REVIEW

Biogenic Amines in Food

J. KAROVIČOVÁ and Z. KOHAJDOVÁ

Department of Food Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, SK-812 37 Bratislava
e-mail: jolana.karovicova@stuba.sk

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Recent trends in food security are promoting an increasing search for trace compounds that can affect human health. Biogenic amines, the so-called natural amines with physiological significance, belong to this group of substances. Their amounts are usually increased during controlled or spontaneous microbial fermentation of food or in the course of food spoilage. Several methods exist for isolation, identifying, and determination of biogenic amines in food.

1. BIOGENIC AMINES

Biogenic amines (BA) are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones [1]. BA are generated in course of microbial, vegetable, and animal metabolisms [2]. The chemical structure of BA can either be: aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine), heterocyclic (histamine, tryptamine). Several authors had classified cadaverine, putrescine, spermine, and spermidine among polyamines [3].

1.1. Function of BA

BA are sources of nitrogen and precursors for the synthesis of hormones, alkaloides, nucleic acids, and proteins [4]. They can also influence the processes in the organism such as the regulation of body temperature, intake of nutrition, increase or decrease of blood pressure [2].

In plants, the diamine putrescine and the polyamines spermidine and spermine are implicated in a number of physiological processes, such as cell division, flowering, fruit development, response to stress and senescence [5].

Polyamines are important for the growth, renovation, and metabolism of every organ in the body and essential for maintaining the high metabolic activity of the normal functioning and immunological system of gut [4, 6].

Because of the diversity of the roles of polyamines in cellular metabolism and growth, the requirement for polyamines is particularly high in rapidly growing tissues. Indeed, the importance of putrescine, spermidine, and spermine in tumour growth is widely recognized, and the inhibition of polyamine biosynthesis in tumour-bearing individuals is one of the major targets of cancer therapy research [6].

BA are potential precursors for the formation of carcinogenic *N*-nitroso compounds [3]. The reaction of nitrosating agents with primary amines produces short-lived alkylating species that react with other components in the food matrix to generate products (mainly alcohols) devoid of toxic activity in the relevant contents. The nitrosable secondary amines (agmatine, spermine, spermidine, *etc.*) can form nitrosamines by reaction with nitrite, while tertiary amines produce a range of labile *N*-nitroso products [5].

In fatty foods, such as bacon, at high temperature and in the presence of water, the carcinogen *N*-nitrosopyrrolidine can be formed from putrescine or spermidine [7].

Some BA such as putrescine, cadaverine, spermidine can act as free radical scavengers [8]. Tyramine has a remarkable antioxidative activity, which increases with its content. Thus, inhibiting effect depends on amino and hydroxy groups [5]. The spermine is able to regenerate tocopherol from the tocopheroxyl radical through hydrogenic donor from amino group. The spermine radical next binds lipid or peroxide radicals into a lipid complex [9].

Some BA contribute to the flavour and taste of food [5].

1.2.Conditions for Formation of BA

Although amino acid decarboxylases are not widely distributed among bacteria, species of many genera such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, *Photobacterium* and the lactic acid bacteria *Lactobacillus*, *Pediococcus,* and *Streptococcus* are capable of decarboxylating one or more amino acids [4, 5].

Because the BA in food products are mainly generated by decarboxylation of the corresponding amino acids precursors, thought bacterial decarboxylases, for formation of BA are necessary these conditions [2, 10]:

– availability of free amino acid, but not always leading to amine formation,

– presence of decarboxylase-positive microorganisms,

– conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity.

Free amino acids either occur as such in foods, or may be liberated through proteolysis. Microbial strains with high proteolytic enzyme activity also potentially increase the risk for BA formation in food systems, by increasing the availability of free amino acids.

Removal of the α-carboxyl group from a proteinous amino acid leads to the corresponding BA. The names of many BA correspond to the names of their originating amino acids: histamine from histidine, tyramine from tyrosine, β-phenylethylamine from phenylethylalanine, tryptamine from tryptophane [11]. In plants and some microorganisms, alternative pathway exists to produce putrescine from arginine *via* agmatine. Lysine is decarboxylated by lysine decarboxylase (EC 4.1.1.18) to produce cadaverine, although it can also be formed by ornitine decarboxylase (EC 4.1.1.17) if the content of ornitine is low, but that of lysine is high [7].

Amino acid decarboxylase activity is stronger in an acidic environment, the optimum pH being between 4.0 and 5.5 [5]. Furthermore, in such an environment bacteria are more strongly encouraged to produce these enzymes, as a part of their defence mechanisms against the acidity [12].

The attention is paid to the influence of glucono-δ-lactone (GDL) on the production of BA in dry sausages. GDL causes decreasing of pH in sausages, which results in increasing decarboxylase activity of bacteria [13]. In these conditions bacteria produced more decarboxylases as part of their protective mechanism [14]. The production of histamine, tyramine and putrescine is increased [13].

The presence of fermentable carbohydrate, such as D-glucose, enhances both growth and amino acid decarboxylase activity in bacteria. D-Glucose content in the range of 0.5–2.0 % has been reported to be optimal, while levels in excess of 3 % inhibited enzyme formation [6].

