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Vertical protein spot chains – proteomic indicators of proteolysis in dry-cured ham?

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Abstract. Proteomic profile of Slovenian "*Kraški pršut"* dry-cured ham matured for 14 months, was studied. Insoluble protein fraction was extracted from dry-cured *Biceps femoris* muscles. 2-dimensional SDS PAGE gels (24 samples in three technical repetitions) were made and the protein pattern analysed. Several distinctive protein spot patterns (*i.e.* protein spot chains containing spots that differed in molecular weight but not in isoelectric point) were observed. The patterns were highly repeatable between the technical repetitions. The subsequent identifications showed the same protein inside one spot chain. Differences in estimated molecular weight between the spots from the same chain indicate the protein degradation, however, it could not be confirmed by the mass spectrometry (lacking accuracy). For firmer confirmation of our hypothesis, a comparison of proteomic profile of hams in different processing phases is needed.

Keywords. Dry-cured ham – Proteolysis – Proteomic profile – Vertical spot chains.

Des chapelets verticaux de protéines : indicateurs protéomiques de la protéolyse dans le jambon sec ?

Résumé. *Le profil protéomique du jambon sec slovène "Kraški pršut" a été établi après 14 mois de séchage. La fraction de protéines insolubles a été extraite du mucle* Biceps femoris*. Les gels d'électrophorèse bidimensionelle SDS PAGE (24 échantillons en trois répétitions techniques) ont été réalisés ainsi que l'analyse d'image des gels. Plusieurs chaînes verticales de protéines distinctes (c'est-à-dire des spots protéiques différant en poids moléculaire, mais pas en point isoélectrique) ont été observées. L'apparition de ces chaînes était très reproductible entre les répétitions techniques. Les identifications ultérieures ont montré qu'il s'agit d'une même protéine à l'intérieur d'une chaîne. Les différences en poids moléculaire estimé entre protéines de la même chaîne indiquent leur dégradation progressive. Pour confirmer notre hypothèse, un suivi du profil protéomique des jambons au cours du séchage est nécessaire.*

Mots-clés. *Jambon sec – Protéolyse – Profil protéomique – Chapelets verticaux*.

I – Introduction

The proteolysis of muscle proteins by endogenous enzymes is one of the most important reactions that take place during dry-cured ham processing and is largely responsible for its sensory quality. The process itself begins already early *post mortem* with the breakdown of large cytoskeletal proteins (by calpains) and proceedes through degradation of myofibrilar proteins (mainly by cathepsins) and generation of great amount of small peptides and free aminoacids (by aminopeptidases), which may last for several months during the ham processing (Toldra and Flores, 1998). The degree of proteolysis can be assessed either directly by monitoring a degradation of several large proteins (in particular myofibrilar proteins) or indirectly by protein degradation products (shorter peptides, free amino acids and other amines, overall non-protein nitrogen). To evaluate degree of proteolysis chemical analysis of free amino acids or non protein nitrogen has thoroughly been used (Buscailhon and Monin, 1994a). One-dimensional protein electrophoresis has been used to follow up degradation of main muscle proteins (Toldra *et al.*, 1993; Tabilo *et al.*, 1999; Larrea *et al.*, 2006); however the method does

not allow separation between different proteins of the same molecular weight. This is possible using a two-dimensional electrophoresis (2DE) which separates proteins according to their molecular weight and isoelectric point and which, coupled by mass spectrometry, enables identification of more than 1000 proteins in one gel (Gorg *et al*., 2000). This so called proteomic analysis represents a valuable tool for identification of molecular markers of food quality. Over the last years, several studies of proteomic research in meat science have been conducted (Hollung *et al.*, 2007). However the studies related to dry-cured ham are rare (Hortos *et al*., 2004; Di Luccia *et al.*, 2005; Sidhu *et al.*, 2005), moreover these studies are mainly preliminary and difficult to compare due to different methodology and approach. In our recent study (Škrlep *et al.*, 2010a) on dry-cured ham proteomic profile, we noticed several distinctive features on the dry-cured *Biceps femoris* gels, among which the vertical protein spot chains attracted the most of our attention. They could be an indication of progressive protein degradation, however, further characterisation would be needed to confirm that hypothesis, which was the aim of the present research.

