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# Histamine and Tyramine in Meat Products: Relationship with Meat Spoilage

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*ABSTRACT*

*Biogenic amines in processed meat products can be useful as indices of poor-quality raw material, but they can also be related with microbial activity involved in fermentation processes. This paper provides new data on this topic. Histamine, tyramine and pH changes were followed during storage/spoilage of beef and pork at both room and refrigerated temperatures. A notable increase in the content of amines was observed at both temperatures studied. A greater and more rapid formation of histamine and tyramine took place in pork than in beef. The increase in the amines occurred before the increase in pH value. A preliminary study about the influence of ripening on histamine and tyramine contents was also carried out. This process seems to exert a greater influence on histamine than on tyramine content. Finally, the histamine and tyramine contents of 63 Spanish meat products were determined. Both amines were detected in all samples, but concentrations varied greatly. Uncooked and ripened meats showed statistically significant higher amounts of both amines than cooked meat products. In cooked meat products, histamine values ranged from 0·25 to 3·90 mg/kg and tyramine from 0·50 to 25·6 mg/kg. For uncooked and ripened meat products, the range of histamine was from 0·25 to 249 mg/kg and of tyramine from 0·45 to 510 mg/kg.*

## INTRODUCTION

The biogenic amines, histamine and tyramine, are sometimes found in certain foods. Their presence in meat products, sometimes in high



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concentrations, has already been noted by several authors (Rice *et al.*, 1975; Vandekerckhove, 1977; Santos-Buelga *et al.*, 1981; Rogowski & Döhla, 1984; Vidaud *et al.*, 1988). It is accepted that these amines are derived from decarboxylation of their precursor amino acids, histidine and tyrosine (Rice *et al.*, 1975; Eintenmiller *et al.*, 1978).

Biogenic amines can be found in processed meat products as a consequence of microbial activity related to the fermentation process involved in the manufacturing of certain sausages, but there is also the possibility that they are found in poor quality raw materials by microbial contamination. Del Prete *et al.* (1979) reported that meat transport, over long distances and prolonged periods of storage in inappropriate conservation conditions, can facilitate contamination by microorganisms which can form biogenic amines. These substances have been proposed as possible 'indicators' of poor hygienic quality of raw materials used and/or of poor production conditions for fish products (Mietz & Karmas, 1977; Hui & Taylor, 1983). Although meat products have not been extensively studied, it has been pointed out that good manufacturing processes for meat products, using fermentation starters, result in formation of very small amounts of biogenic amines (Rice *et al.*, 1975, 1976; Eintenmiller *et al.*, 1978). So under these conditions and assuming that good quality raw materials have been used, large amounts of these substances in meat products would be indicative of the existence of contaminant microorganisms during the manufacturing process (Rice & Koehler, 1976). In order to avoid or reduce the formation of biogenic amines in meat products, the following precautions should be taken (Rice & Koehler, 1976; Cerutti, 1986): supervision of hygienic conditions in production plants, use of starter cultures and adequate thermal treatments on raw materials.

On the other hand, biogenic amines in foods have toxicological implications: (a) 'histamine intoxication' can be provoked by the consumption of foods with high histamine contents (Codex Alimentarius, 1985; Committee on Food Protection, 1985; Taylor, 1985); (b) biogenic amines in the presence of nitrites may produce compounds which can be endogenous precursors of *N*-nitrosamines (Rogowski & Döhla, 1984; Vidaud *et al.*, 1988); (c) ingestion of large amounts of biogenic amines may provoke hypertensive crises in patients under treatment with monoamine-oxidase enzyme-inhibiting drugs (MAOI) (Ponto *et al.*, 1977; Mariné *et al.*, 1986). However, these amines are not considered to be a grave risk for humans, as long as they are present in low concentrations and their metabolism is not blocked or genetically altered (Pechanek *et al.*, 1980).