Oxygen supply also appears to have a significant effect on the biosynthesis of BA. *Enterobacter cloacae* produces about half the quantity of putrescine in anaerobic compared with aerobic conditions, and *Klebsiella pneumoniae* synthesizes significantly less cadaverine but acquires the ability to produce putrescine under anaerobic conditions [5]. The redox potential of the medium also influences BA production. Conditions resulting in a reduced redox potential stimulate histamine production, and histidine decarboxylase activity seems to be inactivated or destroyed in the presence of oxygen [6].

Amine formation by bacteria is decisively influenced by temperature. Temperature between 20 °C and 37 °C is optimal for the growth of the most bacteria containing decarboxylases, decreased temperature stops their growth [12].

Presence of sodium chloride activates tyrosine decarboxylase activity and inhibits histidine decarboxylase activity [4]. At the content of sodium chloride 3.5 % the ability of *Lactobacillus buchneri* to form histamine is partly inhibited and at the content of 5.0 % its formation is stopped [15]. The natrium nitrite activates tyrosine decarboxylase activity [12].

Repression of histidine decarboxylase has also been detected when the amount of histamine was accumulated in the medium. The presence of histamine has an inhibiting effect on the histidine decarboxylation activity of *Photobacterium histaminum* C-8; histamine, agmatine, and putrescine inhibit the histidine decarboxylation activity of *Photobacterium phosphoreum* N-14 [5].

Application of suitable starter cultures with aminooxidase activity decreases formation of BA [16]. Addition of *Micrococcus varians* in dry sausages causes decreasing level of tyramine [17]. *Brevibacterium lineans* decreases content of tyramine and histamine during ripening of cheese [18]. Fermentation of sauerkraut by *Lactobacillus plantarum* is able to suppress the growth of *Leuconostoc mesenteroides* in the first step of fermentation and *Pediococcus cerevisiae* in the third step of fermentation. These bacteria are responsible for putrescine and tyramine production during fermentation of sauerkraut [19, 20].

1.3. Toxicology of BA

BA are organic substances present in food, which induce toxicological risks and health troubles [19]. The most frequent foodborne intoxications caused by BA involve histamine [1, 6]. An intake of 5—10 mg of histamine can be considered as defecting to some sensitive people, 10 mg is considered as tolerable limit, 100 mg induce a medium toxicity and 1000 mg is highly toxic [1, 21]. Histamine is also referred to as scombroid

fish poisoning because of the association of this illness with the consumption of scombroid fish, such as tuna, mackerel, and sardines. Another phenomenon is the cheese reaction caused by high levels of tyramine in cheese [1, 6].

1.4. Histamine

Histamine is contained in mast cells and basophils, and its biological effects are usually seen only when it is released in large amounts in the course of allergic and other reactions. Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal, and haematological/immunological systems and the skin. There are three types of receptors, H_1, H_2, and H_3. The most common symptoms result from action on the cardiovascular system [22]. Histamine causes dilatation of peripheral blood vessels, capillaries and arteries, thus resulting in hypotension, flushing, and headache. Histamine-induced contraction of intestinal smooth muscle, mediated by H_1 receptors, may account for abdominal cramps, diarrhoea, and vomiting. Gastric acid secretion is regulated by histamine through H_2 receptors located on the parietal cells [23, 24]. Pain and itching associated with the urticarial lesions may be due to sensory and motor neuron stimulation through H_1 receptors [22].

The histamine poisoning is possible to overcome by using of antihistaminic drugs [4]. For many years, H_1-receptor antagonists have been available for the treatment of allergic conditions while H_2-receptor antagonists have been available for the treatment of gastric ulcers. By contrast, the H_3-receptor subtype was only discovered in 1983 and H_3-receptor antagonists were used for treatment of central nervous system disorders. The role of H_3 receptors in learning and memory indicates that H_3-receptor antagonists could have a role in the treatment of memory disorders such as Alzheimer's disease [25].

Humans metabolize histidine to urocanic acid through the activity of L-histidine ammonium lyase, to form glutamate and then α-ketoglutarate, which enters the citric acid cycle, or to histamine through the activity of histidine decarboxylase [22].

There are two ways of histamine metabolism in the human body. Nitrogen in the imidazole cycle is methylated by histamine *N*-methyltransferase at the formation of *N*-methylhistamine, which is further oxidized by monoamino oxidase to *N*-methylimidazolylacetic acid [23]. This enzyme is very selective for histamine detoxification and involves *S*-adenosylmethionine as donor of methyl group [22]. Histamine is oxidized by diamino oxidase to imidazolylacetic acid, which is bound to ribose [4, 23].

N-Methylation is the major process responsible for termination of the neurotransmitter actions of histamine in the brain, it is also the major pathway for the metabolism of histamine in bronchial epithelium [26].

Histamine is also converted to inactive acetylhistamine in the intestine, presumably by bacterial enzymes. The human kidney has a considerable capacity for removing histamine from blood. When healthy individuals were infused intravenously with histamine, a large proportion was methylated by the kidney and excreted in the urine and a smaller proportion was excreted unchanged in the urine [22].

1.5. Legal Limits

The Nutritional codex of the Slovak Republic had determined the maximal tolerable limit for the following two BA: histamine (20 mg kg^{-1} in beer and 200 mg kg^{-1} in fish and fish products) and tyramine (200 mg kg^{-1} in cheese) [19].

