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SEPARATION AND QUANTIFICATION OF BIOGENIC AMINES IN BANANAS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: This paper reports separation and quantification of biogenic amines in bananas by high performance liquid chromatography. Fresh and preserved samples (seven days of storage at refrigeration temperature, at room temperature respectively) were analyzed. We obtained the separation of all nine biogenic amines (tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermidine, spermine) with good resolution, including good resolution of serotonin and internal standard (1,7diaminoheptane). Mean biogenic amines levels of 10.344 mg/kg (tryptamine) to 61.502 mg/kg (putrescine) were found in fresh bananas. After seven days of storage at room temperature, 2-phenylethylamine decreases significantly. After seven days of storage at refrigeration temperature, tryptamine increases significantly, whereas phenylethylamine decreases significantly.

Keywords:2-phenylethylamine, bananas, biogenic amines, cadaverine, histamine, HPLC, putrescine, spermidine, spermine, serotonin, tryptamine, tyramine

1. Introduction

Biogenic amines are natural low molecular weight organic bases of aliphatic, aromatic or heterocyclic structure derived from decarboxylation of the corresponding amino acids. Biogenic amines are present in small quantities in some products such as fruits, vegetables, milk etc.

Polyamines such as putrescine, spermidine and spermine have been found in all higher plants and are involved in important physiological processes, such as fruit growth and development [1cited by 2]. Free biogenic amines in fruits and vegetables shape the typical and characteristic taste of mature foods and are precursors of certain aroma compounds [3 cited by 4].

Biogenic amines can be found in relatively large amounts in some fermented/ matured foodstuffs. Also biogenic amines can be used as indicators of quality and freshness, especially for animal raw material and food, as food spoilage is accompanied by increased content of biogenic amines.

Biogenic amines exert physiological and toxicological effects. Some aromatic amines (tyramine, tryptamine, and 2-phenylethylamine) show a vasoconstrictor action while others (histamine and serotonin) present a vasodilator effect.

Despite of low levels of biogenic amines in fruits, vegetables and fruit juices [5, 2, 6, 7 8], quantification of biogenic amines is important due to the existence of risk consumer categories. These include both patients treated with painkillers and drugs used for the treatment of stress and

depression, acting as a blocker of monoamine oxidase - the main route of biogenic amines detoxification [9], and young children and infants, as they are early feed with bananas, at 5 - 6 months of age.
Through our research we aimed at the separation and quantification of biogenic amines in bananas by high performance liquid chromatography, since few data are reported about this topic [10, 11, 7, 12].

2. Materials and methods

2.1. Samples
Bananas were obtained from Romanian retail stores and were analyzed fresh and after preservation: seven days of storage at refrigeration temperature, respectively at room temperature.

2.2. Reagents and standards
Tryptamine (Trp), 2-phenylethylamine (Phe), putrescine (Put) , cadaverine (Cad), histamine (His), serotonin (Ser), tyramine (Tyr), spermidine (Spd), spermine (Spm) and internal standard 1,7 diaminoheptane were purchased from Sigma-Aldrich. Stock solutions of biogenic amines and internal standard (1 mg/ml) were prepared, which were kept in the refrigerator for a month. These solutions were used for the preparation of standard solutions (100μg/ml) used for calibration curves. All other reagents used were p.a. grade and solvents were HPLC grade.

2.3. The analytical procedure has been adapted in the Chemistry and Biochemistry Laboratory of the HORTING Institute [13] following a previously published protocol [14]. In our case chromatographic separation was performed using a BDS Hypersil C18 column (250 * 4.6 mm), 5μ particle size (Thermo Electron Corporation). The mobile phase consisted of ammonium acetate 0.1 mol / l (solvent A) and acetonitrile (solvent B). We modified the gradient elution program proposed by Eerola et al. (1993) [14] (Table 1). The mobile phase flow rate was of 1ml/min and the column temperature was set at 40^0C.
Dansylated derivatives of biogenic amines were UV detected at 254 nm. The control, data acquisition and processing software used ChromQuest 4.2. (ThermoFinnigan).
The results were statistically processed with Student test using GraphPad Prism (version 5.00, GraphPad Software Inc., San Diego, 2007). The differences $p < 0.05$ were considered significant.

