region decrease, expressed in the decrease of RIP intensity and consequent increase in the intensity of the analyte related peak [11].

Being the IMS coupled with GC separation, a 3-dimensional chromatogram is produced, which can be converted into a 2D representation by transforming the z-axis data into a color scale for each detected signal. The higher the detector response the more intense the corresponding detection signals [12].

The main objective of the present work was to develop an experimental method and protocol for direct analysis of volatile organic compounds (VOCs) of biological origin, more precisely BAs, by using the GC-IMS technology. As far as we know, this is the first study where 7 selected biogenic amines were detected using this novel approach.

III. Materials and Methods

A. Apparatus

All experimental analysis was performed by GC-IMS apparatus (G.A.S. GmbH, Dortmund, Germany), equipped with a β-radiation source (low-radiation tritium, ^3H source), coupled to the chromatographic column (MXT-200) with a length of 30 m. The sample inlet lets a continuous stream of air with a flow rate of 50 mL/min pass. Also, a drift gas flow rate of 150 mL/min was applied to provide good ion separation.

Several factors such as gas flow rates, headspace's volume, vapor pressure, temperature and humidity influence the separation process and thus need to be taken in consideration to achieve a better separation and maximization of sensitivity and selectivity. During all measurements both room relative humidity and room temperature of the laboratory air were maintained at 50-70% and 22-24°C respectively.

B. Standards

Histamine dihydrochloride (CAS No. 56-92-8), putrescine (CAS No. 110-60-1), cadaverine (CAS No. 462-94-2), tyramine (CAS No. 51-67-2), tryptamine (CAS No. 61-54-1), phenethylamine (CAS No. 64-04-0), spermine (CAS No. 71-44-3) and spermidine (CAS No. 124-20-9) were obtained from Sigma-Aldrich (Darmstadt, Germany). These amines were studied in their pure form (except histamine) to avoid any possible peaks appearance of unknown or undesired compounds in IMS spectra.

All amines studied were measured at room temperature (23°C) and heated to 40°C to test their volatility or volatile sub-products. Once weighed and placed in 20mL vials, the vials containing the amines were heated in an Analogic Heating Plate from VWR® during a pre-established time (10 minutes) at 40°C and then connected to the device through a needle attached to a syringe containing the headspace to analyze according to each amine (1.0 mL, 2.0 mL, 4.0 mL and 5.0 mL). Between measurements, a cleaning program was used to prevent accumulation of amine and remove any contaminants.

C. Materials

Fresh specimens of some selected fish species: atlantic bonito, atlantic horse mackerel and sardine were purchased from a fish market in Almada, Portugal. Gutting and filleting were manually performed before transportation to the laboratory. To avoid any contaminations, only sterile knives were used. The samples were then packed in polystyrene boxes with ice and transported in a thermal isolated bag to the laboratory within two hours after purchase.

Once in the laboratory, the samples were weighed to establish the weight range of all measurements. Due to the high sensitivity of the device and strong characteristic fish odor, the weight of the fish muscle samples to be analyzed should not surpass the mass unit gram, the average weight being 0.1540 g. Fish muscle samples were then placed into separate vials immediately after weighing. The vials were then sealed and placed in the laboratory exposed to room temperature for 4 days to promote degradation of fish muscles samples. Measurements were then carried out by analyzing 2ml of headspace of each vial over the four days.

IV. Results and Discussion

All spectra were recorded in the positive ion mode. The data acquisition was processed in the software Laboratory Analytical Viewer, LAV® from G.A.S., capable of providing 2D or 3D representation of spectra. In order to verify if the observed signals were characteristic of the analyzed amines, room air measurements were also performed. In the 2D spectra recorded the x-axis represents the drift time relative to the RIP or in milliseconds and the y-axis represents the retention time in seconds.

A typical representative spectrum from the performed analysis is shown in Fig. 1. Two peaks characteristic of spermidine were observed in the spectrum (yellow rectangle) at 40°C.

In the present study 12 characteristic peaks were detected for the selected biogenic amines. Using LAV software, it was possible to extract the retention and drift times, allowing the calculation of the ion mobility constant and hence characterize each amine, with a substance-specific pattern and value. The calculation of the reduced ion mobility constant follows (1) and (2).

In Table I are shown the calculated values of reduced ion mobility, as well as the respective drift time for all the detected signals for biogenic amines.

