## FORMATION OF NON-VOLATILE FLAVOR COMPOUNDS IN IBERIAN DRY-CURED HAM DURING PROCESSING

Lourdes Martín;Teresa Antequera; Juan José Córdoba; María Luisa Timón and Jesús Ventanas.
Tecnología de los Alimentos, Facultad de Veterinaria, UEX, Avda. Universidad s/n, 10071 Cáceres (Spain)

### BACKGROUND

Iberian dry-cured ham is considered the most valuable meat product in Spain due to the flavour. The development of such flavour requires highly marbled thighs obtained from Iberian pigs fattened in pasture with acorns, which should be processed following a traditional method that requires at least 18 months. The processing involves salting for 15 days, postsalting (resting for 3 months under low temperatures), followed by a period of increasing temperatures (drying) for 1-2 months during the summertime. Finally, the hams are left in a cellar (ageing) for a period that generally exceeds 12 additional months.

The chemical and biochemical changes occurring during the successive steps of processing have been investigated as well as the volatile and non-volatile compounds responsible for the desirable flavour (García *et al.*, 1991; Antequera *et al.*, 1992; Ventanas *et al.*, 1992; Córdoba *et al.*, 1994). The proteolysis that takes place during the ripening is an important source of flavour compounds (free amino acids, small peptides and Maillard-reaction products) in dry-cured hams such as Serrano (Aristoy and Toldrá, 1995), Parma (Careri *et al.*, 1993) and French hams (Buscailhon *et al.*, 1994). These modifications should be more marked in Iberian ham due to the long ageing time and the high temperatures that characterise some periods of its ripening. Thus, a large increase of amino acid nitrogen fraction takes place during processing of traditional Iberian ham, being the most abundant (73%) among the non-protein nitrogen fractions (Ventanas *et al.*, 1992; Córdoba *et al.*, 1994). From published results (Martín *et al.*, 1996; Martín *et al* 1998a; Martín *et al.*, 1998b) it is observed that the processing conditions (temperature and salt content) could determine the level and the kind of compounds released from the protein breakdown during the dry-curing of Iberian hams. Due to the actual tendency to consume low salted products, many industries processing Iberian hams have reduced the salting period to 9-10 days. A lower salt concentration seems to contribute to generate different quantity in the non-protein nitrogen fraction in dry-cured Iberian hams. In this sense, Martín *et al.* (1998b) found a lower rate of amino acid nitrogen (only reached 48% of the overall non-protein nitrogen) and an increase in peptide nitrogen in lower salted Iberian hams. More information about the nature and proportions of non-volatile taste-active compounds would be very important to understand the mechanisms of flavor development and could provide the means of enhancing the sensory quality of Iberian cured hams.

### OBJECTIVES

As the non-volatile compounds generated from proteins may be of great importance could be the sensory quality in dry-cured hams, the aim of our work was to investigate the low molecular weight compounds different to free amino acids existing in the water soluble fraction of low-salted Iberian hams, and to study their evolution during ripening.

### METHODS

Thirty-one thighs were obtained from Iberian pigs (160 kg liveweight) fattened extensively including acorns from *Quercus ilex* and *Quercus suber*, and processed for 22 months. The steps included in the sampling procedure and the number of hams removed for testing at each stage were as follows: 1. Green state (n=5), 2. Salting-postsalting (n=5), 3. Drying (n=6), 4. Four months cellar (n=6), 5. Eight months cellar (n=5), 6. Fully matured ham (n=4).

Samples of *Biceps femoris* were taken and used for the following analysis:

**Salt content** was estimated as chlorides, which were extracted with water-ethanol (60:40 v/v) and quantified by the Carpentier Volhard method (AOAC, 1984).