3. Results and Discussion

By modifying the gradient elution programme proposed by Eerola et al. (1993) [14] we found that resolution of serotonin and the internal standard was good (Fig. 1). Eerola et al. (1993) [14] asserted that the overlapping of serotonin and internal standard peaks did not interfere with the quantitation because the samples investigated contained no serotonin or only very small amounts.
All biogenic amines investigated were present in fresh samples, but putrescine was predominant (Table 2). Kalac & Krausova (2005) [6] and Okamoto (1997) [5] reported that the putrescine content was predominant in grapefruit and apple, and orange respectively. Cirilio et al. (2003) [2] reported that the predominant amines in green coffee were serotonin and putrescine. Putrescine concentration in fresh samples (61.502 mg/kg) was comparable with the values reported by Kalac & Krausova (2005) [6] in grapefruit and fresh green pepper (62.1 respectively 70 mg/kg). Moret et al. (2005) [4] carried out a survey on free biogenic amine contents in fresh vegetable products and reported that putrescine was one of the most represented amines. Adao & et al. (2005) [11] found a putrescine content around 0.75mg/100g in bananas.

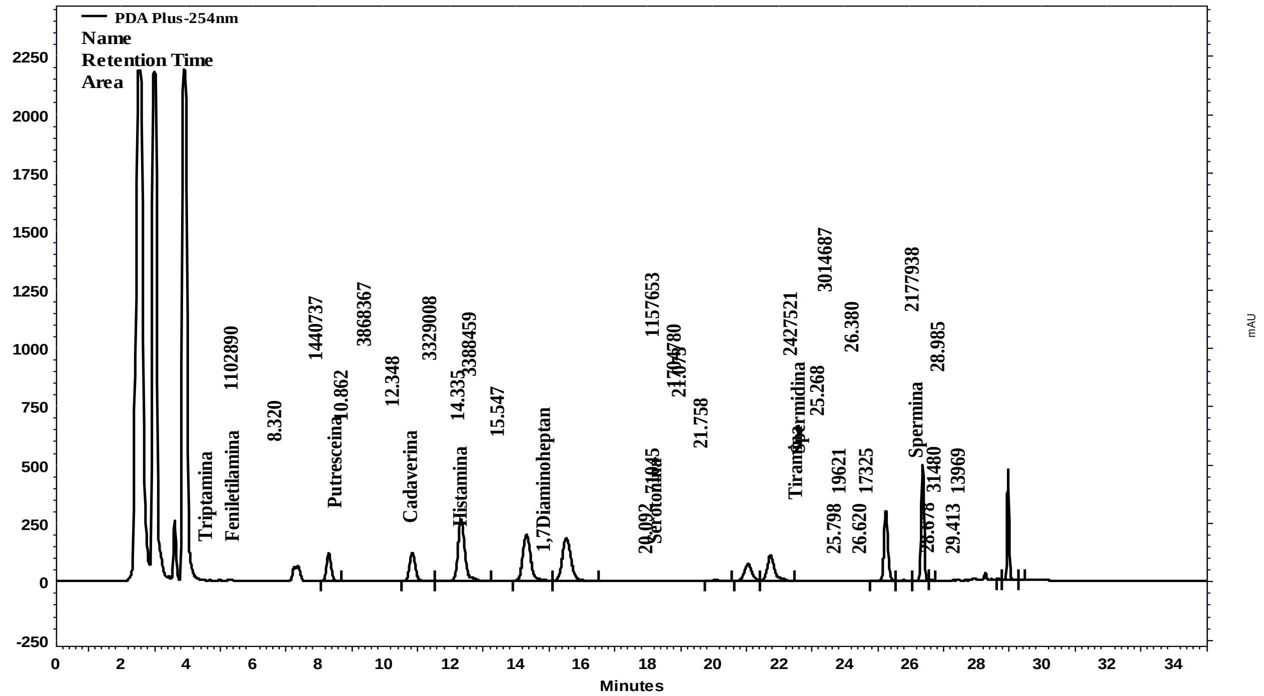

Fig. 1. Chromatographic separation of dansyl derivatives of biogenic amines (2 µg/ml) by gradient elution

