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# Levels of biogenic amines in typical vegetable products

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**Abstract**

Biogenic amines were determined in frozen spinach purée, ketchup, concentrated tomato pasta and frozen green pea as N-benzamides, by micellar electrokinetic capillary chromatography. Putrescine and spermidine were observed in most samples at detectable levels, while histamine, spermine and cadaverine concentrations were frequently below detection limits. Maximum mean levels (33.6 and 52.5 mg $kg^{-1}$ of tyramine and putrescine, respectively) were found in ketchup and 46.6 mg $kg^{-1}$ of spermidine in pea. Levels of histamine and spermine were low, and those of cadaverine and tryptamine low or medium. Thus, biogenic amines in the observed vegetable products should not be of a risk for healthy consumers. 

*Keywords:* Biogenic amines; Vegetable products; Spinach; Ketchup; Tomato pasta; Green pea

## 1. Introduction

Increased levels of biogenic amines (BAs) in foods have been of interest from several points of view. Histamine (HI) and tyramine (TY) are amines with the best known deleterious psychoactive and/or vasoactive effects, while the polyamines, putrescine (PUT), spermidine (SPD) and spermine (SPM), are considered as constituents affecting several roles in cellular metabolism. They are involved in growth of cells, tissues and organs and their intake in foods may be beneficial under some physiological conditions (Bardócz et al., 1995). Minimum information has been available on health effects of tryptamine (TR). Putrescine and cadaverine (CAD) may potentiate vasoactive effects of other amines.

Most amines in foods originate from corresponding amino acids which have undergone decarboxylation by putrefactive bacteria (mainly PUT, CAD and HI) or lactic acid bacteria (mainly TY). Several criteria for food quality assessment are based on BAs determination.

Increased and high BAs levels have been observed in proteinaceous and/or fermented foods, such as scombroid fish, cheeses, ripened salami, sauerkraut, wine and beer (for detailed review see Beutling, 1996). Different opinions exist about BAs levels in non-fermented vegetables and fruit products. Data from the 1960's and 1970's, often cited in reviews and respected by nutritionists and physicians, suggested relatively high levels of HI and/or TY, while few results in later papers were auspicious (Maxa & Brandes, 1993; Simon-Sarkadi, Holzapfel, & Halasz, 1994).

The objective of the present work was to survey contents of seven BAs in several commonly consumed vegetable products.

## 2. Materials and methods

### 2.1. Sampling

In total, 89 samples of four products were taken from supermarkets and retail shops from March 1999 to February 2001: 32 samples of frozen spinach purée, 24 ketchup samples, 19 samples of concentrated tomato pasta in tins and 14 samples of frozen green pea. Foods were produced by Czech, Hungarian, Italian and Polish manufacturers. Harvest years were not regularly declared; however, nearly all products were sampled during the initial half of their recommended shelf lives.

Prior to analysis, frozen products were thawed in original packages using running warm water; thawed pea was homogenized in a mortar with small amount of 0.6 M perchloric acid.

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Table 1
Statistical characteristics of amine concentrations (mg $kg^{-1}$) in frozen spinach purée ($n = 32$)

| Amine | $n >$ DL[a] | Mean for > DL[b] | $s_x$ | $x_{min}$ | $x_{max}$ |
|---|---|---|---|---|---|
| Histamine | 8 | 3.4 | 2.4 | 2.1 | 9.8 |
| Tyramine | 17 | 10.2 | 6.5 | 3.5 | 31.8 |
| Tryptamine | 16 | 7.4 | 4.7 | 1.4 | 14.3 |
| Putrescine | 25 | 12.9 | 23.7 | 2.5 | 119 |
| Cadaverine | 8 | 3.8 | 2.0 | 1.9 | 7.7 |
| Spermidine | 31 | 7.3 | 3.8 | 1.3 | 15.4 |
| Spermine | 11 | 2.2 | 0.8 | 1.4 | 3.8 |

[a] Number of samples with concentrations above detection limit.
[b] Mean values for samples with concentrations above detection limit.

Table 2
Statistical characteristics of amine concentrations (mg $kg^{-1}$) in ketchup ($n = 24$)

| Amine | $n >$ DL | Mean for > DL | $s_x$ | $x_{min}$ | $x_{max}$ |
|---|---|---|---|---|---|
| Histamine | 9 | 6.5 | 4.7 | 2.0 | 18.0 |
| Tyramine | 20 | 33.6 | 36.6 | 4.5 | 149 |
| Tryptamine | 22 | 20.5 | 20.9 | 1.3 | 72.5 |
| Putrescine | 22 | 52.5 | 54.1 | 2.4 | 165 |
| Cadaverine | 11 | 30.7 | 39.2 | 1.4 | 131 |
| Spermidine | 15 | 6.1 | 9.0 | 1.1 | 33.4 |
| Spermine | 8 | 5.0 | 3.1 | 1.4 | 12.1 |

Table 3
Statistical characteristics of amine concentrations (mg $kg^{-1}$) in concentrated tomato pasta ($n = 19$)

| Amine | $n >$ DL | Mean for > DL | $s_x$ | $x_{min}$ | $x_{max}$ |
|---|---|---|---|---|---|
| Histamine | 5 | 4.6 | 2.8 | 2.0 | 10.1 |
| Tyramine | 14 | 10.4 | 7.4 | 3.0 | 31.1 |
| Tryptamine | 15 | 8.4 | 5.2 | 2.6 | 19.8 |
| Putrescine | 19 | 25.9 | 8.2 | 7.9 | 41.1 |
| Cadaverine | 12 | 5.8 | 5.1 | 1.4 | 17.6 |
| Spermidine | 17 | 8.4 | 3.7 | 2.7 | 15.8 |
| Spermine | 4 | 2.1 | 0.5 | 2.1 | 2.9 |

