CHANGES IN EXTRACTABILITY OF PROTEIN AND LIPIDS FROM DRY CURED HAM DURING RIPENING.

CORDOBA, J. J.; ANTEQUERA, T.; GARCIA, C.; LOPEZ BOTE, C.; VENTANAS, J. and ASENSIO, M. A.

Food Science and Technology. Faculty of Veterinary Science. 10071 - Caceres. Spain.

SUMMARY

In a study of the evolution of different components of dry cured hams along ripening, an unexpected decrease in protein and lipid content during the salting/post-salting stage was observed. Contrary to the demonstrated positive effect of low salt concentrations on protein solubility, protein as well as lipid extractability showed minimum values when salt levels were of about 4%. A part of the unextracted proteins, mainly actin, α-actinin and myosin as revealed by SDS-PAGE, could be recovered treating the samples with 6 M urea. However, as it was clear from microscopic observations, apparently intact groups of muscle fibres were present in the sediment after protein extraction from samples aged for 75 and 120 days. Therefore, some kind of resistant structure that keeps muscle fibre reasonably unaltered has to be effective during homogenization and extraction with 6 M urea, such as those derived from the formation of a stable gel or from changes in collagen fibres that made it more resistant to chemical attack.

INTRODUCTION

Dry cured hams processed following the typical Spanish country style are uncooked, salted and dried hind legs from Iberian pigs that are salted and left to mature for a total period of about 18 months. As a part of a study where the changes that take place in this product throughout maturation (see Ventanas et al., 1989), changes in protein and lipid composition were studied. The present work arose as a consequence of a decrease in the total amount of these components obtained during the salting and post-salting stages, that would be an important drawback if unextracted compounds had different characteristics to the

extracted ones.

Even though the interaction of protein and salt in meat products is rather complex, there seems to be a clear and positive effect of low salt concentrations on protein solubility (Grabowska and Hamm, 1979; Knight and Parsons, 1988; Kenney and Hunt, 1990) and an irreversible denaturation of myosin at high salt concentrations (Knight and Parsons, 1988). In addition, as it has been reviewed by Tarrant (1982), there are some other functional properties of myofibrillar proteins that could be related with protein solubility and extractability, such as dissolved proteins acting as emulsifying agents in sausage emulsions or the formation of a stable gel in comminuted meat products. Finally, other changes in muscle tissue could also interfere with protein extraction. For example, those derived from the decrease in the amount of reducible crosslinks in collagen that could be responsible for a lower solubility and increased resistance to chemical and enzymatic attack of collagen (Bailey and Robins, 1976). Some preliminary attempts have been done to elucidate what the reasons of these changes are and to try to solve this problem.

MATERIALS AND METHODS

The process followed to obtain the dry cured hams is described in Ventanas et al. (1989). Batches of 4 hams each were drown during the initial steps of the processing at 0 (48 h post-mortem), 15, 75 and 120 days after slaughter. All results given are mean values of four determinations. Biceps femoris (Bf) and Semimembranosus (Sm) muscles were extracted from the thighs, ground separately in a Moulinex meat grinder, and stored at -18°C until analysis.

To estimate the salt content, chlorides were extracted with water/ethanol (60/40, v/v) and quantified by the Carpentier-Vohlard method (AOAC, 1984a).

Sarcoplasmic and myofibrillar protein extractions were carried out following the method of Helander (1957). For this, 2 g samples were consecutively homogenized in a Sorvall Omni-mixer for 30 sec at maximum speed with 2 different solvents: 40 ml of 0.03 M pH 7.4 phosphate buffer and 1.1 M IK pH 7.4 + 0.1 M

phosphate (0.6 M) buffer. In addition, a further extraction with 6 M urea was also used to recover as many unextracted proteins as possible. The nitrogen extracted in every step was quantified by the Kjeldahl method according to (AOAC, 1984b).

The proteins obtained in these 3 fractions were characterized by SDS-PAGE following the procedure of Weber and Osborn (1969). For this, 5 ml aliquots were dialyzed against a 0.01 M pH 7.1 phosphate buffer and incubated with 1.5% Sodium Dodecyl Sulfate, 1% mercarptoethanol, 0.01M pH 7.1 phosphate buffer. Ten microliters of a 1.5 µg/µl solution of protein were loaded per slot of the electrophoresis gel.

Non protein nitrogen (NNP) was determined by the method of Johnson (1941), after protein precipitation with 0.6 N perchloric acid, as described by De Ketelaere et al. (1974).

