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Traditional and Fast Salting Effect on Physico-Chemical and Ultrastructural Properties of Spanish Dry-Cured Ham

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Abstract: The elaboration techonology of dry-cured ham is directed to get a right stability product with sensorial desirable properties. This a long process, which has three principal stages: salting, post-salting and dry-maduration. The salting is the first stability stage of the product. Two types of salting (traditional and fast) were studied. The physis (Lightness), chemical (moisture and salt content), physicochemical (pH and water activity) and structural properties of the different muscles (Semimembranosus (SM), Semitendinosus (ST) and Biceps femoris (BF)) were measured in the two types of salting. All parameters studies showed significant differences between two types of salting. These differences were due at Semimembranosus muscles. The electron microscopy study showed that in this muscle were observed only structural changes a comparasion with fresh muscle.

Key words: Traditional, salting, physico-chemical, ultrastructural, dry cured

Introduction

Dry-cured ham is one of the meat products with high prestige in the latin gastronomic culture. It is a raw, dry, maduration and nonhomogeneous products (Girard, 1991). Spain is the first producer and consumer nation of this product, with a total of 39.5 million pieces of dry-cured ham in 2002 (Cruz, 2003), followed by Italy and France in a lower rate.

The elaboration technology of dry-cured ham can change on the basis of the production area and the industrial or craft system, but is principally directed to get a right stability product with sensorial desirable properties (Sayas-Barberá, 1997). This is a long process, which has three principal stages: salting, postsalting and dry-maduration (Sayas-Barberá, 1997), several physical (Forcén *et al.*, 1993; Fernández-López *et al.*, 1994: Montero-Alonso, 1995), chemical (Baldini *et al.*, 1977 and Sayas-Barberá, 1997), biochemical (Motilva *et al.*, 1994 and Rosell and Toldrá, 1996) and ultrastructural (Sayas-Barberá, 1997) changes take place during its process.

Nowadays great changes in dry-cured ham sensorial properties have been carried out , among other things, by the use of fast production techniques. This techonology has higher economic advantages but some of the sensorial properties are not as good as those which are used in the traditional cured method (Toldrá, 1996).

Salting is the first stability stage of the product. In this stage salt diffusion is used (due to the factor, that the piece is completely covered with this additive) so that the piece can acquire the appropiate amount to protect its storage, but it should not acquire enough quantitives in orden not to damage its sensorial properties.

Salt makes several actions in meat products such as free water amount reduction, microbiotic selection and muscle protein straction, which are solubility in salt solution and realize very important functions related to the texture of these products.

The salt content of the piece depends on the length of time of this stage which, in addition to the influence in flavour and the control of the microbiological growth by its bacteriostactic effect, controls the biochemical reactions responsible for flavour (de Prado, 1988).

In Spain salting is, usually, made according to the traditional dry-cured method. In this stage, the pieces of ham are alternated with salt layers and ensure that the height does not exceed the range of 6-8 hams, avoiding the contact between pieces (Sayas-Barberá, 1997). The higroscopics properties of salt on the surface of the meat make exude on inner fluide towards outside. Part of the salt, which covers the ham, is dissolved into this liquid so it can penetrate by difussion into the meat (Prändl, 1994).

As a consequence of the dry-salting technology the surface of the pieces are temporarily exposed to a high concentration of salt, which would be on the decrease during the following cured stages, whereas salt concentration would be on the increase step by step in the most internal zones of the ham. The achievement of this process must be important for the release of proteins and changes in the structure (Knight and Parsons, 1988). Fernández-López (1998) reported that lightness (L*) is related to the muscle structure in meat products, however the effects of the process of structural changes in dry-cured meat products have scarcely been analysed.

Salting time of the pieces differs from one process to another; it depends on the raw material, maker´s experience and used technology. Both salting processes (traditional and fast) have not fixed rules; every manufacturer makes them according to their experience. Although the salting type can change between 1 to 1.5 days per kg of raw

ham in the traditional cured method, there are different points of view which range from 0.7 day to 1 day per kg of ham in the fast salting method.
The general purpose of the research was to study the effect of traditional and fast salting on the physis (lightness), chemical (moisture and salt content), physicochemical (pH and water activity) and structural properties of the different muscles (Semimembranosus, Semitendinosus y Bicepes femoris) of the Spanish dry-cured ham.

