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Food Control 13 (2002) 519–524

FOOD CONTROL

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# Biogenic amines: quality index of freshness in red and white meat

G. Vinci [a,*], M.L. Antonelli [b]

[a] Department of Control and Management of Goods and of their Impact on Environment, University "La Sapienza", Via del Castro Laurenziano, 9-00161 Rome, Italy

[b] Department of Chemistry, University "La Sapienza", P.le A. Moro, 5-00185 Rome, Italy

Received 26 November 2001; received in revised form 19 February 2002; accepted 21 February 2002

**Abstract**

Biogenic amine (BA) content in meat can be considered as a freshness marker or as a bad conservation marker. In particular the study of BA quantities in meat as a function of conservation time, could be a useful tool to control meat spoilage. In fact, the formation of some amines and concentration increase of those already existing in meat, are due to degrading processes in food, which are promoted by enzymatic reactions caused by external microbial activity or by endogenous tissue activities. The amines considered are: tryptamine, putrescine, cadaverine, serotonin, tyramine, spermidine, spermine. Their quantitative determination was carried out by means of HPLC, with spectrophotometric-UV detection, on pre-treated meat samples, both "red" (adult bovine) and "white" (chicken). The amines were extracted in acid aqueous solution ($HClO_4$) and then derivatised by dansylchloride. The trend of BA concentrations as a function of time was also investigated, in a period of 36 days, at the conservation temperature of $4 \pm 1$ °C. The proposed method is linear in the range of concentrations between 0.01 and 5.0 μg/ml. For all the amines considered recoveries were $\geqslant 93\%$. The CV values for all the measures ranged between 1.47% and 2.94%. The results show that in red meat the BA levels are still low until 9 days of storage ($\leqslant 30$ mg/kg) and that over 36 days only cadaverine and tyramine concentrations become very high ($\geqslant 120$ mg/kg). In white meat all the BA levels remain quite low ($\leqslant 40$ mg/kg) all over the 36 days, instead of the cadaverine content which gains 50 mg/kg at the seventh day of storage.


*Keywords:* Biogenic amines; Freshness markers; HPLC; Meat

## 1. Introduction

Knowledge of the biogenic amine (BA) levels in food is important for assessing health hazards, for example, they can cause some neurotransmission disorders due to their action as false neurotransmitters (Silla Santos, 1996). Moreover the presence of BAs can cause headaches, nausea and palpitations, especially if some monoamine oxidase (MAO) inhibitors are also ingested as drugs or alcohols (Arlorio, Coissonm, & Martelli, 1998). In particular tyramine excess could cause hypertension, meanwhile serotonin is a vasoconstrictor (Lehninger, 1975, Chap. 25). In particular BAs are produced in foods where high levels of protein are present, for example in meat (Askar & Treptow, 1989).

The formation of BAs is primarily a consequence of the enzymatic decarboxylation of specific amino acids due to microbial enzymes or tissue activity (Halasz, Barath, Simon-Sarkadi, & Holzapfel, 1994). In any case the action of micro-organisms action is very complex (Leuschner, Kurthara, & Hammes, 1998a) because it involves different enzymatic reactions. The quantity of BAs is also to be considered a marker of the level of microbiological contamination in food (Leuschner, Kurthara, & Hammes, 1999). However it is not an absolute criterion because these amines, too, could be degraded by some micro-organisms (Leuschner, Heidel, & Hammes, 1998b).

BA determination in meat is therefore suitable for detecting incipient spoilage and their quantities can be related to the freshness of the meat.

"Red meat" (adult bovine) and "white meat" (chicken) are particularly susceptible to protein degradation, which takes place under appropriate conditions. So that the levels of BAs in these two kinds of meat can be related to spoilage and sometimes to their protein degradation.

* Corresponding author. Tel.: +39-0649766514.

*E-mail addresses:* giulivin@scec.eco.uniroma1.it (G. Vinci), marta.antonelli@uniroma1.it (M.L. Antonelli).