The aim of the present work was to study the changes in histamine and tyramine contents during storage/spoilage of beef and pork meat, at both refrigerated and room temperatures, in order to obtain data to establish any

possible relationship between biogenic amine content of meat products and poor-quality raw materials (meats) used in their production. We have also studied the influence of ripening on biogenic amines in ripened meat products. Finally, this paper provides data on the histamine and tyramine content in some Spanish meat products. Data on this topic are scarce in meat products, especially concerning tyramine, which is of the same toxicological and technological interest as histamine.

## MATERIALS AND METHODS

### Samples

To study the histamine and tyramine evolution during storage/spoilage of beef and pork meat, we used whole meat pieces of approximately 0·5 kg, which were triturated and homogenized in the laboratory. Trituration of meat favours its decomposition, but it is a necessary operation to assure sample homogeneity. Moreover, many meat products are manufactured using minced meat. Triturated and homogenized beef and pork meat were divided into two portions; one of these was kept at room temperature (20–22°C) for 40 h, samples being taken every 8 h and the other was refrigerated (4–5°C) for 110 h, with samples being taken every 12 h.

To study the influence of ripening on biogenic amine content, six samples of a single ripened sausage ('sobrasada') of the same commercial brand and processed in the same factory were used. However, these samples came from different manufacturing batches and had different ripening times. Sample 1 corresponded to a recently manufactured product (ripening time zero) and the remaining five samples had progressively increasing ripening times (15, 36, 111, 142 and 217 days, respectively). Samples were stored at −18°C until analyzed.

Histamine and tyramine contents in 63 Spanish meat products were determined. Most samples studied consisted of mixtures of pork, or pork and beef, seasoned with salt, curing substances and spices and were packed in natural and/or artificial casings. We analyzed: (a) 11 samples of 'mortadela', a meat product in which manufacturing includes a cooking process, but is not ripened, (b) five samples of cooked ham, (c) 38 samples of uncooked and ripened meat products (19 samples of 'salchichón', 11 of 'chorizo', three of 'sobrasada' and five of 'salami'), which are similar in composition but differ in the spices used in their production and in the way that they are ripened and (d) nine samples of country-cured ham. All samples were stored at −18°C prior to analysis.

## Methods

Histamine was determined according to the spectrofluorometric method of Vidal-Carou *et al.* (1989*a*) with some modifications to make it suitable for meat products (Vidal-Carou *et al.*, 1989*b*). The method consists of the following successive steps: (1) grinding and homogenization of the sample and extraction of histamine with $HClO_4$, (2) alkalinization of 5 ml of the perchloric acid extract and extraction of histamine with *n*-butanol, (3) transferring the histamine to HCl, (4) fluorescent complex formation with *o*-phthaldehyde, and (4) spectrofluorometric reading at 350 nm excitation wavelength and 430 nm emission wavelength. Reliability of this method in terms of precision was CV = 5·42% for a 36·60 ± 2·02 mg/kg histamine content ($n = 10$), and recovery was 96·20% ($n = 20$).

Tyramine determination was carried out according to the spectrofluorometric method of Santos-Buelga *et al.* (1981), which consists of the following steps: (1) grinding, alkalinization and homogenization of sample with washed fine grain sand and anhydrous $Na_2SO_4$, (2) extraction of tyramine with ethyl acetate in a glass column, (3) transference of the tyramine to HCl, (4) fluorescent complex formation with α-nitroso-β-naphthol and (5) spectrofluorometric reading at 450 nm excitation wavelength, and 540 nm emission wavelength. Its reliability was also verified for precision, CV = 6·46% for a tyramine content of 112·9 ± 7·30 mg/kg ($n = 10$) and recovery, 96·90% ($n = 20$).

pH was measured by direct reading in a pH meter, after trituration and homogenization of samples with distilled water (Sanchidrián, 1985).

# RESULTS AND DISCUSSION

## Biogenic amines evolution during storage/spoilage of meat

Changes in histamine, tyramine and pH levels of pork meat and beef were followed as a function of storage/spoilage time; the experiments at room temperature (18–22°C) and refrigerated temperature (4–5°C) are shown in Figs 1 and 2, respectively. Formation of both amines was observed in both types of meat and at both temperatures. At the beginning of the experiments (time zero), the histamine and tyramine concentrations in both types of meat were very low ($\leq 2$ mg/kg). The levels of amines at room temperature were higher than at refrigerated temperature for the same time elapsed, and the increases in their levels occurred much more quickly at room temperature. In all cases the histamine and/or tyramine increases occurred before a significant increase in pH value was detected.

