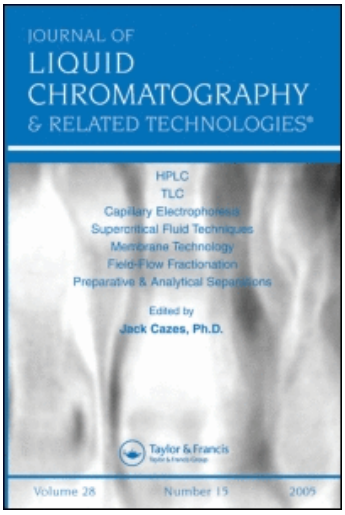

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ABSTRACT

An high-performance liquid chromatography (HPLC) method is described for specific and sensitive quantification of seven biogenic amines in coffee (putrescine, cadaverine, histamine, serotonine, tyramine, spermidine, and spermine). They are extracted from the coffee matrix with trichloroacetic acid, followed by an ion-pair clean-up procedure with *bis*-2-ethylhexylphosphate. Finally, they are converted to the corresponding dansyl derivatives for HPLC analysis. The factors that influence the determination were carefully adjusted according to coffee matrix characteristics (extraction, clean-up, and derivatization conditions).

Chromatographic separation is performed in a Kromasil C_{18} column, using a 0.5 mM phosphoric acid/acetonitrile/methanol gradient, at a flow rate of 1 mL/min. The effluent is monitored by a series arrangement of a diode-array detector followed by a fluorimetric detector.

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The present analytical method proved to be accurate, linear, and with lower limits of quantification (less than 0.5 mg/kg). Data concerning recovery and repeatability of the method are also reported.

INTRODUCTION

Biogenic amines are naturally occurring compounds, ubiquitous in animals and plants. These low molecular weight bases can be either aliphatic (putrescine, cadaverine, spermidine, spermine), or aromatic (tyramine, histamine, serotonine). Although many of these active substances play normal roles in mammalian physiology, they can also cause unnatural or toxic effects when consumed in large amounts, especially if their metabolism is blocked or genetically altered.[1,2]

Polyamines (putrescine, spermine and spermidine) fulfill an array of roles in cellular metabolism and are involved in protein, RNA, and DNA synthesis. Their most important function seems to be the mediating action of all known hormones and growth factors.[2] All plant cells, tissues, and organs provide the biosynthetic machinery and capacity to produce aliphatic polyamines in some specific organs and tissues. Polyamines are described as being accumulated in high concentration in storage organs, such as seeds. Biogenic amines are also food aroma components and potential precursors of the carcinogenic *N*-nitroso compounds.[3]

Biogenic amines have been extensively analyzed in food products, such as cheese, meat, fish, vegetables, and feedstuffs.[4] Data about these compounds in coffee are, however, quite scarce in the literature.[5–7]

Several chromatographic methods have been proposed for the quantitative determination of amines, including thin-layer chromatography, gas chromatography, capillary electrophoresis, micellar electrokinetic chromatography and, more frequently, high-performance liquid chromatography (HPLC).[8]

Biogenic amines are a group of compounds of difficult simultaneous analysis because of their structural diversity and lack of pronounced UV-Vis absorption. The usual approach, therefore, has been the derivatization of free amines with an easily detectable group in order to increase the sensitivity of the method. This derivatization step usually reduces the basicity of the nitrogen atom and the use of reversed phases is usually considered the most suitable technique for this analytical purpose.

Several methods for separation, identification, and determination of amines by HPLC have been published throughout during the last two decades. All methods involve three main steps, i.e., amines extraction, purification of the extract, and derivatization. Among the more described reagents for the derivatization is dansyl chloride.[8–12] It has the advantage, comparing with fluorescamine or ortho-phthaldehyde, to originate derivatives with both primary and secondary amines

which are stable enough for subsequent spectroscopic analysis. Dansyl chloride is a particularly well established reagent and gives high fluorescent sulfonamide derivatives with improved chromatographic properties.