0956-7135/02/$ - see front matter 
PII: S0956-7135(02)00031-2

Red and white meat differ regarding their nutritional value, production processes, economic aspects and spoilage (Bachrach, 1973; Cohen, 1971; Davidek & Davidek, 1995).

Various methods have been developed for the analysis of BAs in foods. All analytical methods employ two steps: extraction of the amines and their quantitative determination.

Many different solvents have been used for the extraction of BAs from the matrix, such as hydrochloric acid (Rice, Eitenmiller, & Koehler, 1975), trichloroacetic acid (Karmas & Mietz, 1978; Suzuki, Kobayashi, Noda, Suzuki, & Takama, 1990), perchloric acid (Minocha, Minocha, & Robie, 1990), methanol and other organic solvents (Redmond & Tseng, 1979). The extraction of amines represents the critical step of the process and it influences negatively the analytical recoveries.

Different chromatographic methods for quantitative determination of BAs in foods have been employed: thin-layer chromatography (Abdel-Monem & Ohno, 1975; Shalaby, 1995; Spinelli, Lakritz, & Wasserman, 1974), gas chromatography (Gaget, Wolf, Heintzelmann, & Wagner, 1987; Staruszkiewicz & Bond, 1981; Yamamoto, Itano, Kataoka, & Makita, 1982) and high performance liquid chromatography (HPLC) (Abdel-Monem & Ohno, 1975; Desiderio, Davalli, & Perin, 1987; Mietz & Karmas, 1977; Suzuki et al., 1990).

In order to find a correlation between the amines levels and the spoilage of the meat, an analytical study was carried out on red and white meat for determining quantitatively some BAs. The amines considered are: tryptamine, putrescine, cadaverine, serotonin, tyramine, spermidine, spermine. These BAs were determined in red (adult bovine) and white (chicken) meat and their levels were controlled during storage time. The main aspect studied was the variation, as a function of time, of amine levels. The study was carried out on fresh meat samples, stored at $T = 4 \pm 1$ °C for 36 days. The quantitative determination of BAs was performed by HPLC with a gradient elution program after extraction with perchloric acid and derivatisation with dansylchloride (Ruggieri, Botré, D'Alessandro, Mele & Vinci, 1995).

## 2. Materials and methods

### 2.1. Apparatus

Liquid chromatograph Varian 5000, Perkin–Elmer Model LC 75 variable wavelength detector connected to a PE Nelson Model 1020 and to an Epson LX-400 printer was used, with a Supelcosil LC-18 ($250 \times 4.6$ mm$^2$, 5 μm) column equipped with a Supelguard LC-18 (Supelco) pre-column. A homogeniser Universal Laboratory Aid MPW-309, a centrifuge ALC 4236, a filter Whatman mod. syringe filter 0.45 μm and an ultrasonic bath Elgasonic (Swiss made) were also employed.

### 2.2. Reagents

Tryptamine, putrescine, cadaverine, serotonin, tyramine, spermidine, spermine and dansylchloride were from Sigma Chemical; sodium hydroxide, sodium bicarbonate, disodium hydrogen phosphate, ammonium acetate, ammonium hydroxide, perchloric acid from Carlo Erba; acetonitrile and acetone for HPLC from Merck; ultrapure water was obtained with a Milli-Q system (Millipore).

### 2.3. Chromatographic conditions

Column temperature ($T_{col}$) = 40 °C; flow rate = 1.2 ml/min; injected volume = 10 μl; detection wavelength ($\lambda$) = 254 nm were used. The mobile phase was a gradient elution program with a binary mixture of 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B), as follows:

| Gradient elution program | |
|---|---|
| Time (min) | % A |
| 0.0 | 65 |
| 12.0 | 90 |
| 18.0 | 90 |
| 25.0 | 65 |

Each HPLC run took about 18 min and afterwards the column must be conditioned again with a mixture of 65% solvent A and 35% solvent B.

