Monitoring of Biologically Active Amines in Cereals and Cereal Based Food Products by HPLC

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Key Words

Column liquid chromatography
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Summary

Biologically active amines (putreanine sulphate, N-acetyl putrescine, putrescine, cadaverine, histamine, agmatine, N-acetyl spermidine, spermidine, spermine) were separated and quantified in cereal flour and cereal products by a liquid chromatographic method. The method consists of the separation of ion pairs formed between biologically active amines and octanesulphonic acid on a reversed-phase column, postcolumn derivatization with o-phtalaldehyde-2-mercapthoethanol and spectrofluorometric detection. Results of the reliability study were satisfactory. The method was linear for each amine at 1–10 mg L^{-1}. Putrescine and spermidine were the only amines always detected in cereal flour and cereal products, ranging from 2.45 to 47.83 mg kg^{-1} for putrescine and 3.27 to 37.14 mg kg^{-1} for spermidine. The most important differences among types of samples were found in polyamine derivatives.

Introduction

Biologically active amines are ubiquitous substances that occur in every living cell [1]. They fulfil an array of roles in cellular metabolism [2, 3] and are involved in many steps of protein, RNA and DNA synthesis, from the control and initiation of translation [4] regulation of its fidelity [5] stimulation of ribosome subunit association [6], through enhancement of RNA [7] and DNA synthesis [8] stabilization of the structure of tRNA [9] and reduction of the rate of RNA degradation [10] and involvement in the condensation of DNA [11] to the covalent modification of proteins [12]. On account of their specificity, polyamines are essential for growth and cell proliferation.

Several methods to isolate and estimate biogenic amines in food have been reported; although, some of them have limitations, such as detection of only a few amines and poor resolution [13]. The aim of our research was to apply and study the reliability of a liquid chromatographic method for biologically active amine determination in cereals and cereal products. The analytical method applied derived from a liquid chromatographic procedure by Seiler and Knödgen [14]. Some modifications have been made by us, such as gradient elution and the inclusion of one more biologically active amine (agmatine). The method turned out to be efficient, rapid, reproducible and sufficiently simple to allow routine application.

In spite of the undoubted importance of polyamines in growth, there is little information on the polyamine content and composition of even the most common foods. Here we would like to determine the polyamine content of cereals and cereal products (different bread samples) because of the main role of cereals (wheat, oats, barley) and bakers' wares in healthy eating. We measured different varieties of cereal flours to monitor and compare their biologically active amine contents.

Experimental

Chromatographic System

A Waters high-pressure liquid chromatograph (Model 2690) was used. The actual separations were performed with a μBondapak C_{18} column (300 × 3.9 mm; 10 μm) (Waters, Budapest, Hungary). Column effluent and *o*-phthalaldehyde reagent were mixed in a T-piece. A Waters 474 Scanning Fluorescence Detector was used equipped with a 150 W xenon arc lamp. Fluorescence excitation was at 345 nm, and emission at 455 nm. The signal of the fluorescence detector was recorded at two sensitivities using a two channel recorder (Waters SAT/IN module, Budapest, Hungary). The reagent was

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supplied by a piston pump (Waters 501 pump system, Budapest, Hungary) pulses being damped by a bulb trap between pump and mixing T-piece.

Chemicals

Sodium acetate ($CH_3COONa \times 3H_2O$), boric acid, potassium hydroxide, 2-mercaptoethanol, acetic acid and the wetting agent Brij-35 were from Reanal (Budapest, Hungary). o-Phthalaldehyde was from Fluka (Budapest, Hungary) acetonitrile and other common chemicals were from Merck (Budapest, Hungary). 1-Octanesulfonic acid (sodium salt) was a product of Romil Pure Chemistry (Cambridge, England). Putrescine dihydrocloride, cadaverine dihydrocloride, spermidine phosphate, spermine phosphate, agmatine sulphate, histamine dihydrocloride, putreanine sulphate, 1,7 diamino heptane and acetyl derivatives were from Sigma (Budapest, Hungary).