The objective of this work was to optimize a method to quantify biogenic amines in coffee by reversed-phase HPLC with dansyl chloride derivatization. As the complexity of the coffee matrix may influence the analytical determination, the behavior of biogenic amines in some steps of the procedure (extraction, clean-up, dansylation conditions, etc,) was extensively studied. Also, a standard addition method was used.

EXPERIMENTAL

Equipment

The HPLC equipment consisted of an integrated system with two model PU-980 pumps, a AS-950 automated injector, a MD-910 multiwavelength diode array detector (DAD), and a FP-920 fluorimetric detector (Jasco, Japan). Data was analyzed using a Borwin-PDA Controller Software (JMBS, France).

The column used was a reversed-phase Kromasil 100 C_{18} (5 μm), 250 × 4.6 mm (Teknokroma, Spain) operating at ambient temperature.

Standards and Reagents

Standard Solutions

Putrescine dihydrochloride (put), cadaverine dihydrochloride (cad), histamine dihydrochloride (his), tyramine hydrochloride (tyr), spermidine trihydrochloride (spd), and spermine tetrahydrochloride (spm), serotonine (ser) (Sigma Chemical Co, USA) at 10 mg/mL free base in water, stored at 5°C, and diluted to working solutions as necessary.

Internal Standard

1,7-Diaminoheptane (IS) (Aldrich, USA) at 10 mg/mL, stored at 5°C.

Dansyl Chloride Solution

Acetone, 7.5 mg/mL, (Sigma), prepared daily and stored at −20°C protected from light.

L-Proline

L-proline (Sigma) was prepared at 100 mg/mL in water, kept refrigerated.

Amine Extraction

For amine extraction, trichloroacetic acid (TCA), perchloric acid (PCA), hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), ammonia, chloroform, and toluene were from Merck (Darmstadt, Germany), *bis*-2-ethylhexylphosphate (BEHPA) was from Aldrich.

High Performance Liquid Chromatography Analyses

For HPLC analyses, phosphoric acid, acetonitrile, and methanol (LiChrosolv—gradient grade) (Merck, Germany), and water purified with a "Seral" system (SeralPur Pro 90 CN), where used throughout after being filtered and degassed.

Sample Preparation

Arabica and robusta coffee beans from several geographical origins were analyzed, both raw and after a standard roast procedure (160–220°C, 15 min). All coffee sample beans were ground and powdered to pass through a 0.75 mm sieve and sample moisture was immediately determined by drying at 103 ± 2°C until constant weight.

Extraction and Derivatization Procedure

The internal standard (1,7-diaminoheptane) was added to a 3 g portion of each powdered coffee bean sample. Two extractions were performed with 5% TCA in a screw cap plastic tube, with a total of 25 mL, stirring each fraction for 10 min. After separation by means of centrifugation at 4000 rpm, the extracts were combined and a 2 mL portion was subjected to an ion-pair clean-up procedure with BEHPA as follows: the pH was adjusted to 7.4 in a screw cap centrifuge tube and the volume adjusted to 3 mL with phosphate buffer pH 7.4 (0.2 M). The buffered sample was extracted with 2 mL 0.1 M BEHPA solution in chloroform, by vortex mixing, and centrifuged at 4000 rpm; the chloroformic

phase was taken to a second tube and back-extracted with 2 mL of HCl 0.1 M, upon vortex mixing and centrifugation as described above.

The derivatization was performed in a screw cup plastic tube as follows: 400 μL extract, 1 mL saturated solution of Na_2CO_3 and pH adjustment to 11.0–11.2. The derivatizing agent was added as a 1 mL volume of 7.5 mg/mL solution in acetone. After rapid vortex-mixing, the samples were closed and placed for 12 min at 60°C in a thermostatically controlled water bath, in the absence of light. Then, proline solution (100 μL) was added, homogenized with a vortex mixer, and rest for 15 min in darkness, at room temperature. Extraction of the derivatized biogenic amines was performed with 1500 μL toluene. After 15 min at −18°C, 1 mL of the organic phase was recovered into a Eppendorf tube and evaporated to dryness under a gentle stream of nitrogen (±40°C). The pellet was then ressuspended in 300 μL acetonitrile (with vortex mixer), centrifuged at 13,000 rpm for 5 min and transferred to appropriate vials with 200 μL inserts for use in the automatic sampler.

