

Proteomic characterisation of normal and pasty dry-cured hams

Hortós M., Santé-Lhoutellier V., Arnau J., Monin G.

in

Audiot A. (ed.), Casabianca F. (ed.), Monin G. (ed.).
5. International Symposium on the Mediterranean Pig

Zaragoza : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 76

2007

pages 245-249

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=800593>

To cite this article / Pour citer cet article

Hortós M., Santé-Lhoutellier V., Arnau J., Monin G. **Proteomic characterisation of normal and pasty dry-cured hams**. In : Audiot A. (ed.), Casabianca F. (ed.), Monin G. (ed.). 5. *International Symposium on the Mediterranean Pig*. Zaragoza : CIHEAM, 2007. p. 245-249 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 76)



<http://www.ciheam.org/>
<http://om.ciheam.org/>

Proteomic characterisation of normal and pasty dry-cured hams

M. Hortós*, V. Santé-Lhoutellier**, J. Arnau* and G. Monin**

*IRTA-Centre de Tecnologia de la Carn. Granja Camps i Armet, s/n. 17121 Monells, Spain

**INRA Station de Recherches sur la Viande. 63122 Saint Genès Champanelle, France

SUMMARY – 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, the proteins involved have not been identified. The development of new separation techniques, such as 2-D electrophoresis, enables the study of those mechanisms related to meat and meat product quality. 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. 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 on 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 using the Maldi-tof spectrometry.

Keywords: Dry-cured ham, pastiness, proteomics.

RESUME – "Caractérisation protéomique de jambons secs à texture normale et pâteuse". La texture du jambon sec est la résultante de l'ensemble des modifications qui affectent la solubilité et l'intégrité des protéines du muscle. La protéolyse est plus intense dans les premiers mois de sèche et tend à diminuer avec l'augmentation de la teneur en sel dans les muscles. La mesure de l'azote non protéique (acides aminés + peptides) est traditionnellement effectuée et constitue la mesure de référence d'estimation de la protéolyse dans le jambon sec. Cependant, de nouvelles techniques telles que l'électrophorèse bidimensionnelle devraient permettre une approche plus globale de caractérisation d'un jambon sec. La comparaison des profils protéiques de jambon sec de texture différente a été entreprise. Pour cela, nous avons utilisé des extraits protéiques solubles à faible force ionique. Les profils protéiques obtenus diffèrent selon la qualité de texture des jambons mais ils sont aussi influencés par le temps d'affinage des jambons secs. Les profils protéiques des jambons secs dont la texture est soit pâteuse soit molle présentent des spots caractéristiques dans la zone des poids moléculaires élevés et une absence de spots protéiques dans la zone centrale du gel soit autour d'un poids moléculaire de 30 kDa et des pHi voisins de 6-7. Cela traduit en fait une augmentation de la solubilité de ces protéines. Pour le process d'affinage moyen, les spots caractéristiques se situent dans la zone de poids moléculaire compris entre 30 et 40 kDa. Ces derniers résultats confirmeraient la protéolyse très intense souvent décrite dans les jambons à texture pâteuse (Parolari *et al.*, 1988). L'approche protéomique de la qualité du jambon sec est complémentaire des mesures classiques de l'azote non protéique. Ses développements, couplés à la spectrométrie de masse, devraient permettre d'identifier les protéines cibles de la protéolyse et à terme définir des marqueurs biologiques de la qualité.

Mots-clés : Jambon sec, caractère pâteux, protéomique.

Introduction

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; García-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.

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

Hundred and fifty mg of muscle were added to 1.5 ml of extraction buffer (150 mM NaCl, 25 mM KCl, 3 mM MgCl₂ 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 stained 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 noticeable 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.