Elution Buffer

The elution system provided a gradient prepared from two buffer solutions. Buffer A: 0.1 *M* sodium acetate adjusted to pH 4.5 with acetic acid and containing 10 m*M* octane sulfonate and buffer B: 0.2 *M* sodium acetate (adjusted to pH 4.5 with acetic acid) plus acetonitrile (10:3, v/v), containing 10 m*M* octane sulfonate.

o-Phthalaldehyde Reagent

This was prepared by dissolving 50 g boric acid, 44 g potassium hydroxide and 3 mL of the Brij-35 solution per litre of distilled water. To this solution 2 mL 2-mercaptoethanol and 400 mg o-phthalaldehyde dissolved in 5 mL distilled methanol were added before use.

Sample Preparation

Samples were accurately weighed, and 5 mL 0.2 M perchloric acid (PCA, containing 0.5 μM 1,7-diaminoheptane dihydrocloride as internal standard) was added and mixed thoroughly over a magnetic stirring plate for 10 min. After centrifugation at 3000 rpm for 10 min and separation of the two phases, 5 mL 0.6 M PCA was added and mixed thoroughly for 10 min, and centrifugation repeated. The two PCA extracts were combined.

Chromatographic Separation

The buffer flow-rate was 0.5 mL min^{-1}. Before each run the column was equilibrated for 10 min with buffer A. The gradient program was implemented as follows: time = 0 min, 100 % A, 0 % B; time = 13 min, 100 % A, 0 % B; time = 17 min, 60 % A, 40 % B; time = 41 min, 40 % A, 60 % B; time = 61 min, 0 % A, 100 % B; time = 90 min, 0 % A, 100 % B; time = 92 min, 100 % A, 0 % B (Table I.). Column eluent and o-phthalaldehyde were mixed in a 1:1 ratio.

Table I. Gradient elution program.

		Solvent[a]	
Operation	Elution time, min	A, %	B, %
Equilibration	0	100	0
Elution period	13	100	0
	17	40	60
	41	60	40
	61	0	100
	90	0	100
Return	92	100	0

[a] Changes of A % and B % following a linear function.

Results and Discussion

Many reports are now available on various aspects of the o-phthalaldehyde procedure as a method of continuous post-column derivatization of polyamines [2, 14]. Method reliability, in terms of linearity, precision, recovery and sensitivity was studied.

The elution program was developed to provide chromatograms of high-resolution peaks (Figure 1). According to literature reports, TCA (trichloric acid), methanol, and PCA can be used to extract polyamines. Jover et al. [13] showed that more interference was present in chromatograms when TCA or methanol was used. Lack of interference from volatile amines, amino acids, and dipeptides was verified. All these substances eluted in the first 10 min. Amine identification was on the basis of retention time by comparison with standard solutions.

Linearity

Detector response in the corresponding calibration curves (1.00 to 10.00 mg L^{-1}) was linear. Linearity was verified by analysis of the variance of the regression. Least-squares analysis produced a correlation coefficient of $r > 0.9990$ for putrescine, spermidine, spermine, N-acetyl putrescine, putreanin sulphate, and cadaverine ($p < 0.001$) and of $r > 0.9975$ for N-acetyl spermidine, histamine and agmatine ($p < 0.001$). The coefficient of determination (r^2) was better than 99.50 % for all standard curves.

Precision

To study method precision, 7 determinations of rye bread ("Bajor") samples were made using the same reagents and apparatus. The RSD values for all amines are shown in Table II.