Chromatography

Elution was performed at 1 mL/min with a linear gradient of A—0.05 M phosphoric acid, and B—methanol/acetonitrile (1 : 1) flushed at 1 mL/min with a gradient elution program as developed by Hornero-Mendez and Garrido-Fernandez.[12] The detection was performed by both the DAD detector, with chromatograms recorded at 254 nm, connected in series with a fluorimetric detector programmed for excitation at 252 nm and emission at 500 nm.

The compounds under study were identified by chromatographic comparisons with authentic standard derivatives, by co-elution and by the UV spectral characteristics of their dansyl derivatives. Peak purity tests were also performed with the DAD.

Identification

The biogenic amines' presence was confirmed by GC-MS after derivatization with heptafluorobutyric anhydride.[13] Although suspecting the presence of tryptamine, this was not confirmed by GC-MS and its analysis was not carried out.

RESULTS AND DISCUSSION

There are many methods published in the literature for the selective extraction of biogenic amines from various matrices. As the matrix itself greatly

influences the results, a method was developed specifically for coffee in order to obtain the highest levels of recovery and also the absence of co-eluting interferences. For this purpose, a green arabica coffee sample from Brazil was used throughout this study.

Choice of Extracting Solvent

In plants, in addition to free polyamines, many polyamines are conjugated to hydroxycinnamic acids, the hydroxycinnamic amines, that only rarely are represented outside the plant kingdom.[14] As water extracts only free amines, we have tested several acidic media in order to also extract amines linked to other matrix components. After literature review,[8–12,15,16] three acids were tested: 0.1 M HCl, 0.4 M or 0.6 M PCA, and 5% or 10% TCA. Each extraction was performed in duplicate and the chromatograms compared in terms of peak areas.

Although cited with relative frequency, especially for cheese, HCl originated a turbid extract with badly resolved peaks and was, therefore, eliminated. Perchloric acid provided abnormal higher peak areas for His and Tyr when compared with TCA, under the fluorimetric detection. A peak purity test performed with the DAD revealed extremely impure peaks. Cad peak seemed, also, to be impure in all the extractions tested.

The other compounds' areas were quite similar, as were also similar the relative areas to the IS. The results achieved in terms of relative areas are presented in Table 1. The mean results were obtained after elimination of His and Tyr for the reasons described above. Facing these inconclusive results, the clean-up studies have proceeded with both 0.4 M PCA and 5% TCA.

Table 1. Relative Areas Achieved Under Several Extracting Conditions

	0.6 M PCA	0.4 M PCA	10% TCA	5% TCA
Putrescine	100	100	89	92
Cadaverine	56	93	94	100
Histamine	(35)	(100)	(4)	(8)
Serotonine	79	85	88	100
Tyramine	(75)	(100)	(47)	(36)
Spermidine	100	94	89	94
Spermidine	91	100	91	100
Mean[a]	85	94	90	97

[a]Without His and Tyr.

Clean-Up Procedures

The extract purification treatments can be divided into two main groups: liquid–liquid extraction with organic solvents,[8–9,12,13,16,17] and solid-phase extraction.[18] For this work, a liquid–liquid extraction was chosen and several methods were tested based on literature reports. Method A uses diethylether, method B requires butanol, method C uses butanol/chloroform, and method D uses a ion-pairing reagent (BEHPA). The results were compared with the unclean extracts (E) in terms of areas (DAD and fluorimetric), relative areas, and peak purity.

Method A did not eliminate the co-eluting interference with His. After the clean-up procedure, an extra peak was observed co-eluting with Cad. This compound was also present in the blank, being, therefore, attributed to reagent contamination.