II – Materials and methods

Material included in the present experiment originated from an extensive study on dry hams performed within EU project Truefood and experimental details are provided in our previous study (Škrlep *et al*., 2010b). The investigation included also the proteomic analysis of dry-cured *biceps femoris* (BF) muscle, for which a subsample of 24 hams was selected. Sample preparation, protein extraction and two dimensional electrophoresis (2DE) procedure is described in Škrlep *et al*. (2010a) and was performed according to the modified method developed at INRA (Theron *et al*., 2010). Shortly, insoluble protein fraction was extracted with repeated washing in low ionic strength buffer and loaded (1000 µg) on immobilised pH gradient strips for isoelectric focusing (70.000 Vh). For each sample three technical repetitions were made. SDS-PAGE was performed on 12.5% polyacrylamide gels. For the assessment of molecular weight (MW), protein MW marker #SM0431 (Fermentas Life Sciences, Glen Burnie, MD, USA) was applied prior to running second dimension. The gels were stained with Coomassie Brilliant Blue G250. The gel images were digitalized and spots automatically detected using ImageMaster 2D Platinum 6 software (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The comparison of the images from the present experiment to the images of fresh meat proteome from the available literature revealed some interesting differences, among which several vertical protein spot chains were the most distinctive (see Fig. 1). For the purpose of mass-spectrometry analysis, the spots of interest (spots from protein spot chains) were excised, destained, dehydrated, digested by trypsin and analysed (by peptide masss fingerprinting) using a Voyager DE Pro MALDITOF-MS (Applied Biosystems, Courtaboeuf, France) as previously described (Laville *et al.*, 2009). The obtained spectra were then compared to those from NCBInr databases susscrofa (20090623, 21575 seq) or mammalia (20090623, 1263710 seq) using Mascot Software (Matrix Sciences London, V2.2, home license),

III – Results and discussion

A representative 2DE gel image of insoluble muscle protein fraction of dry-cured BF muscle is shown on Fig. 1. In this article we focused on vertical chains of spots (also designated on Fig. 1). Such patterns could not be seen when compared to the corresponding regions of the gels reported for the fresh pig muscle (Morzel *et al*., 2004; Hwang *et al*., 2005; Laville *et al*., 2005). The chains of spots had almost the same isoelectrical point and different (app 0.5 – 1.0 kDa) estimated molecular weight (see Fig. 1). Some of the most distinctive spots from the chains were subsequently excised (n=16) and analysed by mass spectrometry. The results of the protein identification and peptide matching against the database records are shown in Fig. 2. In the first case (chain 1) all five spots (2932, 2357, 2404, 2423 and 2433) were identified as the

same protein – myosin light chain (MLC1f). Three spots from the second chain (spots 2604, 2626 and 2653) were identified as another myosin light chain (HUMMLC2B) and one spot (2676) as fast skeletal myosin alkali light chain 1. For the remaining two analysed spot chains (spots 2457A, 2457, 2475, 2519 and 2558 in chain 3; spots 2576, 2617 and 2627 in chain 4) were again identified as the same protein, namely α-B-crystallin (chain 3) and myoglobin (chain 4).

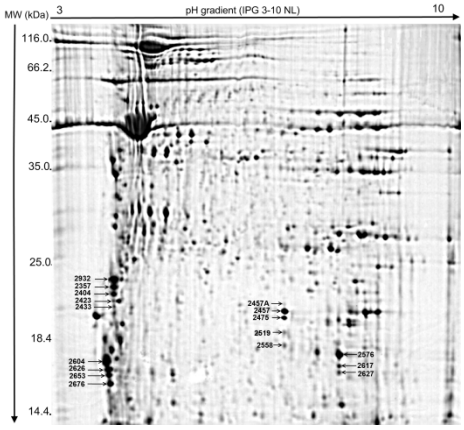

Fig. 1. Representative gel image of unsoluble protein fraction of dry-cured *Biceps femoris* muscle. Identified protein spots from vertical spot chains are denoted with arrows and reference numbers.

Since the pattern of vertical spot chains was repeatable between the technical repetitions (results not shown) showing clear separation of the spots with progressive decreasing of the estimated molecular weight, one explanation for that could be the gradual proteolytic degradation of the protein molecules. In agreement with our results several studies reported, that dry-cured ham proteins are prone to intensive hydrolysis during the processing period (Cordoba *et al*., 1994; 1993, Monin *et al*., 1997; Tabilo *et al*., 1999). More detailed studies, using one- and two- dimensional electrophoresis for monitoring the course of proteolysis (Di Luccia *et al*., 2005; Larrea *et al.*, 2006) reported notable degradation or even complete disappearance of several specific myifibrillar proteins (including myosin light chain) during the course of dry-cured ham processing, but direct comparison with the present study is not possible.