For lipid extraction the method described by Bligh and Dyer (1959) was followed. From the extract so obtained free fatty acids (FFA) were determined as described in the AOAC methods (1984c).

The changes in the microstructure of the sediment obtained after protein extraction with buffers of increasing molar strength were monitored by light microscopy. After staining with hematoxilin-esosin, 1 um thick samples were observed with a Nikon Labophot.

Statistical analysis was performed using Tukey analysis of variance of Statgraphics. The confidence level was set at 99% to determine differences in means.

RESULTS AND DISCUSSION

As it can be seen in Fig. 1, total lipids extracted by the Bligh and Dyer (1959) method from either Bf or Sm samples showed a drastic reduction through the salting/post-salting stages of dry cured ham processing. Even though according to the increase in the acid degree (Fig. 1) some lipolysis took place during this period, it was not important enough to explain satisfactorily the phenomenon in its whole extent. Statistical analysis revealed that the reduction in lipid extractability showed significant values before in Bf than in Sm muscle.

Similarly, important decreases in the amount of proteins extracted from either Bf or Sm samples with 0.03 M and 0.6 M

buffers (Fig. 2) were also observed. Again, this could not be explained by the respective increase in non protein nitrogen (Fig. 2). In this case, the decrease in the amount of protein extracted with the 0.6 M buffer was always significant in both muscles studied, while extractions with 0.03 M buffer showed only small and occasionally significant differences.

Given that the maximum positive effect on protein extractability has been obtained at NaCl levels of 4% (Kenney and Hunt, 1990) and 5.8% (Knight and Parsons, 1988), the possible relationship between these two factors was studied. In spite of the more external situation of Bf, salt content showed a faster increase in Sm (Fig. 1) due to the thick layer of fat that covers the former reaching in both muscles final values very close to the optimum for protein extractability. Therefore, neither a positive correlation of salt contents of about 4-6% with higher protein extractability nor lower extractability in Bf after being subjected to salt levels higher than 6% has been found.

As protein extractability does not seem to be directly correlated to salt content, some kind of physical interaction between these components, such as emulsion formation during sample homogenization, was thought to be responsible for these phenomena throughout salt equalization. As it is shown in Fig. 2, part but not all the protein that in all samples had not been extracted with the 0.6 M buffer could be recovered from the pellet with 6 M urea. Electrophoretic analysis of this fraction (Fig. 3) revealed that the main proteins present were myosin, actin and α-actinin. In addition, the same treatment with 6 M urea applied to some sediments obtained after lipid extraction by the Bligh and Dyer method allowed to recover only a part of the unextracted fat (Data not shown). Therefore, treatment of the samples with 6 M urea would help to collect a higher proportion of lipids and myofibrillar proteins from the samples, but can not be regarded as the best way of solving the problem.

In some way these results could support the emulsion formation hypothesis, given that the extraction of a fraction of the proteins that interact with water and fat would release a part of the latter. On the contrary, microscopic observation of the sediment obtained before and after extraction with 6 M urea

points to something quite different. As it is shown in Fig. 4, the typical structure of muscle tissue of samples drawn in the first sampling time is severely damaged after homogenization and extraction with the 0.6 M buffer. As it was expected, after extraction with 6 M urea no sign of muscle structure was evident in these samples. However, the sediment obtained at the two last sampling times after protein extraction with the 0.6 M buffer and even with 6 M urea revealed that there were fractions where tissue structure was virtually intact.

As a consequence, the differences in extractability could derive from the formation of a resistant structure that kept the muscle fibres reasonably unaltered has to be effective even after homogenization and extraction with 6 M urea. Examples of possible causes are the formation of a stable gel, as it has been shown in comminuted meat products, being the heavy chain portion of the myosin molecule the only muscle protein apparently involved (Schmidt, 1979). Another reason for the high resistance to homogenization could be related to changes in the connective tissue. As it has been reviewed by Bailey and Robins (1976), collagen fibres become less soluble and more resistant to chemical attack with age in a process recognized as normal maturation where reducible crosslinks were found to decrease. In addition, the rate of disappearance of these compounds seems to be faster in _in-vitro_ ageing than _in vivo_. Therefore, to clarify if any of these or some others are the reasons for the lower extractability observed further investigation is needed.

REFERENCES

AOAC (1984a). AOAC Official Methods of Analysis, 3, n. 24010: 431.

AOAC (1984b). AOAC Official Methods of Analysis, 3, n. 24038: 435.

AOAC (1984c). AOAC Official Methods of Analysis, 3, n. 28032: 508.