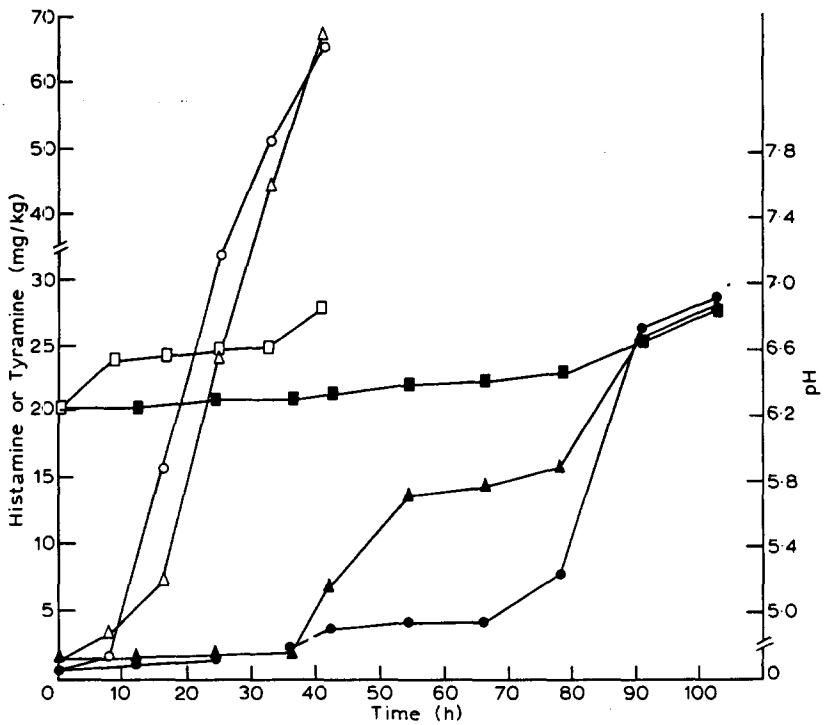


**Fig. 1.** Changes in histamine, tyramine and pH during storage/spoilage of pork meat at both refrigerated and room temperature. Refrigerated temperature: ●, histamine; ▲, tyramine; ■, pH. Room temperature: ○, histamine; △, tyramine; □, pH.

*Room temperature experiments*

Higher levels of both amines were reached in pork meat (approx. 65 mg/kg for each one) than in beef (approx. 5 mg/kg of histamine and 25 mg/kg of tyramine). Histamine and tyramine formation were similar in pork meat (Fig. 1) so, after 8 h, there was a strong increase in both amines and rapid increase continued throughout the whole period of the study. On the other hand, histamine and tyramine evolution in beef were different (Fig. 2); a constant increase in tyramine levels was observed throughout the entire time period of study, whereas histamine did not show much increase during this time (from 1·50 mg/kg to 5·90 mg/kg).

*Refrigerated temperature experiments*

The levels of histamine and tyramine reached at the end of this study were similar in pork meat (Fig. 1) although their evolution profiles were different. A marked increase of tyramine was observed after 40 h, whereas no great increase in histamine occurred until after 80 h. The evolution of both amines in beef (Fig. 2) was similar to pork until 54 h but, after this time, histamine showed a more rapid and quantitatively more significant increase than