**Non-volatile low-molecular weight compounds** (LMW) was determined homogenising 10 g of sample with perchloric acid 0.6N. The homogenates were then centrifuged at 15300*g* for 10 min and filtered through Whatman No. 54 paper. After the pH of the filtrates was adjusted to 6 with potassium hydroxide 30%, they were filtered again through Whatman No. 54 paper. 20 $\mu$l of filtrate were used for the HPLC analysis, using an ODS column, 25cmx4.6mm (5$\mu$m particle size) and UV detector (214nm). The eluents used were (A) water HPLC grade and (B) acetonitrile with 0.1% of trifluoroacetic acid.

**Statistical method** was performed by the analysis of the variance and differences between stages were analysed using the Bonferroni test.

### RESULTS AND DISCUSSIONS

The intense proteolytic breakdown leads to the formation of several non-volatile low molecular weight substances (LMW) in the Iberian hams. Fig. 1 shows a representative chromatogram of the reverse-phase HPLC separation of the perchloric acid soluble compounds, where fourteen peaks exhibiting absorbance at 214 nm were detected (P1 to P14). The chromatographic procedure allowed to elute early in the same run (5.5 min) the free amino acids existing also in the extract, avoiding their interference in the analysis. Further identification of these 14 characteristic peaks by LC- MS analysis will be necessary in order to verify the proteinic nature of these compounds. However, their presence in this fraction and the polarity exhibited in the chromatography analysis indicated that probably they can be considered peptides of small molecular weight. Its higher retention behaviour in the reverse-phase HPLC separation appeared to depend on their larger molecular weight, comparing to free amino acids.

Fig. 2 shows the typical chromatograms of extracts from raw meat and cured hams at different stages of processing. There was an obvious relationship between the successive curing steps and the formation of LMW compounds. The biggest increase in the peaks area took place during the drying cycle, which was significant for most of the peaks ($p<0.05$). Also, during the latter step of processing, while the hams were in the cellar, the rate of formation was activated, showing a significant increase ($p<0.05$) from drying. This two-step increase was in close agreement with the growth of the overall non-protein nitrogen (NPN) and free amino acids

reported previously for low-salted Iberian hams (Martín *et al.*, 1996; Martín *et al.*, 1998b), and was consistent with the relative high temperatures (20-25°C) reached during these two phases (Fig. 3).

As free amino acids are ultimate products of proteolysis, the peaks measured would represent a dynamic intermediate in which both formation and breakdown took place. However, a more detailed view to the increase in the 14 individual peaks along processing showed it was not as linear as expected (Fig. 4). Some authors reported that the pattern of liberation of free amino acids during ripening of Iberian hams was not selective, increasing significantly according to their proportion in the porcine skeletal muscle (Córdoba *et al.*, 1994). Nevertheless, some of the studied peaks in this work which were absent in raw meat (P3, P9) increased along the ripening, what indicates an accumulation of new proteolysis products during processing. P10 decreased ($p<0.05$) and almost disappeared and P13 showed a slight reduction, which could be due to an inhibition of the proteases that produced them from a longer protein and further proteolysis. On the other hand, P5, P6, P8 and P14 displayed small amounts in the green state and experimented a sudden increase during drying. The proteolytic system responsible for their generation (oligopeptidases) seemed to continue the action during the whole dry-curing process, being the most abundant peaks in the final product. Thus, an increase of LMW compounds in the long processed hams would be expected.

## CONCLUSION

The rate of LMW compounds is strongly influenced by temperature of the drying stage and tend to increase with processing time and temperature of the final step in cellar. It is important to continue these studies to identify these non-volatile low-molecular weight compounds as due to their nature they must be somehow responsible for the specific flavour in Iberian dry-cured ham.

## PERTINENT LITERATURE


Antequera, T.; López-Bote, C.; Córdoba, J.J.; García, C.; Asensio, M.A.; Ventanas, J.; García-Regueiro, J.A. and Díaz, I. (1992). *Food Chem.* **45**, 105.

Aristoy, M.C. and Toldrá, F. (1995). In *Food Flavors: Generation, Analysis and Process Influence*. G. Charalambous. Elsevier Sci. B.V. Amsterdam. 1323.