Table 1. Number of selected spots

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

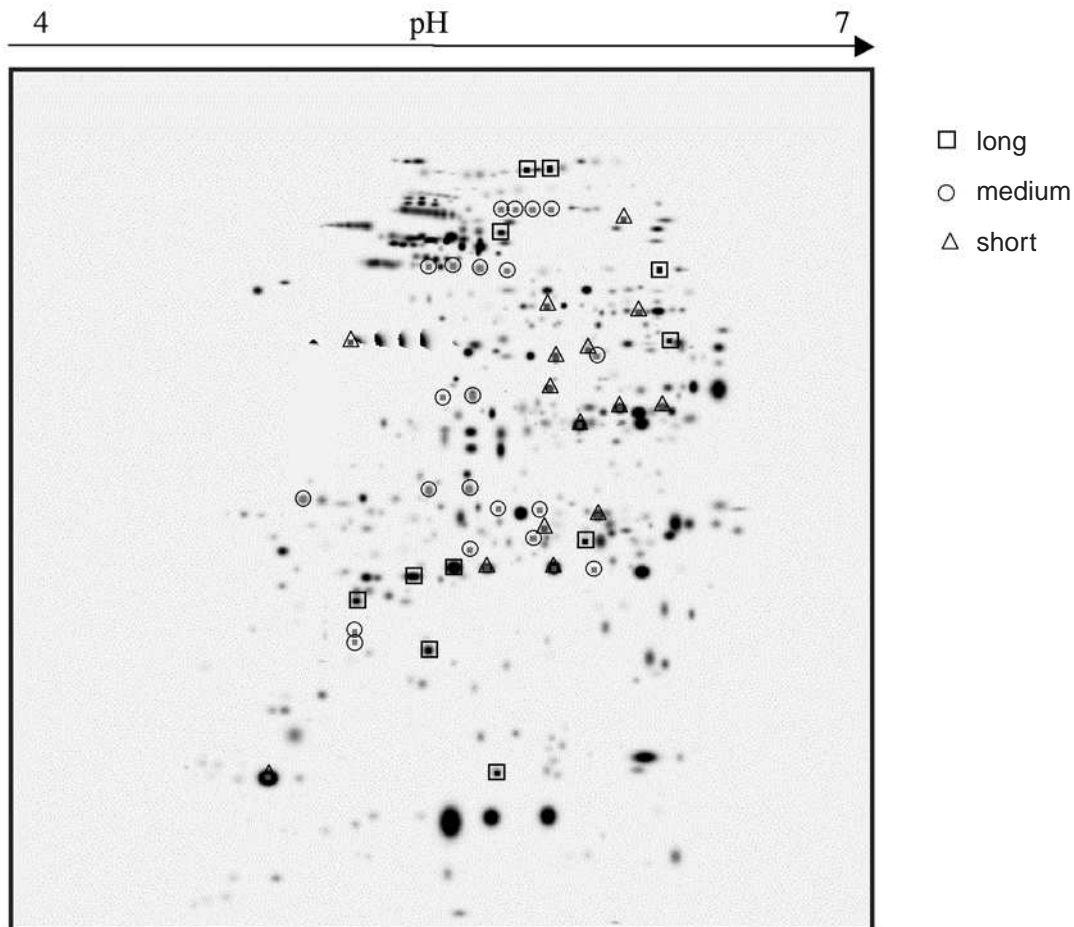


Fig. 1. 2D electrophoresis – characteristic spots for hams with long, medium and short processing times.

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.