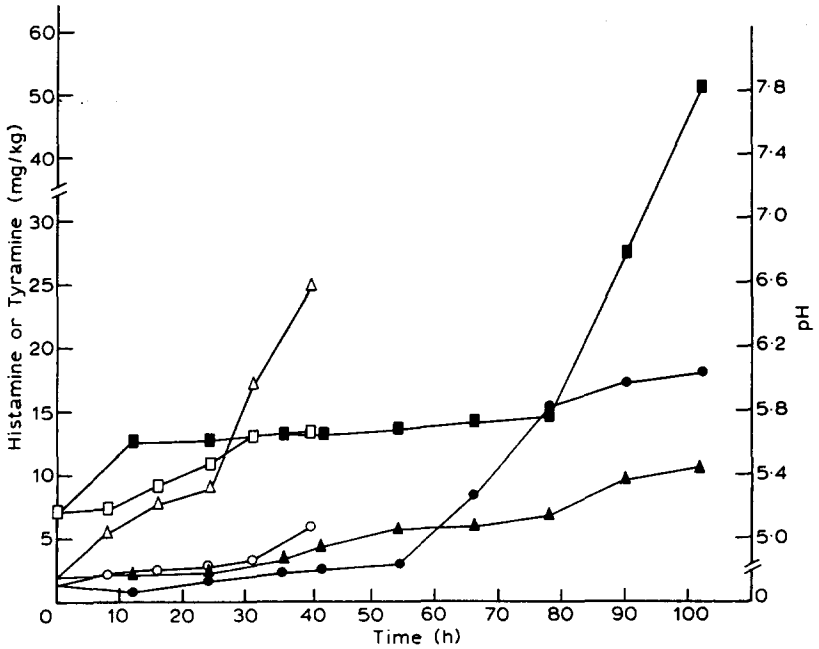


**Fig. 2.** Changes in histamine, tyramine and pH during storage/spoilage of beef at both refrigerated and room temperature. Refrigerated temperature: ●, histamine; ▲, tyramine; ■, pH. Room temperature: ○, histamine; △, tyramine; □, pH.

tyramine. A sharp increase in the pH value of beef was observed after 80 h which, nevertheless, was not matched by a marked increase in amine content.

By comparing the development of histamine and tyramine in both meats it is notable that: (a) similar concentrations of both amines were reached at each temperature by the end of the storage in pork meat and (b) for beef, more tyramine than histamine was formed at room temperature, whereas more histamine than tyramine was formed under refrigeration. The time elapsed from the beginning of the experiment to the point where a significant increase occurred in one or the other amine was different for each type of meat, being shorter for pork than for beef. Thus, 'latency time' at room temperature was approximately 10 h for pork, whereas it was approximately 25 h for beef. At refrigeration temperature, this 'time' was approximately 40 h for pork and approximately 60 h for beef.

The results indicate that inadequate refrigeration and prolonged holding of meat can lead to a significant formation of biogenic amines. These results agree with similar previous studies on the subject (Wortberg & Woller, 1982; Sayem-El-Daher *et al.*, 1984). Nevertheless, there are still few studies on this topic and thus definitive conclusions on the role of biogenic amines as indicators of the poor quality raw materials used in the manufacturing of meat products cannot be made.

## Influence of ripening time on the histamine and tyramine content of a ripened sausage ('Sobrasada')

To establish the role of biogenic amines as indicators of the use of poor quality raw materials in the manufacturing of meat products, it is also necessary to check whether or not the ripening process results in amine formation. We have studied the histamine and tyramine contents in six 'sobrasadas' of the same commercial brand, produced by the same factory, but which have different ripening times. The size of the 'sobrasada' was approximately 7–8 cm × 40 cm. Different histamine and tyramine contents could be found depending on the part of piece considered. In order to check this possibility, two aliquots of each piece were taken for analysis: one from the centre, and one from the end of each piece, as shown in Fig. 3. Table 1 shows both amines' contents in the two parts of those products, as well as the ripening time corresponding to each sample. In most samples, a greater amount of histamine was observed in the centre than in the end of the piece, but for tyramine this difference was not so pronounced. This fact could be related to the type of microorganisms responsible for amine formation. Results obtained indicate that, under anaerobic conditions (centre of the piece), formation of histamine is higher than in the end of the piece. This behaviour was not observed for tyramine. Figure 4 shows the average (centre and end) values of histamine and tyramine content for each sample. Ripening time seemed to exercise a greater influence on histamine content than on tyramine content, since in the first samples we found lower levels of histamine than in samples with a longer ripening time. No clear difference was observed in tyramine concentrations as a function of ripening time. Results of this study should be considered orientative or preliminary, and it will be necessary to confirm them in products for which (a) the

