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Emerging Trends in Biogenic Amines Analysis

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Abstract

Biogenic amines are low-molecular-mass substances, essential for proper health for all organisms. These compounds could be detrimental to human health with various toxicological effects when they are present in high concentrations. Therefore, biogenic amines monitoring in food samples is a matter of utmost importance, and their accurate determination is considered indispensable. Under this context, we provide an overview over the most widely employed analytical techniques for biogenic amines determination such as chromatographic techniques and biosensors, emphasizing on new approaches. A critical comparison of the techniques is also given, presenting their advantages and drawbacks regarding important analytical characteristics such as sensitivity. Finally, we focus on foods in which biogenic amines mainly occur such as fish, meat and wine and other fermented products.

Keywords: biogenic amines, analysis, food, chromatography, biosensors

1. Introduction

Biogenic amines (BAs) are small molecular organic nitrogenous compounds (bases), polar or semipolar. The most common BAs in food are putrescine, cadaverine, spermine and spermidine with an aliphatic structure; tyramine, tryptamine and β -phenylethylamine with an aromatic structure and histamine with a heterocyclic structure [1, 2]. These compounds can produce a wide range of toxicological effects [3]. Theoretically, BAs occurrence could be expected in all foods that contain free amino acids (AAs) or protein and are exposed to conditions enabling microbial or biochemical activity [4]. Biogenic amines share common characteristics with their precursors, AAs, and that is taken into consideration when we try to come up with analytical methods for their effective determination.

In low concentrations, these nitrogenous organic bases are essential for good health, acting as hormones or neurotransmitters and generally being important for growth, temperature regulation and high metabolic activity of the normal functioning and immunological system of gut. By and large, BAs constitute sources of nitrogen and precursors which lead to the synthesis of many specific compounds such as hormones, alkaloids, proteins and nucleic acids. Also, they control several processes in the organism such as the regulation of body temperature, intake of nutrition and increase/decrease of blood pressure [5].

On the other hand, in high concentrations, BAs are considered quite hazardous and able to cause health problems to consumers, especially to sensitive persons.

Histamine is the most widely studied biogenic amine due to its ability to cause headaches, nausea, hypotension, digestive problems and skin allergy, while tyramine is often associated with migraine and hypertension [6]. It has also been proved that tyramine is more cytotoxic than histamine on an in vitro model of the human intestinal epithelium. Tyramine caused a cell necrosis, while histamine induced apoptosis [7]. Referring to polyamines such as putrescine and cadaverine, they are less pharmacologically active; however, they could interact with the amine oxidases and potentiate the effects of histamine and tyramine. Besides, these polyamines can react with nitrite to form potentially carcinogenic nitrosamines [8]. At this point, it should be noted that microbiological spoilage cannot occur in salted products since BAs accumulation can occur before salting. Moreover, if sea salt, rock salt or other preservatives contain nitrate and nitrite as impurities, BAs in salted products may react with nitrites to form nitrosamines, as mentioned above [9, 10]. Tyramine, cadaverine, putrescine and histamine are the most common BAs in meat and meat products. The concentration of histamine is usually quantitatively lower than that found in fish [11].

As for the BAs level regulations, histamine is currently the only BAs having official limits in fish products, despite the fact that BAs have been described as having a certain potential toxicity in food products in general. The European Food Safety Authority confirmed histamine and tyramine as the most toxic and particularly relevant for food safety [11]. The presence of other amines has been found to enhance histamine toxicity [9, 10]. The maximum acceptable histamine levels in fish have been established in many countries; in the USA, the Food and Drug Administration established a maximum limit of 50 mg kg^{-1} at the port and 100 mg kg^{-1} in pickled fish for species prone to form histamine [11, 12]. The European Union has established regulations according to which histamine levels should be below 100 mg kg^{-1} in raw fish and below 200 mg kg^{-1} in salted fish [13], regarding species belonging in the Coryphaenidae, Engraulidae, Pomatomidae, Clupeidae and Scombridae families. In Brazil, the Regulation of Industrial and Sanitary Inspection of Animal Products does not mention the amine maximum level allowed in products of animal origin. However, the MERCOSUR institute, co-run by Argentina, Brazil, Paraguay and Uruguay, established a maximum level of 100 mg kg^{-1} of histamine in the muscles of species of the Clupeidae, Pomatomidae, Scombridae, Scomberesocidae and Coryphaenidae families [14]. Scombroid poisoning is a type of fish poisoning developed by eating spoiled fish. Since most fish species are rich in free histidine, scombroid poisoning is accepted as mainly caused by elevated histamine levels in fish, generated by bacterial enzymatic conversion of free histidine [9]. Fish products head the list of foods most studied from the point of view of BAs.

Some European countries have recommended the establishment limits for histamine in wine: Germany (2 mg L^{-1}), Belgium ($5\text{--}6 \text{ mg L}^{-1}$) and France (8 mg L^{-1}) [15, 16]. Switzerland established an upper limit of 10 mg L^{-1} of histamine, which was rejected later [17]. In Slovak Republic, histamine is regulated as 20 mg kg^{-1} in beer, 200 mg kg^{-1} in fish/fish products and tyramine as 200 mg kg^{-1} in cheese [18]. The Institute of Dairy Research in the Netherlands and the Czech Republic has proposed an upper limit of $100\text{--}200 \text{ mg kg}^{-1}$ for histamine in meat products. There are no official establishments regarding the standards for cadaverine, putrescine or other BAs, except for some proposals. About tyramine, the recommended limit is in the range of $100\text{--}800 \text{ mg kg}^{-1}$ of food. A figure of 30 mg kg^{-1} for β -phenylethylamine has been considered toxic dose in food [1].

Since the occurrence of BAs has been detected in a broad range of products (fish, fish products, meat, meat products, beer, wine, cheese, milk, dairy products, various beverages, condiments, fruits, vegetables, vinegar, tea, chocolate and coffee), it is an urgent need to develop new analytical methods or improve the current methods for BAs analysis in terms of rapidity and reliability as far as food safety is concerned.

