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**Differences in peptide oxidation between muscles in 12 months Spanish
dry-cured ham**

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Abstract

Oxidative modifications of proteins and peptides can negatively affect nutritional, sensory and quality characteristics of dry-cured hams. The aim of this study was to use a peptidomics strategy for the identification and relative quantification of oxidised peptides in two different muscles, the external *Semimembranosus* (SM) and the internal *Biceps femoris* (BF), from dry-cured hams of 12 months of curing. The analysis by nanoliquid chromatography-tandem mass spectrometry identified peptides showing oxidation at different amino acid residues such as methionine, cysteine, histidine, tryptophan, and phenylalanine. The highest percentages of oxidised peptides identified in SM and BF muscles were from pyruvate kinase protein (16.7 and 28.6 %, respectively). The relative quantitation of oxidised peptides using mass spectrometry in tandem label-free methodology evidenced significant differences between muscles, with myosin and troponin fragments as main responsible peptides for the clustering of data. The existing differences between BF and SM peptides were confirmed using MALDI imaging mass spectrometry technique. The obtained results suggest that differences in physicochemical characteristics such as water and salt contents between SM and BF muscles could affect the quantity of peptide oxidation. This peptidomics approach has allowed to analyse differences in peptide oxidation between dry-cured ham muscles as well as to improve the knowledge about the oxidative processes that occur in dry-cured hams.

Keywords: Oxidised peptides, mass spectrometry, label-free quantification, peptidomics, MALDI imaging.

1. Introduction

The transformation of pork meat into dry-cured ham is mainly due to the absorption and diffusion of salt and the progressive dehydration of the meat, leading to different muscle modifications regarding physicochemical characteristics such as pH, NaCl concentration, moisture content and water activity. Moreover, several biochemical reactions such as proteolysis and lipolysis take place during the dry-cured ham processing, which involve changes in colour, flavour, taste, and texture giving rise to the typical characteristics of the final product (Toldrá, 2004; Petrova, Aansen, Rustad, & Eikevik, 2015).

The characteristics of dry-cured hams can be affected by oxidative modifications of proteins and peptides that commonly occur during their processing and storage (Estévez, 2011; Koutina, Jongberg, & Skibsted, 2012; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). Protein oxidation results in different chemical modifications including the formation of carbonyl derivatives, cleavage of protein backbones, protein crosslinking and modification of amino acid side chains. These oxidative modifications can give rise to structural changes in proteins affecting their physical, chemical, functional and nutritional properties (Lund, Heinonen, Baron, & Estévez, 2011; Zhang, Xiao, & Ahn, 2013).

The analysis of protein oxidation can be performed by different methodologies depending on the oxidative product to be measured. The formation of protein carbonyls is one of the most notable modification resulting from protein oxidation, so that the analytical procedures applied are usually based on the quantification of these compounds by using the 2,4-dinitrophenylhydrazine (DNPH) derivatisation method, fluorescence spectroscopy techniques or mass spectrometry (MS)-based methodologies (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009; Zhang et al., 2013). The

combination of liquid chromatography (LC) with tandem MS is a valuable tool to determine the specific oxidised amino acid and the nature of oxidative post-translational modifications. This methodology allows the sensitive and accurate identification and quantification of oxidised peptides as they have predictable mass shifts due to chemical modifications of amino acid side chains and thus can be identified in the fragmentation pattern (Schey & Finley, 2000). Nevertheless, the analysis of oxidative modifications continues to be challenging due to the complexity of the oxidative mechanisms that generate a wide variety of oxidation products as well as the low abundance and instability of some of them under the conditions of sample preparation and/or analysis (Silva, Vitorino, Domingues, Spickett, & Domingues, 2013; Verrastro, Pasha, Jensen, Pitt, & Spickett, 2015).

Few studies have been focused on studying the generation of oxidised peptides during dry-cured ham processing to date (Gallego, Mora, Fraser, Aristoy, & Toldrá, 2014; Gallego, Mora, Aristoy, & Toldrá, 2015a; Gallego, Mora, & Toldrá, 2018), and to our knowledge there are no studies evaluating the oxidation of peptides in different muscles. The aim of this study was to assess potential differences in peptide oxidation between *Semimembranosus* and *Biceps femoris* muscles of Spanish dry-cured ham of 12 months of processing. To this end, a peptidomic approach has been used for the identification and relative quantification of oxidised peptides in order to evaluate whether differences between muscles affect peptide oxidation.

