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

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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 ± 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 $\geq 93\%$. 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 (≤ 30 mg/kg) and that over 36 days only cadaverine and tyramine concentrations become very high (≥ 120 mg/kg). In white meat all the BA levels remain quite low (≤ 40 mg/kg) all over the 36 days, instead of the cadaverine content which gains 50 mg/kg at the seventh day of storage.
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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.

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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 (250×4.6 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 (λ) = 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

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