### 2.4. Preparation of standard solutions

Amine standard solutions were prepared by dissolving separately 70 mg of putrescine, 50 mg of cadaverine, 70 mg of spermidine, 70 mg of spermine, 70 mg of tryptamine, 80 mg of serotonin and 70 mg of tyramine in 50 ml of purified water. Stock solutions of the various compounds were diluted with $HClO_4$ (0.4 M) to obtain the necessary final concentration (2.5 μg/ml for cadaverine, 4.0 μg/ml for putrescine, spermine, serotonin and spermidine, 5.0 μg/ml for tyramine and tryptamine). Two hundred μl of NaOH 2 N were added to 1 ml portions of standard solution of amines to make it alkaline, then buffered by adding 300 μl of saturated $NaHCO_3$ solution and then 2 ml of dansylchloride solution (10 mg/ml in acetone) were added. The dansylation reaction proceedes at room temperature (Ruggieri et al., 1995) in darkness. One hundred μl of $NH_4$ OH were added after 15 min to stop the reaction and to remove residual dansylchloride. The final volume was adjusted to 5 ml by adding acetonitrile. The dansylated

Table 1
Mean recoveries of biogenic amines (cadaverine, tryptamine, putrescine, serotonin, tyramine, spermidine, spermine) from meat samples (adult bovine); data are expressed as mean values of three replicates ± S.D.

| Amine | Amount found in sample (mg/kg) | Amount of standard added (mg/kg) | Amount found in sample after addition (mg/kg) | Recovery (%) |
|---|---|---|---|---|
| Tryptamine | 20.42 ± 0.60 | 13.20 | 32.76 ± 0.68 | 93.5 |
| Putrescine | 2.08 ± 0.05 | 6.08 | 7.86 ± 0.18 | 95.1 |
| Cadaverine | 18.54 ± 0.54 | 11.84 | 29.76 ± 0.60 | 94.8 |
| Serotonin | 2.83 ± 0.08 | 5.60 | 8.27 ± 0.24 | 97.2 |
| Tyramine | 10.71 ± 0.31 | 11.65 | 22.10 ± 0.37 | 97.8 |
| Spermidine | 2.20 ± 0.06 | 5.22 | 7.24 ± 0.18 | 96.5 |
| Spermine | 27.15 ± 0.40 | 13.30 | 40.26 ± 0.77 | 98.6 |

amine solution obtained was filtered and injected into the chromatograph.

### 2.5. Preparation of sample solutions

All samples were obtained from the same butcher's shop. Red meat samples were from adult bovine thigh, all pieces were taken for analyses 15 days post mortem. White meat samples were cut from chicken breast at 2 days post mortem.

Sample treatments were the same for each kind of meat (adult bovine and chicken). To an amount of 5 g of minced meat were added 20 ml of 0.4 M $HClO_4$, the samples were homogenised and then centrifuged for 5 min at 2500 rpm. The sedimented solid portion was re-extracted by the same procedure as above. The two supernatants were pooled and diluted to 50 ml with 0.4 M $HClO_4$. One milliliter of the centrifuged acid extract was derivatised as previously described for the standard solutions.

## 3. Results and discussion

### 3.1. Method's performances

The proposed method for BA determination in meat, by means of chromatographic separation of their dansylchloride derivatives, is linear over the range of concentrations between 0.01 and 5.0 μg/ml. The detection limits were 2.0 mg/kg for tryptamine and serotonin, 1.0 mg/kg for putrescine and cadaverine and 0.5 mg/kg for tyramine, spermidine and spermine. The reported values refer to the BA quantities detected taking into account the injection volume and the dilution effects due to the extraction and derivatisation steps.