Methods B and C presented also interfering peaks coincident with His and Cad, confirmed by both purity peak analysis with the DAD and absence of characteristic dansyl derivatives' fluorescence. Gradient adjustment proved unsuccessful to separate these impurities. Also, these two methods require saturated salt solutions in order to improve extraction coefficients and to prevent mixing of the aqueous and organic phases during the liquid–liquid extraction. The use of Na_2CO_3 for this purpose, although frequently referred, makes it difficult to standardize, as its solubility is greatly influenced by temperature and by other compounds present in the extract, causing pH variations.

Method D-ion-pair extraction with BEHPA-has proved to be a powerful and very efficient agent for the extraction of various organic bases, leading to increased recovery and enhanced purification for the extracts obtained from complex matrices. Although more time consuming, the chromatograms obtained were clearer, with basically no differences between PCA and TCA extracts.

Based on these observations, we have chosen method D. Concerning the extract, 5% TCA represented, always, a slightly better choice. A blank analysis was performed with the chosen method and no peaks coincident with the studied biogenic amines were observed.

Optimization of Derivatization Conditions

There is no agreement in the literature on the derivatization conditions that should be used for biogenic amines; instead, a wide variety of conditions is proposed. It was necessary to adjust the derivatization conditions in order to find the optimal dansyl chloride concentration, incubation pH, temperature, and reaction time.

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Influence of pH

During the derivatization step, the pH is usually controlled, as it greatly influences the partition equilibrium of amines between the two phases. Sodium carbonate is frequently used for this purpose. Several pH's were tested from 10.0 to 12.0, as represented in Fig. 1. The optimum pH is related to the amines being determined, as their behaviors vary. pH 11 represented a compromise situation and was, therefore, chosen for this step.

Dansyl Chloride Concentration

Tests were performed in order to find the optimal dansyl concentration on the derivatization medium. The effect of dansyl chloride concentration on the derivatization is presented in Fig. 2. Concentrations higher than 3 mg/mL did not increase the detector response and introduced impurities coeluting with the smaller peaks. Therefore, this was the chosen concentration corresponding to the addition of 1 mL dansyl chloride at 7.5 mg/mL.

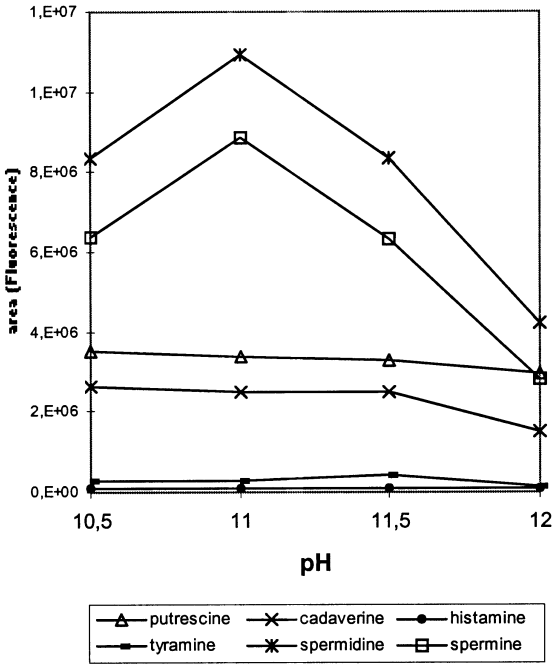

Figure 1. pH influence during the derivatization step.