Materials and Methods

Materials: Eighteen hams were selected from 6 month old pigs (Large White x Landrace (female) and White Belgian (male). The selection was made according to their pH (5.6-6.0) which was measured in Semimembranosus muscle with an average weight of 8 ± 1 kg .
After the surface of the hams had immediately been nitrified with a dry salt mixture (0.9g of NaCl, 0.6g of $NaNO_2$ and 0.4g of $NaNO_3$ per kg of ham) and put in cold storage room (T: 2 ± 1°C), for 24 hours. After that, the pieces were completely covered with salt in cold storage room (T: 3 ± 0.5°C y R H: 90 ± 5%).
The half pieces remained covered with salt for 15 days (traditional method) and the other half pieces for 9 days (fast method).
At the end of the salting stage the excess salt was brushed off and the hams were washed with cool water (< 10°C).
Two cross cuts were made to obtain 6 cm thick slices (center section) in each ham (Schwartz *et al.*, 1983; Ferná ndez-López, 1994, Sayas-Barberá, 1997). 3 muscles were selected in each slice: Semimembranosus (SM), Semitendinosus (ST) and Biceps femoris (BF) (Fig. 1).

Physicochemical Analysis

pH: pH determinations were taken using Crison 507 pHmeter and a Crison CAT. n° 52-32 (Crison Instruments, S.A., Alella, Barcelona, Spain).

Water activity (aw): The measurements were made in a Novasina Thermoconstanter TH2 (Zurich Switzerland) at a working temperature of 25 °C.

Chemical analysis

Moisture content: The moisture content was determined according to the ISO method (1975a). Results were expressed as water (g)/ 100 g tissue).

Chloride concentration: Chloride concentration was determined according to the ISO method(1975b). Results were expressed as NaCl (g)/ 100 g tissue).
All physicochemical and chemical analysis were performed in triplicate.

Physical analysis

Lightness(L*): The lightness color co-ordinate was determined using a Minolta CM-2002 spectrophotometer (Minolta Camera Co. Ltd., Osaka, Japan), with D65 as light source and 10° as standard observer (Cassens *et al.*, 1995). A low reflectance glass was interposed between the samples and spectrophotometer (CR-A51 1829-752, Minolta Camera, Co. Osaka, Japon. 9 measurements was determined by each sample.

Electron microscopic study: For this study small samples (1mm x 1mm) were taken from each muscle and were immersed in a fixative solution (3% glutaraldehyde-M/15 phosphate buffer) at 7.03 pH, overnight, followed by a postfixation in 1% osmiun tetraoxide- M/15 phosphate buffer, for 5 hours. After fixation, samples were dehytrated in different solutions of acetone (from 30 to 100%) and embedded in epon. These sections were made with an ultramicrotome (Reichert Jung) and stained with 1% uranyl acetate. Electron micrographs were obtained with a transmission electron microscope (Zeiss EM109).

Statistics: Conventional statistical methods were used to calculate means and standars deviations. Statistical analyses (ANOVA) were applied to the data to determine statistically significant differences ($P < 0.01$ and 0.05) between types of salting (two levels) and muscles (three levels). When significant statistical differences were found in factors, an orthogonal contrast was carried out between each level ($P < 0.05$). All statistical analysis were made using BMDP Stadistical Software, 1993 (BMDP, 1993).

Results and Discussion

Table 1 reflects the means of each chemical, physicochemical and physical parameters in muscles at the final step of traditional and fast salting.

pH: The ANOVA results of this parameter pointed out significant differences between the two salting types and muscles ($P<0.01$).
Tukey test applied to the type of salting showed the highest values for fast salting. This behaviour could be

Table 1:Mean values of chemical, physical and physicochemical parameters in semimembranosus (sm), semitendinosus (st) and biceps femoris (bf) muscles, for the traditional and fast salting.