AOAC (1984). *Official Methods of Analysis* (3rd edn). 24010, 432.

Buscailhon, S.; Berdagué, J.L.; Bousset, J.; Cornet, M.; Gandemer, G.; Touraille, C. and Monin, G. (1994). *Meat Sci.* **37**, 229.

Careri, M.; Mangia, A.; Barbieri, G.; Bolzoni, L.; Virgili, R. and Parolari, G. (1993). *J. Food Sci.* **58**, 968.

Córdoba, J.J.; Antequera, T.; García, C.; Ventanas, J; López-Bote, C. and Asensio, M.A. (1994). *J. Agric. Food Chem.* **42**, 2296.

García, C.; Berdagué, J.J.; Antequera, T.; López-Bote, C.; Córdoba, J.J. and Ventanas, J. (1991). *Food Chem.* **41**, 23.

Martín, L.; Córdoba, J.J.; García, C.; Timón, M.L.; Tejeda, J.F. and Ventanas, J. (1996). *42th IcoMST*. Norway.

Martín, L.; Antequera, T.; Ruiz, J.; Cava, R.; Tejeda, J.F. and Córdoba, J.J. (1998a). *Food Sci.Tech.Intern* **4**, 17.

Martín, L.; Córdoba, J.J.; Antequera, T.; Timón, M.L. and Ventanas, J. (1998b). *Meat Sci.* (in press).

Ventanas, J.; Córdoba, J.J.; Antequera, T.; García, C; López-Bote, C. and Asensio, M.A. (1992). *J. Food Sci.* **57**, 813.


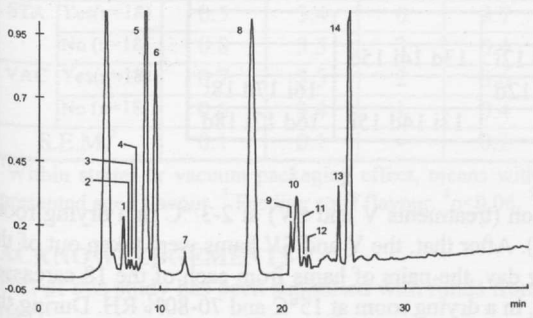


Fig. 1. Chromatogram of the non-volatile soluble compounds in *Biceps femoris* muscle of Iberian ham.

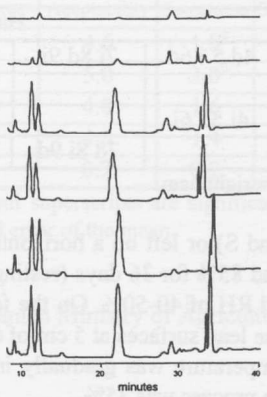


Fig. 2. Chromatograms of extracts from Iberian ham in green state, after postsalting, after drying, at four and eight months of cellar and fully matured ham (from top to bottom, respectively).

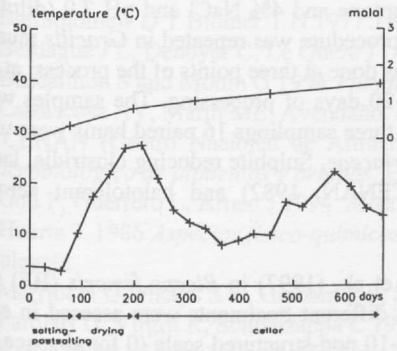


Fig. 3. Environmental temperature and *Biceps femoris* chloride content during processing.

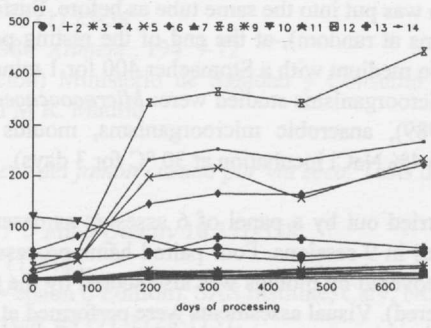


Fig. 4. Development of non-volatile soluble compounds in *Biceps femoris*