However, there are also several facts that speak against our hypothesis. The cleavage of protein spots is expected to cause the shift in isoelectric point, which is not the case for vertical spot chains. This could happen if the cleaved peptide (or aminoacids) were neutral or nonpolar. Furthermore, it could not be undoubtedly confirmed by the mass spectrometry results associated with protein database query that the spots were fragments (see Fig. 2), although the results of matching may indicate such conclusions (e.g. progressive decrease in sequence coverage and number of matched peptides), especially in the case of chains 1 (MLC) and 4 (myoglobin). It is also worth mentioning, that in the case of chain 1, the observed molecular MW of all five spots exceeds the MW of the theoretical match. The database query also did not give any myosin molecules, which would match our search closer in MW, making explanation even more difficult. However, Larrea *et al*. (2006) in a study supported by monodimensional SDS-PAGE, reported comparable myosin light chain of 24.75 kDa, which could match ours. It is worth noting, that we have not made any control gels on fresh ham muscle or muscle during different processing stages, which would be useful to clarify the origin of vertical spot chains.

Fig. 2. Peptide matching against the database records in case of all four identified protein spot chains (matched sequences designated in bald and underlined).

IV – Conclusions

Although we could not undoubtedly prove that protein spot chains indicate proteolytic degradation of several dry-cured ham proteins, there is a strong indication towards our hypothesis. However, confirmation of our results is needed, by comparing the proteomic profiles of dry-cured ham in different processing stages.

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References

Buscailhon S. and Monin G., 1994. Déterminisme des qualités sensorielles du jambon sec. Chaptre 1, Evolution de la composition et des qualités sensorielles du jambon au cours de la fabrication. In: *Viandes Prod. Carnes*, 15: 23-34.

Córdoba J.J., Antequera T. and García C. *et al*., 1994. Evolution of free amino acids and amines during ripening of Iberian dry-cured ham. In: *J. Agric. Food Chem*. 42: 2296-2301.

Di Luccia A., Picariello G. and Cacace G. *et al*., 2005. Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. In: *Meat Sci.* 69: 479-491**.**

Gorg A., Obermeier C. and Bought G. *et al*., 2000. The current state of two-dimensional electrophoresis with immobilized pH gradients. In: *Electrophoresis*, 21: 1037-1053**.**

Hollung K., Veiseth E. and Jia X. *et al*., 2007. Application of proteomics to understand the molecular mechanisms behind meat quality. In: *Meat Sci*. 77: 97-104.

Hortos M., Santé-Lhoutellier V.and Arnau J. *et al*., 2004. Proteomic characterisation of normal and pasty dry-cured hams. In: *Proceedings of 5th International Symposium on the Mediterranean Pig*, 16-19. November, Tarbes, France. p. 245-249.

Hwang I.H., Park B.Y.and Kim J.H. *et al*., 2005. Assessment of post-mortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. In: *Meat Sci*. 69: 79-91.

Larrea V., Hernando I.and Quiles A. *et al*., 2006. Changes in proteins during Teruel dry-cured ham processing. In: *Meat Sci*. 74: 586-593.

Laville E., Sayd T. and Santé-Lhoutellier V. *et al*., 2005. Characterisation of PSE zones in semimembranosus pig muscle. In: *Meat Sci*. 70: 167-172.

Laville E., Sayd T. and Morzel M. *et al*., 2009. Proteome changes during meat ageing in tough and tender beef suggests the importance of apoptosis and protein solubility for beef aging and tenderization. In: *J. Agric. Food Chem*. 57: 10755.10764.

Monin G., Marinova P.and Talmant A. *et al*., 1997. Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the dehairing technique. In: *Meat Sci*. 47: 29-47.

Morzel M., Chambon C.and Hamelin M. *et al*., 2004. Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. In: *Meat Sci*. 67: 689-696.

Sidhu S.M., Hollung K. and Berg P., 2005. Proteolysis in Norwegian dry-cured hams; preliminary results. In: *Proceedings of the 51st ICOMST*. 7-12. August, Baltimore, MD, USA. p. 1031-1036.

Škrlep M, Mandelc S.and Javornik B. *et al*., 2010a. Green ham pH value affects proteomic profile of dry-cured ham. In: *It. J. Anim. Sci*. 9: 153-156.

Škrlep M., Čandek-Potokar M. and Kavar T. *et al*., 2010b. Association of PRKAG3 and CAST genetic polymorphism with traits of interest in dry-cured ham production: comparative study in France, Slovenia and Spain. In: *Livest. Sci*. 128: 60-66.

Tabilo G., Flores M. and Aristoy M.-C. *et al*., 1999. Post-mortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham. In: *Meat Sci.* 51: 255-260.

Theron L., Sayd T. and Pinguet J. *et al*., 2010. Proteomic analysis of semimembranosus and biceps femoris muscles from Bayonne dry-cured ham. *Meat Sci*. article submitted.

Toldra F., Rico E. and Flores J., 1993. Cathepsins B, D, H and L activities in the processing of dry-cured ham. In: *J. Sci. Food Agric*. 62: 157-161.

Toldra F. and Flores M., 1998. The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. In: *Crit. Rev. Food Sci. Nutr*. 38: 331-352.