Salting	Muscle	pH	aw	Moisture	Chloride	L*
Traditional	SM	5.75b	0.983 a	65.30a	7.06c	37.94a
	ST	5.78c	0.995c	72.89d	0.79a	42.87c
	BF	5.67a	0.995c	71.14c	1.06a	45.80d
Fast	SM	5.70b	0.990b	67.89b	4.47b	41.09b
	ST	5.64a	0.997c	73.12d	0.50a	43.25c
	BF	5.79c	0.996c	71.58c	0.76a	47.50d

a-d For each variable, means within the same column with different superscripts differ significantly ($P<0,05$)
aw (water activity), Moisture (moisture content), chloride (chloride concentration), L* (Lightness)

because of a higher phosphate loss a higher contact time with salt, a higher salt enter and finally due to a higher loss of other basics compounds, in the traditional salting (Arnau *et al.*, 1995; Nayak *et al.*, 1996).
Both traditional and fast salting, showed significant differences between all muscles studies (SM, ST y BF).
Other researches about dry-cured products, reported a pH decrease, in comparison with raw values, because of the incorporation of this additive during the salting (Migaud and Frentz, 1978; Ventanas *et al.*, 1989; Córdoba, 1990; Arnau *et al.*, 1995; Pérez-Alvarez *et al.*, 1997b; Sayas-Barberá, 1997).

Water activity (aw): The ANOVA results for these two kinds of salting indicated significant differences between traditional and fast salting and between the muscles ($P<0.01$). Tukey test applied to theses types of salting, showed, in average, the hams of traditional salting had lower values, because they were longer time in contact with salt.
No significant statistical differences ($P>0.05$) were found in both muscles: ST and BF, but statistical differences ($P<0.01$) were found berween these two muscles and the SM (Table 1).
The differences between the two kinds of salting must be at SM muscle, which has a higher surface in contact with salt and it receives, mainly, the entrance of salt-. In the hams from traditional salting this muscle showed a lower values (0.983 ± 0.003) than the same in the hams from fast salting ($0.990 \pm 0,003$).
Aw values obtained in the ST and BF muscles agree with those found by Sayas-Barberá y Pérez-Alvarez (1989) in fresh muscle. The BF muscle, in spite of presenting an average salt levels 0.91%, has not the enough amount to affect this parameter (aw). This behavior may be due to " barriers" as skin, conjunctive tisue and subcutane fat that protect this muscle from dehydration.
No muscles in the two salting methods reached aw values to inhibit the growth of microrganisms that cause the decay of these products, specially the growth of Clostridium botulinum (Sayas-Barberá and Pérez-Alvarez, 1989; Leistner, 1994). Other investigations about salting stage (Fernández-López *et al.*, 1994; Muñoz *et al.*, 1994; Montero-Alonso, 1995) found that conditions don' t exit (nitrite, salt concentration, oxide-reduction potential, etc.) for the stability of the product, especially, to inhibite the Clostridium botulinum growth. As a consequence, the process will need auxiliary mechanisms such as temperature.

Moisture: Significant differences were found between fast and traditional salting ($P<0.01$) and between muscles ($P<0.01$).
Tukey test pointed out significant differences ($P<0.05$) between all the muscles (SM, ST and BF) for both salting methods. Moisture average was lower in the SM muscle, because of the higher osmotic effect produced by salt that covered the surface of the ham. ST muscle showed the highest moisture values (Table 1).
Hams from traditional salting presented lower moisture values (Tukey test, $P<0.05$). This behavior could be because to the pieces were covered with salt for a period of 15 days in the traditional salting method, as a consequence of that, the osmotic effect was higher. These differences between the salting processes was due to SM muscle, so the days of salting didn´t affect the inside muscle (ST and BF).

Chloride concentration: Significant statistical differences in chloride concentration were between both salting processes($P<0.05$) and muscles ($P<0.01$).
Tukey test showed that hams from traditional salting had a higher chloride concentration ($P<0.05$), because of the hams remained in salt longer. These differences between both salting methods are due to the SM muscle.
Tukey test concerning the muscle factor showed that both salting methods did not present significant statistical

differences between the ST and BF muscles ($P>0.05$) but significant differences were found between these and the SM ($P<0.05$).
The small salt concentration in the BF muscle suggests the diffusion of salt through the skin, fat and conjunctive tissue, in small quantities.
In other meat products with anatomic integrity like " lomo embuchado", it has been observated that the conjunctive tissue affects the salt diffusion (Pérez-Alvarez, 1996).