The aim of this chapter is to summarize, discuss and compare the most widely employed analytical techniques/approaches such as chromatographic, capillary electrophoresis and biosensors for BAs. Moreover, we provide the emerging trends and the recent advances on BAs analytical methods.

2. Techniques

2.1 Chromatographic

The determination of biogenic amines is not simple at all, due to the variety of their chemical structures and their presence at relatively low levels in matrices which are usually complex. However, a precise identification of these compounds and the detection of even slight changes in their profile are urgent regarding both the quality control and the consumers' health. The monitoring and determination of BAs in food matrices is based on new analytical approaches which combine higher accuracy, sensitivity, reproducibility and rapidity and are also inexpensive and convenient and thus can be adopted by laboratories worldwide, applied in numerous applications. In this part, the most widely employed analytical techniques are described and discussed. Moreover, newly applied methods are presented, compared and evaluated, based on innovations and technical possibilities that they propose.

The quantification of BAs in food samples has mainly been accomplished by an array of chromatographic methods, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), ultra-performance liquid chromatography (UPLC), ion chromatography (IC), thin-layer chromatography (TLC), ion-pair liquid chromatography (IPLC) and capillary electrophoresis (CE). However, in recent years, many sensors have been developed for the BAs analysis as alternatives to the expensive instrumentation of chromatographic techniques [19]. What is more, it should be noted that the employment of a sensitive and efficient detector is a crucial issue as it ensures the trustworthiness of the analytical method as a whole. There are several detection processes having been reported in BAs detection studies, such as ultraviolet (UV) [20], indirect UV [21], mass spectrometry (MS) [22], electrochemical [23], conductometric [24], enzymatic, immunoassay and polymerase chain reaction (PCR) processes [25]. Generally, after derivatization, the approaches usually used for BAs determination are UV, fluorescence and MS.

Classical reversed-phase high-performance liquid chromatography (RP-HPLC) using C-18 columns has been indistinctively employed for the BAs quantitative determination in different types of food because of its sensitivity, high resolution, great versatility and relatively simple sample treatment [26]. Yet, a previous solvent extraction step and a chemical derivatization step is required prior to final separation. The former aims to remove some potentially interfering compounds and also to concentrate the analytes of interest. The latter reduces the BAs polarity and improves resolution in RP columns, making them more sensitive towards detection. Also, the BAs polarity needs to be reduced because this high polar character results in a greater solubility in water rather than in the organic solvents which are frequently used in the majority of the techniques.

Solid phase extraction (SPE) is a widely used technique for sample clean-up and proper isolation of BAs, while it is the most common extraction method for BAs determination in beverages [27]. Also, solid phase microextraction (SPME) [28], molecularly imprinted solid phase extraction (MISPE) [29], dispersive liquid-liquid microextraction (DLLME) [30], vortex-assisted surfactant-enhanced emulsification liquid-liquid microextraction (VSLLE) [31], hollow-fibre liquid-phase

microextraction (HF-LPME) [32], salting-out assisted liquid-liquid extraction (SALLE) [33] and cloud point extraction (CPE) [34] have been used in many different BAs determination studies. Also, the addition of specific chemical substances is sometimes required so as to ensure the retention of potentially interfering substances such as lipids, proteins and polyphenols. These compounds have similar structures to BAs, thereby posing problems for the derivatization reaction, making the BAs quantification and detection difficult. The chemical substances added usually are trichloroacetic acid (TCA), ethyl acetate, hydrochloric acid, perchloric acid, diethyl ether and polyvinylpyrrolidone (PVP). Hence, the matrix interferences are minimized, up to an extent.

The selection of an effective derivatization agent is a matter of utmost importance in order to decrease the derivatization time and increase the derivatization reaction efficiency. There are many different HPLC studies discussed, aiming to BAs determination and using numerous derivatization agents such as 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) [35], dansyl chloride (Dns-Cl) [36–39], O-phthalaldehyde (OPA) [19], 2,6-dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester (DMQC-Osu) [40], ethyl-acridine-sulfonyl chloride (EAC) [41], O-phthalaldehyde/N-acetyl-L-cysteine (OPA/NAC) [42], O-phthalaldehyde/mercaptoethanol (OPA/MCE) [43] or 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiazas-indacene (TMBBSu) [44] coupled with fluorescence detection. It is obvious that the obtained limits of detection (LODs) were quite satisfying. Also, there are some HPLC studies presented, employing benzoyl chloride [45], 2-chloro-1,3-dinitro-5-(trifluoromethyl)-benzene (CNBF) [46, 47], diethyl ethoxymethylenemalonate (DEEMM) [48], 9-fluorenylmethyl chloroformate (FMOC) [31], 1-naphthylisothiocyanate (NITC) [49], phenyl isothiocyanate (PITC) [50] or dansyl chloride (Dns-Cl) [51] with UV detection, obtaining low LODs, too.

By and large, the LODs values of analytical methods for BAs usually lie at ppm (mg L^{-1}) levels. In some cases, SPE processes prior to chromatographic analysis can decrease the LODs values even to ppb ($\mu\text{g L}^{-1}$) levels, and the ultra-trace analysis of BAs can be accomplished. For instance, in a study of Basheer et al. [28], the synthesized hydrazone-based ligands were physically trapped in a silica sol-gel matrix and used for micro-SPE of the dansylated BAs. The technique was applied to the pre-concentration of BAs in orange juice, before HPLC analysis and UV detection. The sol-gel sorbent that contained benzophenone 2,4-dinitrophenylhydrazone ligand showed great affinity to the target analytes. The obtained LODs were 3.82–31.3 ng L^{-1} . Apart from that, the LODs of 0.25–50 $\mu\text{g L}^{-1}$ were obtained by Huang et al. [40], applying the IL-based ultrasonic-assisted liquid-liquid microextraction method (IL-UALLME) with DMQC-OSu as derivatization agent. Also the LODs of 8.82–40.4 ng L^{-1} were obtained in a study by Gao et al. with TMBBSu as a derivatization agent [44].