2. Materials and methods

2.1 Dry-cured ham processing

Spanish dry-cured hams of 12 months of processing from 6-months-old pigs (Landrace × Large White) were prepared according to the traditional procedure described by

[Escudero et al. \(2013\)](#). It comprises a pre-salting period using a mixture of salt (NaCl), nitrate and nitrite for 30 min; a salting stage where hams were buried in salt and piled up at 2-4 °C and 90-95% relative humidity (RH) for 12 days; a post-salting stage for 60 days at 4-5 °C and 75-85% RH; and finally a ripening-drying period at temperatures increasing to 13-20 °C and RH decreasing to 70%. Two different muscles, the external *Semimembranosus* (SM) and the internal *Biceps femoris* (BF) muscles were selected for the analysis (n=3).

2.2 Determination of moisture and salt contents

The moisture content of each muscle was determined in 2 g of minced sample by drying to constant weight at 175 °C using a halogen moisture analyser HB43 (Mettler Toledo, Switzerland).

The determination of salt content in each muscle was done by ion chromatography according to the methodology described by [Armenteros, Aristoy, and Toldrá \(2012\)](#). For that, 5 g of minced sample was homogenised in water (1:10; w/v) in a stomacher (IUL Instruments, Barcelona, Spain) for 10 min at 4 °C, and centrifuged (12000 xg, 20 min, 4 °C). The supernatant was filtered through glass wool, diluted in water (1:100), and immediately frozen at -20 °C until use. Then, thawed samples were filtered through a 0.45 µm nylon membrane filter (Millipore, Bedford, MA, USA), and injected into the ion chromatograph (Metrohm Advanced compact IC-861, Metrohm® Ltd., Herisau, Switzerland) to determine the sodium (Na⁺) content. The column was a Metrosep C3 column (250×0.4 mm; Metrohm® Ltd.), and 3 mM HNO₃ with 5% acetone was used as mobile phase at a flow rate of 1.2 mL/min. Collection and processing of data were done using IC Net 2.3 software (Metrohm® Ltd.), and the concentration of the cation in each sample was determined by plotting the peak areas against a calibration curve prepared with standard solutions of Na⁺ (0.5 - 100 ppm) (Fluka, Switzerland, Sigma, St. Louis,

MO, USA). Results of salt contents were expressed as percentage of sodium on dry weight basis of sample.

2.3 Extraction of peptides

Peptide extraction was carried out according to the method described by Escudero et al. (2013), with some modifications. A total of 20 g sample of each sample (SM and BF muscles) were minced and homogenised with 80 mL of 0.01 N HCl for 8 min in a stomacher (IUL Instrument, Barcelona, Spain) after removing the free O₂ with N₂ gas injection. The homogenate was centrifuged (12000 xg, 20 min, 4 °C) and filtered through glass wool. Then, a total of 250 µl of the supernatant was taken and deproteinised by adding 3 volumes of ethanol (2 h, -20 °C), centrifuged (12000 xg, 20 min, 4 °C), and finally dried in a rotatory evaporator and lyophilised.

2.4 nLC-MS/MS analysis

The identification of the peptides was performed by nanoliquid chromatography-tandem mass spectrometry (nLC-MS/MS) using an Eksigent Nano-LC Ultra 1D Plus system (Eksigent of AB Sciex, CA, USA) coupled to the quadrupole/time-of-flight (Q-ToF) TripleTOF® 5600+ system (AB Sciex Instruments, MA, USA) with a nanoelectrospray ionisation source (nESI).

Lyophilised samples were resuspended (10 mg/mL) in water with 0.1% of trifluoroacetic acid (TFA). Then 15 µL of each sample was cleaned and concentrated using Zip-Tip C₁₈ with standard bed format (Millipore Corporation, Bedford, MA) according to manufacturer's guidelines. Peptide solutions were dried and resuspended in 20 µL of 0.1% TFA, injecting a total of 5 µL in the LC-MS/MS system. The sample was preconcentrated on an Eksigent C18 trap column (3 µm, 350 µm × 0.5 mm; Eksigent of AB Sciex, CA, USA) using 0.1% TFA as mobile phase at a flow rate of 3 µL/min for 5 min. The trap column was automatically switched in-line onto a nano-

HPLC capillary column (3 μm , 75 $\mu\text{m} \times 12.3 \text{ cm}$, C18; Nikkyo Technos Co., Ltd., Japan), using 0.1% formic acid in water ~~was used~~ as solvent A, and 0.1% formic acid in acetonitrile as solvent B. Chromatographic conditions were a linear gradient from 5% to 35% of solvent B over 90 min, and 10 min from 35% to 65% of solvent B, at a flow rate of 0.3 $\mu\text{L}/\text{min}$ and 30 $^{\circ}\text{C}$. The column outlet was coupled to nESI. The Q/ToF operated in positive polarity and information-dependent acquisition mode, with 0.25 s MS scan from 100 to 1250 m/z and 0.05 s product ion scans from 100 to 1500 m/z on the 50 most intense 1 - 5 charged ions.