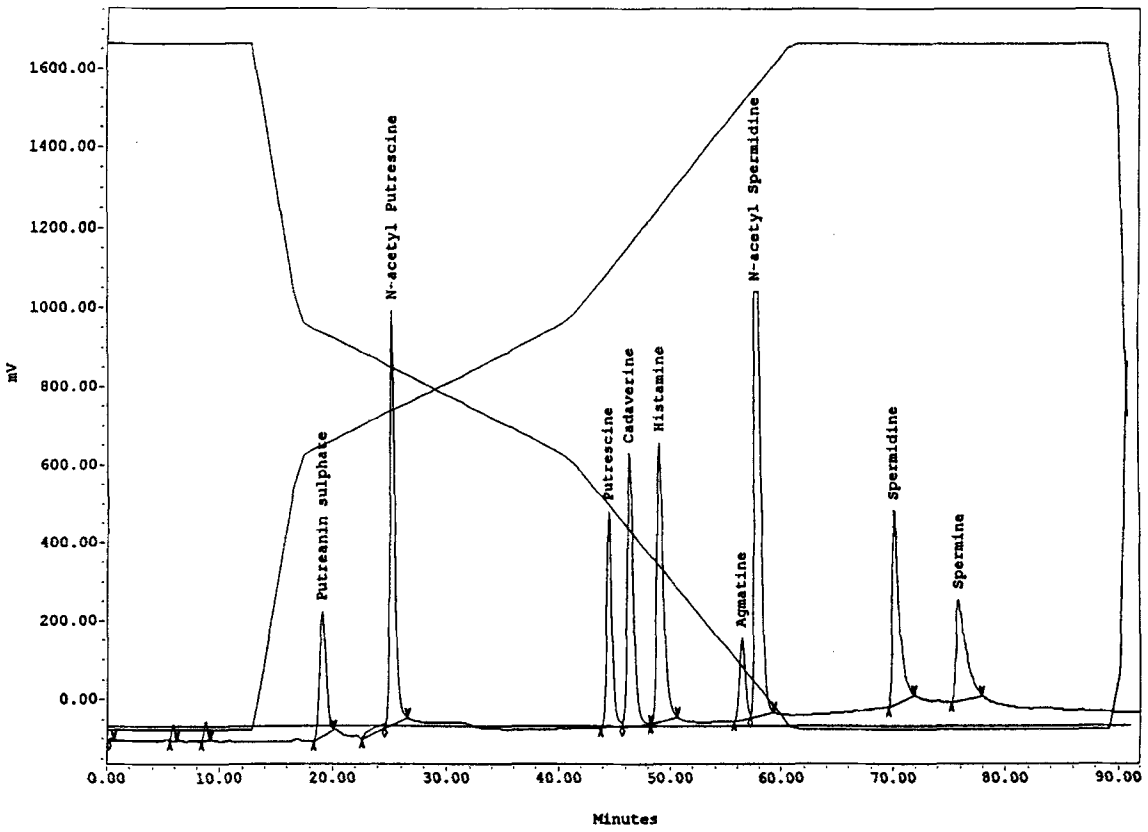

Figure 1

Chromatogram standard solution of biologically active amines. Peaks: putreanin-sulphate, N-acetyl putrescine, putrescine, cadaverine, histamine, agmatine, N-acetyl spermidine, spermidine, spermine. Separation conditions see Experimental section.