The European Union (EU) has established regulations for histamine levels, such that they should be below 100 mg kg^{-1} in raw fish, and below 200 mg kg^{-1} in salted fish for species belonging to the *Scombridae* and *Clupeidae* families [27].

A recommended upper limit of 100 to 200 mg kg^{-1} for histamine in meat products has been proposed by the Netherlands Institute of Dairy Research and by the Czech Republic [28].

1.6. Detoxification of BA

In the intestinal tract of mammals affects a detoxification system which is capable of metabolizing normal dietary intake of BA [4]. Under normal conditions in humans exogenous amines absorbed from food are rapidly detoxified by the action of amine oxidase or conjugation, but in the case of allergic individuals or if monoamine inhibitors are applied or when too high levels are consumed the detoxification process is disturbed and BA accumulate in the body [5].

The enzymes monoamino oxidase (MAO, EC 1.4.3.4) and diamino oxidase (DAO, EC 1.4.3.6) play an important role in detoxification process. However, upon intake of high loads of BA in food, this detoxification system is unable to eliminate BA sufficiently.

MAO and DAO occur in the gut epithel and thus oxidation products of BA are getting into the blood circulation [3]. Polyamines are usually in the first place acetylated and consequently oxidized by DAO or polyamino oxidases [1, 23].

People with gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, stomach and colonic ulcers) are also at risk because the activity of oxidases in their intestines is usually lower than that in healthy individuals. In women, there is premenstrual decrease in the activity of B-type MAO and this can also be a problem [7]. Patients, who are taking medicines with inhibiting effect to MAO and DAO such as antihistamines, antimalaria agents, psy-

chopharmaceutics, might have a changed metabolism of BA, which can cause healthy problem [2, 4, 5]. Some amines, especially putrescine and cadaverine, inhibit histamine detoxifying enzymes and thus act as potentiators of histamine toxicity [5]. These amines in the intestinal tract preferably react with MAO and DAO that tend to increase the level of histamine in blood [2]. Aminoguanidine, anserine, carnosine, agmatine, and tyramine inhibit DAO and phenylethylamine, tryptamine, octopamine have inhibiting effect on *N*-methyltransferase. Also, injuries of intestinal mucosa can reduce the function of BA detoxification enzymes [22, 23].

2. OCCURRENCE OF BA IN FOOD

In virtually all foods that contain protein or free amino acids and are subject to conditions enabling microbial or biochemical activity BA can be expected [5].

BA are present in fermented products (*e.g.* cheese 5—4500 mg kg^{-1}, wine 5—130 mg dm^{-3}, beer 2.8—13 mg dm^{-3}, sauerkraut 110—300 mg kg^{-1}) and in improperly kept food (such as fish 2400—5000 mg kg^{-1}, beef liver about 340 mg kg^{-1}, prepared meats 10—700 mg kg^{-1}) [29]. In nonfermented foods these compounds were found useful as indicators and markers of food decomposition [30]. Spoiled foods are also rich in BA and usually contain high levels of putrescine and cadaverine [7].

The tissues of scombroid fish contain high levels of free histidine, which may be converted to histamine by associated microorganisms [6]. Tuna fish and other fish species of the *Scombridae* and *Clupeidae* families have been commonly found to contain high levels of histamine, as a result of inadequate handling and preservation [27]. The quantification of histamine in canned products indicates the thermostability of the molecule [31]. Odours that normally signal decomposition to the organoleptic analyst may be modified, reduced, or eliminated by thermal processing, therefore histamine is a useful indicator of decomposition in scombroid and certain other fish [32].

The formation of high levels of histamine in fish products can be fairly rapid and develops on the number of microorganisms present. Several bacteria are involved in toxicity, such as *Proteus morganii, Hafnia alvei, Acromonas hydrophila, Vibrio alginolyticus, Pseudomonas sp., Klebsiella sp.*, *etc.* These bacteria are capable of producing hazardous amounts of histamine in very short period of time when fish are held at elevated temperatures [31]. Low storage temperatures are used in the fishery industry to control bacterial histamine production [33].

The Biogenic Amine Index (BAI) was proposed for measurement of the quality of raw and processed seafood: BAI = (mg kg^{-1} histamine + mg kg^{-1} putrescine + mg kg^{-1} cadaverine)/1 + mg kg^{-1} spermine + mg kg^{-1} spermidine. A BAI value exceeding 10 is regarded as representing some kind of loss in quality [13].

The manufacturing of sauerkraut coursed in three steps characterized by active microorganisms, which produced BA [19, 34]:

1. Leuconostoc mesenteroides, producing putrescine in content about 250 mg kg^{-1},

2. Lactobacillus sp., producing putrescine and tyramine,

3. Pediococcus cerevisiae, producing histamine in content about 200 mg kg^{-1}.

The BA, especially putrescine, accumulate in sauerkraut brine [6].

The cadaverine, histamine, putrescine, spermidine, and tyramine were found in the lactic acid fermented vegetables (such as carrot and red beet) in content ranging from 1 to 15 mg kg^{-1} [35].

Reports on the hygienic status of leafy vegetables indicate the association of high microbial numbers and the presence of BA, both with the fresh and packed products. Number of the dominant groups, *Pseudomonadaceae* and *Enterobacteriaceae*, typically range between 10^5 cfu g^{-1} and 10^7 cfu g^{-1} for fresh salads and up to 10^8 cfu g^{-1} for processed packed lettuce and salad mixtures [36].