All in all, the methods using a fluorescent detector are more sensitive than those with a UV-vis detector, regardless of the reagent employed. In two studies in Chilean young and reserved wine samples, conducted by the same laboratory and using the same protocol, the LODs using a fluorescence detector were 1–90 $\mu\text{g L}^{-1}$, whereas the LODs using a UV detector were 90–300 $\mu\text{g L}^{-1}$ [36, 37]. In the same way, in a HPLC study in fish products, the LODs with fluorescence detection were 20–240 $\mu\text{g kg}^{-1}$, while the LODs with UV detection were 567–1800 $\mu\text{g kg}^{-1}$ [38, 52]. However, the study of Tameem et al. [53] using a UV-vis detector and obtaining sensibly low LODs (20–60 ng L^{-1}) represents an exception to the above mentioned. The authors stated that this accomplishment was because of the large sample volume (50 mL) injected into the chromatograph.

In some cases, the derivatization step can be phased out when HPLC is coupled with MS detectors. Hence, the analysis time can be much smaller. Sagratini et al.

elaborated a method with underivatized BAs in fish tissues using LC-MS/MS analysis after SPE [54]. The obtained LODs were 20–250 $\mu\text{g kg}^{-1}$. On the whole, due to the considerable sensitivity and the specific structural information for the derivatized amines that MS or MS/MS detectors can provide, they are the most efficient detection tools for metabolites which are usually present in low concentrations [55]. Furthermore, they are very helpful in identifying co-eluting peaks in real sample analysis. What is more, tandem mass spectrometry (MS/MS) was employed in a study where isotopically labeled BAs were added as internal standards. Isotopically labeled internal standards have been proven to minimize matrix interferences in complex matrices. The limits of quantification (LOQs) were at 50 ng kg^{-1} level [56]. Finally, in a very recent study, Jastrzębska et al. (2018) used 3,5-Bis-(trifluoromethyl)phenyl isothiocyanate (BPI) to produce the BAs-BPI derivatives which were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The obtained LODs ranged from 2.0 to 4.3 ng L^{-1} [57].

In another method, the separation of BAs with HPLC was followed by evaporative light-scattering detection (ELSD) [58]. The detector's response was based on the amount of light scattered by analyte particles created by evaporating a solvent while it passed through a light beam. There was no need for the use of chromophores for target analytes; hence no derivatization was required. This LC-ELSD method was compared to a LC-UV method applied in the same study and was found less sensitive. Yet, it was good enough for the BAs detection in cheese samples. The LODs were 1.4–3.6 mg L^{-1} . In general, ELS detectors are considered more affordable than mass spectrometers with the same characteristics.

In a BAs determination study in beer samples, dairy beverage samples and herb tea and vinegar samples, the analytes were separated by ion-pair liquid chromatography and detected by a chemiluminescent nitrogen detector (CLND) [59]. In comparison with a HPLC-UV and a HPLC-charged aerosol detector (CAD) method for 14 BAs in the same study, the HPLC-CLND method gave narrower peaks, with highly improved resolutions. The LODs were 0.1–0.4 mg L^{-1} . Sun et al. [59] optimized and validated this method using nonafluoropentanoic acid (NFPA), which is an ideal agent as it was tested and selected as the finest ion-pair reagent amongst many other perfluorocarboxylic acids tried. In another implementation of ion-pair liquid chromatography (IPLC), the chaotropic salt KPF_6 was applied in vinegar samples [60].

It should be noted that the time of analysis depends heavily on the number of analytes and usually varies between 5 and 85 min when the conventional LC method with C18 columns is employed. In the vast majority of studies with simultaneous determination of BAs, the run time lasted more than 30 min. In many cases lately, the BAs determination has been performed by UPLC, which outweighs HPLC, mainly in terms of solvent consumption, analysis time, better resolution and increased peak capacities [61]. Since 2004, new generations of stationary phases compatible with LC systems have been widely used under the trade name Ultra Performance Liquid Chromatography (UPLC) [62]. The separation time of BAs is greatly decreased when the UPLC technique with short columns (5 cm) packed with smaller particles ($<2 \mu\text{m}$) and high flow rates is employed [19]. Jia et al. [63] published a study where they developed an ultra-performance LC/quadrupole time-of-flight mass spectrometry method (UPLC/Q-TOFMS) for dansylated BAs along with 23 amino acids in cheese, beer and sausage samples. The separation of all analytes was completed in 25–30 min. The LODs were 5–20 $\mu\text{g L}^{-1}$. The UPLC/Q-TOFMS method was also used for BAs detection in another study [64] with less analysis time than the conventional HPLC method (13 min) and LODs 3–15 $\mu\text{g L}^{-1}$. In another UPLC-MS/MS method, the elution time was also very short ($<8.5 \text{ min}$) [65]. In a more recent study by Lee et al. [66], the UPLC-MS/MS (ESI+) method

was applied for the determination of 9 BAs in rice wine samples with run time 21 min and LODs 0.1–4.6 $\mu\text{g L}^{-1}$.

Next, in some cases, BAs have been determined by ion chromatography (IC). The LODs were not very low, yet this technique does not require a derivatization step. The LODs in fruit juice samples were 56–1630 $\mu\text{g L}^{-1}$ [67], whereas the LODs in wine samples were 23–68 $\mu\text{g kg}^{-1}$ [68]. Ion chromatography (IC) coupled with pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD) has been reported in many studies. All the same, the use of acids and salts in conjunction with the use of organic solvents in high concentrations is sometimes essential for the separation of strongly retained amines, such as spermidine and spermine [69]. Organic solvents can cause decomposition by-products resulting in potential interferences. Hence, longer retention times and poor resolution or peak shapes have been reported [70].