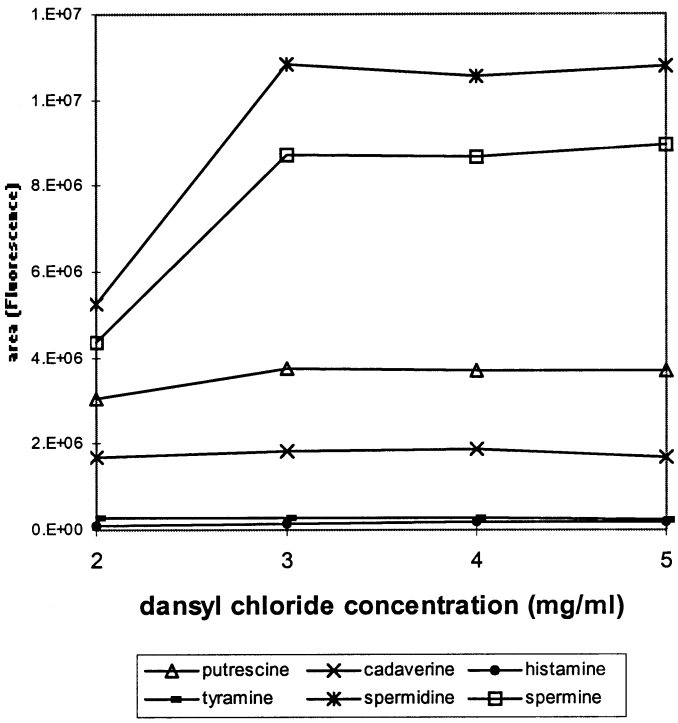

Figure 2. Effect of dansyl chloride concentration on the derivatization conditions.

Incubation Conditions

Several temperature/time combinations were tested in order to find the fastest and still accurate combination. Derivatization temperature was studied at 40°C (15, 30, 45 min), and 60°C for (3, 5, 8, 12, 15 min) in a water bath. The data obtained are represented in Table 2; they show that the best derivatization conditions were between 12 and 15 min at 60°C. Higher incubation times gave inconstant compound losses (not represented). Incubation for 12 min at 60°C produced better results for the three principal amines (put, spd, and spm) and was, therefore, chosen.

Neutralization of the Dansyl Chloride

Some recent works describe the neutralization of the excessive dansyl chloride in order to obtain stable derivative solutions and more reproducible

Table 2. Incubation Condition Tested (Relative Yields in Percentage)

Temperature:	40°C			60°C				
Time:	15	30	45	3	5	8	12	15
Putrescine	66	94	93	100	89	99	97	92
Cadaverine	40	100	73	46	62	85	86	89
Histamine	36	42	38	39	31	28	94	100
Tyramine	52	55	47	66	86	40	100	99
Spermidine	74	86	86	72	80	86	100	94
Spermine	66	81	75	76	67	74	100	95
Mean	56	77	69	66	69	69	96	95

results, with fewer secondary peaks. For this purpose, ammonia and *L*-proline were tested. A 100 μL portion was sufficient to neutralize the excess dansyl chloride, as described by Duflos et al.[11] and no differences were observed between the two compounds tested.

Validation: Linearity, Precision, Recovery, Quantification Limit

The first calibration curves were constructed with standard solutions submitted to the entire protocol as described in the Experimental section. The correlation coefficients (r^2) were always higher than 0.999 with both detectors.

As our objective was to quantify coffee biogenic amines by the standard addition method, the same arabica coffee sample used throughout this study was extracted, in duplicate, after spiking with four different concentrations of each of the biogenic amines. Correlation coefficients (r^2) of standard addition calibration curves were higher than 0.99, except for serotonine. The tested range for each biogenic amine, selected according to the expected values for coffee, is summarized in Table 3.

The results from the validation of the method are listed in Table 3. The method proved to be precise (within-day precision ranging from 1.2 to 12.5% and inter-day precision ranging from 2.3 to 13.2%).

Detection limits (three times signal-to-noise ratio under the DAD detector) were lower than 0.06 μg/g. The lower limit of quantification was investigated in coffee samples. Recovery was tested by the standard addition procedure; three addition levels were used for each amine. Mean extraction recoveries are also represented in Table 3.

Figure 3 represents chromatograms obtained with standards, with the DAD detector (A) and the fluorimetric detector (B).

