# OXIDATION OF PEPTIDES DERIVED FROM CREATINE KINASE PROTEIN IN DRY-CURED HAM

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**Abstract – This study evidences the presence of oxidised peptides arising from the degradation of the creatine kinase sarcoplasmic protein during the processing of 14 months dry-cured ham. A total of 112 peptides have been identified by nanoliquid chromatography coupled to tandem mass spectrometry (nLC-MS/MS), and among them, 17 peptides showed methionine oxidation in their sequence, corresponding to 15% of peptide oxidation. So, this peptidomic approach provides useful information for a better knowledge of oxidation processes in this sarcoplasmic protein during dry-cured ham processing.**

**Key Words – Methionine oxidation, proteomics, mass spectrometry.**

## I. INTRODUCTION

Dry-cured ham is a traditional high-quality product with unique organoleptic characteristics developed throughout its long processing. Proteolysis is the most important reaction, that generates large amounts of peptides and free amino acids from the enzymatic degradation of sarcoplasmic and myofibrillar proteins [1, 2].

However, other chemical reactions like oxidative changes of proteins and peptides take place along the dry-curing ham process. Oxidation processes occur mainly along the salting stage and lead to certain loss of quality in the final product [3, 4]. So, protein oxidation comprises flavour, colour and texture deterioration as well as changes in conformation and functionality of proteins and loss of essential amino acids [3, 5]. Nevertheless, peptide oxidation has been poorly described.

Methionine oxidation is the main modification that occurs during the dry-cured processing, because of the high susceptibility of this amino acid to react with oxygen species. As a result, methionine sulfoxide and methionine sulfone are generated, although this process can be reversible by reductase enzymes [5, 6].

In this study, a peptidomic approach has been used to identify those peptides showing methionine oxidation derived from the degradation of creatine kinase protein along the dry-cured ham processing.

## II. MATERIALS AND METHODS

The study was carried out in Protected Designation of Origin of Teruel dry-cured hams (PDO Teruel hams) with a total time of processing of fourteen months. Peptides were extracted by homogenising in an Ultra-Turrax® T-25 (IKA®-Werke, Germany) with 0.01 N HCl, and after decanting at 4 ºC overnight, the supernatant was filtered. Then, the sample was desalted by solid phase extraction using an Oasis® HLB cartridge (35 cc, Waters, Ireland) and concentrated by using Zip-Tip C18 with standard bed format (Millipore Corporation, Bedford, MA). The identification of peptides was done by nanoliquid chromatography and tandem mass spectrometry (TripleTOF® 5600+ system, AB Sciex Instruments, MA, USA) with a nanoelectrospray ionisation source, according to the methodology described by Gallego *et al.* [7]. Finally, Mascot Distiller v2.5.1. software (Matrix Science, Inc., Boston, MA, USA) and Uniprot database were used to analyse the data [7].

## III. RESULTS AND DISCUSSION

Dry-cured ham extracts have been analysed by using nLC-MS/MS to identify those peptides showing methionine oxidation. In this study, only peptides derived from creatine kinase were selected, detecting a total of 112 peptides that include 17 of them with oxidations of methionine in their sequence.

Table 1 shows the sequences of oxidised peptides together with the observed masses and the charge states. All of the sequenced peptides contain only one oxidised methionine, excepting the peptide 1

(MEKGGNMKEVFRR), which shows two oxidations. The oxidised methionines are indicated in bold on the sequences, and those peptides detected both with and without methionine oxidation are marked with an asterisk.

Table 1. Oxidised peptides from creatine kinase protein identified by nLC-MS/MS.