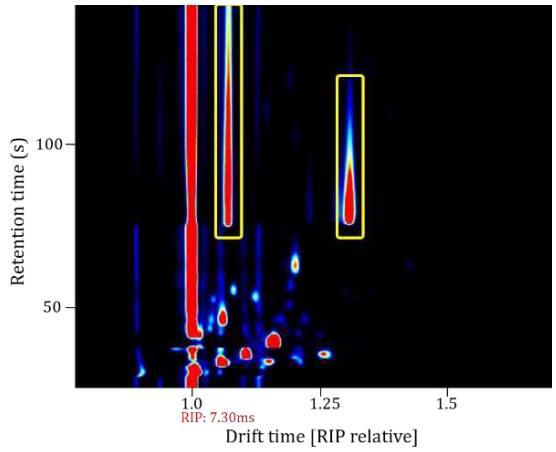


Figure 1. *Representative spectrum obtained for spermidine at 40°C.*

The presence of biogenic amines is currently the most reliable indicator of fish decomposition, due to their association with organoleptic evaluation of fish freshness. Very low molecular weight compounds such as ammonia, dimethylamine (DMA) and trimethylamine (TMA) are often present in fish tissues when spoilage takes place [13][14].

Ammonia was identified in all the fish species analyzed: atlantic bonito, atlantic horse mackerel and sardine. Mackarel presented the higher concentration of all three, which might be explained by its high spoilage rate.

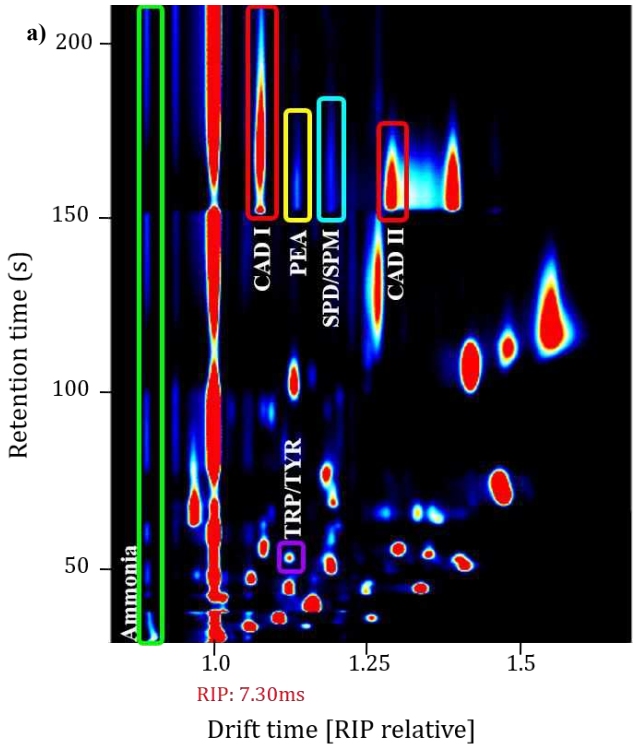
Possible traces of some biogenic amines were found in all fish species, however with both drift and retention deviations from the peaks identified for pure amine's samples. Sardine only presented traces of cadaverine, tryptamine and tyramine.

On the third day of all measurements, an unpleasant odor took place, indicating the fish decay and spoilage. Although TMA is the main responsible for the "fishy" odor, other compounds may also contribute [15]. It was then stablished that in the third day after fish purchase, the fish is considered spoiled.

Atlantic bonito presents traces of six amines in the second day of measurements and with a higher concentration than the amines identified in sardine and mackerel. (Fig. 2 a)). Fig. 2 b) represents the intensity of the detected amines for atlantic bonito over time.

TABLE I. ION MOBILITY CONSTANT FOR THE DETECTED BIOGENIC AMINES.

Biogenic Amine	*CAS No.*	*Drift time (ms)*	*K ($cm^2V^{-1}s^{-1}$)*	*K_0 ($cm^2V^{-1}s^{-1}$)*
TYR	51-67-2	8.338	2.3037	0.0138
		7.936	2.4204	0.0145
TRP	61-54-1	8.389	2.2900	0.0137
		7.051	2.7241	0.0163
SPM	71-44-3	7.771	2.4718	0.0148
		9.506	2.0206	0.0121
SPD	124-20-9	7.809	2.4597	0.0147
		9.552	2.0109	0.0120
CAD	462-94-2	8.361	2.2971	0.0137
		10.072	1.9071	0.0114
PUT	110-60-1	7.706	2.3037	0.0138
PEA	64-04-0	7.804	2.4613	0.0147