BAILEY, A. J. and ROBINS, S. P. (1976) Sci. Prog., Oxf., 63: 419.

BLIGH E. G. and DYER, W. J. (1959). Can. J. Biochem. Physiol., 37: 911.

GRABOWSKA, J. and HAMM, R. (1979). Fleischwirtschaft, 59: 1166.

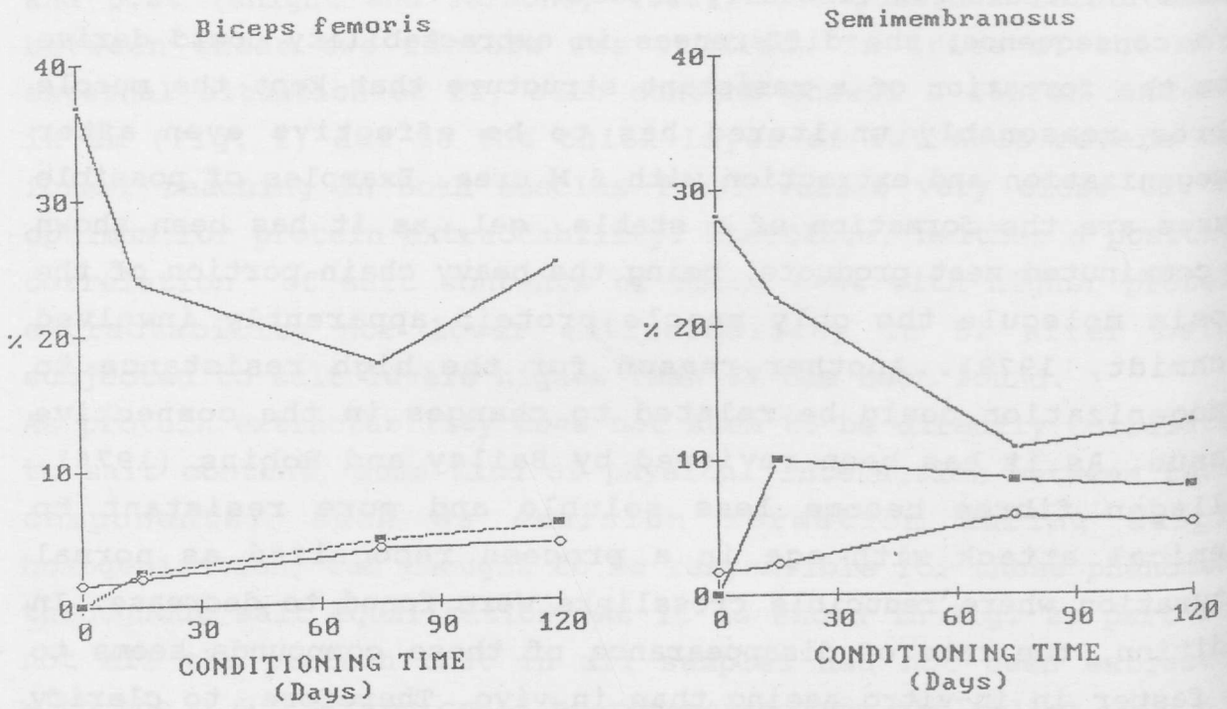

Figure 1. Evolution of total lipids extracted (▬) (% Dry Matter), acid degree (O) (% oleic acid) and salt content (■) (% Wett Matter) throughout salting/post-salting stages of Iberian ham maturation.