Thin-layer chromatography (TLC) exemplifies an alternative to LC methods. No special equipment is required, and several samples can be analysed at the same time. Notwithstanding, this method is semi-quantitative and the analysis can be relatively long [71, 72]. An economic TLC/densitometry for BAs detection in wine samples was validated [71]. The potential interferences were avoided with the use of PVPP. Furthermore, isohexane was used for the extraction of the dansylated derivatives, before TLC separation. The analysis was completed in 90 min and the LODs were 600–700 $\mu\text{g L}^{-1}$.

Gas chromatography (GC) methods are also used in some studies, yet not to the same extent that LC or capillary electrophoresis (CE) does, due to the lack of volatility of BAs. Apart from decreasing the polarity of BAs, the derivatization step in GC is essential so as to increase the volatile properties of these analytes. Mass spectrometers are used for detection in the majority of studies. Almeida et al. validated a dispersive liquid-liquid microextraction (DLLME) method followed by GC-MS for the determination of 18 BAs in beer samples. The DLLME procedure was performed simultaneously with the derivatization process. The LODs were 0.3–2.9 $\mu\text{g L}^{-1}$ [73]. Also, Cunha et al. elaborated a GC-MS method and used IBCF to determine the content of 22 BAs in grape juice and wine samples, with toluene as an extraction solvent and LODs lying at the level of 1 $\mu\text{g L}^{-1}$. The derivatization was carried out in a two-phase reaction system, eliminating the need for a previous extraction procedure [74]. The IBCF derivatization was also performed in a BAs determination study in home-made fermented alcoholic drinks by Plotka-Wasyłka et al. [75] involving in situ derivatization-DLLME combined with GC-MS. The LODs were 1.1–4.1 $\mu\text{g L}^{-1}$ [75]. In a more recent study, Huang et al. performed an environmentally friendly SPME coupled with GC-MS for the determination of biogenic amines in fish samples. The LODs were 2.98–45.3 $\mu\text{g kg}^{-1}$ [76]. The analysis time was shorter in the GC methods than the LC methods, and the obtained LODs were generally satisfying.

2.2 Capillary electrophoresis

Capillary electrophoresis (CE) is a separation technique which is widely performed, following HPLC regarding the extent of its application in biogenic amine analysis. This technique renders possible the analysis of a vast range of compounds which occur in low concentration levels and is also characterized by rapidity, separation efficiency, sensitivity and shorter analysis time than LC methods and less solvent consumption. Another important fact is that CE is suitable for analytes which cannot be analysed with GC due to thermal instability. Yet, it should be noted that the number of separated BAs and their precursors, AAs, is usually much smaller than HPLC methods [69]. There are different CE methods employed in BAs

studies, such as micellar electrokinetic chromatography (MEKC), capillary gel electrophoresis (CGE), capillary zone electrophoresis (CZE), capillary isotachopheresis (CITP) and capillary isoelectric focusing (CIEF) [77].

Fluorometric methods are frequently employed by virtue of the fluorescence of BAs at some pH range their reaction with proper agents. Many fluorescence derivatization reagents have been used in CE studies, like fluorescein isothiocyanate (FITC), 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein (DTAF), naphthalene-2,3-dicarboxaldehyde (NDA), OPA and 3-(2-furoyl)-quinoline-2-carboxaldehyde (FQ). In a study involving FITC, proposed by Uzaşçı et al., a fast separation of seven biogenic amines in wine samples was performed, and significantly low LODs down to 57.6–113 ng L⁻¹ were reported [78]. The authors proposed a novel nonionic micellar electrokinetic chromatography method (MEKC) through FITC coupled to laser-induced fluorescence detector (LIF). The separation was completed in 9 min. Also, in a study by Zhang et al. involving the combination of laser-induced fluorescence (LIF) detector and CE separation, a quite satisfying improvement in detection limits was observed [79].

A CE-MS/MS method was reported for the quantitative determination of BAs in beer and wine samples [80]. The migration time for the 9 BAs was very short (<10 min), and the LODs were in the range of 1–2 µg L⁻¹ for wine and 3–8 µg L⁻¹ for beer samples. The main drawback regarding the use of a MS detector for CE is its higher price compared with conventional UV or LIF detection and the limitation in the type of running buffers that can be used as they have to be volatile and compatible with ESI.

Offline precolumn derivatization is the most widely used application, while the occurring BAs derivatives are then injected into the CE. Generally, UV detection sensitivities in CE are lower than those of HPLC and can be increased by novel online pre-concentration procedures along with derivatization developments or coupling CE with isotachopheresis (ITP) [19].

In a study, the online coupling of capillary zone electrophoresis (CZE) with capillary isotachopheresis (CITP) and UV detection increased the sensitivity of the method. The BAs were online pre-concentrated in the ITP step, separated and detected in the CZE step. The study was conducted for the determination of histamine, phenylethylamine and tyramine in wine samples. The LODs were 0.35 mg L⁻¹ for histamine, 0.33 mg L⁻¹ for phenylethylamine and 0.37 mg L⁻¹ for tyramine [81]. In a more recent CITP method with a conductometric detector in beer and wine samples by Jastrzębska et al. [82], the derivatization step was eliminated, and the LODs 200–480 µg L⁻¹ were lower in comparison with an LC method by the same authors. In the latter, dansylated BAs derivatives were synthesized and a UV detector was employed.

Finally, in a method elaborated by Dossi et al., the separation of BAs and AAs in beer samples was performed by microchip CE and followed by amperometric detection with the use of ruthenium oxide/hexacyanoruthenate polymeric films, electrochemically deposited onto glassy carbon electrodes. The separation of single amines and AAs was not possible through this method. Hence, the analytes were co-eluted in groups. The LODs were 1.4–6.8 mg L⁻¹ [83].

