PROTEOMIC CHARACTERISATION OF NORMAL AND PASTY SPANISH DRY-CURED HAMS

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Background

Tenderness is an important quality trait of dry-cured ham for which changes in the solubility and integrity of the muscle structural proteins are considered to be the main mechanisms responsible for the tenderization of hams during dry-curing. Monin *et al*. (1997) pointed out that the hardness and chewiness of hams increase during the initial stages of processing due to a decrease in protein solubility and water content and subsequently which decreases as proteolysis progresses. However, intense proteolysis can induce textural defects that may reduce the acceptability to consumers.
The relationship between excessive proteolysis and defective texture was first reported in Italian dry-cured hams (Parolari *et al*. 1994, Virgili *et al.* 1995). However, although pastiness and softness have been related to increased proteolysis measured as amino acid and peptide contents (Parolari *et al.* 1988, Careri *et al*. 1993, Virgili *et al.* 1999), but the proteins implicated have not been identified. The endogenous lysosomal cathepsins of muscle are thought to be the enzymes mainly responsible in this process (Parolari *et al*. 1988, Schivazappa *et al.* 2002). However, several factors have been reported to affect proteolysis: genetic type, sex, age, meat pH and processing (salting, temperature and time) (Buscailhon *et al*. 1994, Arnau *et al*. 1998, Garcia-Garrido *et al*. 1999, Tabilo *et al*. 1999).

The development of new separation techniques, such as 2-D electrophoresis, enables the study of those mechanisms related to meat and meat products quality.

Objectives

The study was carried out to compare protein 2D electrophoretic patterns of pasty and soft dry-cured ham to those from normal hams to find markers related to textural defects. To achieve this, low molecular ionic strength extracts were used to find markers related to the defective texture of dry-cured hams.

Material and methods

The normal-texture dry-cured hams (control) processed with different processing times: short (6 months), medium (8-12 months) or long (18 months); were selected at a Spanish company. The defectiveness samples (3) were selected according to their textural characteristics: soft (1), pasty (1) and very pasty (2). The analyses were performed on the *Biceps femoris* muscle.

Protein extraction/fractionation

150 mg of muscle were added to 1.5 ml of extraction buffer (150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$ and 4 mM EDTA) in an Eppendorf tube containing a glass bead. Homogenisation was performed using a Retsch MM2 agitator (Retsch, Haan, Germany) for 30 min at 4°C. Homogenates were centrifuged at 10,000g for 10 min at 10°C. The supernatant was collected and protein content was measured using Bio-Rad Bradford Protein Assay kit.

2D electrophoresis

2D electrophoresis was performed on both normal and defective quality samples. Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (BioRad), using BioRad strip, 17 cm pH 4-7. 90 µg of soluble proteins were loaded onto the strips for analytical gels. Protein loading on strips, IEF and SDS-PAGE were performed according to Morzel *et al*. (2004). Gels, in duplicate, were silver stainned following the protocol of Yan *et al*. (2000). Gel images were acquired using GS-800 densitometer and analysed using the PDQuest software (Bio-Rad).

Results and discussion

Different 2DE patterns were detected between dry-cured ham quality, although the profiles were also clearly influenced by the total processing time.

Actually, differences in profile evolution of the spot intensity were detected between processing times. However, a noticable number of spots were characteristic for middle (n=21 spots) or long (n= 11 spots) processing times (Table 1). Moreover, the intensity of a significant number of spots was also higher in both the long and the middle processing times. Such evolution could be explained by a higher protein solubility development as the processing time increased. And the relative number of characteristic spots to the higher intensity spots, for both the long and medium processes, is probably related to the different solubilities and degradation patterns of those proteins implicated. A high protein solubility would increase the intensity and/or the number of spots, while more degradation would produce progressively a decrease of protein molecular weight. This is consistent with the different spot pattern observed in gels between the processing times. For the long processing time, spots were located either at the upper zone of the gel or at the lower zone and at a pH area about 5 or 6 (Fig 1). In contrast, for medium processing, the characteristic spots were above 30-40 kDa.

Furthermore, the lower protein solubility from the short processing time was also consistent with an absence of any characteristic spot. But we observed numerous spots having lower intensity, mainly distributed between around pH 6 – 6.5, showing a decrease in solubility of the molecules having a pI close the dry-cured ham pH.

	Processing time		
Process	Long	Medium	Short
Characteristic	11	21	-
Higher Intensity	24	7	4
Lower Intensity	1	11	15

Table 1. Number of selected spots

The characteristic spots of the softness profile were mainly detected in the middle (2104-2201--5112-5113-5210) and bottom (2013-7019) parts of the gel. However, the soft hams had a very similar spot pattern to the normal quality ham pattern. Also several spots were detected in both normal and soft dry-cured hams but were more intense from soft hams (5707-5715-5717-5718-6712-6713-6714-6716-6717-7716).

Both softness and pastiness patterns had common spots at the upper zone of the gels, on the anode (3624-4517) side. The softness and pastiness pattern did not show the several low molecular weight spots characteristic of normal hams (Fig 2). These results are in agreement with the intense proteolysis previously reported (Parolari *et al*. 1988), assuming that these spots (protein or fragment) result from proteolysis.

