

ACTIN FRAGMENTS GENERATED DURING DRY-CURED HAM PROCESSING

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Introduction

Dry-cured ham is a traditional food requiring a long processing period for development of its appreciated texture and flavour characteristics. During this time, there is an intense degradation of muscle proteins due to the action of endogenous proteolytic enzymes. This gives rise to an important generation of free amino acids and oligopeptides that contribute directly or indirectly to flavour characteristics of the final product. The nature and properties of free amino acids has been well established but, on the contrary, little is known about the identity of the peptide fraction at the end of curing. The main objective of the present work was to gather more information on this peptide fraction, and to relate the results to flavour development in dry-cured meat products.

Materials and Methods

Twenty-five grams of muscle *semimembranosus* were taken from a Spanish Serrano dry-cured ham in order to obtain a deproteinised ham extract. Five ml of this extract were fractionated on a Sephadex G-25 size exclusion column, pooling fractions eluting in the range 215-230 ml. These fractions were concentrated to a final volume of 2 ml that was subsequently injected on a SymmetryPrep C18 (7.8 x 300 mm) reverse phase column. Peptide separation was made by a linear gradient from 0 to 50 acetonitrile in 55 min at a flow rate of 3 ml/min. Separation was monitored at $\lambda=214$ nm and prominent peaks were collected manually, dried and stored at -20 °C. The selected fraction was redissolved in 6 μ l of 50 % acetonitrile containing 1% TFA, then spotting 1 μ l on a MALDI plate and mixed with an equal volume of a α -cyano-4-hydroxycinnamic acid solution. Molecular mass determinations were performed in an Applied Biosystems MALDI-TOF mass spectrometer, operated in delayed extraction and positive reflector mode.

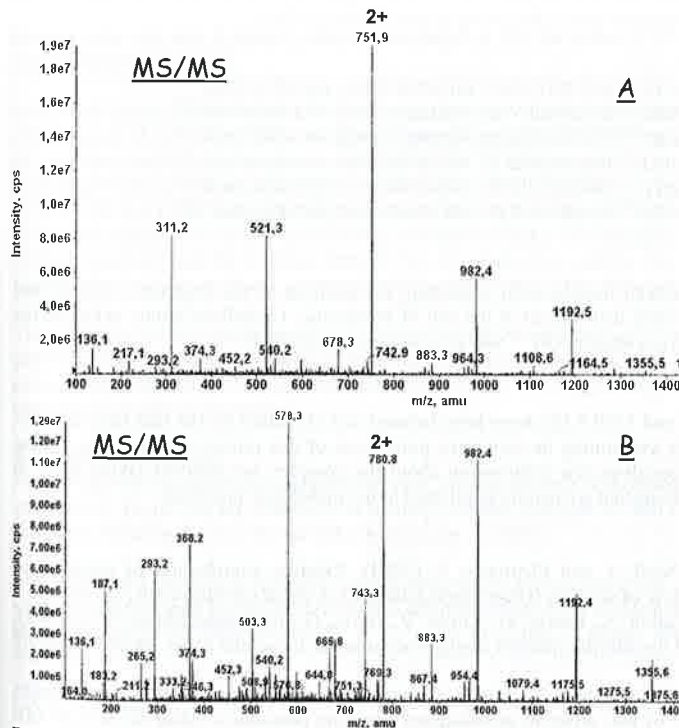


Figure 1: MS/MS spectrum of singly-charged isolated ions 1501.5 (A) and 1559.5 (B). Peptide sequence matching to each one of these fragmentation patterns is shown in capital letters.

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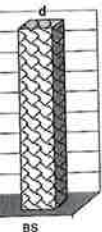
1.80^a

0.69

5.10^c

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acts evaluated



Determination of peptide sequence. Relevant peptide ions obtained from MALDI-TOF were subjected to ESI mass spectrometry analysis using a Qtrap mass spectrometer (Applied Biosystems). Doubly-charged ions were fragmented using the Enhanced Product Ion with Q₀ trapping option. Enhanced resolution was performed at 250 amu/s across the entire mass range.