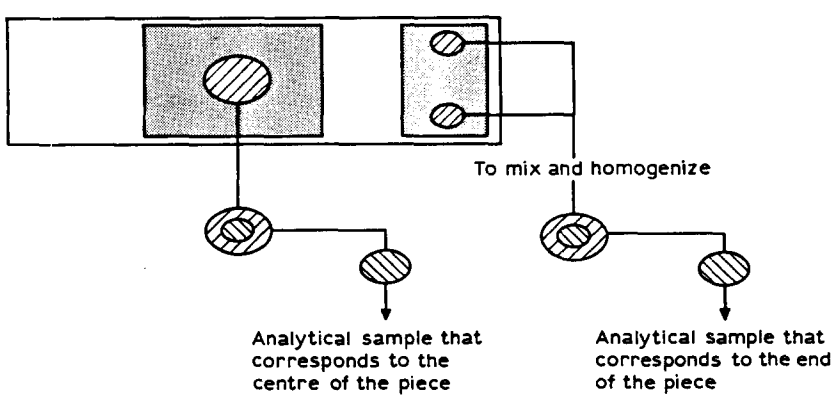


**Fig. 3.** Analytical sampling scheme for histamine and tyramine determinations in pieces of 'sobrasadas' with different time of ripening.

**TABLE 1**
Histamine and Tyramine Contents in Centre and End of 'Sobrasadas' with Different Times of Ripening

| *Sample number* | *Time of ripening (days)* | *Histamine (mg/kg)* | | *Tyramine (mg/kg)* | |
|---|---|---|---|---|---|
| | | *Centre* | *End* | *Centre* | *End* |
| 1 | 0 | 2·60 | 2·90 | 3·50 | 6·70 |
| 2 | 15 | 11·2 | 5·90 | 21·5 | 6·90 |
| 3 | 36 | 27·5 | 13·7 | 2·35 | 3·30 |
| 4 | 111 | 53·2 | 30·7 | 153·0 | 140·0 |
| 5 | 142 | 27·2 | 41·0 | 20·2 | 7·35 |
| 6 | 217 | 42·5 | 27·8 | 13·6 | 14·8 |

hygienic quality of the raw materials used, and (b) possible differences in the microbial flora present in the centre and the end of the meat product are known.

## Histamine and tyramine contents in some Spanish meat products

Both amines were detected in the 63 samples studied. The concentrations of histamine and tyramine varied greatly between the different types of meat products as well as among samples of a single product type. Table 2 shows

