BIOGENIC AMINES IN TWO PROCESSES OF SPANISH DRY-CURED HAM

M. Hortós and J. A. García Regueiro
IRTA. Food Chemistry Unit. Centre de Tecnologia de la Carn. Granja Camps i Armet s/n. 17121 Monells (Spain)

Background

Biogenic amines are produced by amino acid decarboxylation during the processing of different kinds of foods which are subject to microbial activity during ripening or storage. The use of biogenic amines as indicators for food freshness and hygiene in meat and meat products has been investigated by several workers (Sayem-el-Daher et al., 1984). Putrescine, Cadaverine, Tyramine and Histamine have been observed at levels lower than 10mg/Kg in fresh meat (Rogowski and Döhla, 1984). Putrescine and Cadaverine have been related with meat spoilage (Jay, 1994). The formation of biogenic amines in some technological processes, especially in fermented and dry-cured products, is favoured by the release of amino acids. A NPN increase during processing of fermented products has been reported by several workers (Cantoni et al., 1974; Astiasaran et al., 1990). The formation of biogenic amines was reported in fermented sausages (Cantoni et al. 1974, Vidal-Carou et al. 1990; Hernández, 1996). There are few data about the concentration of biogenic amines in dry-cured ham that has been reported to have low concentrations of these compounds (Cantoni et al. 1970, Cordoba et al. 1994).

Objectives

Evaluation of the content of biogenic amines in Spanish dry-cured ham produced by short (6 months) and long (12 months) processes.

Methods

Sampling

80 female pigs were sampled in a commercial slaughterhouse previous selection was carried out by pH and quality meter measurements to avoid PSE and DFD conditions.

Ham curing

Hams were cured with a mixture (40 g/Kg) of salt and nitrate in a ratio 100:1. Fifteen days later the hams were washed and hung at 5° C for 30 days. The temperature was increased 1,5°C/weekly until the sixth month in the short process and 0,6°C/weekly until the twelth month in long process. Five samples of *Semimembranosus* and *Biceps femoris* muscles were taken for analysis at different aging period: fresh ham and salting were common for both processes; after in short procedure were taken in post-salting (T2) and 2 (T3), 4 (T4) and 6 (T5) months, and in the long procedure in post-salting (T2) and at 4 (T3), 6 (T4) and 12 (T5) months

Amine analysis

The method used was described by Hui and Taylor (1983) but it was applied with some modifications. 5 g of of whole minced muscles were homogenized with 50 ml of water by means of an Ultraturrax (*13000xg*) and 25 ml of $HCLO_4$ 2.4 M and water were added to reach the final volume up to 100 ml. The extract was stored at 4°C during 1 hour and filtered through a paper filter (Whatman n° 52). 75 ml of the solution were taken and KOH 0.1M was added until the pH reached to 7 and water was added to reach the volume up to100 ml. The solution was stored at 4°C overnight and after was filtered through a paper filter (Whatman n° 52). An aliquot of 5 ml were taken for amine derivatization. The solution was dried adding acetone and the residue was redissolved in 2 ml of a saturated sodium bicarbonate; 1ml of dansyl chloride solution and 10µl of internal standard (diaminoheptane) were added, the mixture was kept overnight in the dark at room temperature. Acetone was evaporated in a stream of nitrogen. Dansylated amines were extracted with three portions of 3 ml diethyl ether. The residue was redissolved in 2ml acetonitrile and 10µl were injected for HPLC analysis. HPLC mobile phase was: solvent **A** methanol:acetonitrile 1:1, solvent **B** 0.33 mM phosphoric acid in distilled water. A linear gradient 60% **A** to 80% **A** in 20 minutes was applied at 35°C with a flow of 1.5 ml/min. UV detector was used at 254 nm. Samples and standards were injected via a Rheodyne valve. The instrument used was a Waters (Millipore, USA).

Statistical analysis

An ANOVA with Tukey test was applied. SAS was used to carry out the statistical analysis.

Results and Discussion

A comparative study of amine extraction in meat and meat products was reported by Zee et al. (1983). Percloric extracts were chosen because of a high recovery was observed. The pre-column derivatization of amines with dansylchloride was used due to allow a easier separation and detection by using fluorescence detection. Ether extraction was necessary to remove interference compounds in order to obtain more accurate results. HPLC separation of biogenic amines was achieved with complete resolution for the biogenic amines studied. The concentrations of biogenic amines detected were in the range 5-1 µg/g at the end of both processes. The concentrations were lower than those described for fermented meat products (Hernández, 1996; Vidal-Carou et al. 1990). Short process showed, in both muscles, higher concentrations than long process (Figure 1); when muscles were compared *Biceps femoris* presented lower concentrations in long process; this result agree with the aminoacid concentrations that were higher in this muscle. Short process was characterized by higher temperature during the drying steps, this factor could increase the production of biogenic amines. Spermidine and spermine were not detected in this work (<1µg/g). Biogenic amines were produced from four to twelve months of processing

(steps T4 and T5). Putrescine and Cadaverine presented the highest concentrations. These biogenic amines showed an important increase between steps T4 and T5 in both processes. A small decrease or not change was observed for histamine.