b) Intensity evolution of the detected amines over time

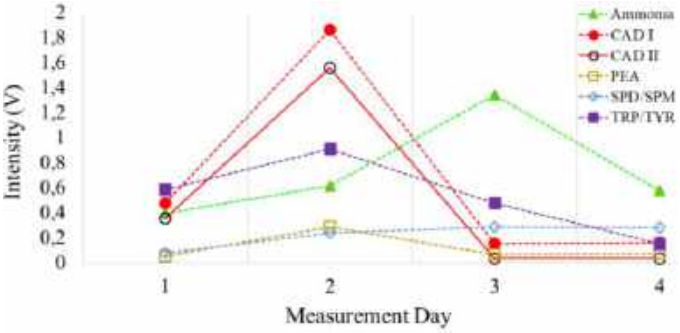


Figure 2. a) *Representative spectrum obtained for atlantic bonito at the second day of measurements, at room temperature (23°C). CAD I and CAD II correspond to cadaverine monomer and dimer respectively.*
b) *Representative graphic of the intensity of the detected amines for atlantic bonito over time, at room temperature (23°C).*

In the first day of fish measurements (Fig. 3), it is possible to verify a correlation between all three fish species regarding the signals from the emitted VOCs. Compounds concentrations may differ due to sample weight differences and distinct fish matrix according to each species. The latter may also explain the deviation of retention times of the peaks.

V. CONCLUSIONS

For the first time, the VOC patterns of seven of the most important biogenic amines in food were successfully obtained by GC-IMS. A developed protocol allows to create a GC-IMS library for seven amines from the chemical standards analysis and its further direct determination in fish tissues.

Being responsible for the most serious foodborne intoxications, histamine represents the most important biogenic amine. However, histamine analysis did not produce satisfactory results most likely due to the low amounts of volatile metabolites.

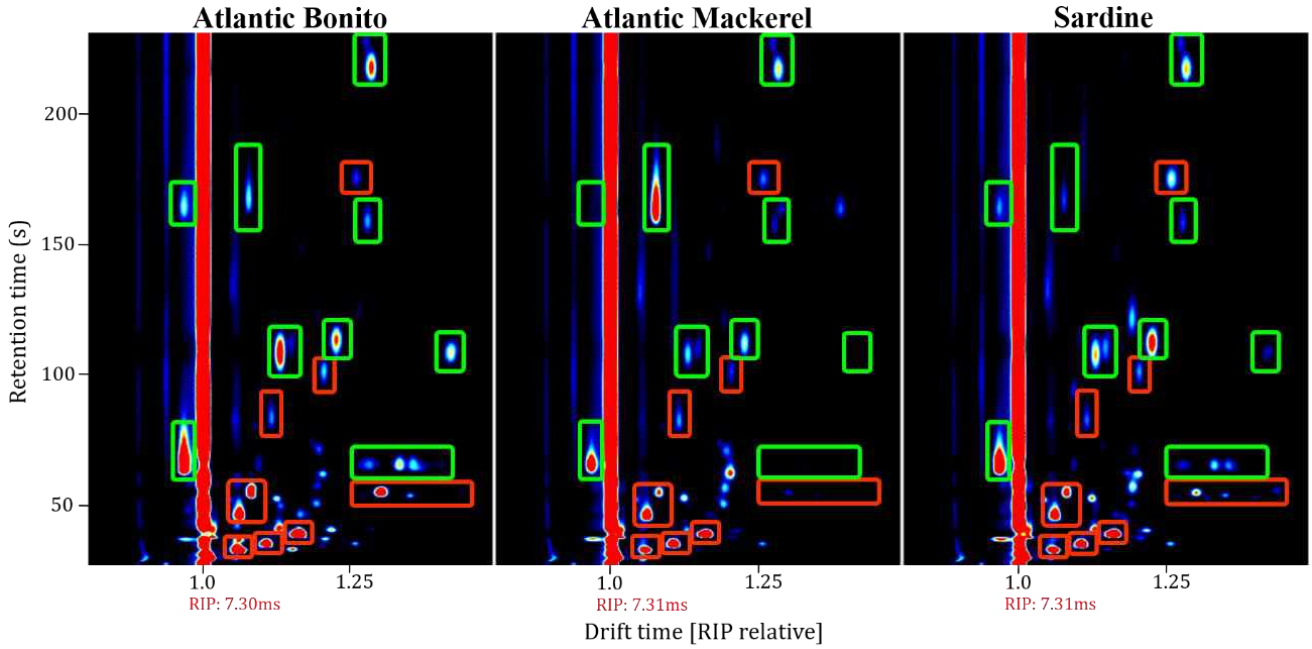


Figure 3. *Representative spectra obtained on the first day of measurements for the three species at room temperature (23°C). The signals of room air are identified by orange rectangles, while fish characteristic compounds are within green rectangles.*

The GC-IMS technique has proven to have a high sensitivity for short analysis time with an exceptional repeatability, being ideal for monitoring food freshness and decomposition, and thus providing an alternative to other monitoring devices such as electronic noses.

