Ultrastructure Analysis in Spanish Dry-Cured Ham:
Dry-cured effect upon principal muscles in ham during postsalting stage.

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SUMMARY: Structural disorganization (M line, H zone, and A,I bands) in the principal muscles of ham during the postsalting stage of the dry-cured ham process were studied. The muscles under study were Gracilis, Semimembranosus, Semitendinosus, and Biceps femoris during the postsalting stage.
The ultrastructure of muscle varied with depth and dry cured time. Gracilis had a total disorganization at 7 days. Semimembranosus the disorganization appeared at 14 days whereas for Semitendinosus did not occur at 28 days, and Biceps femoris did not show any important disruption at 28 days. We supose that these changes in muscle fibers can be explained by NaCl diffusion, cell dehydration and proteolytic activity.

INTRODUCTION: Dry-cured ham is a well know product in Spain, the consumption of dry-cured ham is high already 2.7 Kg / person / year, and the production is 52,500 Tm per year, this makes of Spain one of the main producers of ham in the world together with USA and Italy. In Spain this high production has a great technological and economic importance. The process of dry-cured ham takes a very long time. The process can be divided in three important stages as follows : Salting, Postsalting, and Dry-maturation.
Salting.- The fresh ham is placed in salt 1-2 days per Kg of weight, in this stage the salt diffuse into muscles. High concentrations of salt is found in the upper muscles (Carrascosa, A. V.; Cornejo, I., 1989)
Postsalting.- This period is very important. The salt diffuses into the muscles by osmotic preasure, only the salt absorved during the salting stage diffuses into the deepper muscles. Here the temperature is a very important factor for ham conservation since the physical-chemical parameters (water activity (Aw), Cl^-, NO_2^-) are not enough for the ham conservation (Sayas-Barbera, E., Pèrez-Alvarez, J. A., 1989). In this period the ham remains for 21 days in a refrigerated camera. At the end of this period the temperature can be gradually elevated.
Dry-maturation.- This stage takes a long time. Homogeneous distribution of salt takes place in this period. Lipolytic and proteolytic activity generates the sensorial characteristics of dry-cured ham (Nieto, P., 1988).
The present study was carried out to study the ultrastructural changes during the postsalting stage in the principal muscles of ham.

MATERIALS AND METHODS : The present study was made with 10 female hams. The selectionated carcass weighted 76 kg. All carcass were clasificated for the Spanish Inspection as IB. The zone of ham under study was located around femur bone. The samples were taken perpendicularlly to that bone. To obtain the samples we use one cylinder with 3.8 cm of inside diameter and 18 cm of lenght adapted in a drill. The samples obtained were selectionated to separate the different muscles (Gracilis, Semimembranosus, Semitendinosus and Biceps femoris). The samples were taken at 0, 8, 14, 28 days of process (8 day is the begining of postsalting stage). All samples were cut into strips (3 mm x 1 mm) of longitudinal sections of muscle fibers, before subsequent fixation. Tissue samples were inmediately inmersed in fixative solution (25 %

glutaraldehyde, 3 % NaCl buffered at pH = 7.03 with M / 15 Sorensen phosphate). Small pieces of muscle tissue were then washed for 12 hours with wash solution (5.4 % Glucose, 3 % NaCl, M / 15 Sorensen phosphate buffered at pH = 7.03). The samples were postfixed for 5 hours in 1 % Osmium Tetroxide solution in wash solution. After fixation, the samples were then dehydrated in a graded series of acetone and embedding in Araldite. The cubes were polimerized at 60 C. Silver sections (aproximately 60-80 nm thick) were cut on a Reichert Jung Ultracut. Sections were mounted on uncoated cooper grids. A Phillips EM 40 Transmission electron microscope was used for observing the sections. Representative photographs of each sample were taken (Sjostrand, F. S., 1967)