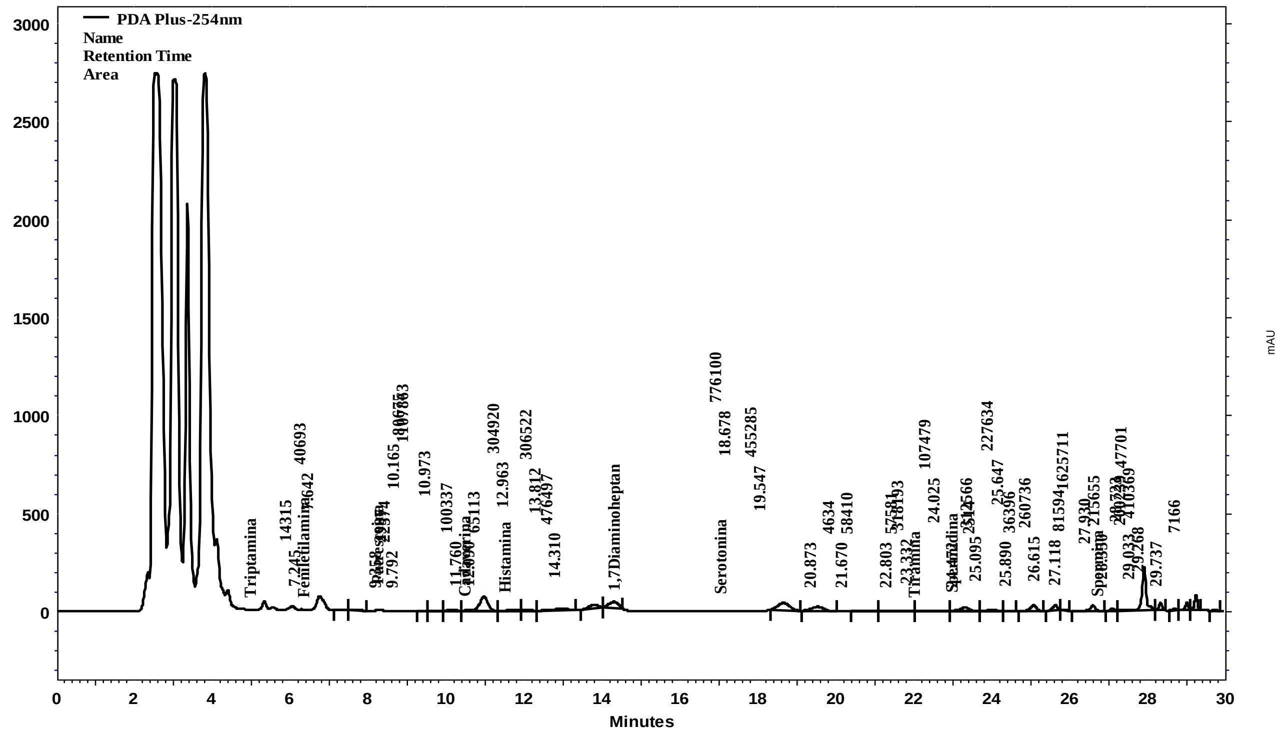

Fig. 2. Chromatogram of biogenic amines extracted from bananas after 7 days of storage at room temperature

Table 1.
Gradient elution programme for the separation of biogenic amines in bananas