Data were processed using ProteinPilotTM v4.5 software (AB Sciex, MA, USA) for the identification and quantification of peptides. The Paragon algorithm of ProteinPilot was used to search in NCBI database with non-enzyme specificity, *Sus scrofa* taxonomy, and including all possible post-translational modifications.

The relative quantification of peptides was performed by using the label-free methodology described by [Gallego, Mora, Aristoy, and Toldrá \(2015b\)](#). This approach is based on the measurement of the integrated areas of extracted ion chromatograms (XICs) from three replicates to determine the ratios for individual peptides. Peptides were quantified using PeakView v1.1 software (AB Sciex, Framingham, MA, USA).

2.5 MALDI-IMS

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) was used to study the spatial distribution of peptides in the two muscles (SM and BF) of dry-cured ham tissue. After defining the two muscle areas in the tissue, frozen samples stored at -80°C were cut on a microtome at a 10 μm thickness onto indium-tin-oxide coated glass slides (Sigma, St. Louis, MO, USA). Sections were subjected to washing steps using 70% ethanol and twice 100% ethanol to remove lipids, and then dried in a desiccator for 30 minutes. Matrix CHCA, which contains 7 mg/mL of saturated α -

cyano-4-hydroxycinnamic acid (Bruker Daltonics, Germany) in acetonitrile:water (60:40) with 0.2% TFA, was applied using the HTX TM-Sprayer (HTX Technologies, Chapel Hill, NC, USA). The plate was introduced in a 5800 MALDI-TOF/TOF (AB Sciex, MA, USA) that was used in automatic positive-ion reflector mode for mass analysis between 650 and 1800 Da. Previously, the plate and the acquisition method were calibrated with a calibration mixture containing [Glu¹]-Fibrinopeptide B and Angiotensin II (Sigma, St. Louis, MO, USA) at a concentration of 1 nmol/μL. Image analysis was performed using TissueView v1.1 software (AB Sciex, MA, USA), and the analysis of data was done using mMass software (<http://www.mmass.org>).

2.6 Statistical analysis

Statistical analysis including one-way analysis of variance (ANOVA) and Fisher's multiple range tests were performed using XLSTAT 2011 v5.01 software (Addinsoft, Barcelona, Spain) in order to determine significant differences in the contents of moisture and salt between muscles. Results were the mean of 3 replicates ± standard error of mean, and differences were considered significantly at $p < 0.05$.

Data obtained from the relative quantification of oxidised peptides were statistically analysed using MarkerView v1.3 software (AB Sciex, Framingham, MA, USA), performing Principal Component Analysis (PCA) and Student's t-test ($p < 0.1$) for testing differences between dry-cured ham muscles.

3. Results and discussion

3.1. Moisture and salt contents

The moisture and salt contents were determined in SM and BF muscles of dry-cured hams at the end of the processing (12 months) ~~and shown in Table 1.~~ Values obtained for the external SM muscle were 50.20 ± 5.06 % of water and 8.62 ± 1.89 % of salt

content, whereas for the internal BF muscle were 60.16 ± 0.91 % of water and 12.28 ± 1.23 % of salt. Statistical analysis of these results indicated significant differences ($P < 0.05$) between muscles for both parameters. BF muscle presents higher moisture and salt contents than SM muscle, because the dynamics of water loss and salt diffusion take place from the surface (SM muscle) to the inner location (BF muscle). Processing conditions and properties of hams such as weight, adiposity and muscle pH determine the rate of dehydration, salt penetration and consequently the activity of proteolytic enzymes, which play a major role in textural and sensory properties of dry-cured hams (Toldrá, Rico, & Flores, 1992; Ruiz-Ramírez, Arnau, Serra, & Gou, 2005, 2006; Giovanelli, Buratti, Laureati, & Pagliarini, 2016). ~~During-In~~ the ~~first-initial step-stages~~ of the ~~cured-ham~~dry-cured ham processing, SM muscle presents a high salt content that can induce changes in solubility of sarcoplasmic proteins due to their denaturation (Théron et al., 2011; Bermúdez, Franco, Carballo, Sentandreu, & Lorenzo, 2014). On the other hand, the delayed salt penetration and water migration in the internal BF muscle would lead to a high degradation of sarcoplasmic and myofibrillar proteins as the action of proteases is not affected by these processing conditions (Théron et al., 2011; Bermúdez et al., 2014).