2.3 Biosensors

A biosensor is widely considered as an analytical platform that converts a biological response into a quantifiable and processable signal. The individual parts of a biosensor were well addressed and comprehensively discussed in [84], which is recommended for further understanding of biosensors function. Even though instrumental methods are quite accurate and sensitive, their high operational cost and time-consuming protocols showcase the need for analytical alternatives. In this

way, biosensors can improve the current situation as they feature highly desired characteristic such as simplicity, rapidness, cost-effectiveness and portability. In BAs analysis, chromatographic techniques are mostly used as the numerous compounds of food matrices need to be separated to accurately detect the analytes [26]. However, biosensors, instead of separation, utilize various selective recognition elements such as antibodies, enzymes, molecularly imprinted polymers (MIPs), aptamers and nucleic acids to bind with the target molecule.

As it is already shown, the utilisation of biosensors in BAs analysis is a novel concept that finds more and more applications in food analysis. Under this context, we indicatively discuss some published studies to provide an overview on the various recognition elements and detection methods. To begin with, various sensing elements have been used (**Table 1**), including classic approaches such as antibodies or enzymes and also pioneering cases such MIPs or nanotechnological applications. Despite the excellent recognition capability of MIPs or the proven superior electronic properties of nanocomposite materials, antibodies and enzymes are still mostly used because of the laborious development process of those recognition elements. Concerning the detection method, electrochemical (EC) sensors were commonly used as they did not require time-consuming sample pretreatment and could be easily miniaturized and used in situ. However, EC sensors usually lacked long-term stability. Alternatively, spectroscopic methods, namely chemiluminescence (CL) and photoluminescence (PL), were also applied because they combined simple formats with zero background measurements for increased sensitivity. Interestingly, digital cameras were also used for the colorimetric detection of BAs. The combination of image data analysis with dipsticks [85] or immunoassays [86] is a trend focusing towards portable and on-site detection of various food contaminants. A striking example of this is the EU-funded FoodSmartphone project, <http://foodsmartphone.eu/> (last visited 5/8/2018), in which several smartphone-based assays for the detection of various food contaminants including allergens,

Analyte	Recognition element	Detection	Matrix	LODs	Ref.
Tyramine	Antibody	ELISA	Meat and fish	1.2 mg kg ⁻¹	[87]
Putrescine	Unsaturated complex of Cu(II)	CL	Shrimp	0.0178 mg L ⁻¹	[88]
Histamine	Cu@Pd core-shell nanostructures	EC	Tuna fish	0.3 ng kg ⁻¹	[89]
Tryptamine	Oxidation	EC	Banana, tomato cheese, sausages	0.12 ng L ⁻¹	[90]
Tyramine	Fe ³⁺ ion complex of FONs	Ratiometric PL	Solutions	0.5 mg L ⁻¹	[91]
Tyramine	Dye Py-1	Digital camera	Shrimp	1.37 mg kg ⁻¹	[92]
Tyramine	Tyrosinase	EC	Cheese, sauerkraut, banana	0.21 mg L ⁻¹	[93]
Histamine	Antibody	EC	Fish	1.25 ng L ⁻¹	[94]
Tryptamine	Covalent immobilization of tryptamine on nanofiber	PL	Beer	6 ng L ⁻¹	[95]
Tyramine	MIPs	EC	Milk	0.32 ng L ⁻¹	[96]

Table 1.
Biosensors application in BAs analysis.

pesticides, marine toxins etc. are being developed. Hence, this approach may also find application for the BAs in the future. Regarding biosensors sensitivity, we can notice that they provided satisfactory detection limits, but in several studies there were no validation data in the food matrix. All in all, biosensors have the potential to improve and simplify the current situation and move towards the on-site BAs determination.

3. Conclusions

The determination of BAs in food is a matter of utmost importance regarding both the consumers' health and the quality control. Regulatory bodies, industries and consumers demand efficient detection of BAs in food products. Thus, the scientific community tries to develop new analytical methods or to improve the current methods concerning their sensitivity and reliability in different food matrices. Yet, it should be noted that the legislation for BAs regulations may differ amongst different countries; there are different production, processing and storage methods, as well as different climate conditions which can either cause or inhibit the BAs formation in food [97, 98]. Classical reversed-phase high-performance liquid chromatography (RP-HPLC) using C-18 columns has been the most commonly employed technique for the BAs quantitative determination because of its sensitivity, high resolution, great versatility and relatively simple sample treatment. However, CE exemplifies a good alternative to HPLC, with high separation efficiency and relatively low running costs. Lastly, the development on sensors has led to the elaboration of new methods characterized by low cost, short analysis time and simplicity. Neither special instrumentation nor sample clean-up and derivatization are required.

Acknowledgements

G.P. Danezis was funded through a postdoctoral studies scholarship, from the Hellenic State Scholarship Foundation (IKY) that is gratefully acknowledged. This research is supported through IKY scholarships programme and co-financed by the European Union (European Social Fund—ESF) and Greek national funds through the action entitled “Reinforcement of Postdoctoral Researchers”, in the framework of the Operational Programme “Human Resources Development Program, Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) 2014–2020.

