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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-àdire 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 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 seg) 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  $\alpha$ -B-crystallin (chain 3) and myoglobin (chain 4).

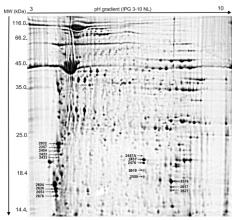


Fig. 1. Representative gel image of unsoluble protein fraction of drycured *Biceps femoris* muscle. Identified protein spots from vertical spot chains are denoted with arrows and reference numbers.

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 mylifibrillar 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.

CHA	AIN 1.						
	2932 (gi 117660	874, MLC1f)					
	MAPKKDVKKP		APAPAPAPAP	PKEEKIDLSA	IKIEFSKEQQ	DEFKEAFLLF	DRTGECKITL
	SQVGDVLRAL						VEGLRVFDKE
	GNGTVMGAEL		MKEEEVEALM	AGQEDSNGCI	NYEAFVKHIM	SI	
	2357 (gi 117660						
	MAPKKDVKKP						
	SQVGDVLRAL GNGTVMGAEL						VEGLEVFDEE
	2404 (gi 117660		PIREEEVEALPI	AGQEDSNGCI	NIEAPVRAIM	51	
	MAPKKDVKKP		АРАРАРАРАР	PKEEKIDLSA	IKIEFSKEOO	DEFKEAFLLF	DRTGECKITL
	SQVGDVLRAL						
141	GNGTVMGAEL	RHVLATLGEK	MKEEEVEALM	AGQEDSNGCI	NYEAFVKHIM	SI	
Spot	2423 (gi 117660	874, MLC1f)					
1	MAPKKDVKKP	АААААРАРАР	APAPAPAPAP	PKEEKIDLSA	IKIEFSKEQQ	DEFKEAFLLF	DRTGECKITL
	SQVGDVLRAL						VEGLRVFDKE
	GNGTVMGAEL		MKEEEVEALM	AGQEDSNGCI	NYEAFVKHIM	SI	
	2433 (gi 117660			DUDDUTDIO	TETRACEROO	DDDVDADI I D	DDBGDGWTB
71	MAPKKDVKKP	CTNDTNA FUK	APAPAPAPAPAP	PREEKIDLSA	FIDMIONTEN	DEFREAFLLF	VEGLEVEDE
141	SQVGDVLRAL GNGTVMGAEL	RHVLATLORK	MKEEEVEALM	ACOEDSNOCT	NVEAEVKHIM	ST	VEGLEVEDEE
141	GNGIVHGREL	RHVHRIDGER	PIRESEVERUPI	AGGEDSNGCI	NIERFVRIIM	51	
1.000	AIN 2.						
	t 2604 (gi 117666			KRAFTUTDON	RDGTTDEEDT	RDTEAMORT	NUKNEELDAM
71	MAPKNAKRRA MKEASGPINF	TVFLTMFGEK	LKGADPEDVI	TGAFKVLDPE	GKGTIKKHFL	EELLTTOCDR	FSOEEIKNMW
141	AAFPPDVGGN	VDYKNICYVI	THGDAKDOE				
Spot	t 2626 (gi 117666	0856, HUMML	C2B)				
1	MAPKNAKRRA	AAEGSSNVFS	MFDQTQIQEF				
71	MKEASGPINF	TVFLTMFGEK	LKGADPEDVI	TGAFKVLDPE	GKGTIKKHFL	EELLTTQCDR	FSQEEIKNMW
	AAFPPDVGGN						
	t 2653 (gi 117666			WELL PROVIDENT	PROTTOWER	PP	MININE DAM
1	MAPKNAKRRA	AAEGSSNVFS	MFDQTQTQEF	KEAFTVIDQN	RDGIIDKEDL	RDTFAAMGRL	NVKNEELDAM
141	MKEASGPINF	VDVKNTCVVT	THODAKDOF	IGAFRVLDPE	GRGIIRRHFL	BELLITYCDK	FSQEEIKNMW
	t 2676 (gi 117666			light chain 1)			
1	MSFSADQIAE	FKEAFLLFDR	TGECKITLSQ	VGDVLRALGT	NPTNAEVKKV	LGNPSNEEMN	AKKIEFEQFL
71	PMLQAISNNK	DQGSYEDFVE	GLRVFDKEGN	GTVMGAELRH	VLATLGEKMK	EEEVEALMAG	QEDSNGCINY
141	EAFVKHIMSI			1			
CHA	AIN 3.						