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Table 3. Method Validation Parameters

	Rt		Linearity	Limits		Precision		Recovery
	min	cv% ($n=10$)	Range (mg/ml)	Detection µg/g	Quantification µg/g	Within-Day CV% ($n=3$)	Inter-Day CV% ($n=3$)	Mean% ($n=3$)
Putrescine	16.2	0.4	1.50–30.00	0.009	0.036	1.6	2.3	98.5
Cadaverine	17.2	0.4	0.05–1.00	0.001	0.002	6.2	11.4	100.2
Histamine	18.6	0.3	0.05–1.00	0.007	0.030	8.3	12.6	98.2
Serotonine	21.3	0.4	0.05–1.00	0.062	0.377	12.5	13.2	95.5
Tyramine	23.8	0.4	0.05–1.00	0.025	0.145	11.0	10.2	100.2
Spermidine	25.4	0.3	1.50–30.00	0.010	0.040	1.2	5.7	90.4
Spermine	28.6	0.6	1.50–30.00	0.011	0.043	4.3	3.5	90.8

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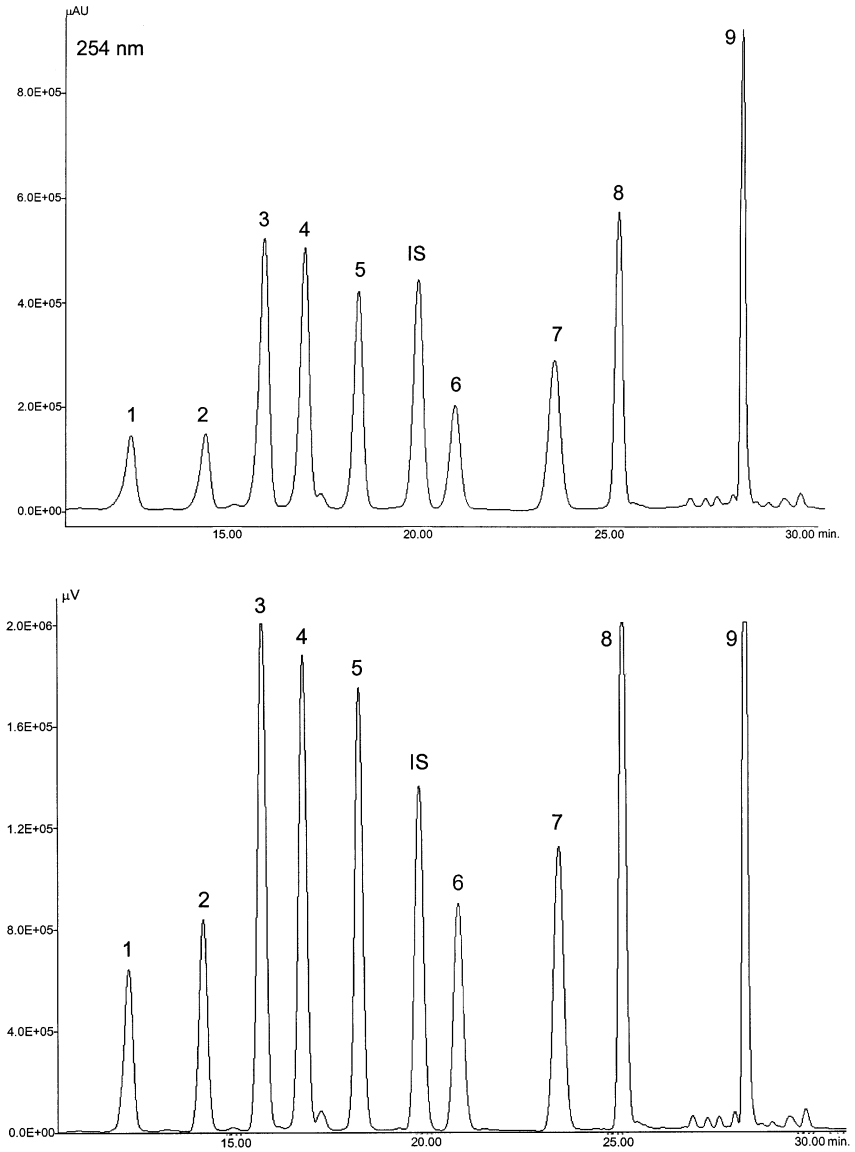

Figure 3. (A) Typical standards chromatogram obtained with the DAD at 254 nm: 1—tryptamine, 2—phenylethylamine, 3—putrescine, 4—cadaverine, 5—histamine, 6—serotonine, 7—spermidine, 8—spermine. For chromatographic conditions see Experimental section. (B) Typical standard chromatogram obtained with the fluorescence detector. Identification as in Fig. 3A.