Occurrence of BA in the milk is low, about 1 mg dm^{-3}, but in the cheese their content achieves already 1 g kg^{-1} [18]. Cheese contains proteins, enzymes, cofactors, water, salt, and bacteria, and therefore represents an ideal environment for BA production from free amino acids by decarboxylating enzymes of microorganisms during cheese ripening [37]. Large amounts of BA in cheese could indicate a failure, from a hygienic point of view, in the milk used for cheese products or during the cheese making [38].

Fresh and processed pork contains high levels of adrenaline, spermidine, and spermine but low levels of noradrenaline, putrescine, histamine, cadaverine, and tyramine [5]. Large amounts of cadaverine present in beef have been associated with heavy contamination by *Enterobacteriaceae*. High production of tyramine (100 mg kg^{-1}) in sausages was associated with lactic acid bacteria contamination [39]. The occurrence of BA in fermented sausages may originate from contaminated raw material or from the fermentation process itself. For example, *Carnobacterium divergens* was found responsible for tyramine formation in vacuum-packed meat, the formation of putrescine and cadaverine was caused by *Enterobacteriaceae* or strains of *Pseudomonas* [40].

There are three possible origins for BA in wines. They can be present in the must, can be formed by yeasts during malolactic fermentation or result from the action of bacteria involved in malolactic fermentation [41]. Predominant BA in wine are histamine, tyramine, putrescine, isophenylamine, and β-phenylethylamine. In wines from different European

origin maximum levels were recently reported as high as 16.6 mg dm^{-3} for histamine, 20.2 mg dm^{-3} for tyramine, and 76 mg dm^{-3} for putrescine. Mean levels of histamine were at 3.63 mg dm^{-3} for French wines, 2.19 mg dm^{-3} for Italian wines, and 5.02 mg dm^{-3} for Spanish wines [11].

3. DETERMINATION OF BA

For determination of BA numbers of analytical methods were developed [4].

The complex matrix sample, the presence of potentially interfering compounds, and the occurrence of several BA simultaneously are typical problems encountered in the analysis [42].

Preclean-up includes extraction of sample with suitable extracting reagent [4]. The following solvents have been suggested for the extraction of BA: 0.6 M-perchloric acid, 5—10 % trichloroacetic acid, and 0.1M-HCl [6]. For milk product is suitable extraction of BA with methanol at the increased temperature (60 °C) [4]. Some authors suggest extraction with butanol or butanol—chloroform at basic pH for the clean-up of samples. Some parameters significantly influence the extraction and recovery of BA (pH and the degree of saturation of the extracting solution by salts) [43]. The relative extraction efficiencies of these solvents depend on type and nature of the BA and the food from which they are being extracted [6]. The solid-phase extraction (SPE) has provided a more efficient choice than classical liquid-liquid extraction by virtue of the wide availability of sorbent materials and of the fact that the need to dispose of organic solvents is avoided [44].

The analytical methods used for separation and quantification of BA are mainly based on chromatographic methods: gas chromatography (GC), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) with precolumn or postcolumn derivatization techniques [45].

Aliphatic BA do not show pronounced absorption bands in the UV VIS region, so that usual spectrometric detectors cannot be used [46]. The direct analysis of BA without derivatization by means of ion-pair chromatography has been suggested using octylamine or heptanesulfonate as ion-pair reagents [43]. For the separation of ion pairs of the BA the usual reversed-phase columns with C_{12}—C_{18} aliphatic chains phenyl residues bound to a silica core are suitable [47].

The HPLC procedures involve pre- or postcolumn derivatization step [30]. Different chemical reagents have been used for the BA analysis, for example ninhydrine and *o*-phthalaldehyde, as a postcolumn derivatization reagent, dansyl and dabsyl chloride, benzoyl chloride, fluoresceine, 9-fluorenylmethyl chloroformate with precolumn derivatization [2, 32, 46, 48].

Dansyl chloride has been the most widely used reagent for derivatization of BA prior to HPLC. Light sensitivity and limited stability of dansyl chloride lead some authors to the use of different derivatization agents. Benzoyl chloride is an inexpensive, stable, easily accessible chemical and its purity is less critical than that of dansyl chloride. Benzamides are not sensitive to light, reaction proceeds at room temperature in alkaline media and no buffers are required [30].

For the detection fluorescence, UV, and electrochemical detectors are used. Electrochemical detectors are based on the oxidation of amino groups [48].

A review of preseparation procedures as well as HPLC conditions to be used for determination of BA in food is in Table 1.

TLC method is especially popular in plant biochemistry [30]. The TLC procedure is of value for semiquantitative screening of food [67]. TLC with preclean-up of sample and derivatization of BA can be used to detect chlorides, 3,5-dinitrobenzamides dansyl and fluorescein derivatives of BA [1]. Dansyl chloride reacts with primary and secondary amino groups and fluorescein reacts only with primary amino groups [47].