RESULTS AND DISCUSSION : Figure 1 shows a tipical ultrastructure of striated muscle, and the characteristic banding pattern of A and I band, Z line are evident, and the thin and thick filaments maintaned their structural integrity, in all muscles under study (this electron micrograph is concerning at fresh muscle). Figure 2 shows the Gracilis muscle at 8 days of process. This micrograph develops great changes in ultrastructure of this muscle. Gracilis is the muscle more outside in ham. This micrographs evidence a loose of structural integrity of myofilaments. The salt generates a solubilization action upon myofibrilar proteins disappeared the banding pattern. This view is the same in all postsalting and maturation stage. The average salt concentration in this muscle was 13.4 %. Figure 3 shows the Semimembranosus muscle in the begining of postsalting stage. In this stage there is a little alteration in the morphological appearence. This minor changes include the disappearence of the H zone and some losses of definition at A and I junction. The average salt concentration in this muscle was 3.10 %. Figure 4 shows at 14 days the Semimembranosus muscle. Gross morphological changes occurs in this muscles, the A and I band are now vaguely discernible. The Z line have ruptures at several points, and the filaments aspect of myofibrilar protein have dissapeared. The rest of micrographs are the same like Gracilis. The average salt concentration was 8.45 %. Figure 5 shows Semitendinosus muscle at the beginig of postsalting stage. Swelling of myofibrilar as feature more characteristic. All banding pattern are undamage. At the 14 days the same image is view. The average salt concentration at the begining of postsalting stage was 1.08 %, while at 14 days the salt concentration was 1.42 %. Figure 6 shows the Semitendinosus muscle at 28 days. The micrograph develops a structural disorganization of myofilaments. The Z line is visible but not defined like fresh muscle. The average salt concentration was 2.42 %. Figure 7 shows Biceps femoris at the begining of postsalting stage, this micrographs develops that any difference are found with the fresh muscle. Little disruption are found in Z line, the cause of this disruption was by enzymatic activity of meat. All the banding patterns of muscle are view in this micrograph. The average salt concentration was 0.33 %. Figure 8 shows the Biceps femoris at 28 days of postsalting stage. This micrograph develops a sarcomers swelling at A band. A and I band are enough visible. H band shows a diffuse image. The average salt concentration was 1. 13 %. Figure 9 shows the aspect of all muscles in ham. This micrograph is representative for all muscles. At the finish of dry-cured process, the filamentous aspect of myofilaments disappeared, only is observed Z line with disruptions and contraction of sarcomeres.

According with micrographs studied, we saw that myofibrils had several changes

in ultrastructure at all dry-cured time. Comparing the micrographs of fresh (Figure 1) and dry-cured (Figure 9) muscles, one can view some significative differences :

1.- Dissappearance of characteristics banding pattern on fresh muscles.
2.- Structure filamentous disorganization in myofibrils.
3.- Cell contraction in all sarcomeres. The contraction during dry cured process is about 15.3 % of original sarcomer of fresh muscle (Sayas-Barberà, E.; Pèrez-Alvarez, J. A., 1989).

The principal factors causing these changes are : Salt diffusion into ham muscles. Cell dehydration. Proteolysis (Lawrie, R., 1983; Nieto, P., 1988) The curing salt produce a solubilization on myofibrilar proteins, this generates a dispersion of myofilaments. The Cl^- ions bind the filaments and increased the electrostatic repulsive forces between them, this join create a swelling of sarcomeres, this can be observed in figure 8. When high concentration of Cl^- ions the transversal unions between the thick and thin filaments become weak by electrostatic repulsive forces producing dispersion of transversal union and depolarization of filaments. These can be observed in figures 2, 4, 5, 9. (Hamm, R., 1960, 1975, 1981, 1982; Offer, G.; Trinick, J., 1983).

Figure 1.- Electron micrograph of Gracilis muscle of fresh ham (x 10000)

