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Analytical Methods

# A review of the liquid chromatographic methods for the determination of biogenic amines in foods

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ABSTRACT

Biogenic amines (BAs) are biologically active molecules which have aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures. They can be detected in raw and processed foods which are formed and degraded through several pathways during the metabolic processes of animals, plants and microorganisms. The identification and quantitation procedures of BAs in food samples are very important, because BAs are considered as the indicators of food quality and freshness. The determination of BAs are commonly achieved by separation techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). In this article, analysis of BAs in foods were reviewed from 2007 to present.


## 1. Introduction

Biogenic amines (BAs) are organic bases endowed with biological activity, which are frequently found in fermented foods and beverages (Novella-Rodríguez, Veciana-Nogués, Trujillo-Mesa, & Vidal-Carou, 2002). They mainly come from the decarboxylation of amino acids (ten Brink, Damink, Joosten, & Huis in 't Veld, 1990). In low concentrations, biogenic amines are essential for many physiological functions (Teti, Visalli, & McNair, 2002). However, if these compounds are consumed in high quantities, several toxicological problems arise (Pérez-Serradilla & Luque de Castro, 2008; Saaid et al., 2009a; Shalaby, 1996).

BAs are classified as mono- or polyamines (PAs) according to their amine content. PAs are involved in nearly each step of nucleic acid and protein synthesis, because of this every organ of the body requires them for its growth, renewal and metabolism (Shalaby, 1996). Therefore the requirement for PAs increases rapidly in growing tissues. Besides, the unneccessary PA intake causes tumour growth. One of the directions in cancer therapy is to limit the intake of PAs (Moinard, Cynober, & de Bandt, 2005).

Also some of BAs (i.e. putrescine, cadaverine, spermidine and spermine) may react with nitrite and produce volatile nitrosamines which are defined as carcinogenic compounds (Wei et al., 2009). Therefore, formation of nitrosamines in the presence of BAs in a wide range of foods, especially in meat and meat products bring about an additional toxicological risk.

Table 1 shows the chemical properties of the most important BAs occurring in foods (Histamine, putrescine, cadaverine, tyramine, tryptamine, b-phenylethylamine, spermine, and spermidine) such as molecular structure, molecular weight (http://pubchem.ncbi.nlm.nih.gov), pK value (Kvasnička & Voldřich, 2006; Saaid et al., 2009a; and Takeda et al., 1983).

BAs in foods are of great interest not only due to their toxicity, they can also be used as good indicators of spoilage (De Borba and Rohrer, 2007). For this reason, it is important to monitor BA levels in foods. There are various analytical methods for the quantification of BAs in foods such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and electro analytical methods.

The presented article describes recent analytical approaches for the analysis of BAs from 2007 to 2011, as a complementary to previous review (Onal, 2007).

## 2. Analytical methods

### 2.1. Liquid chromatographic methods

Liquid chromatographic separation methods are popular and useful for selective and sensitive determination of BAs in foods. Various detection techniques are used for the quantitation of BAs in food samples.

#### 2.1.1. Ultraviolet or fluorescence detection

The most frequently used methods for BA analysis are based on derivatization reactions and separation processes followed by

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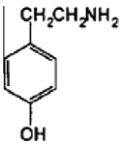
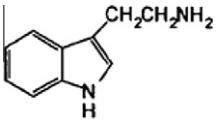
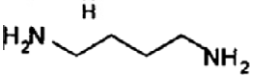
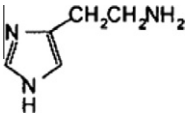
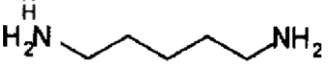
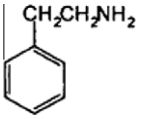
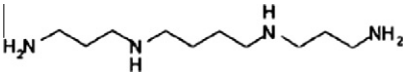
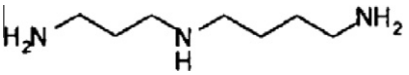
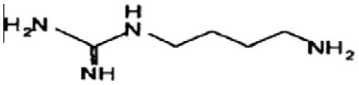
**Table 1**
Some chemical properties of biogenic amines [a] http://pubchem.ncbi.nlm.nih.gov/, [b] Kvasnička and Voldřich (2006); Saaid et al. (2009a), and Takeda et al. (1983).