3.2. Identification of peptides

Peptide extracts from SM and BF muscles were analysed by nLC-MS/MS for the identification of peptides. More than 5,000 peptides from different proteins were identified in the peptide extracts of both muscles, and the study was focused on those showing oxidative modifications. Table 2-1 shows main proteins from which the identified peptides were derived, including myofibrillar proteins such as actin, myosin, titin, and troponin, and sarcoplasmic proteins such as glycolytic enzymes. The numbers of oxidised and unoxidised peptides identified for each protein as well as the total

percentage of oxidised peptides are given in [Table 21](#). In SM muscle, oxidised peptides were mainly derived from myosin, creatine kinase and beta-enolase proteins. However, pyruvate kinase and phosphoglycerate kinase showed the highest percentages of oxidised peptides (16.67 % and 7.14 %, respectively). In BF muscle, peptides showing oxidation were mainly derived from actin, myosin and beta-enolase, whereas the highest percentage of oxidised peptides was also observed for pyruvate kinase (28.57 %).

It has been reported that the salt used in the dry-cured ham processing could exert a pro-oxidative effect because sodium chloride can initiate protein oxidation by lipid oxidative products, change the structure and solubility of proteins increasing their susceptibility to be oxidised, and activate heme pigments that would promote carbonylation and cross-linking of proteins ([Souza et al., 2013](#); [Soladoye et al., 2015](#)).

In this regard, the study carried out by [Wang et al. \(2011\)](#) evidenced the intense degradation of proteins in SM and BF muscles and the increase of protein oxidation during the salting stage of dry-cured Xuanwei ham, showing higher values of carbonyl contents at the end of salting in SM than BF due to the external muscle is exposed to oxygen more easily. Also [Koutina et al. \(2012\)](#) reported an extended formation of protein radicals and protein carbonyls during the first stages of Parma dry-cured ham processing, faster for SM than BF due to the better availability of oxygen and higher salt content in the external SM muscle. However, these oxidative processes tended to be stabilised toward the final stages of maturation and similar values were found in both SM and BF muscles at the end of the processing ([Koutina et al., 2012](#)). The results of the present study also showed minor differences between muscles in terms of the number of peptides with oxidative modifications and main proteins from which they were derived despite the significant differences in salt content between muscles. In fact, the identified oxidised peptides could derive from the degradation of other peptides

showing this modification in earlier processing steps such as post-salting stage (Gallego et al., 2014).

Some of the identified peptides in this study showed several oxidative modifications in their sequences. So, Table 3-2 indicates the number of oxidised peptides identified according to the addition of one, two, or three oxygen atoms at different amino acid residues such as methionine (Met), cysteine (Cys), histidine (His), tryptophan (Trp), and phenylalanine (Phe). Met was the residue that underwent more oxidative modifications, representing around 77 % of the oxidised peptides identified in SM muscle and 84 % of those identified in BF muscle. Cys residues were mainly modified by the addition of two oxygen atoms in the SM muscle, and even trioxidations on this residue were observed in both muscles. His and Trp were oxidised to a lesser degree, and oxidation of Phe was only detected in peptides from BF muscle.

Several studies have identified amino acid oxidations in peptides derived from different myofibrillar proteins in dry-cured hams, mainly involving Met (Gallego et al., 2014; Gallego et al., 2015a), but also proline (Pro) and Trp residues (Gallego et al., 2018).

Some amino acid residues have an intrinsic susceptibility to be oxidised due to its structure, but their neighboring residues and position within the peptide sequence also determine their oxidation (Estévez, 2011; Ghesquière & Gevaert, 2014). The amino acids Met and Cys are very susceptible to oxidative modifications due to the reactive sulfur atoms contained in their structures. The oxidation of Met generates sulfoxide as main oxidative product, which can be further oxidised to sulfone (Stadtman, Moskovitz, & Levine, 2003). On the other hand, Cys can be oxidised to sulfenic acid, sulfinic acid and sulfonic acid by addition of one, two or three oxygen atoms, respectively (Silva et al., 2013; Zhang et al., 2013). Some of the oxidative modifications of Met and Cys residues are reversible and have been described to be involved in antioxidant

mechanisms and changes on conformation, solubility, functionality, and proteolytic susceptibility of peptides (Stadtman et al., 2003). Aromatic amino acids such as His, Trp, and Phe need more severe conditions to be oxidised, and mainly undergo hydroxylation reactions (Stadtman & Levine, 2003).