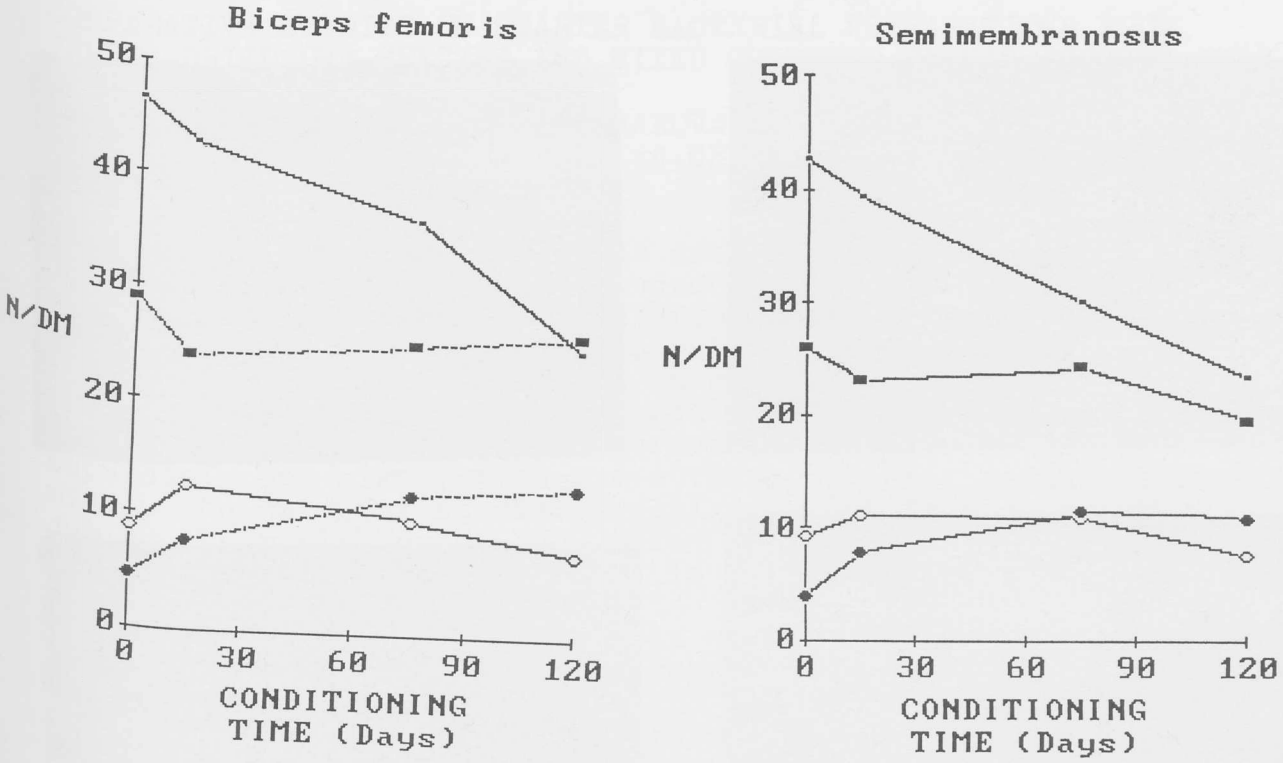

Figure 2.- Evolution of Protein Nitrogen extracted with 0.03 M buffer (■), 0.6 M buffer (-) and 6 M urea (O), and NPN (◀▶) throughout salting/post-salting stages of Iberian ham maturation. Al data are given as mg of N/ g Dry Matter.

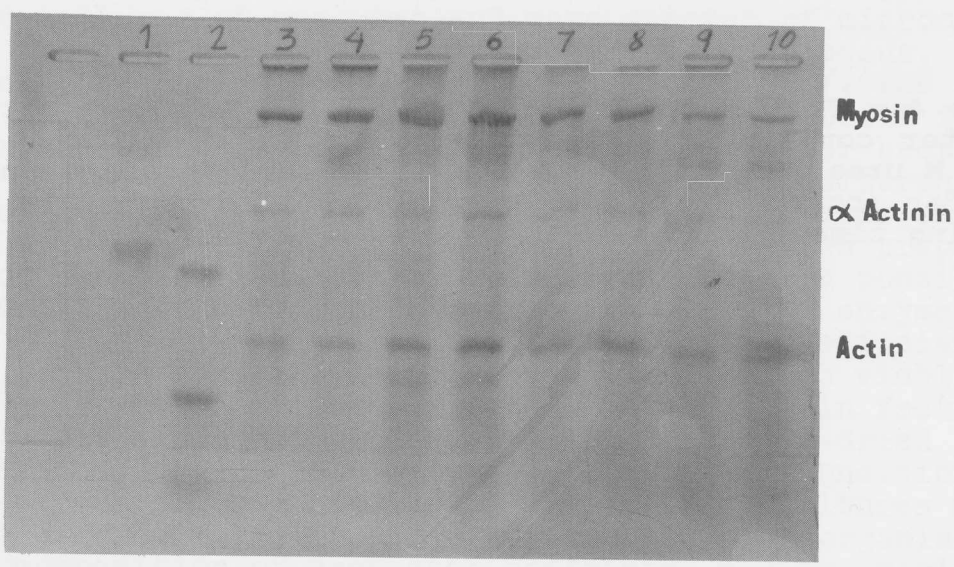

Figure 3.- SDS-PAGE of proteins extracted with 6 M urea after a previous extraction with 0.6 M buffer from the first (lanes 3 and 4), second (lanes 5 and 6), third (lanes 7 and 8) and fourth (lines 9 and 10) sampling times. Lane 1, molecular weight standard: Hemocyanin cross-linked (210,000; 140,000; 70,000); Lane 2, molecular weight standard: bovine serum albumin (66,000), carbonic anhydrase (29,000), cytochrome oxidase C (12,500).