| Name | Abbreviation | Molecular formula[a] | Structure formula | p$K$[b] | Molecular weight[a] |
|---|---|---|---|---|---|
| Tyramine | TYR | $C_8H_{11}NO$ |  | p$K$ = 9.6 | 137.2 |
| Tyrptamine | TRP | $C_{10}H_{12}N_2$ |  | p$K$ = 10.2 | 160.2 |
| Putrescine | PUT | $C_4H_{12}N_2$ |  | p$K_1$ = 10.8 p$K_2$ = 9.4 | 88.2 |
| Histamine | HIS | $C_5H_{10}N_3$ |  | p$K_1$ = 9.8 p$K_2$ = 6.0 | 111.1 |
| Cadaverine | CAD | $C_5H_{14}N_2$ |  | p$K_1$ = 11.0 p$K_2$ = 9.9 | 202.2 |
| Phenylethylamine | PEA | $C_8H_{11}N$ |  | p$K$ = 10.0 | 121.2 |
| Spermine | SPM | $C_{10}H_{26}N_4$ |  | p$K_1$ = 11.50, p$K_2$ = 10.95 p$K_3$ = 9.79 p$K_4$ = 8.90 | 202.3 |
| Spermidine | SPD | $C_7H_{19}N_3$ |  | p$K_1$ = 9.5, p$K_2$ = 10.8 p$K_3$ = 11.6 | 145.3 |
| Agmatine | AGM | $C_5H_{14}N_4$ |  | p$K_1$ = 12.5 | 130.2 |

ultraviolet (UV) or fluorescence (FL) detection. Derivatization is essential for such kind of detections because most of BAs are lack of chromophore. The derivatization reactions occur via amino group with various types of tagging reagents, such as *o*-phthaldialdehyde (OPA), dansyl chloride (dansyl-Cl), 4-chloro-3,5-dinitrobenzotrifluoride (CNBF), 1,2-naphthoquinone-4-sulfonate (NQS), 6-aminoquinolyl-*N*-hydroxysuccinimidy (AQC), *N*-hydroxysuccinimide ester (DMQC-Osu).

*2.1.1.1. Ultraviolet detection.* Soufleros, Bouloumpasi, Zotou, and Loukou (2007) investigated the BA levels in Greek wines. Samples were analysed by reverse phase (RP)-HPLC with UV detection after pre-column derivatization with dansyl-Cl and subsequent solid-phase extraction (SPE) of the derivatives through C18 cartridges. It was revealed that ninety percent of the wine samples were at acceptable levels of BA content. BA levels of sucuk (Turkish dry fermented sausage) were determined by using HPLC method with diode array detector (DAD) after pre-column derivatization with dansyl-Cl. Putrescine and cadaverine are detected as 93% and 87% of the samples, respectively. Spermine and spermidine were detected in ranges from not detected to 16.4 and from not detected to 10.7 mg $kg^{-1}$, respectively. Histamine was found to be between 50 and 100 mg $kg^{-1}$ as 17% of the samples. Tryptamine was detected in the range of 1.2–82.3 mg $kg^{-1}$. Tyramine contents of all samples were within the acceptable level. Phenylethylamine was found in 17 of the 30 samples and levels in all detected samples were found to be lower than 25 mg $kg^{-1}$ (Gençcelep, Kaban, Aksu, Öz, & Kaya, 2008). BAs except histamine were determined by HPLC after derivatisation with dansyl-Cl conjugates at 55 °C in wine samples. The mobile phase consisted of ethanol, acetonitrile, water, tris buffer at pH 8 and a gradient elution programme was used. Histamine was determined by a radio-immuno assay method. Amine concentrations were similar in 100 selected high-quality red wines made from seven different cultivars except two ones, which showed significantly higher tryptamine and cadaverine levels. (Konakovsky et al., 2011).

HPLC–UV analysis of BAs were carried out using hollow fibre liquid-phase microextraction (LPME) with in situ derivatization using dansyl-Cl in shrimp sauce and tomato ketchup samples. The samples were made alkaline (pH 9.5) with saturated sodium hydrogen carbonate before derivatization. The derivative was extracted with liquid–liquid extraction (LLE) technique with dihexyl ether (Saaid et al., 2009a). The BAs of Herby cheese were determined with HPLC method as their dansyl derivatives on 30 samples obtained from retail markets in East Anatolia Region, Turkey. The tyramine (range 18.0–1125.5 mg $kg^{-1}$) and cadaverine varied from not detected to 1844.5 mg $kg^{-1}$ were the most important BAs. Histamine content was found higher than 100 mg $kg^{-1}$. The concentration of amines in some cheeses was found much higher than the toxic dose limits. The analysis were performed with HPLC combined to DAD. The samples determined as their dansyl-Cl derivatives. Acetonitrile and ammonium acetate solutions were used as mobile phase with a flow rate 1 ml $min^{-1}$ (Andiç, Genççelep & Köse, 2010). Pre-column (derivatization with dansyl-Cl) and post-column (derivatization with OPA) derivatization methods for HPLC were modified using column particles of 1.8–3 μm in diameter for the identification and quantification of nine BAs in lean canned tuna and fatty frozen herring by Simat and Dalgaard. The methods were more rapid with reduced amount of eluent compared to the corresponding classical HPLC methods (Simat & Dalgaard, 2011). BA contents were analyzed in boza samples (traditional fermented beverage in Turkey) by HPLC with C18 column after derivatization