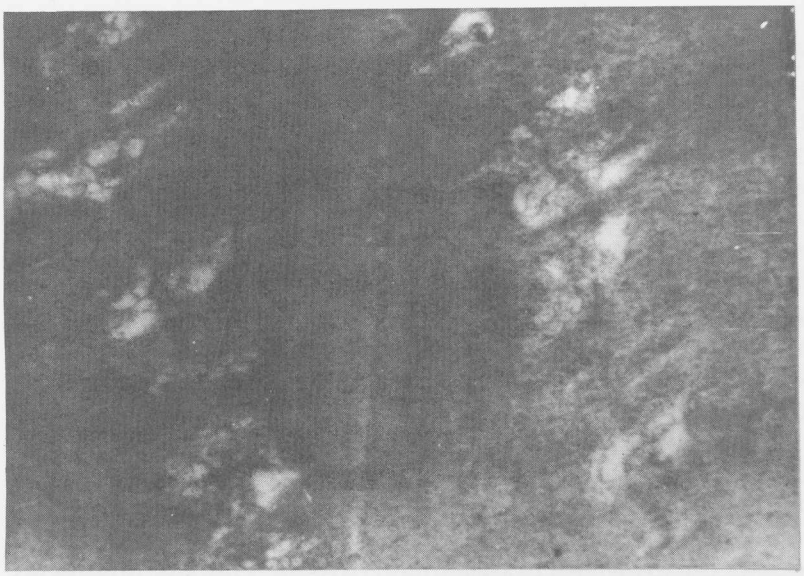

Figure 2.- Electron micrograph of Gracilis muscle at 8 days of process (x 12500)

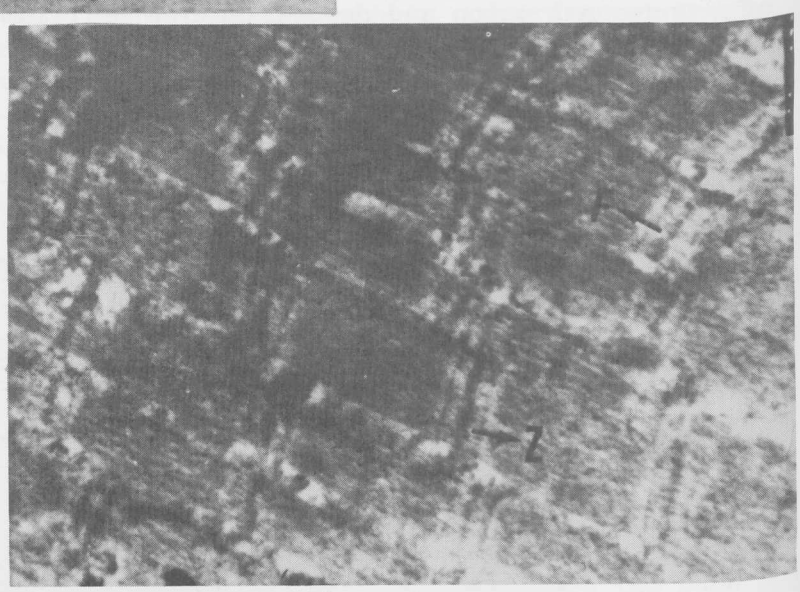

Figure 3.- Electron micrograph of Semimembranosus muscle at 8 days of process (x 10000)

Z = Z line
F = fissures

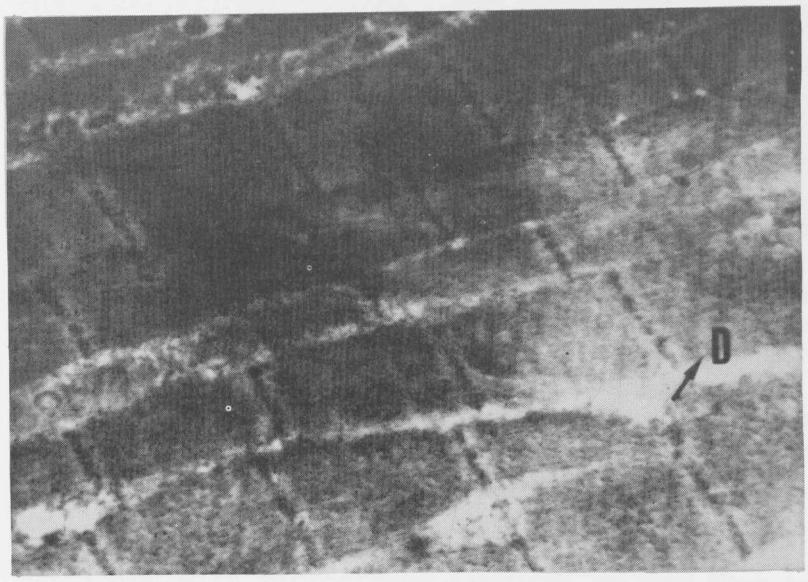

Figure 4.- Electron micrograph of Semimembranosus muscle at 14 days of process (x 10000)
Z = Z line
D = Discontinuity

