QUANTITATIVE DETERMINATION OF BIOGENIC AMINES IN PORTUGUESE HAMS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF THE DANSYL DERIVATIVES

C. Alfaia[1,2], M. Castro[1,3], A. Correia[2], A. Dias[4]
[1] Secção de Química Analítica, Faculdade de Farmácia, UL, 1600 Lisboa, Portugal
[2] Secção de Bioquímica, Faculdade de Medicina Veterinária, UTL, Alto Ajuda, 1300 Lisboa, Portugal
[3] Centro de Farmacologia Experimental e Clínica, Faculdade de Medicina, UL, 1600 Lisboa, Portugal
[4] Alicontrol – Tecnologia e Controlo de Alimentos, Lda, Lisboa, Portugal

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Background

Biogenic amines are low molecular organic bases usually produced during the final processes of protein breakdown by bacterial enzymatic descarboxylation of the precursor amino acids. During the manufacture of dry-cured ham muscle proteins undergo an intense proteolysis leading to small peptides and higher levels of free amino acids, which may eventually be transformed to biogenic amines: putrescine (Put), cadaverine (Cad), histamine (His), tyramine (Tyr), spermidine (Spd) and spermine (Spm). Several authors have also reported liquid chromatographic procedures for the determination of amines in other foods, such as wine, sausage and fish (1-5). The presence of high levels of biogenic amines in foods assumes a relevant role in public health and the main concern is related to their toxicological effects.

Objective

The aim of this study was to apply and to study the reliability of a liquid chromatographic method for the quantitative determination of six biogenic amines in dry-cured ham.

Methods

All samples of dry-cured hams were purchased from a manufacturer and stored at -20°C prior to analysis. For the determination of biogenic amines, the samples were ground and mixed and extracted with 0.4 M perchloric acid (PCA) in Ultraturrax blender. An appropriate amount of 1,7-diaminoheptane was added to the samples before homogenization as an internal standard. After desproteinization the filtered and alkalinized extracts were derivatized with dansyl chloride solution and the mixture was allowed to react at 40°C for 45 minutes. The derivatised sample extract was filtered through a 0.45 μm filter and an aliquot (20 μl) was injected into the HPLC column. Extraction and derivatization procedures were modified basically from the method of Eerola *et al.*, (1993). Liquid chromatographic separations were performed on RP-C18 ODS column with an ammonium acetate-acetonitrile gradient elution and UV detection at 254 nm. Gradient begins at 50% and ends at 90% acetonitrile in 25 minutes. The system was equilibrated 5 min before next analysis.

Results and discussion

All the amines were well separated and the elution time was less than 25 min. Each amine was identified by the relative retention time values compared to the internal standard. Figure 1 shows a chromatogram of six dansylated amines standard and the internal standard at 1 μg/ml. The 1,7-diaminoheptane used as internal standard (ISTD) was eluted between histamine and tyramine. Typical chromatogram of dry-cured ham is shown in Figure 2.

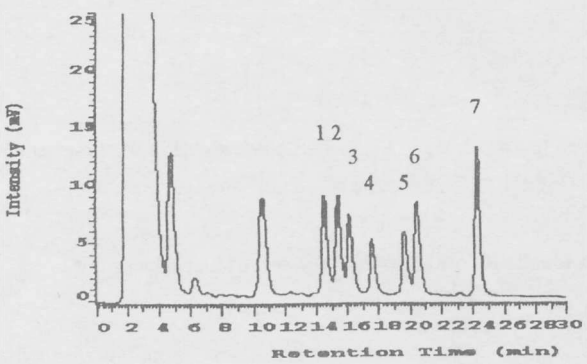

Figure 1. Chromatogram of dansyl derivatives of biogenic amines standard solution of 10 μg/ml. Peak identification and retention time: (1) Put, 14.47; (2) Cad, 15.34; (3) His, 16.09; (4) ISTD, 17.51; (5) Tyr, 19.59; (6) Spd, 20.38; (7) Spm, 24.24

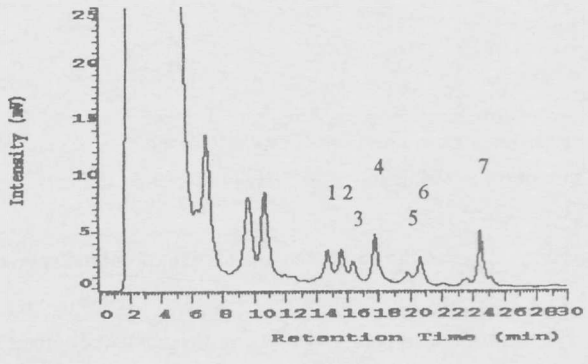

Figure 2. Chromatogram of dansyl derivatives of biogenic amines from dry-cured ham sample. Peak identification (1) Put; (2) Cad; (3) His; (4) ISTD; (5) Tyr; (6) Spd; (7) Spm

Validation of the method

Standard curves were prepared using standards in concentration range 0.05-10 μg/ml. Dansylated amines showed good linear responses and the correlation coefficients were >0.999. The limits of determination were 1 mg/kg for cadaverine and tyramine; 2 mg/kg for histamine and putrescine; and 3 mg/kg for spermine and spermidine.