Dansylated BA emit the energy of absorbed long-wave UV light as fluorescent light, enabling the analyst to detect these compounds at low levels on the chromatogram. The natural fluorescence (under UV light) of the separated spots of dansylated BA from sample extract can be compared with that of standard spot by eye [67]. The fluorescent dansyl derivative zones are visualized and marked with the aid of a suitable UV-light source (360 nm) [68]. TLC one-dimensional developing techniques enable to give sufficient separation of BA, it is because other interfering compounds, such as amino acids also moved along with the analyzed BA [47]. The multidimensional developing technique improved resolution of BA from each other and from interfering materials, and compact and intense spots were obtained. For visual detection of eluated BA, various systems of detecting agents such as ninhydrin, *o*-phthalaldehyde (for chloride of BA) solution of ethanol, and naphthylamine can be used [67].

GC is not so often applied for the determination of BA. Because of inherent tailing problems, derivatization is also frequently used [30]. The BA are determined in derived forms as trifluoroacetyl, trimethylsilyl or 2,4-dinitrophenyl derivatives [1]. The columns used in the GC are capillary or filling. The capillary columns allowed better separation of BA. The detectors for the determination of BA by GC are healthy-conductivity, flame ionization, and electron capture detector [1, 4].

Reports dealing with separation of BA by capillary electrophoresis (CE) are not numerous to data. There are three possible approaches to solve this task [30]:

1. Aromatic or heterocyclic BA can be separated in selected buffer systems without derivatization.

2. Polyamines are determined either derivatized

Table 1. Preseparation Procedures as well as HPLC Conditions to be Used for Determination of BA in Food

Amine	Sample	Sample treatment	Derivatization	Column/ Stationary phase	Mobile phase	Detection λ_i	Ref.
Histamine	Fish, sauerkraut, wine	Extraction with 6 % perchloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(250 mm × 4.6 mm), 5 μm INERSIL ODS 2	Phosphate buffer (pH 7) and acetonitrile (φ_r = 825 + 152)	Fluorimetric (excitation 340 nm, emission 455 nm)	[48]
Putrescine Cadaverine Histamine Spermidine Spermine	Fish	Extraction with 5 % trichloroacetic acid	Precolumn derivatization with dansyl chloride (60 min, 55 °C)	(250 mm × 3 mm), 10 μm RP-8 LICHROSORB	Gradient elution with methanol, acetonitrile and 0.02 M-acetic acid	UV (254 nm)	[49]
Various amines	Fish	Extraction with 5 % trichloracetic acid (heating 60 °C for 15 min)	Precolumn derivatization with danzyl chloride (60 min, 55 °C)	(250 mm × 4.6 mm) ALTEX ULTRASPHERE – Si	Hexane—ethyl acetate (φ_r = 40 : 60) with addition of 0.01 % aminoethanol	Fluorimetric (excitation 333 nm, emission 470 nm)	[50]
Putrescine Cadaverine Histamine Tyramine	Various food, cabbage juice	Extraction with 10 % trichloroacetic acid	Precolumn derivatization with dansyl chloride (20 min, 40 °C)	(250 mm × 8 mm), 4 μm NUCLEOSIL C_{18}	Methanol—acetonitrile—water (φ_r = 2 : 1 : 1)	UV (254 nm)	[2, 10]
Various amines	Cheese	Extraction with 0.1 M-hydrochloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(300 mm × 3.9 mm), 10 μm μ BONDAPAK C_{18}	*A*: 0.2 M-sodium acetate, 10 mM-diethyl ether *B*: ethanol—acetonitrile—sodium octanesulfonate (φ_r = 1 : 9 : 1)	Fluorimetric (excitation 340 nm, emission 445 nm)	[51]
Tyramine	Meat mixture	Extraction with 10 % trichloroacetic acid	Precolumn derivatization with dansyl chloride	(125 mm × 4 mm), 5 μm SPHERISORB ODS 2	*A*: 0.1 M-ammonium acetate *B*: acetonitrile	UV (254 nm)	[52]
Various amines	Wine	Extraction with 0.1 M-hydrochloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(200 mm × 4.6 mm) NUCLEOSIL 100 $5C_{18}$	0.08 M-acetic acid and acetonitrile	Fluorimetric (excitation 230 nm, emission 440 nm)	[53]
Various amines	Cheese, red wine, sausages	Extraction with 0.1 M-hydrochloric acid	Precolumn derivatization with dansyl chloride (heating to 70 °C for 15 min)	(150 mm × 4.6 mm), 3 μm SPHERISORB ODS 2	*A*: 4 % dihydrogen phosphate, dimethylformamide, 0.18 % trimethylamine (pH 6.55) *B*: 80 % acetonitrile, 10 % *tert*-butyl ether, 10 % water	UV (436 nm)	[47]
Various amines	Meat	Extraction with 0.4 M-perchloric acid	Precolumn derivatization with dansyl chloride (heating to 40 °C for 45 min)	(125 mm × 4 mm), 5 μm SPHERISORB ODS 2	Ammonium acetate and acetonitrile	UV (254 nm)	[13]
Various amines	Meat	Extraction with 0.6 M-perchloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(150 mm × 3.9 mm), 4 μm NOVAPAK C_{18}	Octanesulfonic acid	Fluorimetric (excitation 340 nm, emission 445 nm)	[54]

Table 1. (Continued)