3.3. Quantification of oxidised peptides and MALDI -IMS

In order to evaluate whether the observed differences in salt content between external and internal muscles could affect peptide oxidation at quantitative level, a relative quantification of the oxidised peptides identified in SM and BF muscles was done using a label-free methodology based on peak intensity measurements. A Principal Component Analysis (PCA) score plot was performed to establish statistical differences between muscles according to the influence of the oxidised peptides and variance among them (Figure 1A). Principal component 1 (PC 1) explained the 37.9 % of the variability in the dataset while PC 2 was responsible for the 23.4% of variance within the dataset, allowing the differentiation between SM and BF muscles. Additionally, the loading plot (Figure 1B) revealed the oxidised peptides with the highest influence for the description of the discriminant components. Statistical analysis using Student's t-test showed that peptides AP[Oxi]KKPEPKKDDAKA, APKKP[Oxi]EPKKDDAKAAAKAAPAPAPAPAPEPPKEPE, and APKKP[Oxi]EPKKDDAKAAAKAAPAPAPAPAPEPPKEPEFD derived from the degradation of myosin light chain protein, as well as GDEEKRRHRAITARRQHLKSVM[Oxi]LQIAATELEKE derived from troponin I protein were the main peptides responsible for differences observed between SM and BF muscles ($p < 0.1$). In this regard, peptides derived from myosin protein had already been described as the most influential at the end of the processing of 9 months Spanish dry-cured ham (Gallego, Mora, & Toldrá, 2016; Gallego et al., 2018).

Up to date, little is known about the effect of dry-cured ham processing conditions on peptide oxidation. The present work suggests that differences in physicochemical characteristics such as water and salt contents between SM and BF muscles could affect peptide oxidation at quantitative level. Recently, [Gallego et al. \(2018\)](#) studied the evolution of oxidised peptides throughout the processing of 9 months dry-cured ham, evidencing quantitative differences in peptide oxidation at different processing times. That work suggested that curing salts and specific processing conditions could lead to an increase of oxidative reactions as the processing of dry-cured ham progressed, but further studies are still needed.

MS techniques such as LC-MS/MS allow a fast, sensitive, precise and specific analysis of peptides and post-translational modifications, and peptidomics has proven to be a useful tool to obtain valuable information at peptide level. However, novel strategies such as MS imaging are a very useful tools for the spatio-chemical characterisation of biomolecules from food samples, the localisation of biomarkers, or the increase of knowledge about food metabolomics ([Li et al., 2016](#)). MALDI-IMS is one of the main techniques currently used in food science research to visualise the distribution of nutrients, elements or contaminants in a single measurement. However, protein and peptide imaging is challenging and difficult to standardise and so, few studies on food products have been undertaken to that end yet ([Yoshimura, Goto-Inoue, Moriyama, & Zaima, 2016](#)).

In this work, a preliminary study using MALDI-IMS has been done in order to obtain an overview of the spatial distribution of peptides in the SM and BF muscles of dry-cured ham tissue. [Figure 2A](#) reveals a spatial heterogeneity of the peptide ion intensities between muscles, and [Figure 2B](#) shows the obtained MALDI-TOF MS spectrum from 650 to 1800 m/z . These results indicate a higher amount of peptides in

SM than BF muscle, despite a more intense proteolysis would be expected in the internal muscle. This fact could be due to peptides present in the BF muscle are of a smaller size than those analysed in the selected mass range, so further studies would be needed for better evaluating peptide differences between dry-cured ham muscles. Up to date, MALDI-IMS has only been applied in order to resolve the distribution of lipids in beef meat (Zaima et al., 2011; Dyer et al., 2014), whereas other imaging techniques such as magnetic resonance imaging (MRI) have been used for the characterisation of dry-cured hams in terms of monitoring changes between muscles or during processing (Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009; Bajd, Škrlep, Čandek-Potokar, Vidmar, & Serša, 2016). Despite MRI and IMS can provide complementary data about localization and chemical characterisation of biomolecules, MALDI-IMS seems a promising tool in food-based applications that require fast, cheap and accurate analysis, and the high resolution provided by tandem mass spectrometers is not necessary (Canela, Rodríguez, Baiges, Nadal, & Arola, 2016). ~~Thus, future studies based on imaging of proteins and peptides in dry-cured hams could be used to localise differences between samples associated with tissue types, physicochemical characteristics, processing conditions, genetic differences as well as to assess food quality, safety and authenticity.~~