Table 4. Biogenic Amine Content in Raw Coffee (Dry Base)

	Robusta			Arabica		
mg/g	Ivory Coast	Angola	Uganda	Colombia	Brasil	Mexico
Putrescine	16.845	6.436	12.377	49.258	45.750	52.424
Cadaverine	0.339	0.165	0.197	0.070	0.124	0.239
Histamine	0.793	0.212	0.602	0.510	0.577	0.629
Serotonine	3.122	3.207	2.311	1.815	3.076	3.189
Tyramine	0.840	4.166	0.975	0.554	0.696	0.579
Spermidine	6.867	3.333	5.107	5.798	7.490	6.233
Spermine	8.416	3.683	7.693	7.203	7.588	6.246

Determination of Biogenic Amines in Coffee Samples

Table 4 shows the biogenic amine contents in the green coffee samples analyzed. The biogenic amine contents are in accordance with the few bibliographic references found.[5,7] All biogenic amines were lost during roasting,

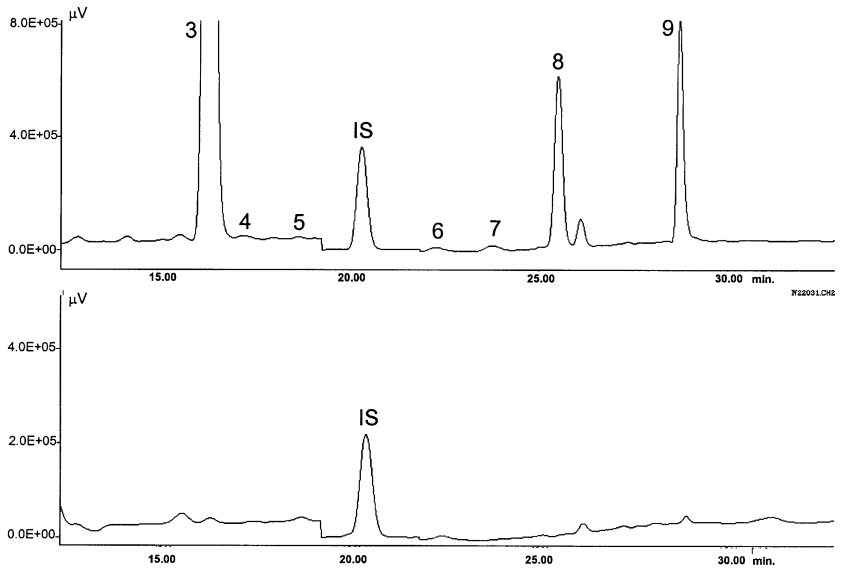

Figure 4. Typical chromatograms of a raw (A), roasted (B) arabica coffee sample. Peak identification as in Fig. 3.

with only vestigial amounts being present, as previously reported by Amorim et al.[5]

Figure 4 represents typical chromatograms of green arabica coffee sample (A) and after roasting (B) with the fluorimetric detector. Both detectors proved equally efficient for the analysis in question.

Based on the results achieved, with apparent differences between arabica and robusta coffee beans, especially for putrescine, it will be interesting to know if green coffee polyamines content can discriminate coffee varieties. Also, the knowledge of their behavior during roasting can probably be important for industrial roasting control. Studies are being conducted with these purposes as a goal.