Amine	Sample	Sample treatment	Derivatization	Column/ Stationary phase	Mobile phase	Detection λ_i	Ref.
Various amines	Food	Extraction with 10 % trichloroacetic acid	Postcolumn derivatization with *o*-phthaldialdehyde	(150 mm × 6 mm) SHIM PAK CLC ODS	*A*: 0.1 M-sodium hydrochloride, 0.01 M-sodium hexanesulfonate *B*: eluent *A* and methanol ($\varphi_r = 1 : 3$)	Fluorimetric (excitation 345 nm, emission 455 nm)	[55]
Putrescine Cadaverine Histamine	Fish	Extraction with 5 % trichloroacetic acid	Precolumn derivatization with fluorescein	(100 mm × 4 mm) PHENOMENEX IB-SIL	0.02 M-phosphate buffer (pH 7.2) and acetonitrile	Fluorimetric (excitation 390 nm, emission 475 nm)	[56]
Various amines	Fermented sausages	Extraction with 0.6 M-perchloric acid	Postcolumn derivatization with *o*-phthaldialdehyde and 3-mercaptopropionic acid	(250 mm × 4.6 mm) NUCLEOSIL 10 7 C18	*A*: 0.05 M-hexanesulfonic acid, 0.1 M-sodium dihydrogenphosphate (pH 3.5) *B*: eluent *A* and acetonitrile ($\varphi_r = 3 : 1$)	Fluorimetric (excitation 340 nm, emission 455 nm)	[57]
Histamine	Sardine	Extraction with 10 % trichloroacetic acid	Postcolumn derivatization with *o*-phthaldialdehyde	(250 mm × 4.6 mm), 5 μm ODS RP 18	Gradient elution with 0.1 M-sodium acetate (pH 6.2)	Fluorimetric (excitation 350 nm, emission 450 nm)	[31]
Various amines	Fish, fish products	Extraction with 0.6 M-perchloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(150 mm × 3.9 mm), 4 μm NOVAPAK C_{18}	Gradient elution with *A*: 0.1 M-sodium acetate and 10 mM-octanesulfonic acid (pH 5.2) *B*: acetonitrile, 0.2 M-sodium acetate and 10 mM-octanesulfonic acid (pH 4.5)	Fluorimetric (excitation 340 nm, emission 495 nm)	[58]
Various amines	Wine	Extraction with toluene	Precolumn derivatization with dansyl chloride	(150 mm × 3 mm), 5 μm RP SEPERON SGX C_{18}	Acetonitrile and ammonium acetate ($\varphi_r = 55 : 45$)	UV (254 nm)	[59]
Various amines	Wine	Extraction with freon 11-dichloromethane and a mixture of butanol, methylene, and chloride	Precolumn derivatization with danzyl chloride	(200 mm × 4.6 mm) NUCLEOSIL $5C_{18}$	*A*: 30 % acetonitrile in water *B*: 60 % acetonitrile in water with 15 % methanol	Fluorimetric (excitation 338 nm, emission 455 nm) or UV (250 nm)	[60]
Various amines	Cheese	Extraction with 0.1 M-hydrochloric acid	Precolumn derivatization with dansyl chloride (heating to 60 °C for 40 min)	(150 mm × 4.6 mm), 3.5 μm SPHERISORB 3STG Waters	Acetonitrile	UV (254 nm)	[61]

Table 1. (Continued)

Amine	Sample	Sample treatment	Derivatization	Column/ Stationary phase	Mobile phase	Detection λ_i	Ref.
Various amines	Milk, cheese	Extraction with 0.6 M-trichloroacetic acid	Postcolumn derivatization with *o*-phthaldialdehyde	(150 mm × 3.9 mm), 4 μm NOVAPAK C_{18}	*A*: 0.1 M-sodium acetate, 10 mM-sodium octanesulfonate (pH 5.3) *B*: acetonitrile, 0.2 M-sodium acetate, 10 mM-sodium octanesulfonate (pH 4.5)	Fluorimetric (excitation 340 nm, emission 445 nm)	[38]
Various amines	Fish, cheese, meat products	Extraction with 0.1 M-hydrochloric acid	Precolumn derivatization with dansyl chloride (heating to 40 °C for 60 min)	(150 mm × 1.6 mm), 3 μm SPHERISORB 3S T6	Mixture of acetonitrile and water	UV (254)	[62]
Various amines	Wine	Extraction with 0.1 M-hydrochloric acid	Precolumn derivatization with dansyl chloride (heating to 70 °C for 21 min)	(244 mm × 4.4 mm), 5 μm LICHROSPHER 100	*A*: 0.02 M-sodium acetate, 10 % dimethylformamide and 0.3 % trimethylamine *B*: acetonitrile, butyl methyl ester, and water (φ_r =87.5 : 10 : 2.5)	UV (446 nm)	[63]
Various amines	Canned fish	Extraction with methanol	Precolumn derivatization with dansyl chloride	(150 mm × 3.9 mm), 4 μm NOVAPAK	Mixture of acetonitrile—methanol—water (φ_r = 1 : 2 : 1)	UV (214 nm)	[64]
Various amines	Wine	Extraction with 0.1 M-hydrochloric acid	Postcolumn derivatization with *o*-phthaldialdehyde	(250 mm × 4 mm), 5 μm LICHROSPHER RP IS	*A*: 0.05 M-sodium acetate (pH 7.2) *B*: methanol and sodium acetate (φ_r = 90 : 10)	Fluorimetric (excitation 340 nm, emission 450 nm)	[65]
Various amines	Lactic acid-fermented vegetable	Extraction with 5 % perchloric acid	Precolumn derivatization with dansyl chloride (heating to 40 °C for 30 min)	(250 mm × 4 mm), 4 μm LICHROSORB RP 18	*A*: acetonitrile and 0.02 M-acetic acid (φ_r = 1 : 9) *B*: acetic acid, methanol, and water (φ_r = 2 : 9 : 9)	UV (254 nm)	[66]

(usually in electrokinetic capillary chromatography mode) or

3. their detection must be indirect.