In Table 1 we report the recoveries of seven BAs in samples of meat. These recoveries were obtained by adding to meat samples different amounts of the standard considered amines. The results showed a good precision and the recovery values were always $\geqslant 93\%$. Parameters which could influence the recovery values were: the solvent (acid chosen), the extraction pH and

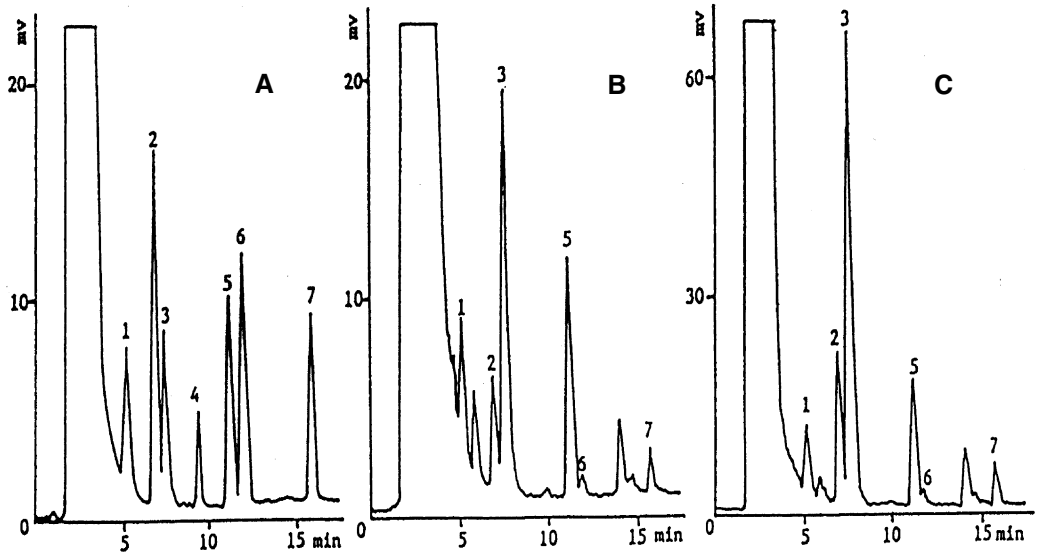


Fig. 1. Chromatograms relative to: (A) standard solution of seven biogenic amines; (B) adult bovine meat sample; (C) chicken meat sample (1 tryptamine, 2 putrescine, 3 cadaverine, 4 serotonin, 5 tyramine, 6 spermidine, 7 spermine).

the conditions chosen for the derivatisation. The reported values are the mean of three determinations of three different portions of the same meat sample. As can be seen the S.D. values ranged between 0.6 and 0.05 and the relative CV values between 1.47% and 2.94% values that are sufficiently low to ensure a good precision of the proposed method. The detection limits for all the considered amines, confirmed that very low quantities of BA could be detected in real samples.

### 3.2. BA determination in meat samples

Fig. 1 shows the chromatograms of: (A) standard solution, containing seven BAs and also those relative to samples of: (B) adult bovine meat and (C) chicken meat. It can be observed that these was a good resolution for peaks relating to all the amines examined in a quite short analysis time (about 15 min). In the chromatograms of real meat samples there were two unknown peaks which did not interfere with those of the amines under examination.

Figs. 2 and 3 show the trends in BA levels as a function of time, determined for the two kinds of meat stored at +4 °C for up to 36 days.

The variations of the BA differed quite markedly and also differed between white and red meats. As can be seen, cadaverine was the amine produced in the greatest quantity in both meats, and this is probably ascribable to the high amount of the precursor lysine in meat. Moreover this amine appeared sooner and increased more rapidly in white meat, where the lysine quantity