Future research in this field will include the analysis of higher histamine concentrations, as well as the testing different heating temperatures for GC-IMS direct analyses. It is also foreseen to perform a quantitative analysis for selected biogenic amines.

ACKNOWLEDGMENT

This work was inserted in the project "3Qs for quality - Development of new devices and techniques for seafood quality assessment", PTDC/MAR-BIO/6044/2014, financed by Foundation for Science and Technology of Portugal.

This work was supported by the Portuguese Foundation for Science and Technology (FCT) in the framework of the Strategic Funding UID/FIS/04559/2013 of LIBPhys.

This work was supported in part by the Unidade de Ciências Biomoleculares Aplicadas – UCIBIO which is financed by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).

We would like to thank NMT – Tecnologia, Inovação e Consultoria, S.A, for allowing the use of the GC-IMS device.

REFERENCES

[1] A. Halász, Á. Baráth, L. Simon-Sarkadi, and W. Holzapfel, "Biogenic amines and their production by microorganisms in food," Trends Food Sci. Technol., vol. 5, no. 2, pp. 42–49, 1994.

[2] I. Ferreira and O. Pinho, "Biogenic Amines in Portuguese Traditional Foods and Wines," J. Food Prot., vol. 69, no. 9, pp. 2293–2303, 2006.

[3] B. Bilgin and H. Gençcelep, "Determination of biogenic amines in fish products," Food Sci. Biotechnol., vol. 24, no. 5, pp. 1907–1913, 2015.

[4] P. Q. Leng, F. L. Zhao, B. C. Yin, and B. C. Ye, "A novel, colorimetric method for biogenic amine detection based on arylalkylamine N-acetyltransferase," Chem. Commun., vol. 51, no. 41, pp. 8712–8714, 2015.

[5] A. R. Shalaby, S. Kurt, and O. Zorba, "Significance of biogenic amines to food safety and human health," Food Res. Int., vol. 29, no. 15, pp. 675–690, 1996.

[6] L. Lehane and J. Olley, "Histamine fish poisoning revisited," Int. J. Food Microbiol., vol. 58, p. 37, 2000.

[7] F. Li, Z. Xie, H. Schmidt, S. Sielemann, and J. Baumbach, "Ion mobility spectrometer for online monitoring of trace compounds," Spectrochim. Acta Part B At. Spectrosc., no. 10, pp. 1563–1574, 2002.

[8] A. Ulanowska, M. Ligor, A. Amann, and B. Buszewski, "Determination of Volatile Organic Compounds in Exhaled Breath by Ion Mobility Spectrometry," Chem. Analityczna, vol. 53, pp. 953–965, 2008.

[9] Z. Karpas, "Applications of ion mobility spectrometry (IMS) in the field of foodomics," Food Res. Int., vol. 54, no. 1, pp. 1146–1151, 2013.

[10] V. Gabelica and E. Marklund, "Fundamentals of ion mobility spectrometry," Curr. Opin. Chem. Biol., vol. 42, pp. 51–59, 2018.

[11] A. Scheinemann, S. Sielemann, J. Walter, and T. Doll, "Evaluation Strategies for Coupled GC-IMS Measurement including the Systematic Use of Parametrized ANN Training Data," Open J. Appl. Sci., vol. 02, no. 04, pp. 257–266, 2012.

[12] L. Vera, E. Companioni, A. Meacham, and H. Gygax, "Real Time Monitoring of VOC and Odours Based on GC-IMS at Wastewater Treatment Plants," Chem. Eng. Trans., vol. 54, pp. 79–84, 2016.

[13] A. P. Snyder, C. S. Harden, D. M. Davis, D. B. Shoff, and W. M. Maswadeh, "Hand-portable Gas Chromatography-Ion Mobility spectrometer for the determination of the freshness of fish," in Inorganic and Physical Chemistry, 1995, pp. 146–166.

[14] L. Y. Chang et al., "One-Minute Fish Freshness Evaluation by Testing the Volatile Amine Gas with an Ultrasensitive Porous-Electrode-Capped Organic Gas Sensor System," ACS Sensors, vol. 2, pp. 531–539, 2017.

[15] G. A. Eiceman, Z. Karpas, and H. H. Hill, Ion Mobility Spectrometry, Third Edit. CRC Press, 2014.