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References

- [1] Papageorgiou M et al. Literature update of analytical methods for biogenic amines determination in food and beverages. *TrAC Trends in Analytical Chemistry*. 2018;**98**:128-142
- [2] Shalaby AR. Significance of biogenic amines to food safety and human health. *Food Research International*. 1996;**29**(7):675-690
- [3] E. P. o. B. Hazards. Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal*. 2011;**9**(10):2393
- [4] Halász A et al. Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology*. 1994;**5**(2):42-49
- [5] Karovičová J, Kohajdová Z. Biogenic amines in food. *Chemical Papers*. 2005;**59**(1):70-79
- [6] Ladero V et al. Toxicological effects of dietary biogenic amines. *Current Nutrition & Food Science*. 2010;**6**(2):145-156
- [7] Linares DM et al. Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. *Food Chemistry*. 2016;**197**:658-663
- [8] Kim M-K, Mah J-H, Hwang H-J. Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chemistry*. 2009;**116**(1):87-95
- [9] Hungerford JM. Scombroid poisoning: A review. *Toxicon*. 2010;**56**(2):231-243
- [10] Bulushi IA et al. Biogenic amines in fish: Roles in intoxication, spoilage, and nitrosamine formation—A review. *Critical Reviews in Food Science and Nutrition*. 2009;**49**(4):369-377
- [11] Ruiz-Capillas C, Jiménez-Colmenero F. Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition*. 2005;**44**(7-8):489-599
- [12] Food and Drug Administration. Fish and Fishery Products Hazards and Controls Guidance. US Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition; 2011
- [13] Directive E. Amending regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Commission regulation (EC) No 1441/2007 of 5 December 2007. *Official Journal of the European Union*. 2007;**322**:12-29
- [14] Rodriguez MBR et al. Bioactive amines: Aspects of quality and safety in food. *Food and Nutrition Sciences*. 2014;**5**(02):138
- [15] Bauza T et al. Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection. *Journal of Chromatography A*. 1995;**707**(2):373-379
- [16] Landete JM et al. Biogenic amines in wines from three Spanish regions. *Journal of Agricultural and Food Chemistry*. 2005;**53**(4):1119-1124
- [17] Konakovsky V et al. Levels of histamine and other biogenic amines in high-quality red wines. *Food Additives and Contaminants*. 2011;**28**(4):408-416
- [18] Halász A, Baráth Á, Holzapfel WH. The influence of starter culture selection on sauerkraut fermentation. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung A*. 1999;**208**(5-6):434-438

- [19] Erim FB. Recent analytical approaches to the analysis of biogenic amines in food samples. *TrAC Trends in Analytical Chemistry*. 2013;**52**:239-247
- [20] Soufleros E et al. Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. *Food Chemistry*. 2007;**101**(2):704-716
- [21] Arce L, Ríos A, Valcárcel M. Direct determination of biogenic amines in wine by integrating continuous flow clean-up and capillary electrophoresis with indirect UV detection. *Journal of Chromatography A*. 1998;**803**(1-2):249-260
- [22] Saccani G et al. Determination of biogenic amines in fresh and processed meat by suppressed ion chromatography-mass spectrometry using a cation-exchange column. *Journal of Chromatography A*. 2005;**1082**(1):43-50
- [23] Pastore P et al. Determination of biogenic amines in chocolate by ion chromatographic separation and pulsed integrated amperometric detection with implemented wave-form at Au disposable electrode. *Journal of Chromatography A*. 2005;**1098**(1-2):111-115
- [24] Kvasnička F, Voldřich M. Determination of biogenic amines by capillary zone electrophoresis with conductometric detection. *Journal of Chromatography A*. 2006;**1103**(1):145-149
- [25] Anlı RE, Bayram M. Biogenic amines in wines. *Food Reviews International*. 2008;**25**(1):86-102
- [26] Proestos C, Loukatos P, Komaitis M. Determination of biogenic amines in wines by HPLC with precolumn dansylation and fluorimetric detection. *Food Chemistry*. 2008;**106**(3):1218-1224
- [27] Molins-Lagua C, Campins-Falcó P. Solid phase extraction of amines. *Analytica Chimica Acta*. 2005;**546**(2):206-220
- [28] Basheer C et al. Hydrazone-based ligands for micro-solid phase extraction-high performance liquid chromatographic determination of biogenic amines in orange juice. *Journal of Chromatography A*. 2011;**1218**(28):4332-4339
- [29] Basozabal I et al. Rational design and chromatographic evaluation of histamine imprinted polymers optimised for solid-phase extraction of wine samples. *Journal of Chromatography A*. 2013;**1308**:45-51
- [30] Rezaee M et al. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *Journal of Chromatography A*. 2006;**1116**(1-2):1-9
- [31] Donthuan J, Yunchalard S, Srijaranai S. Vortex-assisted surfactant-enhanced-emulsification liquid-liquid microextraction of biogenic amines in fermented foods before their simultaneous analysis by high-performance liquid chromatography. *Journal of Separation Science*. 2014;**37**(21):3164-3173
- [32] Pedersen-Bjergaard S, Rasmussen KE. Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid-liquid extraction. *Journal of Chromatography A*. 2008;**1184**(1-2):132-142
- [33] Ramos RM, Valente IM, Rodrigues JA. Analysis of biogenic amines in wines by salting-out assisted liquid-liquid extraction and high-performance liquid chromatography with fluorimetric detection. *Talanta*. 2014;**124**:146-151
- [34] Paleologos E et al. Determination of biogenic amines as their benzoyl