Lightness (L*): The ANOVA results concerning the lightness indicated significant statistical differences ($P<0.01$) between the two salting methods and between muscles. Significant differences between all muscles (Tukey test, $P< 0.05$), in both salting processes, were obtained.
Tukey test showed that the hams from traditional salting had lower lightness values than in fast salting. These differences must mainly due to the SM muscle.
During the salting stage, lightness values disminished by means of the salt entrance, which is more remarked in the SM muscle. This may be due to the fact that this muscle (SM) received a higher salt concentration. The decrease in the lightness values can be due to different phenomena, such as the salt concentration and the increase of the water holding capacity (WHC) (Sayas-Barberá, 1997; Fernández-López, 1998)..
The lower lightness values in traditional salting would be due to a higher salt incorporation, so the movement and wastage of water were larger than in fast salting. The muscles from fast salting, altogether, suffered the salt effects in a smaller degree.
The decrease of the lightness values found when this aditive was incorporated has been reported by others authors in dry-cured sausages model systems and in cooked meat products (Sánchez-Rodríguez *et al.*, 1994; Pérez-Alvarez, 1996; Pérez-Alvarez *et al.*, 1997a; García-Marcos *et al.*, 1996; Zaragoza, 1997; Perlo, 1997; Rosmini, 1997; Fernández-López, 1998; Saez, 1997).
The lightness depends on the factors such as pH, water content (moisture), WHC, additives and species incorporated, muscle structure and the water movement (dehydration) into the piece (Swatland, 1995; Varnam and Sutherland, 1995; van Laak *et al.* 1995; Sayas-Barberá , 1997; Rosmini *et al.*, 1998; Fernandez-López, 1998).

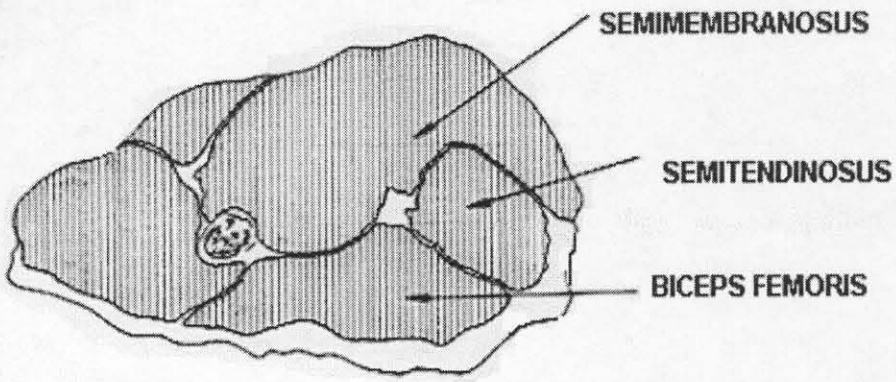

Fig. 1:Longitudinal selection of dry-cured harm

Electron microscopic study: Fig. 2 shows a cross-sectional area of the ST muscle of fresh ham, before the salting stage. In this micrograhs, the typical characteristics of muscle myofibrils are observed with banding pattern. However, there are structural changes in some areas near the Z disc. These alternances could be due to a proteolitic activity during the storage of meat. After slaugthering, these changes are responsible for the increase tenderness (Gil, 1995).
The implicated proteolitic enzymes, in these alterations, will be the cathepsins and calpains (Zeece *et al.*, 1992) The more affected proteins can be the desmine and alfa-actinin, associated to the Z disc (Bandam, 1992). The degradation of these proteins can explain the deorganization near the Z disc.
Fig. 3 shows the myofibril appearance of SM muscle from fast salting. Important changes develop a comparasion with fresh muscles (Fig. 2). In this Fig., the disappearance of the H-band and the loss of intensity A-band can be observed. These could be due to extractions of miosine in a high NaCl concentration during salting stage (Offer and Knight, 1983; de Prado, 1999; Griethusen and Knight, 1991; Cunham *et al.*, 1995).
In the Fig. 3 it is observed that Z disc remains, however, the intensity decreases and has transversal breakings,

