Spanish Dry-Cured Ham : Physicochemical and Ultrastructural Analysis During the Postsalting Stage

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SUMMARY

Physicochemical analysis and Ultrastructural disorganization were studied during the postsalting stage of Dry-Cured process. The muscles under study were : Gracilis, Semimembranosus, Semitendinosus and Biceps femoris. Physicochemical parameters (pH, water activity , salt concentration and residual nitrite) showed that during the postsalting stage reached inhibition values for *Clostridium botulinum*, and Meat Ultrastructure (A and I bands, H zone, M line) showed that all ultrastructure disappeared for Gracilis, Semimembranosus and Semitendinosus at the end of postsalting stage.

INTRODUCTION

The Spanish processed meat industry traditionally made dry-cured products like " Chorizo ", " Salchichón ", but the most important of these products is the Dry-Cured Ham. " DIAZ RUIZ (1990) reported " that the Spaniards have the highest consum of this product in CEE. In 1991 the Dry-Cured Ham industry is going to produce 173,000 Tm.

The dry-cured process can be divided in three fundamental stages : Salting, Postsalting and Dry maturation. The Salting and Postsalting stage take place at low temperature (< 3.3 ºC). The Spanish Dry-Cured Ham has not been sufficiently studied because this product was a craft industry. Actually exist a great interest to know all factors that have influence in this process.

During the postsalting stage physicochemical and ultraestructural changes take place. " SAYAS et al. (1989) reported " that in this stage the physicochemical parameters more important are salt concentration, residual nitrite, water activity and pH, while the ultrastructural changes affect the banding patterns.

The aim of this work was the study of the physicochemical and ultrastructural parameters during the postsalting stage in the principal muscles of ham (Gracilis, Semimembranosus, Semitendinosus and Biceps femoris).

MATERIALS AND METHODS

The present study was carried out with 10 female hams (Large White x Belgium White). All the hams were selectionated for the Spanish Inspection like IB (aproximately 76 Kg). The zone under study were delimited between the central part of the femur bone and the perpendicular zone at that bone. The samples were obtained with a hollow cilinder of stainless steel with an inner diameter of 38 mm and 160 mm of lenght. In the meat cilinder were identified the following muscles : Gracilis, Semimembranosus, Semitendinosus and Biceps femoris. The samples were successively taken at 0, 7, 14, 21 days after the beginning of the process. The physicochemical parameters under study were : pH, water activity (aw), the salt concentration (Cl^-) and the residual nitrite (NO_2^-). The engaged methods were :

- pH.- Ingold Crison 406 Electrode
- aw.- Novasina Thermoconstanter TH2. Humidat recording TH2/ IC11. Working temperature 25ºC.
- Cl^-.- ISO R1841
- NO_2^-.- ISO/DIS 2918. Diode Array HP 8451A. Spectrophotometer.

The samples for ultrastructural analysis were cutted into strips (3mm x 1 mm) of longitudinal sections of muscle fibers, before subsequent fixation. Tissue samples were inmediately fixed in a 2.5 % Glutaraldehyde solution. The samples were postfixed in a 1% Osmiun Tetroxide solution and then were dehydrated with a graded series of acetone and embedding in Araldite. Silver section were cut on a Reichert Jung Ultracut. A Phillips EM 40 Transmission Electron Microscope was used for observing the sections.

RESULTS and DISCUSSION

The tables 1 to 4 show the physicochemical and ultrastructural analysis of the muscles under study during the postsalting stage.

Fig. 1 Electron micrograph of Gracilis muscle at 0 days after the beginning of postsalting stage (x 12 500)

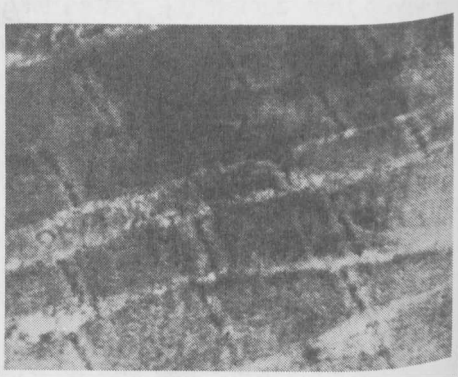

Fig. 2 Electron micrograph of Semimembranosus at 14 days after the beginning of postalting stage (x 10 000) Z = Z line.

process time (days) / parameters	0	7	14	21
pH	5,66 ± 0,04	5,55 ± 0,05	5,60 ± 0,10	5,70 ± 0,08
aw x 10^2	97,76 ± 1,35	96,15 ± 3,25	95,13 ± 2,87	95,65 ± 2,25
% salt in aqueous phase	15,90 ± 2,84	15,56 ± 1,84	13,07 ± 1,69	5,15 ± 0,84
residual nitrite ppm	68,28 ± 8,88	243,5 ± 23,13	273,32 ± 17,08	352,15 ± 14,75
Ultrastructural changes	- At the beginning of Postsalting stage all banding patterns (A and I), H zone and M line disappear. - Filamentous aspect of the sarcomere disappear. - These characteristics can be observed during the postsalting stage.			

Table 1.- Physicochemical parameters and Ultrastructural changes for Gracilis muscle during the postsalting stage.

pH values increased in this stage in all the muscles of ham. This phenomena was in good agree with the " BELLATI et al. (1983) results " for Parma Ham and the results of " HUERTA (1986) " for Spanish Dry-Cured Ham, in these works showed that the pH value at the end of this stage is aproximately 5,9.