Conclusion

The concentrations of biogenic amines in dry-cured ham were low (< 5µg/g). Long process (12 months) produced lower quantities of these compounds than short process (6 months). Putrescine and Cadaverine presented the highest concentrations. The most remarkable change was produced between the two last steps of drying.

References

Astiasarán, I., Lizarraga, T., Melgar, J., Santamaría, I. Villanueva, R. and Bello, J. (1990). Estudio comparativo de las características del chorizo según la tecnología de elaboración empleada. *Alimentaria*, 57-62

Cantoni, C., Bianchi, M. A., Cerutti, F. and Goffi, M.L. (1970). Sulla presenza di ammine non volatili e di aminoacidi liberi in prosciutti staginati normali ed alterati. *Arch. Veter. Ital.*, 21: 89

Cantoni, C., Bianchi, M. A. and Beretta, G. (1974). Variazioni di aminoacidi liberi istamina e iramina in insacatti stagionati (salami). *Industrie Alimentaria*, 13: 75-78

Córdoba, J.J., Antequera. T., García, C., Ventanas, J., López-Bote, C. and Asensio, M. (1994). Evolution of free aminoacid and amines during ripening of Iberian ham. *J. Agric. Food Chem.*, 42: 2296

Hernández, T. (1996). Estudi de l'origen de les amines biògenes en derivats càrnics. *Thesis*. Universitat de Barcelona.

Hui, J. and Taylor, S. (1983). High pressure liquid chromatographic determination of putrefactive amines in foods. *J. AOAC.*, 66: 853

Jay, J. M. (1994). Microbiología moderna de los alimentos. Ed. Acribia. Zaragoza

Rogöwski, B. and Döhla, I. (1984). Bestimmung und gehalt biogener aminen in fleish und felischwaren. *Lebensm. Geritchtl. Chem.*, 38: 20-21

Sayem-El-Daher, N., Simrad, R.E., and Fillion, J. (1984). Changes in the amine content of ground beef during storage and processing. *Lebensm. Wiss. u. Technol.*, 17: 319-323

Vidal-Carou, M. C., Izquierdo Pulido, M., Martín Morro, M. C. and Mariné Font, A. (1990). Histamine and Tyramine in meat products: relationship with meat spoilage. *Food Chem.*, 37: 239-249

Zee, J.A., Simard, R.E. and L'Hereux, L. (1983). Evaluation of analytical methods for determination of biogenic amines in fresh and processed meat. *J. Food Protec.*, 46: 1044-1049

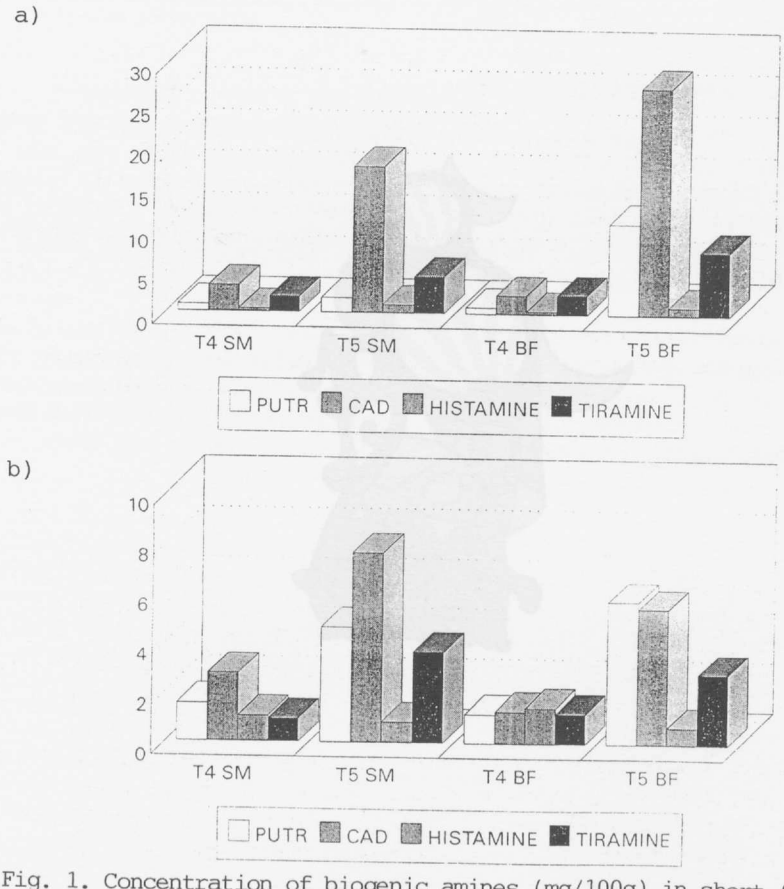

Fig. 1. Concentration of biogenic amines (mg/100g) in short (a) and long (b) in dry-cured ham processes

NOTES