Spot	t 2457A (gi 75063	3982, alpha-B-cr	vstallin)				
1	MDIAIHHPWI EKDR <mark>FSVNLD</mark>	RRPFFPFHSP	SRLFDQFFGE	HLLESDLFPA	STSLSPFYFR	PPSFLRAPSW	IDTGLSEMRL
71	EKDRESVNLD	VKHFSPEELK	VKVLGDVIEV	HGKHEERQDE	HGFISREFHR	KYRIPADVDP	LTITSSLSSD
141	GVLTVNGPRR	QASGPERTIP	ITREEKPAVT	AAPKK			
Spot	t 2457 (gi 750639	82, alpha-B-crys	tallin)				
1	MDIAIHHPWI EKDRFSVNLD GVLTVNGPR <mark>R</mark>	RRPFFPFHSP	SRLFDQFFGE	HLLESDLFPA	STSLSPFYFR	PPSFLRAPSW	IDTGLSEMRL
141	GULTUNGDER	OACOPERTIR	TTPEEKDAUT	AADKK	HGF ISREFAK	KIKIPADVDP	LIIISSLSSD
Spot	t 2475 (gi]750639	82. alpha-B-crvs	tallin)				
1	MDIAIHHPWI	RRPFFPFHSP	SRLFDQFFGE	HLLESDLFPA	STSLSPFYFR	PPSFLRAPSW	IDTGLSEMRL
71	MDIAIHHPWI EKDRFSVNLD	VKHFSPEELK	VKVLGDVIEV	HGKHEERQDE	HGFISREFHR	KYRIPADVDP	LTITSSLSSD
141	GVLTVNGPRR	QASGPERTIP	ITREEKPAVT	AAPKK			
Spot	t 2519 (gi 750639	87 alpha-B-crvs	tallin)				
1	MINTATHUDWT	oz, aipita D erjs					
P2	EVODDOUBUT	RRPFFPFHSP	SRLFDQFFGE	HLLESDLFPA	STSLSPFYFR	PPSFLRAPSW	IDTGLSEMRL
71	EKDRFSVNLD	RRPFFPFHSP VKHFSPEELK	SRLFDQFFGE VKVLGDVIEV	HLLESDLFPA HGKHEERQDE	STSLSPFYFR HGFISREFHR	PPSFLRAPSW KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
		RRPFFPFHSP VKHFSPEELK QASGPERTIP	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT	HLLESDLFPA <mark>HGK</mark> HEERQDE AAPKK	STSLSPFYFR HGFISREFHR	PPSFLRAPSW KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
Spot	t 2558 (gi 750639	RRPFFPFHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin)	HLLESDLEPA	STSLSPEVER	PPSFLRAPSW	TOTGL SEMEL
Spot 1 71	t 2558 (gi 750639 MDIAIHHPWI <u>EK</u> DR <mark>FSVNLD</mark>	RRPFFPFHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys RRPFFPFHSP VKHFSPEELK	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV	HLLESDLFPA HGKHEERQDE	STSLSPEVER	PPSFLRAPSW	TOTGL SEMEL
Spot 1 71	t 2558 (gi 750639	RRPFFPFHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys RRPFFPFHSP VKHFSPEELK	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV	HLLESDLFPA HGKHEERQDE	STSLSPEVER	PPSFLRAPSW	TOTGL SEMEL
Spot 1 71 141	t 2558 (gi 750639 MDIAIHHPWI <u>EK</u> DR <mark>FSVNLD</mark>	RRPFFPFHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys RRPFFPFHSP VKHFSPEELK	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV	HLLESDLFPA HGKHEERQDE	STSLSPEVER	PPSFLRAPSW	TOTGL SEMEL
Spot 1 141 CHA Spot	t 2558 (gi 750639 MDIATHHPWI EKDRFSVNLD GVLTVNGPR AIN 4. 2576 (gi 475235-	REPFPPHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys REPFPPHSP VKHFSPEELK QASGPERTIP 46, myoglobin)	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT	HLLESDLFPA <mark>HGK</mark> HEER <mark>QDE</mark> AAPKK	STSLSPFYFR <mark>HGFISR</mark> EFHR	PPSFLR <mark>APSW</mark> KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