4. Conclusions

The use of peptidomics has allowed the study of differences in peptide oxidation between the external SM muscle and the internal BF muscle from 12 months dry-cured hams. Numerous peptides were identified showing oxidation at different amino acid residues such as methionine, cysteine, histidine, tryptophan, and phenylalanine. Pyruvate kinase was the protein showing the highest percentage of oxidised peptides in

both muscles, whereas the label-free quantification showed that three peptides derived from myosin light chain protein and one peptide derived from troponin I were the main responsible for oxidative differences between SM and BF muscles. On the other hand, MALDI imaging mass spectrometry was used for the simultaneous mapping of peptides present in both sample sections, confirming the potential of this technique to determine differences between samples. Future studies based on imaging of proteins and peptides in dry-cured hams could be used to localise differences between samples associated with tissue types, physicochemical characteristics, processing conditions, genetic differences as well as to assess food quality, safety and authenticity.

The results obtained in this study suggest that differences in physicochemical characteristics between muscles such as water and salt contents could affect oxidation at peptide level, allowing a better understanding of the oxidative processes that take place in the dry-cured ham currently focused on protein oxidation.

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FIGURE CAPTIONS

Figure 1. **A)** Principal Component Analysis (PCA) score plot to assess the variance among the oxidised peptides of *Semimembranosus* (SM) and *Bicep femoris* (BF) muscles in three replicates ($n = 3$). **B)** Loading plot showing the oxidised peptides affecting the score plot distribution. The main peptides responsible for statistical differences between muscles are shown in red.

Figure 2. **A)** MALDI imaging mass spectrometry (IMS) of dry-cured ham tissue, showing the spatial distribution of peptides in *Semimembranosus* (SM) and *Bicep femoris* (BF) muscles. **B)** MALDI-TOF MS spectrum from 650 to 1800 m/z .

Figure 1

Figure 1.

Figure 2

A)

B)

Figure 2.

Table 1

Table 1. Number of oxidised peptides derived from main proteins identified by LC-MS/MS in Si

Dry-cured ham muscle	Protein name	Protein abbreviation
<i>Semimembranosus</i>	Actin	ACTIN
	Myosin	MYO
	Titin	TITIN
	Troponin	TROP
	Creatine kinase	CK
	Pyruvate kinase	PK
	Beta-enolase	ENO
	Glyceraldehyde-3-phosphate dehydrogenase	G3PDH
	Phosphoglycerate kinase	PGK
	Lactate dehydrogenase	LDH
<i>Biceps femoris</i>	Actin	ACTIN
	Myosin	MYO
	Titin	TITIN
	Troponin	TROP
	Creatine kinase	CK
	Pyruvate kinase	PK
	Beta-enolase	ENO
	Glyceraldehyde-3-phosphate dehydrogenase	G3PDH
	Phosphoglycerate kinase	PGK
	Lactate dehydrogenase	LDH

^aNumber of oxidised peptides identified in the protein. ^bNumber of unoxidised peptides identified in

Table 2

Table 2. Number of peptides showing oxidation of different amino acids identified by LC-MS/MS in *Semimembranosus* and *Biceps femoris* muscles.

Dry-cured ham muscle	Amino acid	Monoxidation ^a	Dioxidation ^b	Trioxidation ^c	Total ^d	Percentage (%) ^e
<i>Semimembranosus</i>	Methionine (Met)	52	6		58	77.33
	Cysteine (Cys)	2	6	2	10	13.33
	Histidine (His)	4			4	5.33
	Tryptophan (Trp)	2	1		3	4.00
<i>Biceps femoris</i>	Methionine (Met)	53	6		59	84.29
	Cysteine (Cys)	2	2	1	5	7.14
	Histidine (His)	3			3	4.29
	Tryptophan (Trp)		1		1	1.43
	Phenylalanine (Phe)		2		2	2.86

^{a,b,c} Number of peptides with addition of one, two, or three oxygen atoms at the amino acid residue. ^d Total number of peptides identified showing oxidation of the amino acid. ^e Percentage that represents the number of peptides with the amino acid oxidation in relation to the total of oxidised peptides identified in the muscle.

Dry-cured ham samples

nLC-MS/MS analysis

MALDI-IMS analysis

**Identification and relative
quantification of oxidised
peptides**