Results and Discussion

Dry-cured ham extract was fractionated on Sephadex G-25, taking fractions corresponding to 215-230 ml elution volume. This corresponded to a molecular mass of around 1530 Da according to the curve made with standards of known molecular weight. Pooled fractions were injected on a Symmetry C18 reverse phase column and a peak eluting at a concentration of 14 % acetonitrile was collected and selected for molecular mass determination. MALDI-TOF MS of this fraction yielded two singly-charged ions having a molecular mass of 1501.5 and 1559.5 Da, respectively, which was in accordance to the estimated peptide size obtained with Sephadex G-25. MS/MS spectra of these ions are shown in Figure 1. Doubly-charged forms of these peptides are clearly appreciated at m/z 751.9 (Figure 1A) and 780.8 (Figure 1B), respectively. Interpretation of mass spectra was performed by using MASCOT software (www.matrixscience.com). The fragmentation pattern shown in Figure 1A matched the sequence DSGDGVTHNVPIYE (peptide A), whereas data of Figure 2B gave an unequivocal result the sequence DSGDGVTHNVPIYEG (peptide B). The only difference between the two peptides was a C-terminal Gly present in peptide B but not in peptide A, being the responsible for the difference in their molecular masses. Sequence homology analysis using BLAST search engine (www.bork.embl-heidelberg.de) revealed a 100 % identity of these peptides with an internal region of muscle actin, as illustrated in Figure 2. The present results give clear evidence of the intense actin proteolysis occurring during dry-cured ham processing. Previous works reported the progressive actin degradation during postmortem muscle proteolysis either during meat ageing (Morzel *et al.* 2004) but specially during dry-curing where most myofibrillar proteins are extensively degraded (di Luccia *et al.* 2005). Other authors reported a notable increase of the peptide fraction during dry-cured ham processing, indicative of an intense proteolysis that is closely related to flavour development (Toldrá, 2002). However, this is the first time that small peptides coming from actin are isolated and fully identified from dry-cured ham. The fact that two fragments with and without Gly¹⁷⁰ in C-terminal position have been identified may be indicative of any carboxypeptidase action during the curing period. According to previous observations, degradation of actin and other myofibrillar proteins during postmortem muscle proteolysis could be due to the action of lysosomal cathepsins (Delbarre-Ladrat *et al.*, 2004), a hypothesis that would be supported by their relatively good stability observed during great part of the curing period (Toldrá 2002).

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MCDEDETAL VCDNGSGLVK AGFAGDDAPR30 AVFPISVGRP RHQGVVMVGMG QKDSYVGDEA60
QSKRGILTLK YPIEHGIITN WDDMEKIWHH90 TFYNELRVAP EEHPDLLTEA PLNPKANREK120
MTQIMPETFN VPAMYVAIQV VLSLYASGR150 TGIVLDSGDG VTHNVPIYEG YALPHAIMRL180
DLAGRDLTDY LMKILTERGY SFVTTAEREI210 VRDIKEKLCY VALDFENEMA TAASSSLEK240
SYELPDGQVI TIGNERFRCP ETLFQPSFIG270 MESAGIHETT YNSIMKCDID IRKDLYANNV300
MSGGTTMYPG IADRMQKEIT ALAPSTMKIK330 TIAPPERKYS VWIGGSILAS LSTFQQMWIT360
KQEYDEAGPS IVHRKCF377

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Figure 2: Primary structure of porcine skeletal muscle actin, indicating the position of the fragments isolated and identified in the present work from a dry-cured ham extract at the end of processing. Underlined amino acids are the common sequence of the two peptides, whereas squared Gly¹⁷⁰ was present only in peptide B.

Conclusion

Two actin fragments of small size (1501.5 and 1559.5 Da) have been isolated and identified for the first time in a dry-cured ham extract at the end of processing, confirming the extensive proteolysis of this protein during curing. These findings contribute to meat research by revealing new information about the complex mechanisms taking place in postmortem muscle and also the enzyme groups that are mainly implicated in the proteolytic processes.

References

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