Spot 1 141 CHA Spot	t 2558 (gi 750639 MDIATHHPWI EKDRFSVNLD GVLTVNGPR AIN 4. 2576 (gi 475235-	REPFPPHSP VKHFSPEELK QASGPERTIP 82, alpha-B-crys REPFPPHSP VKHFSPEELK QASGPERTIP 46, myoglobin)	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT	HLLESDLFPA <mark>HGK</mark> HEER <mark>QDE</mark> AAPKK	STSLSPFYFR <mark>HGFISR</mark> EFHR	PPSFLR <mark>APSW</mark> KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
Spot 1 141 CHA Spot 1 71	t 2558 (gi 750639 MDIAIHHPWI <u>EKDRFSVNLD</u> <u>GVLTVNGPR</u> AIN 4. 2576 (gi 475235 MGLSDGEWQL TALGGILKKK	REPFPFHSP VKHFSPEELK QASGPERTIP 82. alpha-B-crys REPFPFHSP VKHFSPEELK QASGPERTIP 46. myoglobin) VLNVWGKVEA GHHEAELTPL	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT	HLLESDLFPA <mark>HGK</mark> HEER <mark>QDE</mark> AAPKK	STSLSPFYFR <mark>HGFISR</mark> EFHR	PPSFLR <mark>APSW</mark> KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
Spot 141 141 CHA Spot 1 71 141	t 2558 (gi 750639 MDIATHHPWI EKDRFSVNLD GVLTVNGPRR AIN 4. 2576 (gi 475235- MGLSDGEWQL TALGGILKKK NDMAAKYKEL	REPFFFFFFF VKHFSPEELK QASGPERTIP 82. alpha-B-crys REPFFFFFS QASGPERTIP 46. myoglobin) VLNVWGKVEA GHHEAELTPL GFQG	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT	HLLESDLFPA <mark>HGK</mark> HEER <mark>QDE</mark> AAPKK	STSLSPFYFR <mark>HGFISR</mark> EFHR	PPSFLR <mark>APSW</mark> KYR <mark>IPADVDP</mark>	IDTGLSEMRL LTITSSLSSD
Spot 1 141 <b>CHA</b> Spot 1 71 141 Spot	t 2558 (gil750639) MDIAIHHPWI EKDRPSVNLD OVLTVNGPR AIN 4. 2576 (gil475235- MGLSDGEWQL TALGGILKKK NDMAAKYKEL 2617 (gil366024)	RRPFPPHSP VKHFSPEELK QASGPERTIP 82. alpha-B-crys VKHFSPEELK QASGPERTIP VLNVWGKVEA GHHEAELTPL GPQG 6. myoglobin)	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVL AQSHATKHKI	HLLESDLFPA HGKHEERQDE AAPKK IRLFKGHPET PVKYLEFISE	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDFGADAQG	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR
Spot 1 141 CHA Spot 1 141 Spot 1 41	t 2558 (gil750639) MDIAIHHPWI EKDRPSVNLD GVLTVNGPRR AIN 4. 2576 (gil475235- MGLSDGEWQL TALGGILKKK NDMAAKYKEL 2617 (gi]366024: GLSDGEWQLV	RRPFPFPHS VKHPSPEELK QASGPERTIP 82. alpha-B-crys RRPFPPFNSP ASGPERTIP 46. myoglobin VLNVWGKVEA GHHEAELTPL GPQG 6. myoglobin) LNVWGKVEAD	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVL AQSHATKHKI	HLLESDLFPA HGKHEERQDE AAPKK IRLFKGHPET RLFKGHPETL	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH EKFDKFKHLK	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDFGADAQG SEDEMKASED	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR LKKHGNTVLT
Spot 1 71 141 CHA Spot 1 71 141 Spot 1 61	t 2558 (gil750639) MDIAIHHPWI EKDRFSVNLD GVLTVNGPR AIN 4. 2576 (gil475235. MGLSDGEWQL TALGGILKKK NDMAAKYKEL 2617 (gil366024. GLSDGEWQLV ALGGILKKKG	REPFPFHSP VKHPSPELK QASGPERTIP 82. alpha-B-crys VKHPSPEELK QASGPERTIP VKHPSPEELK QASGPERTIP VLNVWGKVEA GHEAELTPL GHEAELTPL LNVWGKVEA LNVWGKVEA	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVL AQSHATKHKI	HLLESDLFPA HGKHEERQDE AAPKK IRLFKGHPET RLFKGHPETL	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH EKFDKFKHLK	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDFGADAQG SEDEMKASED	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR LKKHGNTVLT