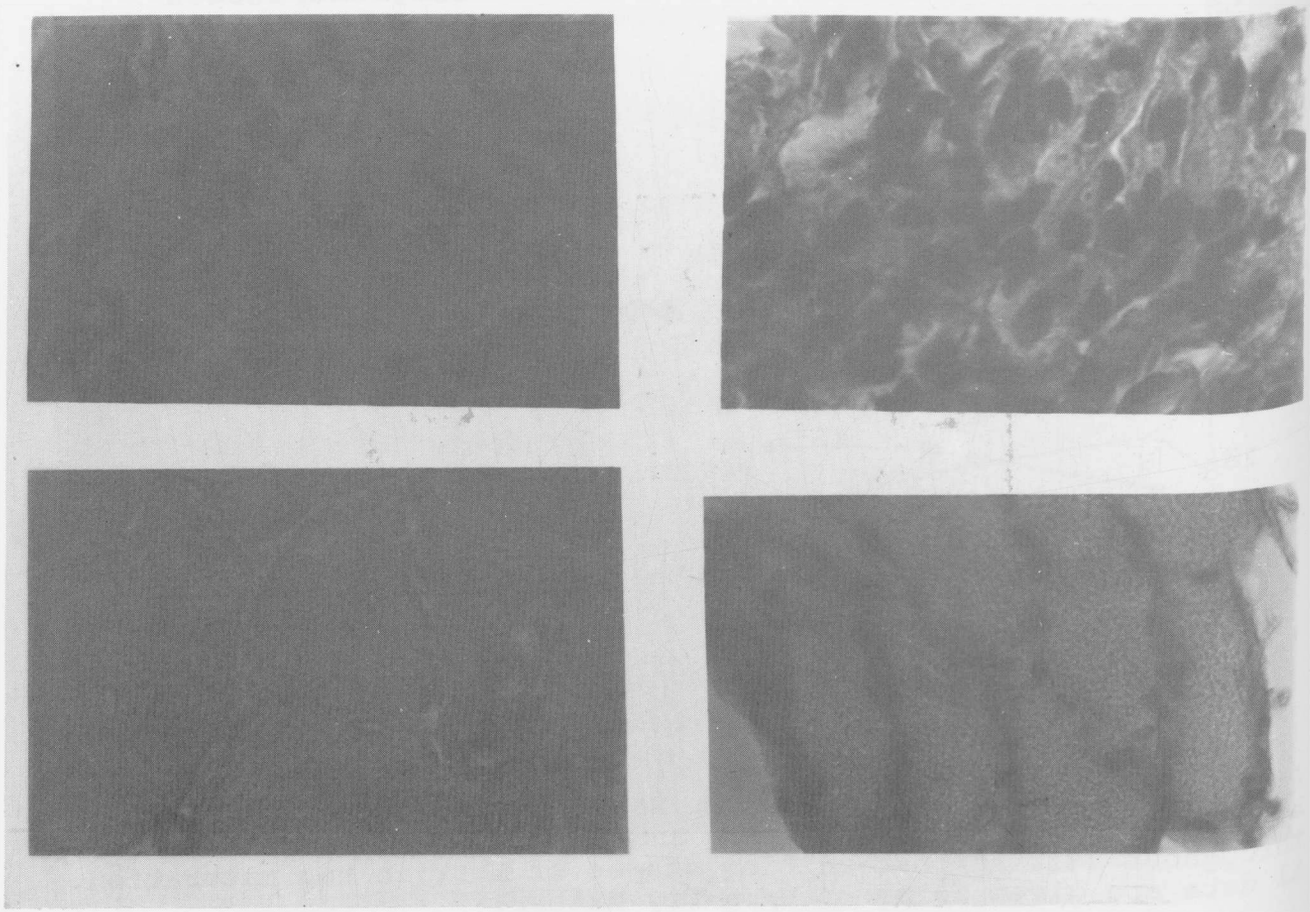

Figure 4.- Typical microphotograph of the sediment obtained from Sm after consecutive extractions with 0.6 M buffer (top left) and 6 M urea (top right) in the first sampling time and with 0.6 M buffer (bottom left) and 6 M urea (bottom right) in the fourth sampling time.