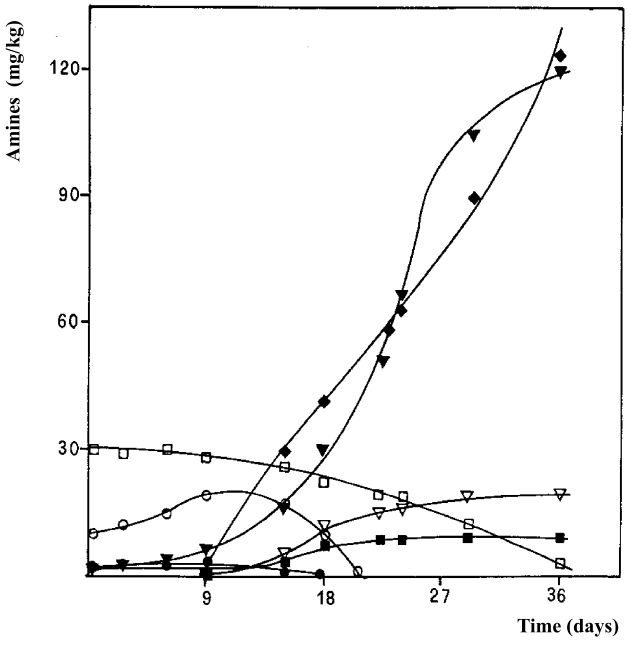


Fig. 2. Amount of biogenic amines in red meat (adult bovine), stored at +4 °C with time (○, tryptamine; ▽, putrescine; ◆, cadaverine; ●, serotonin; ▼, tyramine; ■, spermidine; □, spermine).

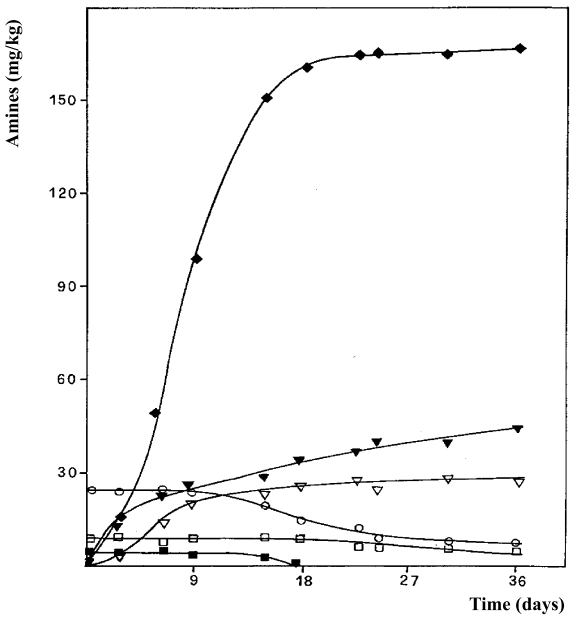


Fig. 3. Amount of biogenic amines in white meat (chicken), stored at +4 °C with time (○, tryptamine; ▽, putrescine; ◆, cadaverine; ●, serotonin; ▼, tyramine; ■, spermidine; □, spermine).

was a little higher than in red meat (Carnovale & Marletta, 1997). Tyramine concentration became considerable in red meat after 15 days and after increased rapidly. In white meat on the contrary it appeared earlier but did not exceed 40 mg/kg even after 30 days of storage. It should be noted that serotonin was present in red meat in a very low quantity and was completely absent in white meat.

In white meat quite all the BA increased earlier than in red meat, probably because in chicken muscles there are shorter fibres, which can be easily attacked by proteolytic enzymes.

In Table 2 the total BA concentrations found in red and white meat samples at the 5th, 15th and 30th day of storage are listed. Because spermidine (spd) and spermine (spm) are physiological polyamines constituents, the table also shows the total amine contents without those two amines. As can be seen, after 30 days the total

Table 2
Total amount of the biogenic amines in red and white meat at different days of storage at +4 °C, calculated with and without spermidine (spd) and spermine (spm) quantities

| Time (days) | Kind of meat | Total amines (mg/kg) | Total amines without spd and spm (mg/kg) |
|---|---|---|---|
| 5 | Red | 52.0 | 20.5 |
| | White | 105.0 | 91.2 |
| 15 | Red | 102.4 | 73.1 |
| | White | 237.2 | 225.4 |
| 30 | Red | 238.1 | 217.4 |
| | White | 248.7 | 243.3 |