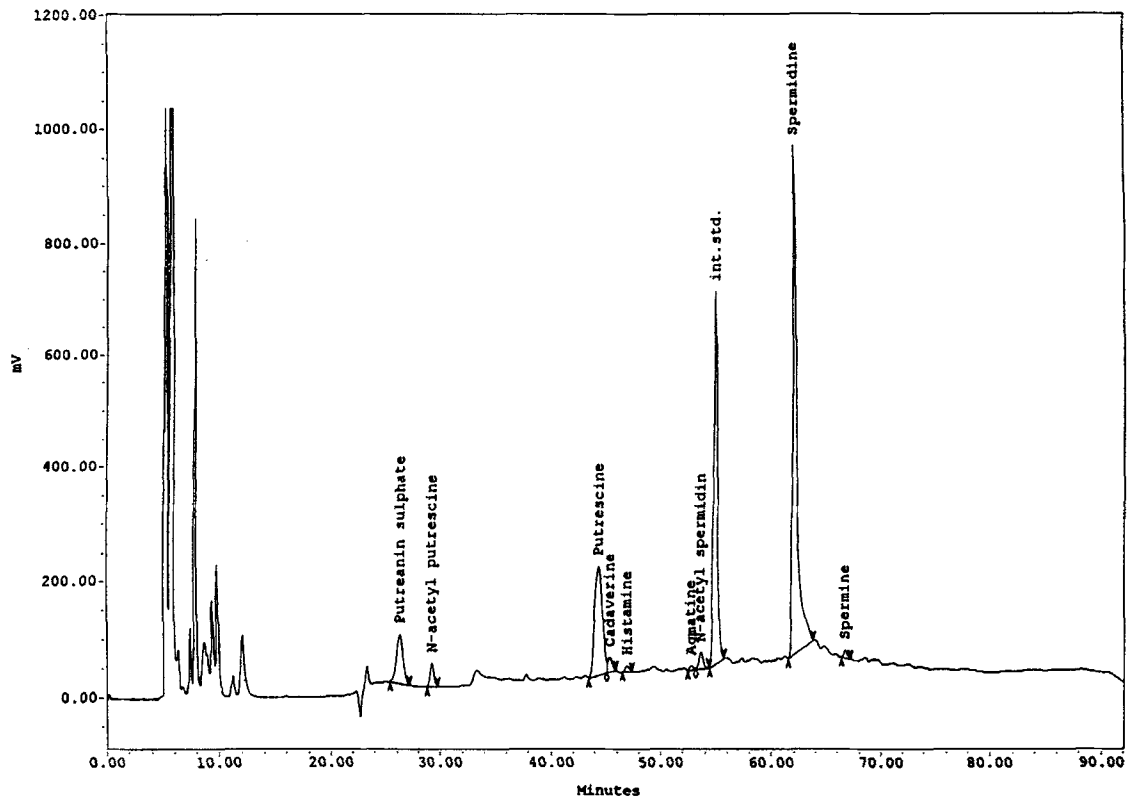

Figure 2

Chromatograms of biologically active amines in "Bajor" rye bread. High content of major polyamines: spermidine and putrescine. Separation conditions see Experimental section.

Table II. Precision of method for determination of biologically active amines in rye bread ("Bajor")(nmol g^{-1}).

Amine	$x_a \pm Sd^a$	RSD, [b] %
Putreanin sulphate	129.6 ± 8.3	6.4
N-acetyl putrescine	34.8 ± 0.6	1.8
Putrescine	293.7 ± 1.5	0.5
Cadaverine	24.6 ± 1.0	4.2
Histamine	7.0 ± 0.3	4.0
Agmatine	17.6 ± 1.2	6.7
N-acetyl spermidine	5.4 ± 0.4	7.1
Spermidine	140.9 ± 0.6	0.4
Spermine	23.8 ± 0.4	1.7

[a] Mean ± standard deviation in nmol g^{-1} (n =7). [b] Relative standard deviation.

Recovery

This was tested by an internal standard (1,7-diaminoheptane)-addition procedure. We used this data to correct our measurements. It was between 93.4 to 98.6 %.

Sensitivity

A linear relationship between amine concentration and fluorimeter signal was established with mixtures of the following compounds: monoacetyl-putrescine, putrescine, histamine, N-acetylspermidine, spermidine and spermine, in the range 50 nM to 5 µM. The smallest amount applied in these experiments was 125 pmole.

Table III. Polyamine content of different types of bread sample (mg kg^{-1} dry sample).

	Putrescine	Cadaverine	Histamine	Agmatine	Spermidine	Spermine
"Bakonyi"	46.7 ± 1.0	N.D.	N.D.	3.4 ± 0.4	37.1 ± 0.9	9.2 ± 0.2
"Kis" white	47.7 ± 0.1	6.6 ± 1.2	1.0 ± 0.6	4.7 ± 0.2	34.8 ± 1.7	8.6 ± 0.2
"Tiroli" rye	47.8 ± 0.1	6.5 ± 0.9	1.0 ± 0.2	3.7 ± 0.6	36.3 ± 0.1	8.1 ± 0.2
French	47.8 ± 1.0	4.6 ± 1.1	0.8 ± 0.1	4.4 ± 0.1	36.1 ± 0.4	8.3 ± 0.0
"Erzsébet"	43.8 ± 2.0	N.D.	0.6 ± 0.1	3.2 ± 0.1	31.9 ± 0.0	8.5 ± 0.1
Potato bread	44.3 ± 0.4	N.D.	N.D.	3.4 ± 0.0	34.7 ± 0.1	8.7 ± 0.3
"Bajor" rye	47.3 ± 0.3	4.3 ± 0.8	0.8 ± 0.1	4.0 ± 0.9	35.9 ± 0.1	8.3 ± 0.1
"Félbarna"	43.7 ± 0.1	N.D.	0.3 ± 0.0	3.4 ± 0.0	34.6 ± 0.1	8.7 ± 0.3
"Bürli"	19.7 ± 2.1	N.D.	N.D.	N.D.	19.7 ± 1.3	N.D.
Wheat flour	5.3 ± 0.8	N.D.	N.D.	N.D.	4.6 ± 0.1	N.D.
Full extracted wheat	2.5 ± 0.2	N.D.	N.D.	N.D.	5.5 ± 0.2	N.D.
Barley flour	2.5 ± 0.5	N.D.	N.D.	N.D.	3.3 ± 0.1	N.D.
Rye flour	5.2 ± 0.2	N.D.	N.D.	N.D	3.9 ± 0.1	N.D.
Oat flour	4.6 ± 0.3	N.D.	N.D.	N.D.	5.3 ± 0.2	N.D.