Spot 1 71 141 CHA Spot 1 71 141 Spot 1 61 141	t 2558 (gil750639) MDIATHHPWI EKDRPSVNLD GVLTVNGPRR AIN 4. 2576 (gil475235- MGLSDGEWQL TALGGILKKK NDMAAKYKEL GLSDGEWQLV ALGGILKKKG DMAAKYKELG	REPFPFHSP VKHPSPEELK QASGPERTIP 82. alpha-B-crys VKHPSPEELK QASGPERTIP VKHPSPEELK QASGPERTIP GASGPERTIP GHUEAELTPL GHUEAELTPLA FQG	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVL AQSHATKHKI	HLLESDLFPA HGKHEERQDE AAPKK IRLFKGHPET PVKYLEFISE RLFKGHPETL	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH EKFDKFKHLK	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDFGADAQG SEDEMKASED	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR LKKHGNTVLT
Spot 1 71 141 CHA Spot 1 141 Spot 1 61 141 Spot	t 2558 (gil750639) MDIAIHHPWI EKDRPSVNLD GVLTVNGPR AIN 4. 2576 (gil475235- MGLSDGEWQL TALGGILKKK MDMAAKYKEL GLSDGEWQLV ALGGILKKKG DMAAKYKELG 2627 (gil475235-	REPFPFHSP VKHPSPEELK QASGPERTIP 82, alpha-B-crys QASGPERTIP 82, alpha-B-crys VKHPSPEELK QASGPERTIP 46, myoglobin) VLNVWGKVEA GHUEAELTPLA FQG 46, myoglobin)	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVL AQSHATKHKI QSHATKHKIP	HLLESDLFPA HGKHEERQDE AAPKK IRLFKGHPET PVK <u>YLEFISE</u> RLFKGHPETL VKYLEFISEA	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH EKFDKFKHLK IIQVLQSKHP	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDFGADAQG SEDEMKASED GDFGADAQGA	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR LKKHGNTVLT MSKALELFRN
Spot 1 71 141 <b>CHA</b> Spot 1 141 Spot 1 141 Spot 1 141 Spot 1 141 141 141 141 141 141 141	t 2558 (gil750639) MDIATHHPWI EKDRFSVNLD GVLTVNGPR AIN 4. 2576 (gil475235. MGLSDGEWQL TALGGILKKK MDMAAKYKEL 2617 (gil366024. GLSDGEWQL DMAAKYKEL 2627 (gil475235. MGLSDGEWQL	REPFPFHSP VKHFSPEELK VKHFSPEELK QASGPERTIP 82. alpha-B-crys VKHFSPEELK QASGPERTIP 46. myoglobin) VLNVWGKVEA GHEAELTPLA GFQG 6. myoglobin) LNWGKVEA HHEAELTPLA FQG 46. myoglobin) VLNWGKVEA	SRLFDQFFGE VKVLGDVIEV ITREEKPAVT tallin) SRLFDQFFGE VKVLGDVIEV ITREEKPAVT DVAGHGQEVLI QSHATKHKIP DVAGHGQEVLI DVAGHGQEVLI	HLLESDLFPA HGKHEERODE AAPKK IRLFKGHPET PVKYLEPISE IRLFKGHPETL IRLFKGHPETL	STSLSPFYFR HGFISREFHR LEKFDKFKHL AIIQVLQSKH IIQVLQSKHP LEKFDKFKHL	PPSFLRAPSW KYRIPADVDP KSEDEMKASE PGDPGADAQG SEDEMKASED GDFGADAQGA KSEDEMKASE	IDTGLSEMRL LTITSSLSSD DLKKHGNTVL AMSKALELFR LKKHGNTVLT MSKALELFRN DLKK <u>HGNTVL</u>
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# 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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