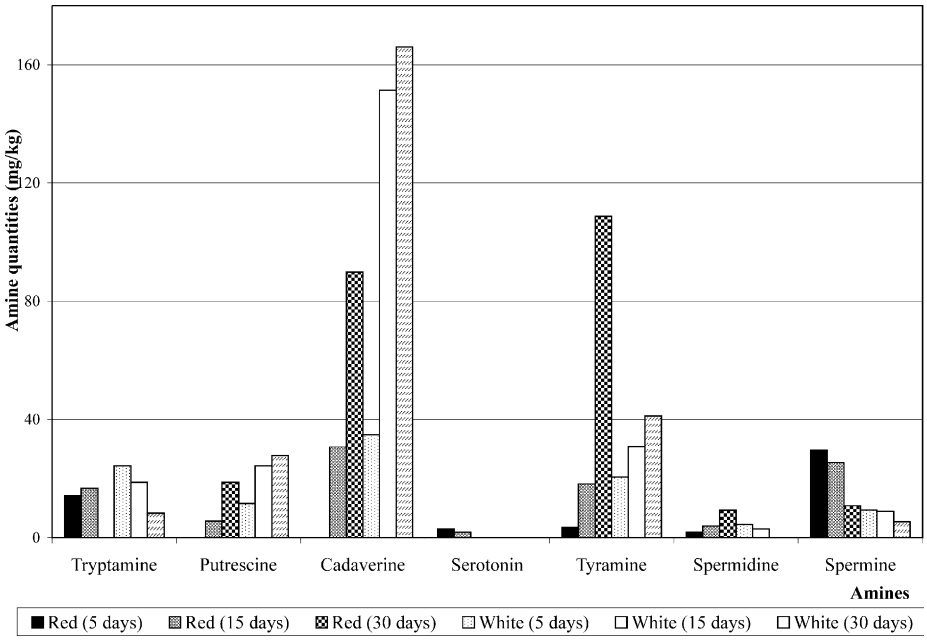


Fig. 4. Histogram relative to the biogenic amine levels in red and white meats monitored at different days of storage at +4 °C.

amount of amines was similar in the two kinds of meat, while after 5 and 15 days the total is about double in white meat. By considering the total without spd and spm, the difference between white and red meat becomes four times at the 5th day and about three times at the 15th day. This means that spd and spm quantities contribute to the total amine level much more in red than in white meat; in other words up until about 15 days of storage, in red meat the quantities of BA, resulting from spoilage, were lower than in white meat. Moreover the total amines levels were similar after 30 days in both meats, but this level was reached after only 15 days in white meat: whether correction was made for the two physiological amines or not. In red meat the contribution to the final total amine quantities, besides cadaverine, was due principally to tyramine, which was low in white meat.

In particular Fig. 4 shows histograms of the relative levels of each amine considered at the 5th, 15th and 30th day of storage, in the same samples. The experimental evidence showed that the great increase of cadaverine, in both kinds of meat, and of tyramine, for red meat, were an indicator of meat spoilage.

## 4. Conclusions

The method proposed here, for biogenic amine (BA) determination in meat samples, showed both a high sensitivity and good precision. The recoveries obtained were also very good, in agreement with those reported in the literature (Antolini, Franciosini, Floridi, & Floridi, 1999; Eerola, Hinkkanen, Lindfors, & Hirvi, 1993).

By using the proposed method the trends of seven BA content were monitored during 30 days in two types of meat samples (red = adult bovine and white = chicken) stored at +4 °C.

The results showed that the BA levels were indicators of spoilage both in red and white meat. In particular the determination of cadaverine concentration could be used to monitor spoilage in both kinds of meat, and also tyramine concentration appeared to be useful to control red meat storage. In general it was observed that chicken meat conservation was critical, because non physiological BA increased earlier and more rapidly than in bovine meat. Probably this was ascribable to the presence of shorter muscular fibres in chicken, and consequently to the presence of proteins with shorter chains compared to those of cow meat (Cappelli & Vannucchi, 1998, Chap. 21). Facilitating attack by proteolytic enzymes and increasing quantities of aminoacid precursors for the biosynthesis of BA.

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