To evaluate the precision of the method 10 determinations of the same sample were performed using the same reagents and apparatus. Intra-assay variations are listed in Table 1; relative standard deviations (RSD) were less than 10%. Inter-assay variation was determined by analyzing the same fortified sample on five consecutive days, to evaluate the run-to-run variation in the method (Table 2).

Table 1. Precision of the method for determination of biogenic amines in dry-cured ham: Intra-assay variations.

Amine	n	Mean value /mg kg^{-1}	RSD (%)
Put	10	12.0	5.5
Cad	10	5.4	7.3
His	10	1.1	8.3
Tyr	10	11.4	4.2
Spd	10	4.9	8.8
Spm	10	42.6	4.5

RSD = relative standard deviation

Table 2. Precision of the method for determination of biogenic amines in dry-cured ham: Inter-assay variations.

day	Put	Cad	His	Tyr	Spd	Spm
1	30.7	168.9	1.4	76.0	3.2	40.5
2	33.0	177.3	1.5	69.1	3.2	38.0
3	28.4	162.2	1.5	69.9	3.0	38.4
4	32.9	177.9	1.7	72.3	3.0	38.1
5	32.8	175.6	1.6	72.3	2.9	36.8
Mean value /mg kg^{-1}	31.6	172.4	1.5	71.9	3.1	38.4
RSD (%)	5.1	3.2	4.6	2.7	3.1	2.3

RSD = relative standard deviation

Perchloric acid extraction was tested by adding working standard solution at 3.0-60 mg/kg to samples before extraction. Six determinations were carried out for each addition level. Mean recovery of the analytes from dry-cured ham samples was between 91.3 and 103.3% (Table 3).

The concentrations of amines in ham were calculated from the peak height relative to the internal standard and the results are listed in Table 4.

Table 3. Recoveries of biogenic amines from dry-cured ham samples

Amine	n	Mean recovery %	RSD (%)
Put	6	91.3	5.2
Cad	6	99.5	3.4
His	6	94.0	13.5
Tyr	6	97.5	6.3
Spd	6	92.1	9.2
Spm	6	103.3	6.1

RSD = relative standard deviation

Table 4. Biogenic amine content in dry-cured ham (9 samples)

Amine	No. positive	[Amine]/mg kg^{-1} in positive samples Range	Mean	SD
Put	5	ND - 7.2	4.3	2.2
Cad	8	ND – 8.6	4.0	2.6
His	8	ND – 5.3	1.9	1.2
Tyr	2	ND - 1.0	0.8	0.2
Spd	9	1.8 – 7.7	4.6	1.4
Spm	9	20.6 – 47.3	35.4	6.4

SD = standard deviation; ND = not detected

Conclusions

This presentation describes a validated method for the quantitative determination of several biogenic amines in dry-cured ham. According to our results, spermine and spermidine always occur in the tested samples. In contrast to the relative uniformity in the levels of these polyamines, the other amines showed wide fluctuations. The levels of tyramine and histamine in dry-cured ham are, in general, too low to elicit direct adverse reactions (1).

References

(1) JOVER. T. H. *et al.* (1997) – Biogenic amine and polyamine contents in meat and meat products. *Journal of Agricultural and Food Chemistry*. 45: 2098-2102.

(2) VECIANA-NOGUES, M. T. *et al.* (1996) – Changes in biogenic amines during the manufacture and storage of semipreserved anchovies. *Journal of Food Protection*. 59 (11): 1218-1222.

(3) CÓRDOBA. J. J. *et al.* (1994) – Evolution of free amino acids and amines during ripening of Iberian cured ham. *Journal of Agricultural and Food Chemistry*. 42: 2296-2301.

(4) EEROLA. S. *et al.* (1993) – Liquid chromatographic determination of biogenic amines in dry sausages. *Journal of AOAC International*. 76 (3): 575-577.

(5) IBE, A. *et al.*, (1991) – Quantitative determination of amines in wine by liquid chromatography. *J. Assoc. Off. Anal. Chem.* 74 (4): 695-698.