Table 4
Statistical characteristics of amine concentrations (mg $kg^{-1}$) in frozen green pea ($n = 14$)

| Amine | $n >$ DL | Mean for > DL | $s_x$ | $x_{min}$ | $x_{max}$ |
|---|---|---|---|---|---|
| Histamine | 2 | 3.1 | – | 2.3 | 3.8 |
| Tyramine | 12 | 8.7 | 3.1 | 4.1 | 16.5 |
| Tryptamine | 13 | 10.5 | 6.2 | 1.3 | 24.4 |
| Putrescine | 14 | 46.3 | 27.0 | 11.7 | 107 |
| Cadaverine | 3 | 3.0 | – | 1.9 | 4.2 |
| Spermidine | 14 | 46.6 | 23.5 | 2.9 | 88.4 |
| Spermine | 11 | 3.8 | 2.0 | 1.6 | 8.5 |

### 2.2. Analytical methods

Acidic extract for BAs determination was prepared from 25.00 g of a product with about 75 ml of 0.6 M $HClO_4$ and shaken in a closed Erlenmeyer flask for 1 h. The mixture was then centrifuged at 4000 rpm for 10 min. Supernatant was filtered through a filter paper, sediment was washed with perchloric acid, centrifuged again, filtered and added to the initial extract. The volume was adjusted to 100 ml. The extracts were stored in a refrigerator until analysis, maximally for 2 weeks.

All analyses were done in duplicate and mean values were used. All chemicals were of analytical grade. Seven observed BAs were determined as N-benzamides after derivatization with benzoylchloride by a method of micellar electrokinetic capillary chromatography, described in detail by Křížek and Pelikánová (1998), using Spectraphoresis 2000 (Thermo Separation Products, Fremont, CA). The detection limits were 1.0, 1.3, 1.4, 1.4, 2.1, 2.1 and 3.5 mg $kg^{-1}$ of a product for SPD, TR, CAD, SPM, PUT, HI and TY, respectively.

Repeatability of the analytical procedure was tested by seven parallel analyses of a frozen spinach purée sample. Relative standard deviations were 9.4, 9.7, 12.4, 12.7 and 14.6% at mean concentrations 11.7, 9.6, 5.1, 9.4 and 2.9 mg $kg^{-1}$ for SPD, PUT, TR, TY and HI, respectively. Concentrations of CAD and SPM were below detection limits.

### 2.3. Statistical methods

Differences in amine concentrations between manufacturers and harvest years were tested by the *t*-test with skewed distribution using Microsoft Excel. Values below the detection limits were used for the calculations as halves of the limits.

## 3. Results and discussion

Summarized data on BAs concentrations of the individual products are given in Tables 1–4. Detectable levels of PUT and SPD were observed in most samples, while HI, SPM and CAD concentrations were often below the detection limits. Maximum mean values 33.6 and 52.5 mg $kg^{-1}$ for tyramine and putrescine, respectively, were observed in ketchup, which also had the highest mean concentrations of CAD and TR. Maximum mean level (46.6 mg $kg^{-1}$) of SPD was found in frozen green pea. Similarly, most of the observed maximum values were in ketchup. Ketchups have been prepared from several materials of plant origin and their

compositions and proportions can differ among manufacturers and types of product.

Low levels of HI, mostly below the detection limit of 2.1 mg $kg^{-1}$ and at maximum 18.0 mg $kg^{-1}$ in a sample of spinach purée, seem to be the most interesting result, opposite to the high levels of 36–400 mg $kg^{-1}$ cited in older papers (e.g. Häberle, 1987; Smith, 1981) for spinach. Similarly, in tomato pasta, we observed considerably lower levels of TY than the 1200 mg $kg^{-1}$ cited for tomato (Davídek & Davídek, 1995). The observed levels of polyamines, PUT and SPD, are comparable with limited data published for spinach and tomato (Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997) and frozen green pea (Ziegler, Hahn, & Wallnöfer, 1994). These amines probably originate from raw vegetables. SPD concentrations are relatively high in green pea. Similarly, TR concentrations in ketchup seem to be relatively high as levels of that amine in foods, except of cheeses, are commonly reported to be very low (e.g. Beutling, 1996; Davídek & Davídek, 1995).

Amine concentrations in the products from manufacturers, with at least five samples, could be only compared statistically. Differences at significance level ($P < 0.05$) were only found for PUT and SPD concentrations in spinach purée from one producer having higher levels than three other manufacturers. Differences in amine concentrations between harvest years were tested only for spinach purée and tomato pasta with declared information. However, no significant differences were found.

Nout (1994) proposed, as acceptable levels for fermented foods, 50–100 and 100–800 mg $kg^{-1}$ for HI and TY, respectively. Similar levels exist in legislation of different countries, also for non-fermented foods. The results of our survey thus indicate that biogenic amines in none of the observed vegetable products are a risk for healthy consumers. However, in the individuals with restricted activity of the detoxification enzyme, monoamine oxidase (MAO, EC 1.4.3.4), such as patients treated with some psychoactive drugs, intake of TY should be calculated, as even 6 mg within a 4-h period can be deleterious (Tailor, Shulman, Walker, Moss, & Gardner, 1994). One serving of spinach or green pea could be up to 150–250 g. Consumption of tomato pasta in soups, sauces and similar meals and also ketchup is lower.

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