EVALUATION OF PROTEIN PROFILES BY SDS-PAGE IN DRY-CURED HAM PROCESSING

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SUMMARY

Biceps femoris muscles from fresh hams, after the salting stage (post-salting) and at the end of a 12 months cruring-process were taken. Salt and soluble proteins were removed from samples using water. Extractability of myofibrillar proteins in high ionic strength buffers (1.1M IK and 0.1M phosphate buffer, pH=7.4) followed by 1% SDS extraction was studied by SDS-PAGE. KCl extracts obtained from all samples were also analyzed. High ionic strength and 1% SDS extracts showed different profiles along the process, whereas profiles from 1% SDS and KCl extracts were similar. Also, a more intense protein breakdown profiles was observed in high ionic strength extracts.

INTRODUCTION

An ultrastructural disorganization of muscles takes place during dry-curing process (Aranda-Catalá et al., 1991), whereas the solubility of muscle proteins changes with time according to the NaCl and temperature levels set in each step. In low ionic media, the extractability is fairly constant or decrease slightly at the end of the process. However, Córdoba (1990) reported a decrease in myofibrillar proteins extractability in high ionic strength (1.1M IK-0.1M phosphate buffer, pH=7.4) during processing. Also the extractability reported by Penedo (1989) in eight different anatomic regions at the end of the Iberian ham processing was in 3% NaCl only slightly higher than in water. A higher decrease in muscle proteins extractability seems to be found in *core* sampling (Flores et al., 1983; Astiasarán et. al, 1989).

The decline in myofibrillar protein extractability throughout the process has been related with a loss on the functional properties of these proteins. So Bellati et al. (1983) and Dazzi et al. (1982) reported a decrease in ATPase activity in Parma hams. However, there is a lack of data about the protein profile involved (Maggi et al., 1977; Penedo, 1989; Córdoba et al., 1990 and Toldrá et al., 1993) although it is one of the more characteristic traits of curing-process.

The aim of this paper is to compare the evolution of myofibrillar protein profiles with time according to their extractability.

MATERIAL AND METHODS

Raw material

Hams from castrated pig males *(Large white)* were salted during 17 days at 2-3 °C and stored at the same temperature during 67 days. Aging of hams was performed in 3 steps: 10-12 °C to 20 °C (75 days), 20-26 °C (111 days) and 20 °C to 14-16 °C (90 days). *Biceps femoris* (BF) muscles were taken from fresh hams, after the salting stage (post-salting) and at the end of the process.

Preparation of protein extracts

To remove NaCl of the samples, ground muscle was mixed 1:20 (w/v) with ice-cold distilled water, left for 1 h at 4 °C and centrifuged at 10.000 x g for 30 min at 4 °C. The washed pellet was resuspended three times in 25 ml of 0.1 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM DTT, 10 mM potassium phosphate at pH 7.0 1:20 and centrifuged at 2000 x g for 5 min (Offer and Trinick, 1983). The pellet constituted the *KCl extract.*

On the other hand, ground muscle was mixed with distilled ice-cold water 1:20 (w/v) and left for 2 h at 4 °C. The mixture was centrifuged at 10.000 x g for 30 min at 4 °C and water-soluble protein-containing supernatant was discarded. Myofibrillar proteins were first extracted by dissolving the washed pellet in ice-cold 0.1 M

phosphate buffer, pH 7.4, containing 1.1 M potassium iodide 1:20 and left at 4°C for 3 h. The mixture was centrifuged again at the same conditions and the supernatant constituted the *high ionic strength extract.*
The pellet was resuspended in 30 ml of 50 mM calcium chloride and centrifuged at 15.000 x g for 30 min at 4°C. The last pellet was dissolved 1:20 in 1% SDS and stirred for 12 h at room temperature. The *1% SDS extract* was obtained by centrifugation (10000 x g, 30 min at 4°C).
All supernatants were filtered throughout glass wool and the protein of each extract was determined by the method of Kjeldahl (Presidencia del Gobierno, 1979). The extractable proteins were precipitated in ice-cold acetone and centrifuged at 5.000 x g for 10 min. The dried samples were then dissolved in rigor (75 mM KCl, 10mM Tris, pH 7.0, 2 mM EGTA, 2 mM $MgCl_2$, 2 mM NaN_3) and sample buffer (8 M urea, 2 M Thiourea, 3% SDS, 75 mM DTT, 25 mM Tris/HCl, pH 6.8) 1:1 to make the appropriate protein concentration (Fritz & Greaser, 1991). The mixture was heated at 50°C for 20 min prior to processing.
Phast Gel Homogeneous 12.5 (Pharmacia Biotec AB) were used to perform the electrophoretic running in a horizontal electrophoresis system (Phast System, Pharmacia Biotec AB). Samples application (1 μl) were made up at 1.0 mA during 1 Vh and running condition at 3.0 mA, 80 Vh, 15 °C. Gels were stained with Coomasie Blue R-350.