CE has several advantages: it is simple, rapid, cost-effective, and reliable, making it a very useful tool for screening a large number of samples in a short period of time [32].

Capillary isotachophoresis is used to quantification of histamine in fish [69] and different BA in the lactic acid-fermented vegetable juices [70].

Fluorometric methods are used owing to fluorescence of BA at some pH and reaction of BA with suitable agents to the fluorescence derivatives. The histamine can be determined by *o*-phthalaldehyde and tyramine by β-naphthol [1].

For determination of BA it is possible to use amino acid analyzer, when at the suitable chosen conditions not only BA, but also their precursors amino acids are determined [19].

Recently due to the commercial availability of enzymes like MAO and putrescine oxidase several groups tried to couple the enzymatic reactions with electrochemical sensors in order to obtain simple and reproducible biosensors. In some cases the BA have been coupled with oxygen sensors or hydrogen peroxide sensors. The biosensor procedure has advantages, such as low cost, short analysis time, simplicity of use and it can be used outside an organized laboratory. The biosensors show a low detection limit with life-time estimated at one month with a 10—30 % loss of sensitivity [45].

Lopéz-Sabater [71] described simple enzymatic

method for evaluation of histamine in fish based on the action of DAO on the histamine causing the formation of hydrogen peroxide. The addition of a second enzyme, a peroxidase, in the presence of hydrogen peroxide and a chromogen (leucocrystal violet) in reduced form (colourless) facilitated its oxidation into crystal violet (coloured). Following a two-hour incubation period, absorbance was measured at $\lambda = 596$ nm.

The principle of immunologic methods is an interaction between antigen and antibody, which leads to formation of a complex [72].

CONCLUSION

The BA represent a group of low-molecular-mass organic bases occurring in all organisms [29]. Enzymatic decarboxylation of free amino acids and other metabolic processes can lead to the presence of BA in food [46].

Under normal conditions in humans exogenous amines absorbed from food are rapidly detoxified by the action of amine oxidases or by conjugation, but in the case of allergic individuals or if MAO inhibitors are applied the detoxification process is disturbed and BA accumulate in the body.

Estimation of BA is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of food [6].

Abbreviations

BA	biogenic amines
MAO	monoamino oxidase
DAO	diamino oxidase
SPE	solid-phase extraction
GC	gas chromatography
TLC	thin-layer chromatography
HPLC	high-performance liquid chromatography
CE	capillary electrophoresis