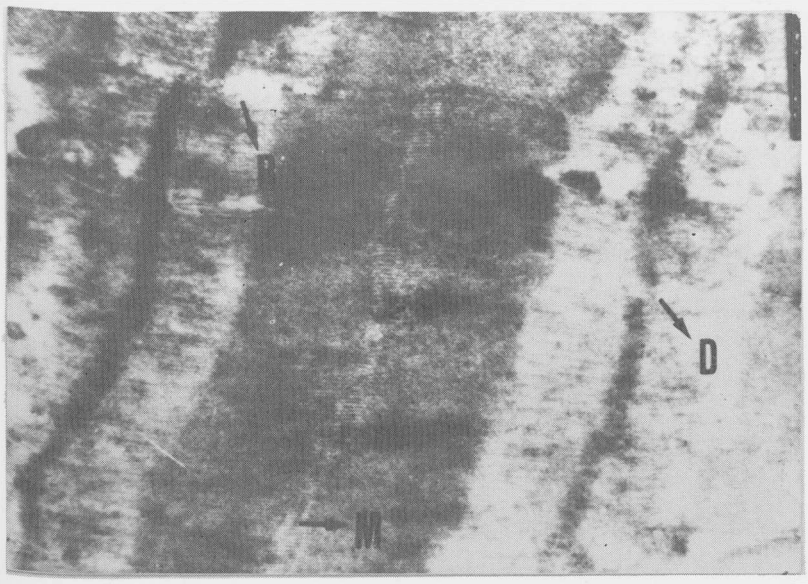

Figure 5.- Electron micrograph of Semitendinosus muscle at 8 days of process (x 16500)
M = M line
D = Discontinuity
R = Z line rupture

Figure 6.- Electron micrograph of Semitendinosus muscle at 28 days of process. (x 16500)
D = Discontinuity
M = M line

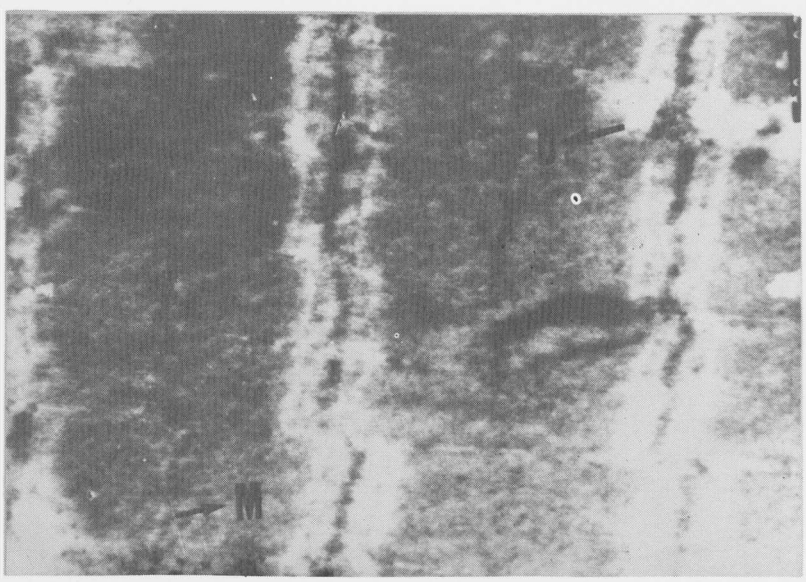

Figure 7.- Electron micrograph of Biceps femoris muscle at 8 days of process (x 21500)
Z = Z line
H = H band
M = M line

Figure 8.- Electron micrograph of Biceps femoris muscle at 28 days of process (x 6000)
Z = Z line
M = Mitochondria

Figure 9.- Electron micrograph of Gracilis dry-cured (x 5000)

CONCLUSIONS : During dry-cured process all muscles have a dehydration with a sarcomeres contraction. The dry-cured process produce a losses of banding patterns. Gracilis is the first muscle in which a total disorganization occurs at 8 days of process. Semimembranosus and Semitendinosus shows during the postsalting stage a evolution to banding pattern disorganization because salt diffusion. Biceps femoris shows a few changes during postsalting stage. During the postsalting stage the banding patterns in Biceps femoris remains.

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