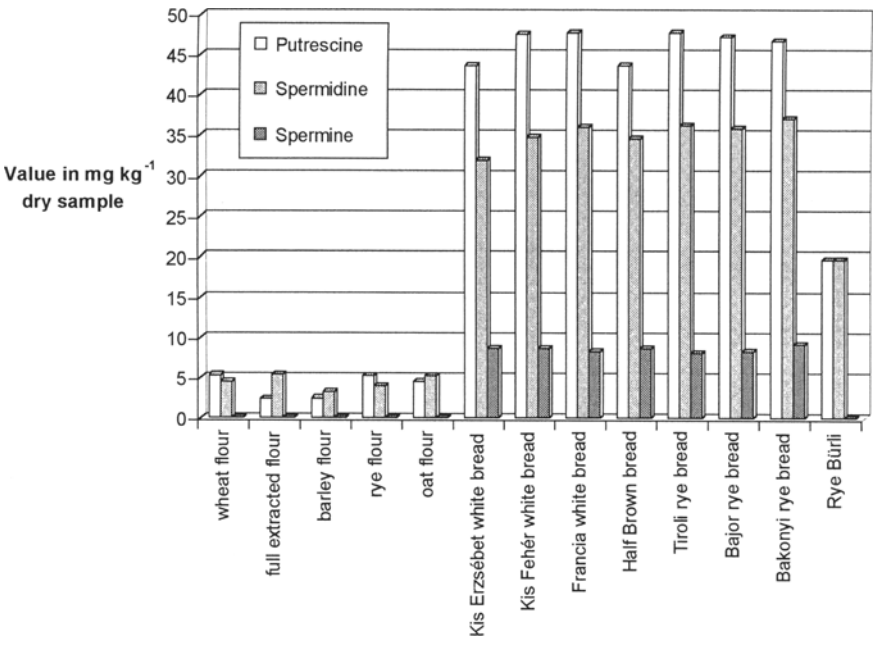

Figure 3

Major polyamines in cereals and cereal products. Eight different breads and bakerys, and five different cereal flours measured.