DISCUSION

The electrophoretic profiles of the myofibrillar proteins extractable in high ionic strength buffers showed a continuos decrease in intensity of several bands with a molecular weight of 36, 28, 25, 21 and 16 kda. However, the intensity of a 34 kda band remained up to the end of the process. Also a decrease in intensity of the MHC in the SDS-PAGE profiles was related initially with the appearance of about 150 kda component. However, whereas the MHC band and 150 kda component nearly disappeared completely at the end of the process, new bands at about 90 kda were detected. Two bands of 58 and 60 kda were also observed throughout the whole processing.
An intense proteolysis or a loss of solubility from the myofibrillar proteins extractable in high ionic strength could be expected according to the decline in nitrogen amount from this fraction. Although the identity of proteins extracted when meat was treated with high salt concentrations has not been completely determined, the decrease in intensity of the myofibrillar proteins with a molecular weight in the range 40-14 kda was probably due to NaCl (Dilber-Van Griethuysen and Knight, 1991). However, according to their minor content in the myobrillar pattern, the amount of nitrogen involved is probably insufficient to explain the marked decline in amount reported by several workers (Córdoba, 1990; Astiasarán et al., 1989).
The myosin breakdown in a 150 kda component was reported in Iberian (Córdoba, 1990) and White hams (Toldrá y col., 1993) during aging steps and minor proteolytic fragments with molecular weight in the range 90-50 kda (Toldrá y col., 1993) and 80, 70 and 67 kda (Córdoba, 1990) were also observed. The disappearance of MHC from these profiles at different steps of processing reported in Parma ham (Maggi et al., 1977), Iberian Ham (Córdoba et al., 1990) and White ham (Toldrá et al., 1993) seems to be related with the technological characteristics of processing. Protein breakdown in small molecular components could explain the decline in amount of nitrogen extractable, but an important increase of soluble nitrogen in low ionic media was not reported.
Initially muscle *biceps femoris* (BF) showed similar protein extractability in high ionic strength and 1% SDS in raw hams. However, whereas the extractability in high ionic strength declined with time, the amount of extractable nitrogen in 1% SDS remained fairly constant throughout the whole processing. So the extractability in 1% SDS since the post-salting step was twofold greater than in high ionic strength. The high molecular weight proteins, myosin and actin were the myofibrillar proteins mainly detected in these SDS-PAGE profiles. A progressive disappearance of the myosin heavy chain (MHC) band and a continuos increase in intensity of a 150 kda fragment were observed in these SDS-PAGE profiles. A significant decrease in amount of the 38 kda band was observed with time. In addition, the 36 kda band increased in intensity and some minor proteolytic components (29 and 27 kda) more noticeable in the post-salting step appeared. Similar protein breakdown products were also reported by Ouali (1987) in the aging of meat. Therefore, other methodologies than SDS-PAGE should be applied to identify some protein breakdown products appeared in these profiles, especially when we take into account the different conditions set between the aging of meat and in dry-cured processing. However, the absence in the 1% SDS profiles of proteolytic products in the range 90-50 kda, similar to the profiles obtained from the KCl extracts, showed a lower accessibility of these proteins to degradation and could be a mechanism to prevent the muscle integrity.

CONCLUSION

A different influence of the dry-curing process on myofibrillar protein breakdown has been observed. Whereas high ionic strength extracts showed a more intense protein breakdown profiles, the 1% SDS and KCl extracts had a similar protein composition.

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Fig. 1. A. Poyacrylamide gel electrophoresis of extractable myofibrillar proteins in high ionic strength. Lanes 1 an 5 standard protein pattern (94, 67, 43, 30, 20.1 and 14.4 kda); lane 2, raw ham; lane 3, post-salting step and lane 4, after 12 months of aging. B. Poyacrylamide gel electrophoresis of extractable myofibrillar proteins in 1% SDS. Lane 4 standard protein pattern (See A); lane 1, raw ham; lane 2, post-salting step and lane 3, after 12 months of aging. C. Poyacrylamide gel electrophoresis of the KCl extracts. Lane 1 standard protein pattern (See A); lane 2, raw ham; lane 3, post-salting step and lane 4, after 12 months of aging.