Time (min)	Gradient		Flow rate (ml/min)	Wavelength (nm)	Column pressure (bar)	Column temperature (°C)	Tray temperature (°C)	Sample volume (µl)
	Ammonium acetate (A) (%)	Acetonitrile (B) (%)						
0.01	40	60	1.00	254	minim 70	40	7	20
15	40	60						
20	30	70						
25	5	95						
30	40	60						

Table 2.
Mean biogenic amines levels (mg/kg) in bananas

	Trp	Phe	Put	Cad	His	Ser	Tyr	Spd	Spm
Fresh samples	10.344 ±0.346	39.42 ±6.091	61.502 ±16.91	32.313 ±4.455	17.991 ±8.71	25.379 ±5.664	14.831 ±0.839	23.303 ±1.199	19.164 ±3.218
Samples preserved 7 days at room temperature	13.759 ±5.652	12.595 ±5.464 p<0.05	51.381 ±27.605	24.784 ±10.268	25.271 ±7.355	25.265 ±19.756	11.537 ±7.567	18.154 ±8.125	6.084 ±3.486
Samples preserved 7 days at refrigeration temperature	17.971 ±1.126 p<0.05	14.300 ±2.621 p<0.05	57.082 ±16.214	29.859 ±1.199	28.053 ±1.352	21.663 ±4.966	13.978 ±0.207	22.436 ±5.418	9.104 ±5.430

Histamine (17.991 mg/kg) was detected in fresh samples. The presence of histamine was not detected in bananas by Lavizzari et al. (2006) [7], but G.G. Mohamed et al. (2009) [12] reported histamine levels of 0–33.10 mg/100g in seven banana samples.

The tiramine concentrations in fresh samples (14.831 mg/kg) were in agreement with those values obtained by Udenfriend et al. (1959) [10] in bananas (0.7 mg/100g).

We detected tryptamine, 2-phenylethylamine, cadaverine in fresh samples, but these biogenic amines have not been detected in bananas by Lavizzari et al. (2006) [7].

Mean serotonin levels of 25.379 mg/kg were found; lower amounts of serotonin were found by Lavizzari et al. (2006) [7], Adao& et al. (2005) [11] in bananas and Cirilio et al. (2003) [2] in green coffee (11.5 mg/kg, around 1.25 mg/100g respectively 1.13 mg/100g).

Mean spermidine and spermine levels of 23.303 respectively 19.164 mg/kg were found. Adao & et al. (2005) [11] found a spermidine content around 1.10 mg/100g in bananas and spermine was present at very low level. Cirilio et al. (2003) [2] also reported prevalence of spermidine to spermine in green coffee with mean levels of 0.60 mg/100g, 0.44 mg/100g respectively.

Moret et al. (2005) [4] reported prevalence of spermidine to spermine in fresh vegetables (0.4–4.5 mg/100 g, a maximum of 1.1 mg/100 g respectively).

The samples analyzed after seven days at room temperature showed significant lower 2-phenylethylamine content (Table 2) and no differences were found for the other biogenic amine levels, while putrescine was the most represented amine.

The samples analyzed after seven days of refrigeration showed a statistical significant higher tryptamine content and a lower phenylethylamine content, and no differences were found for the others biogenic amine levels, while putrescine was the most represented amine (Table 2).

It is widely known that some aromatic biogenic amines (tyramine, tryptamine, 2-phenylethylamine) exert a vasoconstrictor effect, and the quantification of these biogenic amines is important, especially due to the existence of risk consumer categories. With regard to biogenic amines' content modification during refrigeration, Moret et al. (2005) [4] reported that vegetables analyzed after a 3-week refrigeration period showed a lower spermidine content and minor differences were found for concentrations of other amines. Further investigation on more samples is required.