Water activity values (aw) showed notorious differences in the differents muscles under study. The muscles with an important decrease of aw values were Gracilis and Semimembranosus, this was for a high salt concentration that this muscles had at the beginning

Parameters \ Process time (days)	0	7	14	21
pH	5,63 ± 0.28	5,78 ± 0,13	5,65 ± 0,05	5,81 ±0,13
aw x10^2	99,40 ± 0,16	99,25 ± 0,38	98,61 ± 1,27	97,20 ± 0,24
% salt in aqueous phase	2,29 ± 1,03	5,27 ± 1,04	5,38 ± 1,76	4,69 ± 1,44
residual nitrite ppm	38,34 ± 5,83	40,74 ± 7,73	115,94 ± 11,88	126,07 ± 12,05
Ultrastructural changes	- H zone disappear at the beginning of the postsalting stage - Definition lose between juntions A band and I band at 14 days after the beginning of the postsalting stage (Fig. 2). - All banding patterns (A, I bands), H zone and M line disappear at 21 days after the beginning of the postsalting stage.			

Table 2.- Physicochemical parameters and Ultrastructural changes for Semimembranosus muscle during the postsalting stage.

of postsalting stage, at the same time the Semitendinosus and Biceps femoris the decrease of aw values was in function of salt diffusion.

Residual nitrites values increased during the postsalting stage, the higher residual nitrite took place in Gracilis muscle, in the " HUERTA (1986) work reported " that the higher concentration was for the growth of Nitrate reductase flora, at the end of this stage for Gracilis and Semimembranosus muscles reached inhibition concentration of nitrite for the growth of *Clostridium botulinum*, for Semitendinosus and Biceps femoris muscles the effect of salt

Parameters \ process time (days)	0	7	14	21
pH	5,87 ± 0,28	5,80 ± 0,14	5,70 ± 0,07	5,95 ± 0,13
aw x 10^2	99,58 ± 0,09	99,53 ± 0,05	99,48 ± 0,13	99,30 ± 0,09
% salt in aqueous phase	0,67 ± 0,12	1,49 ± 0,39	1,55 ± 0,30	3,32 ± 0,28
residual nitrite ppm	19,74 ± 4,14	33,27 ± 6,22	77,42 ± 10,18	113,56 ± 11,48
Ultrastructural changes	- All banding patterns remain and a myofibrillar swelling occurs at 7 days of the beginning the postsalting stage - Myofilaments structural disorganization and disappeareance of all banding patterns take place at the 21 after the beginning of the postsalting stage			

Table 3.- Physicochemical parameters and Ultrastructural changes for Semitendinosus muscle during the postsalting stage.

concentration, the decrease of water activity and residual nitrite generated the conditions for inhibit the growth of *Clostridium botulinum*.

At the beginning of postsalting stage the muscle with the higher salt concentration was the Gracilis, the other muscles increased salt concentration in function of the process time. At the end of this stage was when it was reached a salt equilibrium in Gracilis and Semimembranosus muscles, whereas for Semitendinosus and Biceps femoris the salt equilibrium did not take place in this stage. The higher salt concentration in Gracilis muscle at the beginning of the postsalting

Parameters \ process time (days)	0	7	14	21
pH	5,62 ± 0,15	5,75 ± 0,23	5,80 ± 0,28	5,88 ± 0,18
aw x 10^2	99,62 ± 0,16	99,56 ± 0,11	99,57 ± 0,22	99,34 ± 0,23
% salt in aqueous phase	1,27 ± 0,40	1,50 ± 0,47	1,87 ± 0,38	2,18 ± 0,60
residual nitrite ppm	16,68 ± 1,87	19,03 ± 2,93	95,25 ± 10,44	102,00 ± 9,91
Ultrastructural changes	- Ultrastructural remain with all characteristics of fresh muscle (all banding patterns remain), development a swelling of sarcomeres at A band, at 21 days after the beginning of the postsalting stage.			

Table 4.- Physicochemical parameters and Ultrastructural changes for Biceps femoris muscle during the postsalting stage.

stage was the cause of ultrastructure disorganization, this disorganization can be observed in fig.1. The salt diffusion to the other muscles made that ultrastructural disorganization took place at different times of the process. In the postsalting stage reached the ultrastructural disorganization in Gracilis, Semimembranosus and Semitendinosus muscles, whereas for Biceps femoris this ultrastructural disorganization took place in the Dry-Maturation stage

CONCLUSIONS

During the postsalting stage the ultrastructural disorganization do not take place at the same time ,the ultrastructural disorganization begins in the upper muscles. Gracilis is the first muscle in that all the banding patterns and myofibrilar structure disappear, these changes occur in the salting stage. In Semimembranosus the total disorganization takes place at 7 days after the beginning of postsalting stage, for Semitendinosus all structural disorganization takes place at 21 days after the beginning of the postsalting stage, and for Biceps femoris during the postsalting stage only shows a swelling at the end of this stage, and all banding patterns remain.

The pysichochemical parameters in the postsalting stage are not yet in equilibrium, in this stage the dry-cured ham does not reach the conditions to be included like an Intermediate Moisture Food, but at the end of this stage the *Clostridium botulinun* can be inhibited in his growth.

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