REFERENCES

1. Ascar, A. and Treptow, H., *Biogene Amine in Lebensmitteln.* Ulmer, Stuttgart, 1986.
2. Greif, G., Greifová, M., and Drdák, M., *Potrav. Vědy 15*, 129 (1997).
3. Křížek, M. and Kalač, P., *Czech. J. Food Sci. 16*, 159 (1998).
4. Silla-Santos, M. H., *Int. J. Food Microbiol. 29*, 213 (1996).
5. Halász, A., Baráth, A., Simon-Sarkadi, L., and Holzapfel, W., *Trends Food Sci. Technol. 5*, 49 (1994).
6. Bardócz, A., *Trends Food Sci. Technol. 6*, 346 (1995).
7. Lovaas, W., *JAOCS 68,* 357 (1991).
8. Ogawa, H., *J. Jpn. Oil Chem. Soc. 45*, 1332 (1996).
9. Greif, G., Greifová, M., Dvoran, J., Karovičová, J., and Buchtová, V., *Czech J. Food Sci. 17*, 21 (1999).
10. Bodmer, S., Imark, C., and Kneubuhl, M., *Inflam. Res. 48*, 300 (1999).
11. Beutling, D., *Arch. Lebensmittelhyg. 47*, 104 (1996).
12. Maijala, R. L., *J. Food Prot. 56*, 129 (1993).
13. Bunčič, S., *Int. J. Food Microbiol. 17*, 309 (1993).
14. Stratton, J. E., Hutkins, R. W., and Taylor, S. L., *J. Food Prot. 54*, 867 (1991).
15. Maijala, R. L., *J. Food Sci. 60*, 1190 (1995).
16. Leuschner, R. G. K. and Hammes, W. P., *Meat Sci. 49*, 296 (1998).
17. Leuschner, R. G. K. and Hammes, W. P., *J. Food Prot. 61*, 878 (1998).
18. Kolesárová, E., *Bull. PV 34*, 122 (1995).
19. Halász, A., Baráth, A., and Holzapfel, W., *Z. Lebensm.–Unters. Forsch. 208,* 438 (1999).
20. Diaz-Cinco, M. E., *J. Food Sci. 57*, 356 (1992).
21. Lehane, L. and Olley, J., *Int. J. Food Microbiol. 58*, 37 (2000).
22. Bentley, S., Bottarelli, A., and Bonardi, S., *Ing. Alimen. 5*, 35 (1995).
23. Stratton, J. E., Hutkins, R. W., and Taylor, S. L., *J. Food Prot. 54*, 466 (1991).
24. Sanson, C., *DDT 5*, 95 (2000).
25. Preuss, Ch. V., Wood, T. C., and Szumlanski, L., *Mol. Pharmacol. 53*, 717 (1998).
26. Gallardo, J. M., Sotelo, C. G., and Perez-Martin, R. I., *Z. Lebensm.–Unters. Forsch. 204*, 340 (1997).
27. Montel, M. Ch., Masson, F., and Talon, R., *Sci. Aliments 19,* 254 (1999).
28. Soufleros, E., Barrios, M. L., and Bertrand, A., *Am. J. Enol. Vitic. 49,* 277 (1998).
29. Křížek, M. and Pelikánová, T., *J. Chromatogr. 815*, 250 (1998).
30. Pacheco-Aquilar, R., *J. Food Comp. Anal. 11*, 195 (1998).
31. Kovács, A., *J. Chromatogr. 836*, 313 (1999).
32. Wei, C. I., *J. Food Sci. 55,* 63 (1990).
33. Kalač, P., *Food Chem. 67,* 280 (1997).
34. Hornero-Méndez, D. and Fernández, A., *J. Food Prot. 60*, 419 (1997).
35. Simon-Sarkadi, L., Holzapfel, W., and Halasz, A. J., *Food Biochem. 17*, 418 (1994).
36. Antolini, F., *Ital. J. Food Sci. 11*, 346 (1999).
37. Rodriquez, S. N., Nogues, M. T., and Vidal-Carou, M., *J. Agric. Food Chem. 48*, 5123 (2000).
38. Masson, F., Talon, R., and Montel, M. C., *Int. J. Food Microbiol. 32*, 207 (1996).
39. Straub, B. W., *Z. Lebensm.–Unters. Forsch. 199*, 12 (1994).
40. Acre, L., Rios, A., and Valcarcel, M., *J. Chromatogr. 803*, 260 (1998).
41. Shalaby, A. R., *Food Chem. 65*, 121 (1999).
42. Arlorio, M., Coisson, J. D., and Martelli, A., *Ital. J. Food Sci. 11*, 360 (1999).
43. Cobo, M. and Silva, M., *J. Chromatogr. 848*, 115 (1999).
44. Carsol, M. A. and Mascini, M., *Talanta 50*, 148 (1999).
45. Casella, I. G., Gatta, M., and Desimoni, E., *Food Chem. 73*, 372 (2001).
46. Seiler, N., *J. Chromatogr. 379*, 176 (1986).
47. Bockhardt, A., Krause, I., and Klostermeyer, K., *Z. Lebensm.–Unters. Forsch. 203*, 70 (1996).
48. Beljaars, P. R., *J. AOAC Int. 81*, 998 (1998).

49. Rosier, J. and Van Peteghem, C., *Z. Lebensm.–Unters. Forsch. 186*, 28 (1988).
50. Lebiedzinska, A., *Z. Lebensm.–Unters. Forsch. 192*, 243 (1991).
51. Vale, S. and Glória, B. A., *Food Chem. 63*, 348 (1998).
52. Masson, F., Johansson, G., and Montel, M. C., *Meat Sci. 52*, 69 (1999).
53. Lethonen, P., *Z. Lebensm.–Unters. Forsch. 194*, 437 (1992).
54. Hernandez-Jover, T., *J. Agric. Food Chem. 44*, 2715 (1996).
55. Suzuki, S., *J. Chromatogr. 508*, 228 (1990).
56. Gingerich, T. M., *J. Food Prot. 62*, 1037 (1999).
57. Straub, A., *Z. Lebensm.–Unters. Forsch. 197*, 232 (1993).
58. Veciana-Nogues, M. T., *J. AOAC Int. 78* 1050 (1995).
59. Belajová, E. and Kolesárová, E., *Bull. PV 35*, 134 (1996).
60. Lethonen, P., *Z. Lebensm.–Unters. Forsch. 183*, 181 (1986).
61. Galgano, F., Suzzi, G., Favati, F., Carusoo, M., Martuscelli, M., and Gardini-Salzano, F., *Int. J. Food Sci. Technol. 36*, 160 (2001).
62. Moret, J. and Conte, L. C., *J. Chromatogr. 729*, 369 (1996).
63. Romero, R., *J. Chromatogr. 871*, 83 (2000).
64. Valls, J. E., *J. Aquat. Food Prod. 8*, 91 (1999).
65. Mafra, I., *Am. J. Enol. Vitic. 50*, 132 (1999).
66. Preib, U., Santos, C. A., Ziegler, W., and Wallnöfer, P. R., *Dtsch. Lebensm.-Rundsch. 95*, 222 (1999).
67. Shalaby, A. R., *Food Chem. 52*, 372 (1995).
68. Shalaby, A. R., *Food Chem. 49*, 310 (1994).
69. Voldřich, M., Hrdlička, J., and Opatová, M., *Potrav. Vědy 6*, 103 (1988).
70. Karovičová, J., Kohajdová, Z., and Lukáčová, D., *Bull. PV 41*, 195 (2002).
71. Lopéz-Sabater, E. I., *Food Addit. Contam. 10*, 602 (1993).
72. Rauch, P., *Potrav. Vědy 9*, 16 (1991).