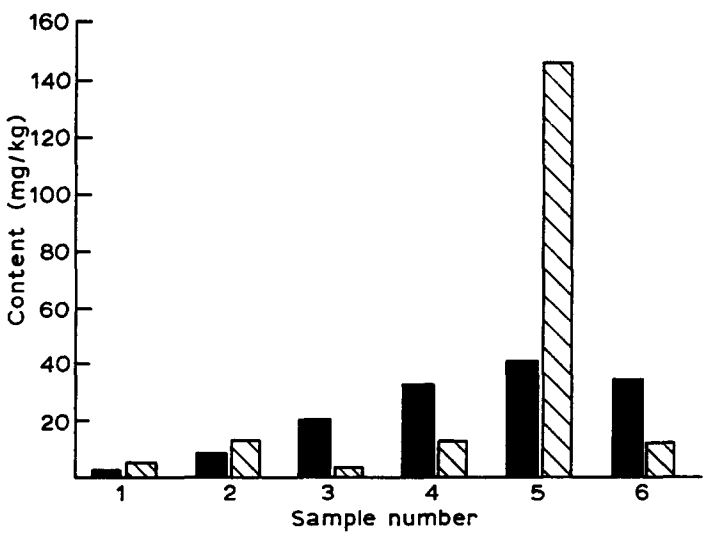


**Fig. 4.** Average contents of (■) histamine and (▨) tyramine in 'sobrasadas' with different time of ripening.

**TABLE 2**
Histamine and Tyramine Contents in the Meat Products Studied

| *Type of meat products* | *Number of samples* | *Histamine (mg/kg)* | | *Tyramine (mg/kg)* | |
|---|---|---|---|---|---|
| | | $\bar{x} \pm SD$ | *Range* | $\bar{x} \pm SD$ | *Range* |
| *Cooked*[a] | ($n = 16$) | 1·45 ± 0·89 | 0·25–3·90 | 8·20 ± 9·42 | 0·50–25·6 |
| 'mortadela' | ($n = 11$) | 1·50 ± 0·55 | 0·55–2·25 | 10·9 ± 9·80 | 0·50–25·6 |
| cooked ham | ($n = 5$) | 1·30 ± 1·30 | 0·25–3·90 | 2·30 ± 1·94 | 0·50–6·0 |
| *Uncooked and ripened*[a] | ($n = 47$) | 29·7 ± 50·9 | 0·25–249 | 108 ± 99·4 | 0·45–509 |
| 'salchichon' | ($n = 19$) | 17·6 ± 26·6 | 0·25–103 | 133 ± 62·3 | 34·5–270 |
| 'chorizo' | ($n = 11$) | 76·3 ± 79·6 | 1·75–249 | 176 ± 150 | 1·55–509 |
| cured country ham | ($n = 9$) | 18·6 ± 41·3 | 1·95–128 | 15·6 ± 27·2 | 0·45–69·5 |
| 'salami' | ($n = 5$) | 6·15 ± 3·30 | 3·45–11·5 | 65·9 ± 38·4 | 2·20–102 |
| 'sobrasada' | ($n = 3$) | 8·40 ± 5·60 | 3·10–14·3 | 55·5 ± 35·8 | 14·2–77·6 |

[a] Data corresponding to all products included in each one of both types.

the range of histamine and tyramine contents, as well as the average values and the standard deviations for both cooked and uncooked and ripened meat products. In general, uncooked and ripened meat products showed higher levels of both amines than cooked meat products. By analysis of variance ($p = 0·05$) it was shown that the differences in histamine and tyramine concentrations were statistically significant (DF = 1, 61; Ft = 4·00; Fe histamine = 4·77; Fe tyramine = 15·68), in spite of the large degree of variability. High biogenic amine contents in cooked meat products, in principle, can only be attributed to the use of poor-quality raw materials, which have high levels of these substances. In ripened meat products, furthermore, biogenic amines may have been formed during the ripening process by microorganisms responsible for this process, or by contaminant microorganisms. This possible double origin could explain the higher concentrations of amines in these products. Other authors have also found higher levels of biogenic amines in uncooked and ripened meat products than in cooked meat products without ripening (Taylor *et al.*, 1978; Santos *et al.*, 1985).

Results of analysis of variance ($p = 0·05$) showed that tyramine contents were higher than histamine contents in cooked meat products (DF = 1, 30; Ft = 4·17; Fe = 8·20), as well as in uncooked and ripened meat products (DF = 1, 92; Ft = 3·95; Fe = 22·83). This fact would suggest a greater tyrosine-decarboxylase activity than histidine-decarboxylase activity in raw materials, or during the manufacturing process. The greater concentration of tyramine as compared to histamine has also been reported by other authors (Rice *et al.*, 1975, 1976; Rogowski & Döhla, 1984).

## CONCLUSIONS

(a) Histamine and tyramine were formed in greater amounts and more quickly in pork meat than in beef, at both refrigerated and room temperatures.
(b) Higher histamine concentrations were found in samples of 'sobrasada' which had undergone a longer ripening period. However, ripening time did not have a clear-cut influence on tyramine content.
(c) Histamine and tyramine concentrations were significantly higher in samples of uncooked and ripened meat products than in samples of cooked meat products. Furthermore, tyramine content was generally higher than histamine content in most of the samples.

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