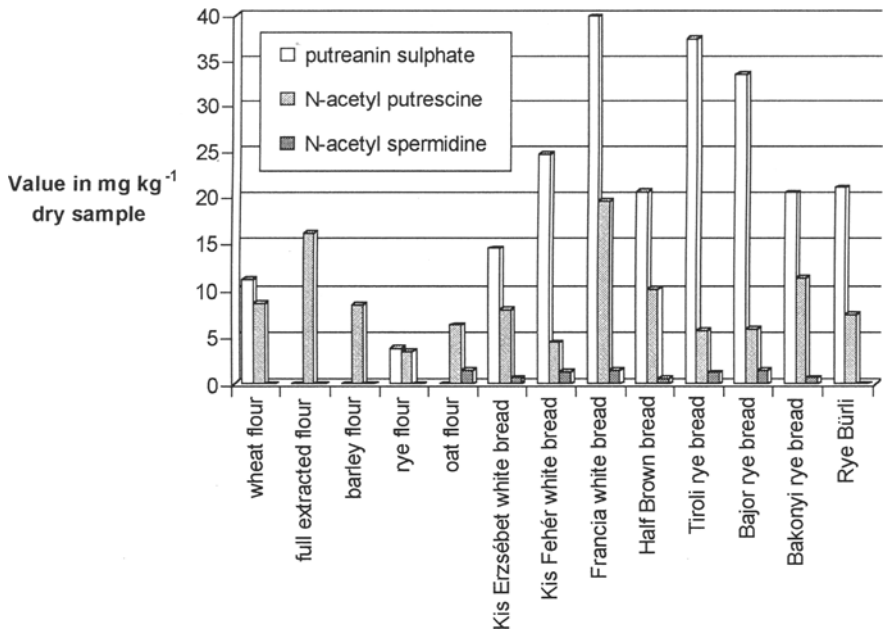

Figure 4

Content of polyamine derivatives in cereals and cereal products. Eight different types of bread and bakerys, five different cereal flours measured.

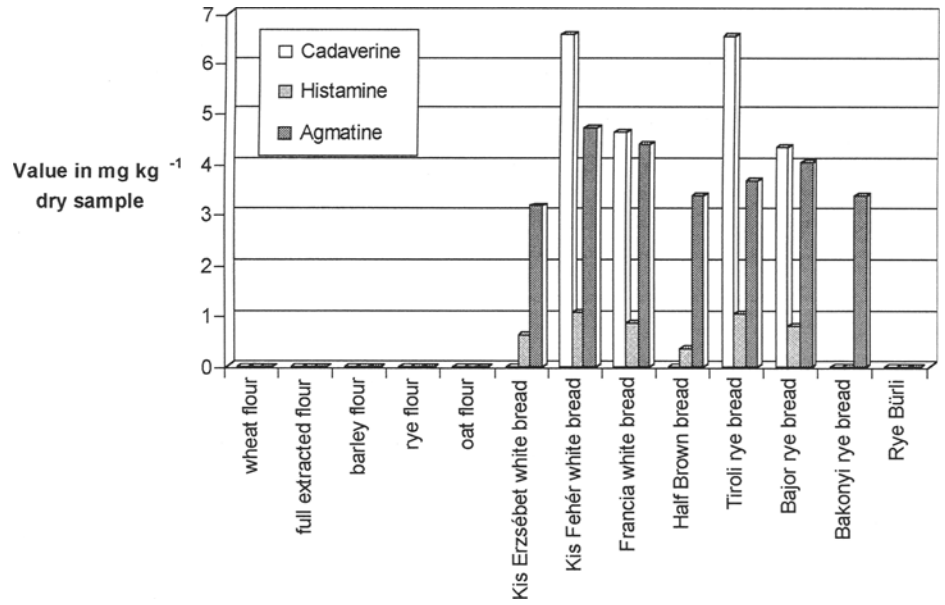

Figure 5

Cadaverine, histamine, agmatine content of cereals and cereal products. Eight different breads and bakerys and five different cereal flours measured.

However, the method allows less than 50 pmoles to be measured.

The content of biologically active amine and their derivatives in cereal products is shown in Table III. Figure 2 shows typical chromatograms of polyamines in cereal product samples. Putrescine and spermidine were always present in cereals and cereal products (Figure 3). Putrescine had the highest average content, most of the samples having values of >40 mg kg^{-1}. Cadaverine, histamine (Figure 5) and N-acetyl spermidine were not always present, but when they were, their content was generally low. Putreanin sulphate and N-acetyl Putrescine were found in all the bakerys analyzed, and they were the amines with the largest fluctuations (Figure 4). The most important differences among types of sample were found in these derivatives.

Conclusions

The LC method was useful for determination of biologically active amines in bread and cereals. It is only necessary to obtain a perchloric extract from samples and to filter it before injection. The LC system effectively separates biologically active amines from possible interference; only filtration of the samples is required before the determinative step. The simple preparation of the sample and the automatic derivatization of the amines considerably reduce analysis time and effort. Furthermore, the method allows a complete pattern of biologically active amines (aromatic, diamines, and polyamines) to be determined in a single run.

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