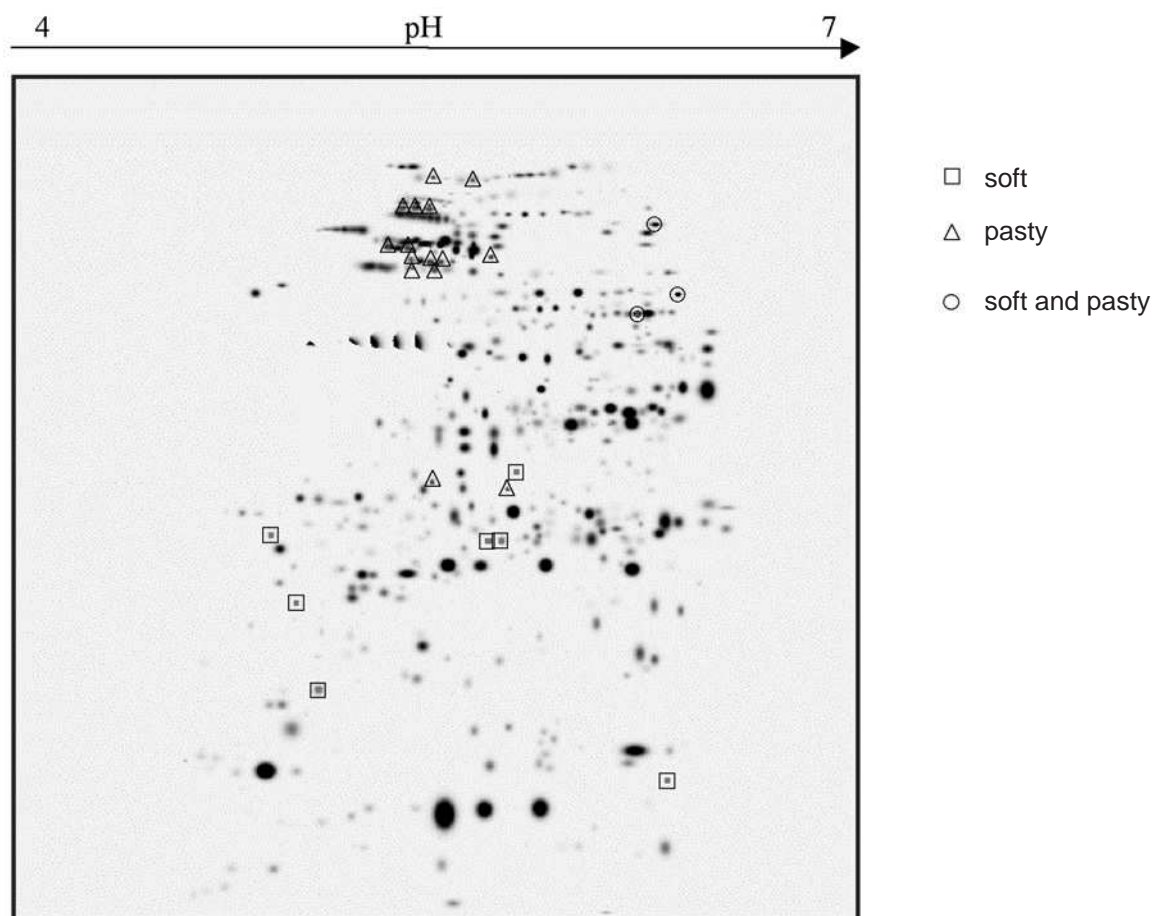


Fig. 2. 2D electrophoresis – characteristic spots for pasty and soft hams.

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.

References

- Arnau, J., Guerrero, L. and Sarraga, C. (1998). The effect of green ham pH and NaCl concentration on cathepsin activities and the sensory characteristics of dry-cured hams. *J. Science Food Agric.*, 77: 387-392.
- Buscailhon, S., Berdagué, J.L., Gandemer, G., Touraille, C. and Monin, G. (1994). Effects of the initial pH on the compositional changes and some sensory traits of French of dry-cured hams. *J. Muscle Food*, 5: 257-270.

- Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R. and Parolari, G. (1993). Sensory properties relationships to chemical data of Italian-type dry-cured ham. *J. Food Science*, 58: 968-972.
- Garcia-Garrido, J.A., Quiles-Zafra, R., Tapiador, J. and Luque de Castro, M.D. (1999). Sensory and analytical properties of Spanish dry-cured ham of normal and defective texture. *Food Chemistry*, 67: 423-427.
- Monin, G., Marinova, P., Talmant, A., Martin, J.F., Cornet, M., Lanore, D. and Grasso, F. (1997). Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the dehairing technique. *Meat Science*, 47: 29-47.
- Morzel, M., Chambon, C., Hamelin, M., Santé-Lhoutellier, V., Sayd, T. and Monin, G. (2004). Proteome changes during pork ageing following use of two different pre-slaughter handling procedures. *Meat Science*, 67: 689-696.
- Parolari, G., Rivaldi, P., Leonelli, C., Bellati, M. and Bovis, N. (1988). Colore e consistenza del prosciutto crudo in rapporto alla materia prima e alla tecnica di stagionatura. *Industria Conserve*, 63: 45-49.
- Parolari, G., Virgili, R. and Schivazappa, C. (1994). Relationship between cathepsin B activity and compositional parameters in dry-cured hams of normal and defective texture. *Meat Science*, 38: 117-122.
- Schivazappa, C., Degni, M., Nanni Costa, L., Russo, V., Butazzoni, L. and Virgili, R. (2002). Analysis of raw meat to predict proteolysis in Parma ham. *Meat Science*, 60: 77-83.
- Tabilo, G., Flores, M., Fiszman, S.M. and Toldra, F. (1999). Post-mortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham. *Meat Science*, 51: 255-260.
- Yan, J., Wait, R., Berkelman, T., Harry, R., Westbrook, J., Wheeler, C. and Dunn, M. (2000). A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption / ionisation and electrospray ionisation mass spectrometry. *Electrophoresis*, 21: 3666-3672.
- Virgili, R., Parolari, G., Schivazappa, C., Soresi Bordini, C. and Borri, M. (1995). Sensory and texture quality of dry-cured hams as affected by endogenous cathepsin B activity and muscle composition. *J. Food Science*, 60: 1183-1186.
- Virgili, R., Parolari, G., Soresi Bordini, C., Schivazappa, C., Cornet, M. and Monin, G. (1999). Free amino acids and dipeptides in dry-cured ham. *J. Muscle Food*, 10: 119-130.