REFERENCES

1. Bardocz, S.; Duguid, T.J.; Brown, D.S.; Grant, G.; Pusztai, A.; White, A.; Ralph, A. The Importance of Dietary Polyamines in Cell Regeneration and Growth. Brit. J. Nutr. **1995**, *73*, 819–828.
2. Igarashi, K.; Kashiwagi, K. Polyamines: Mysterious Modulators of Cellular Functions. Biochem. Biophys. Res. Comm. **2000**, *271*, 559–564.
3. Sen, N.; Seaman, S.; Weber, D. Mass Spectrometric Confirmation of the Presence of N-Nitrosopyrrolidine in Instant Coffee. J. Assoc. Off. Anal. Chem. **1990**, *73* (2), 325–327.
4. Brink, B.; Damink, C.; Joosten, H.; Veld, J. Occurence and Formation of Biologically Active Amines in Foods. Intl. J. Food Microb. **1990**, *11*, 73–84.
5. Amorim, H.V.; Basso, L.C.; Crocomo, O.J.; Teixeira, A.A. Polyamines in Green and Roasted Coffee. J. Agric. Food Chem. **1977**, *25* (4), 957–958.
6. Kele, M.; Ohmacht, R. Determination of Serotonin Released from Coffee Wax by Liquid Chromatography. J. Chromatogr. A. **1996**, *730*, 59–62.
7. Macrae, R. Nitrogenous Components. In *Coffee Vol. I: Chemistry*; Clarke, R.; Macrae, R., Eds.; Elsevier Applied Science: London, 1987; 115–152.
8. Gennaro, M.; Giacosa, D.; Abrigo, C.; Biglini, P.; Campi, E. Organic Bases. In *Handbook of Food Analysis*; Nollet, L., Ed.; Marcel Dekker, Inc.: New York, 1996; Vol. 1, 745–820.
9. Moret, S.; Conte, L. High Performance Liquid Chromatographic Evaluation of Biogenic Amines in Foods. An Analysis of Different Methods of Sample Preparation in Relation to Food Characteristics. J. Chromatogr. A **1996**, *729*, 363–369.
10. Valle, M.; Malle, P. Optimization of a Liquid Chromatographic Method for Determination of Amines in Fish. J. AOAC Intl. **1997**, *80* (1), 49–56.

11. Duflos, G.; Dervin, C.; Malle, P.; Bouquelet, S. Relevance of Matrix Effect in Determination of Biogenic Amines in Plaice (*Pleuronectes Platessa*) and Whiting (*Merlangus merlangus*). J. AOAC Intl. **1999**, *82* (5), 1096–1101.
12. Hornero-Mendez, D.; Garrido-Fernandez, A. Biogenic Amines in Table Olives. Analysis by High-Performance Liquid Chromatography. Analyst **1994**, *119*, 2037–2041.
13. Fernandez, J.O.; Ferreira, M.A. Combined Ion-Pair Extraction and Gas Chromatography-Mass Spectrometry for the Simultaneous Determination of Diamines, Polyamines and Aromatic Amines in Port Wine and Grape Juice. J. Chromatogr. A **2000**, *886*, 183–195.
14. Bagni, N. Metabolism and Accumulation of Biogenic Amines in Plant Food. Amino Acids **1999**, *17*, 57.
15. Veciana-Nogues, M.T.; Hernandez-Jover, T.; Marine-Font, A.; Vidal-Carou, M.C. Liquid Chromatographic Method for Determination of Biogenic Amines in Fish and Fish Products. J. AOAC Intl. **1995**, *78* (4), 1045–1050.
16. Antolini, F.; Franciosini, S.; Floridi, A.L.; Floridi, A. An Ion-Pair HPLC Method for the Determination of Histamine, Tyramine, Tryptamine, β-Phenylethylamine and Their Amino Acid Precursors in Cheeses for Industrial Purposes. Ital. J. Food Sci. **1999**, *11* (4), 335–345.
17. Salazar, M.T.; Smith, T.K.; Harris, A. High-Performance Liquid Chromatographic Method for Determination of Biogenic Amines in Feedstuffs, Complete Feeds, and Animal Tissues. J. Agric. Food Chem. **2000**, *48*, 1708–1712.
18. Mollins-Legua, C.; Campíns-Falco, P.; Sevillano-Cabeza, A.; Pedron-Pons, M. Urine Polyamines Determination Using Dansyl Chloride Derivatization in Solid-Phase Extraction Cartridges and HPLC. Analyst **1999**, *124*, 477–482.

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