| Peptide number | Obs (m/z)[a] | Charge (+) | Sequence |
|---|---|---|---|
| 1 | 404.18 | 2 | **M**EKGGN**M**KEVFRR |
| 2 | 523.76 | 2 | IDD**M**IPAQK |
| 3 | 560.28 | 3 | KGQSIDD**M**IP |
| 4 | 567.28 | 3 | SIDD**M**IPAQK* |
| 5 | 595.79 | 3 | KGQSIDD**M**IPA |
| 6 | 621.29 | 3 | YPLKS**M**TEQE* |
| 7 | 650.37 | 2 | KPVSPLLLASG**M**A* |
| 8 | 650.80 | 2 | GVDNPGHPFI**M**T |
| 9 | 659.77 | 2 | GQSIDD**M**IPAQK |
| 10 | 482.91 | 3 | KGQSIDD**M**IPAQK* |
| 11 | 525.93 | 3 | EKGQSIDD**M**IPAQK* |
| 12 | 563.62 | 3 | LEKGQSIDD**M**IPAQK* |
| 13 | 563.64 | 3 | KKLEKGQSIDD**M**IPA |
| 14 | 567.58 | 3 | EEYPDLSKHNNH**M**A |
| 15 | 544.75 | 4 | FKAEEEYPDLSKHNNH**M**A* |
| 16 | 576.77 | 4 | FKAEEEYPDLSKHNNH**M**AK* |
| 17 | 819.43 | 4 | VVDGVKLMVEMEKKLEKGQSIDD**M**IPAQK* |

[a]Molecular ion mass observed in the nESI-LC-MS/MS analysis calculated in Daltons. *Sequences identified with and without methione oxidation. Oxidised methionines are in bold.

The oxidation of methionines depends on their location in the protein and accessibility to oxidising agents, thus not all the residues have the same level of susceptibility to oxidative changes. In this study, a total of 17 oxidised peptides were identified, which correspond to 15% of the total. As an example of oxidised peptide, Figure 1 shows the MS/MS spectrum of KGQSIDDMIPAGQ (peptide 10).

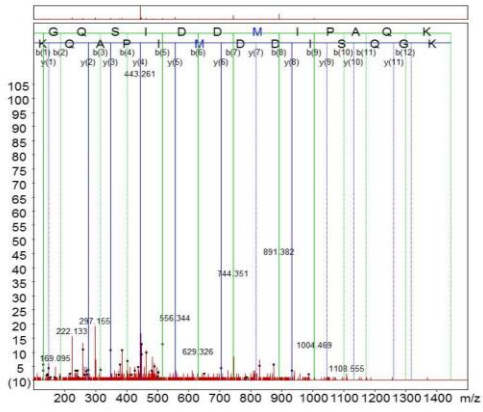


Figure 1. MS/MS spectrum of peptide KGQSIDDMIPAGQ from creatine kinase protein, showing the oxidised methionine in blue.

Moreover, amino acids near to methionine residues also determine their susceptibility to oxidation, so that methionines surrounded by Asp, Glu, Ala, Ser, and Thr are more sensitive [8]. In the present study, all of the identified peptides with oxidation show these amino acid residues, mainly Ala and Glu, next to the oxidised methionines.

In a previous study about creatine kinase done by Mora *et al.* (2009) [9], 58 peptides including 10 of them with methionine oxidation were identified in Spanish dry-cured ham of 9 months of processing. LEKGQSIDDMIPAQK peptide (number 12 of this study) was also previously identified. However, no more coincidences in the identified sequences have been detected although all peptides described in that study have resulted to be fragments of peptides detected in the present work, mainly from the degradation of peptide 17. This fact could be due to the action of muscle enzymes like exopeptidases [1] as the loss of consecutive residues was observed. The important differences detected between both types of dry-cured ham regarding the identified sequences could be due to differences in length and type of processing, that was very specific in the case of PDO Teruel hams. In fact, only 58 peptides were identified in Spanish dry-cured ham (9 months) [9] whereas a total of 112 are shown in this study of PDO Teruel ham (14 months), probably because a higher time of processing results also in a higher proteolysis. However, the ratio of oxidation calculated dividing the number of oxidised peptides and total number of peptides was very similar in both studies, reaching values of 0.17 and 0.15 for Spanish and Teruel dry-cured hams, respectively.

## IV. CONCLUSION

Dry-cured ham peptides showing methionine oxidation have been identified from the degradation of creatine kinase sarcoplasmic protein. This peptidomic analysis becomes very useful to obtain information about characteristics of peptides more likely to be oxidised as well as it allows a better knowledge of the oxidation processes occurred during the dry-cured ham processing and their effects on the quality characteristics of the final product.

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