- derivatives after cloud point extraction with micellar liquid chromatographic separation. *Journal of Chromatography A*. 2003;**1010**(2):217-224
- [35] Peña-Gallego A et al. High-performance liquid chromatography analysis of amines in must and wine: A review. *Food Reviews International*. 2012;**28**(1):71-96
- [36] Pineda A et al. Preliminary evaluation of biogenic amines content in Chilean young varietal wines by HPLC. *Food Control*. 2012;**23**(1):251-257
- [37] Henríquez-Aedo K et al. Evaluation of biogenic amines content in Chilean reserve varietal wines. *Food and Chemical Toxicology*. 2012;**50**(8):2742-2750
- [38] Zhai H et al. Biogenic amines in commercial fish and fish products sold in southern China. *Food Control*. 2012;**25**(1):303-308
- [39] Šimat V, Dalgaard P. Use of small diameter column particles to enhance HPLC determination of histamine and other biogenic amines in seafood. *LWT-Food Science and Technology*. 2011;**44**(2):399-406
- [40] Huang K-J et al. Development of an ionic liquid-based ultrasonic-assisted liquid-liquid microextraction method for sensitive determination of biogenic amines: Application to the analysis of octopamine, tyramine and phenethylamine in beer samples. *Journal of Chromatography B*. 2011;**879**(9-10):579-584
- [41] Li G et al. Simultaneous determination of biogenic amines and estrogens in foodstuff by an improved HPLC method combining with fluorescence labeling. *LWT-Food Science and Technology*. 2014;**55**(1):355-361
- [42] Kelly MT, Blaise A, Larroque M. Rapid automated high performance liquid chromatography method for simultaneous determination of amino acids and biogenic amines in wine, fruit and honey. *Journal of Chromatography A*. 2010;**1217**(47):7385-7392
- [43] Iijima S et al. Optimization of an online post-column derivatization system for ultra high-performance liquid chromatography (UHPLC) and its applications to analysis of biogenic amines. *Analytical Sciences*. 2013;**29**(5):539-545
- [44] Gao PF et al. Determination of trace biogenic amines with 1, 3, 5, 7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiaza-s-indacene derivatization using high-performance liquid chromatography and fluorescence detection. *Journal of Separation Science*. 2011;**34**(12):1383-1390
- [45] Özdestan Ö, Üren A. A method for benzoyl chloride derivatization of biogenic amines for high performance liquid chromatography. *Talanta*. 2009;**78**(4-5):1321-1326
- [46] Kim JY et al. Effects of storage temperature and time on the biogenic amine content and microflora in Korean turbid rice wine, Makgeolli. *Food Chemistry*. 2011;**128**(1):87-92
- [47] Piasta AM et al. New procedure of selected biogenic amines determination in wine samples by HPLC. *Analytica Chimica Acta*. 2014;**834**:58-66
- [48] Bach B et al. Validation of a method for the analysis of biogenic amines: Histamine instability during wine sample storage. *Analytica Chimica Acta*. 2012;**732**:114-119
- [49] Jain A, Gupta M, Verma KK. Salting-out assisted liquid-liquid extraction for the determination of biogenic amines in fruit juices and alcoholic

beverages after derivatization with 1-naphthylisothiocyanate and high performance liquid chromatography. *Journal of Chromatography A*. 2015;**1422**:60-72

[50] Valente IM et al. Application of gas-diffusion microextraction for high-performance liquid chromatographic analysis of aliphatic amines in fermented beverages. *Analytical Methods*. 2012;**4**(8):2569-2573

[51] De Mey E et al. Dabsyl derivatisation as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography. *Food Chemistry*. 2012;**130**(4):1017-1023

[52] Köse S et al. Biogenic amine contents of commercially processed traditional fish products originating from European countries and Turkey. *European Food Research and Technology*. 2012;**235**(4):669-683

[53] Tameem AA et al. A 4-hydroxy-N'-[(E)-(2-hydroxyphenyl) methylidene] benzohydrazide-based sorbent material for the extraction-HPLC determination of biogenic amines in food samples. *Talanta*. 2010;**82**(4):1385-1391

[54] Sagratini G et al. Simultaneous determination of eight underivatized biogenic amines in fish by solid phase extraction and liquid chromatography-tandem mass spectrometry. *Food Chemistry*. 2012;**132**(1):537-543

[55] Papageorgiou M et al. Direct solid phase microextraction combined with gas chromatography-mass spectrometry for the determination of biogenic amines in wine. *Talanta*. 2018;**183**:276-282

[56] Mayr CM, Schieberle P. Development of stable isotope dilution assays for the simultaneous quantitation of biogenic amines and polyamines

in foods by LC-MS/MS. *Journal of Agricultural and Food Chemistry*. 2012;**60**(12):3026-3032

[57] Jastrzębska A et al. Application of 3, 5-bis-(trifluoromethyl) phenyl isothiocyanate for the determination of selected biogenic amines by LC-tandem mass spectrometry and 19F NMR. *Food Chemistry*. 2018;**239**:225-233

[58] Restuccia D et al. A new method for the determination of biogenic amines in cheese by LC with evaporative light scattering detector. *Talanta*. 2011;**85**(1):363-369

[59] Sun J et al. Direct separation and detection of biogenic amines by ion-pair liquid chromatography with chemiluminescent nitrogen detector. *Journal of Chromatography A*. 2011;**1218**(29):4689-4697

[60] Zhong J-J et al. Liquid chromatographic method for toxic biogenic amines in foods using a chaotropic salt. *Journal of Chromatography A*. 2015;**1406**:331-336

[61] Ordóñez JL et al. Recent trends in the determination of biogenic amines in fermented beverages—A review. *Analytica Chimica Acta*. 2016;**939**:10-25

[62] Nguyen DT-T et al. High throughput liquid chromatography with sub-2 μm particles at high pressure and high temperature. *Journal of Chromatography A*. 2007;**1167**(1):76-84

[63] Jia S et al. Simultaneous determination of 23 amino acids and 7 biogenic amines in fermented food samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. *Journal of Chromatography A*. 2011;**1218**(51):9174-9182

[64] Jia S et al. Determination of biogenic amines in Bokbunja (*Rubus coreanus* Miq.) wines using

a novel ultra-performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry. *Food Chemistry*. 2012;**132**(3):1185-1190

[65] Romero-González R et al. Simultaneous determination of four biogenic and three volatile amines in anchovy by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*. 2012;**60**(21):5324-5329

[66] Lee S, Yoo M, Shin D. The identification and quantification of biogenic amines in Korean turbid rice wine, Makgeolli by HPLC with mass spectrometry detection. *LWT-Food Science and Technology*. 2015;**62**(1):350-356

[67] Jastrzębska A, Piasta A, Szłyk E. Application of ion chromatography for the determination of biogenic amines in food samples. *Journal of Analytical Chemistry*. 2015;**70**(9):1131-1138

[68] Palermo C et al. A multiresidual method based on ion-exchange chromatography with conductivity detection for the determination of biogenic amines in food and beverages. *Analytical and Bioanalytical Chemistry*. 2013;**405**(2-3):1015-1023

[69] Mohammed G et al. A critical overview on the chemistry, clean-up and recent advances in analysis of biogenic amines in foodstuffs. *TrAC Trends in Analytical Chemistry*. 2016;**78**:84-94