In the upper gel zone, several characteristic spots (3621-3622-3625-3626-3733-3734-4213-4624-4735-4736-4824-4825-5616) were apparent of the gel from the pastiness ham. Other spots (3735-3736-4627-4628) were present in the pasty ham while in the very pasty hams, they were less intense or absent. In the middle part of the gels, the 5205 spot exhibited a lower intensity in high pasty hams which could be due to a more intense proteolysis in very pasty samples. However, the intensity of the 4116 spot increased as pastiness increased and seemed to be related to the extent of defectiveness.

Conclusions

Different 2DE patterns were detected between dry-cured ham quality, although the profiles were also clearly influenced by the total processing time. The protein solubility increased as the processing time increased.
The decrease in the molecular weight of proteins depended to the processing time.
The pasty and soft texture of dry-cured hams had more spots with high molecular weight.
2-D electrophoresis was effective in characterizing the quality of dry-cured hams. In further work , spots of interest (either proteins or fragments) will be studied by Maldi-tof spectrometry.

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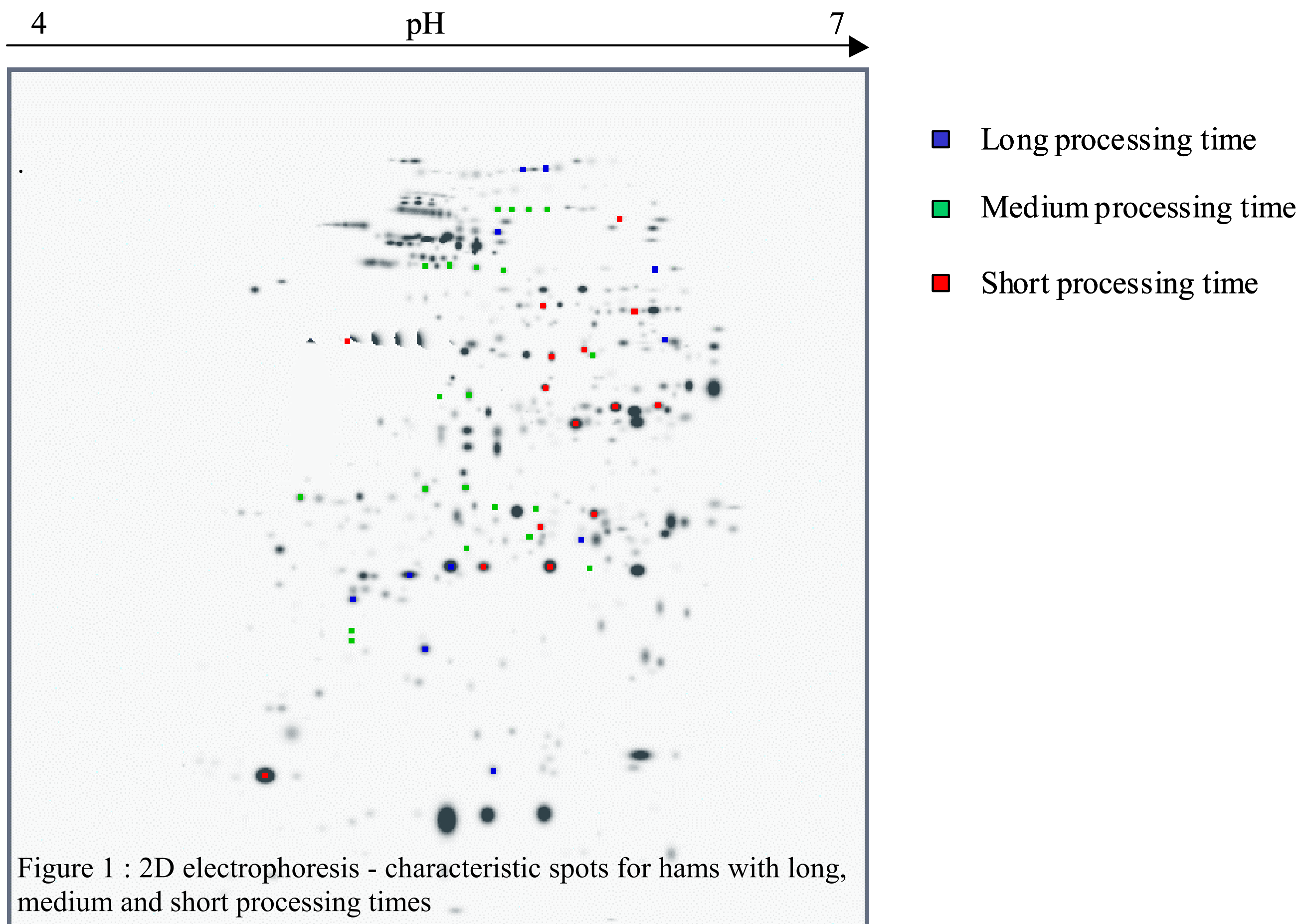

Figure 1 : 2D electrophoresis - characteristic spots for hams with long, medium and short processing times

4 pH 7

Soft ham

Pasty ham

Soft and pasty ham

Figure 2 : 2D electrophoresis - characteristic spots for pasty and soft hams