4. Conclusion

a) We obtained the separation of all nine biogenic amines from bananas by HPLC with good resolution, including good resolution of serotonin and internal standard (1,7 diaminoheptane).
b) Mean biogenic amines levels of 10.344 mg/kg (tryptamine) to 61.502 mg/kg (putrescine) were found in fresh bananas.
c) After seven days of bananas storage at refrigeration temperature, their content in 2-phenylethylamin decreased significantly, while their tryptamine content increased significantly.
d) After seven days of storage at room temperature, 2-phenylethylamine decreased significantly in bananas.

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All the authors declare no conflict of interest.

6. References

[1]. ESTI, M., VOLPE, G., MASSIGNAN, L., COMPAGNONE, D., LA NOTTE, E., PALLESCHI, G. Determination of amines in fresh and modified atmosphere packaged fruits using electrochemical biosensors. Journal of Agricultural and Food Chemistry, 46, 4233–4237 (1998).
[2].CIRILIO, M.P.G., COELHO, A.F.S., ARAUJO, C.M., GONCALVES, F.R.B., NOGUEIRA, F.D., GLORIA, M.B.A. Profile and levels of bioactive amines in green and roasted coffee. Food Chemistry, 82, 397-402 (2003).
[3]. ASKAR, A., TREPTOW, H. Biogene amine in Fleisch-producten. Ernahrung/Nutrition, 13, 425–429 (1989).
[4]. MORET, S., SMELA, D.,POPULIN, T., CONTE, L.S., A survey on free biogenic amine content of fresh and preserved vegetables. Food Chemistry, 89, 355–361 (2005).
[5]. OKAMOTO, A., SUGI, E., KOIZUMI, Y., YANAGIDA, F., UDAKA, S. Polyamine content of ordinary foodstuffs and various fermented foods. Bioscience, Biotechnology and Biochemistry, 61 (9) , 1582-1584 (1997).
[6]. KALAC, P., KRIZEK, M., PELIKANOVA, T., LANGOVA, M., VESKRNA, O. Contents of polyamines in selected foods. Food Chemistry, 90, 561-564 (2005).
[7]. LAVIZZARI, T., VECIANA-NOGUES, M. T., BOVER-CID, S., MARINE-FONT, A., VIDAL-CAROU, M. C. Improved method for the determination of biogenic amines and polyamines in vegetable products by ion-pair high- performance liquid chromatography. Journal of Chromatography A, 1129, 67-72 (2006).
[8]. SAAID, M., SAAD, B., HASHIM, N.H., ALI, A.S.M., SALEH, M.I. Determination of biogenic amines in selected Malaysian food. Food Chemistry, 113(4), 1356-1362 (2009).
[9]. BARDOCZ, S. Polyamines in food and their consequences for food quality and human health. Trends in Food Science & Tehnology, 6, 341-346 (1995).
[10]. UDENFRIEND, S., LOVENBERG, W., SJODERMA, A. Physiologically active amines in common fruits and vegetables. Archives of Biochemistry and Biophysics, 85, 487–490 (1959).
[11]. ADAO, R. C., GLORIA M. B. A. Bioactive amines and carbohydrate changes during ripening of ‘Prata’ banana (*Musa acuminata · M. balbisiana*). Food Chemistry, 90, 705–711 (2005).
[12]. MOHAMED G.G., EL-HAMEED A.K., EL-DIN A.M., EL-DIN L.A. High performance liquid chromatography, thin layer chromatography and spectrophotometric studies on the removal of biogenic amines from some Egyptian foods using organic, inorganic and natural compounds. The Journal of Toxicological Sciences, 35 (2), 175-187 (2010).
[13]. BASTON, O., STROIA, A. L., MOISE, D., BARNA, O. Validation of a HPLC method able to measure biogenic amines in chicken meat. The Annals of the University Dunarea de Jos of Galati. Fascicle VI – Food Technology, New Series Year II (XXXI), 44-50 (2008).
[14]. EEROLA, S., HINKKANEN, R., LINDFORS, E., HIRVI, T. Liquid chromatographic determination of biogenic amines in dry sausages. Journal of AOAC International, 76 (3), 575-577 (1993).