[70] Bilgin B, Genççelep H. Determination of biogenic amines in fish products. *Food Science and Biotechnology*. 2015;**24**(5):1907-1913

[71] Romano A et al. Determination of biogenic amines in wine by thin-layer chromatography/densitometry. *Food Chemistry*. 2012;**135**(3):1392-1396

[72] Önal A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*. 2007;**103**(4):1475-1486

[73] Almeida C, Fernandes J, Cunha S. A novel dispersive liquid-liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC-MS) method for the determination of eighteen biogenic amines in beer. *Food Control*. 2012;**25**(1):380-388

[74] Cunha S, Faria M, Fernandes J. Gas chromatography-mass spectrometry assessment of amines in port wine and grape juice after fast chloroformate extraction/derivatization. *Journal of Agricultural and Food Chemistry*. 2011;**59**(16):8742-8753

[75] Płotka-Wasyłka J, Simeonov V, Namieśnik J. An in situ derivatization-dispersive liquid-liquid microextraction combined with gas-chromatography-mass spectrometry for determining biogenic amines in home-made fermented alcoholic drinks. *Journal of Chromatography A*. 2016;**1453**:10-18

[76] Huang J et al. Environmentally friendly solid-phase microextraction coupled with gas chromatography and mass spectrometry for the determination of biogenic amines in fish samples. *Journal of Separation Science*. 2016;**39**(22):4384-4390

[77] Guo YY et al. Biogenic amines in wine: A review. *International Journal of Food Science and Technology*. 2015;**50**(7):1523-1532

[78] Uzaşçı S, Başkan S, Erim FB. Biogenic amines in wines and pomegranate molasses—A non-ionic micellar electrokinetic chromatography assay with laser-induced fluorescence detection. *Food Analytical Methods*. 2012;**5**(1):104-108

[79] Zhang N et al. Sensitive determination of biogenic amines

by capillary electrophoresis with a new fluorogenic reagent 3-(4-fluorobenzoyl)-2-quinolinecarboxaldehyde. *Talanta*. 2008;**76**(4):791-797

[80] Daniel D et al. Determination of biogenic amines in beer and wine by capillary electrophoresis-tandem mass spectrometry. *Journal of Chromatography A*. 2015;**1416**:121-128

[81] Ginterová P et al. Determination of selected biogenic amines in red wines by automated on-line combination of capillary isotachopheresis-capillary zone electrophoresis. *Journal of Chromatography B*. 2012;**904**:135-139

[82] Jastrzębska A, Piasta A, Szłyk E. Simultaneous determination of selected biogenic amines in alcoholic beverage samples by isotachopheretic and chromatographic methods. *Food Additives & Contaminants: Part A*. 2014;**31**(1):83-92

[83] Dossi N et al. A modified electrode for the electrochemical detection of biogenic amines and their amino acid precursors separated by microchip capillary electrophoresis. *Electrophoresis*. 2011;**32**(8):906-912

[84] Grieshaber D et al. Electrochemical biosensors—Sensor principles and architectures. *Sensors*. 2008;**8**(3):1400-1458

[85] Apilux A et al. Paper-based acetylcholinesterase inhibition assay combining a wet system for organophosphate and carbamate pesticides detection. *EXCLI Journal*. 2015;**14**:307-319

[86] Ludwig SKJ et al. Calling biomarkers in milk using a protein microarray on your smartphone. *PLoS One*. 2015;**10**(8):e0134360

[87] Sheng W et al. Development of an enzyme-linked immunosorbent assay

for the detection of tyramine as an index of freshness in meat and seafood. *Journal of Agricultural and Food Chemistry*. 2016;**64**(46):8944-8949

[88] Chen P et al. A novel chemiluminescence enhanced method for determination of putrescine in shrimp based on the luminol-[Ag(HIO₆)₂]⁵⁻ reaction. *Analytical Methods*. 2016;**8**(5):1151-1156

[89] Gajjala RKR, Palathedath SK. Cu@Pd core-shell nanostructures for highly sensitive and selective amperometric analysis of histamine. *Biosensors and Bioelectronics*. 2018;**102**:242-246

[90] Costa DJE et al. Determination of tryptamine in foods using square wave adsorptive stripping voltammetry. *Talanta*. 2016;**154**:134-140

[91] Kaur N et al. Fe(III) conjugated fluorescent organic nanoparticles for ratiometric detection of tyramine in aqueous medium: A novel method to determine food quality. *Food Chemistry*. 2018;**245**:1257-1261

[92] Yurova NS et al. Functional electrospun nanofibers for multimodal sensitive detection of biogenic amines in food via a simple dipstick assay. *Analytical and Bioanalytical Chemistry*. 2018;**410**(3):1111-1121

[93] Kochana J et al. Mesoporous carbon-containing voltammetric biosensor for determination of tyramine in food products. *Analytical and Bioanalytical Chemistry*. 2016;**408**(19):5199-5210

[94] Dong X-X et al. Portable amperometric immunosensor for histamine detection using Prussian blue-chitosan-gold nanoparticle nanocomposite films. *Biosensors and Bioelectronics*. 2017;**98**:305-309

[95] Ramon-Marquez T et al. Novel optical sensing film based on a

functional nonwoven nanofiber mat for an easy, fast and highly selective and sensitive detection of tryptamine in beer. *Biosensors and Bioelectronics*. 2016;**79**:600-607

[96] Li Y et al. Tyramine detection using PEDOT:PSS/AuNPs/1-methyl-4-mercaptopyridine modified screen-printed carbon electrode with molecularly imprinted polymer solid phase extraction. *Biosensors and Bioelectronics*. 2017;**87**:142-149

[97] Ansorena D et al. Analysis of biogenic amines in northern and southern European sausages and role of flora in amine production. *Meat Science*. 2002;**61**(2):141-147

[98] Visciano P et al. Biogenic amines in raw and processed seafood. *Frontiers in Microbiology*. 2012;**3**:188

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