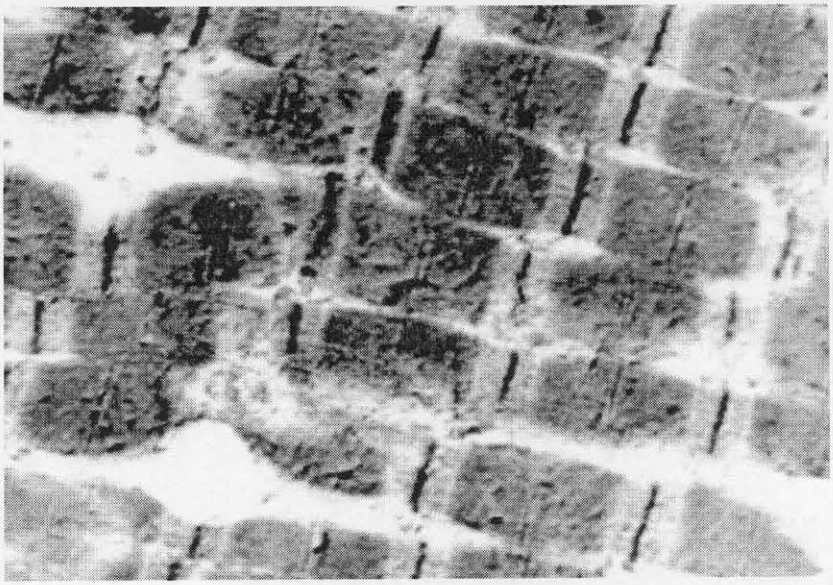

Fig. 2: Electron micrograph of semimembranosus muscle(X7.700)

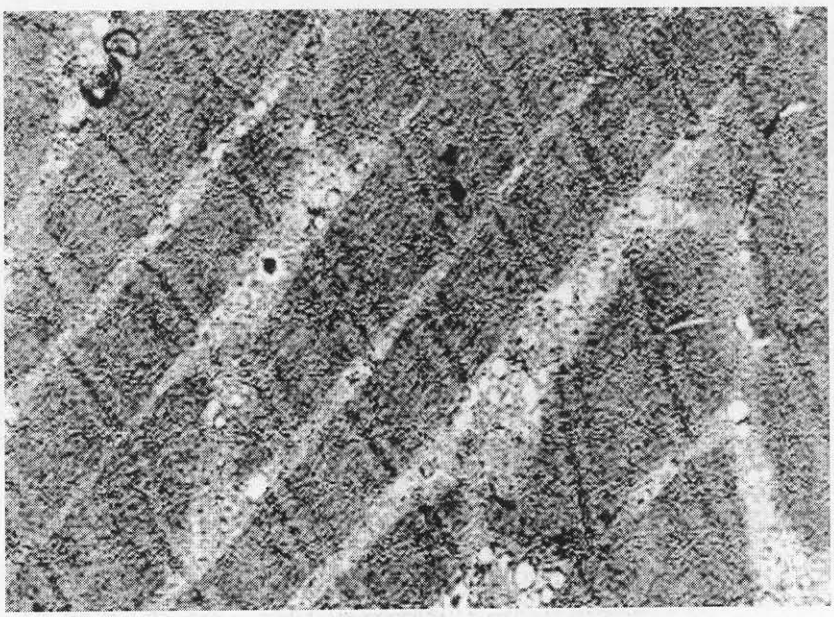

Fig. 3:Electron micrograph of sesmimembranosus muscle from fast salting (X6.800)

which can be due to the enzymatic activity of the meat. During the salting stage of dry-cured ham process calpain activity which is responsible for the degradation of the Z-disc has been detected (Sárraga *et al.*, 1993). Semitendinosus and Biceps femoris muscles did not show any changes. This could be because of its lower salt concentration (Table 1).

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