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**Deamidation post-translational modification in naturally generated
peptides in Spanish dry-cured ham**

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Abstract

A large number of peptides are generated during the processing of dry-cured ham that can be affected by post-translational modifications (PTM). One of the most studied PTMs is the oxidation of susceptible residues but other modifications such as deamidation have been scarcely reported in the literature. This work has been focused on the impact of deamidation in those peptides generated in 12-months dry-cured hams. The results obtained in *Biceps femoris* and *Semimembranosus* muscles showed that 52% and 48% of the identified peptides, respectively, were affected by PTMs, with a total of 277 and 282 sequences showing deamidation in both muscles, respectively. It appears that deamidation is not influenced by the type of muscle but is probably favored by characteristic processing conditions like temperature, pH, and salting of hams. The knowledge of the affected sequences provides novel information for a better characterisation of proteolysis phenomena during the processing of dry-cured ham.

Keywords: Dry-cured ham, peptides, deamidation, proteolysis, post-translational modifications, mass spectrometry.

1. Introduction

40 Dry-cured ham represents a traditional and highly appreciated Mediterranean product. One
41 of the most important biochemical reactions taking place during the processing of dry-cured
42 ham is an intensive proteolysis that affects sarcoplasmic and myofibrillar proteins. This
43 massive protein degradation is mainly caused by endogenous proteolytic enzymes.
44 Endopeptidases, mainly cathepsins and calpains, generate large polypeptides by breaking
45 protein internal sites, and exopeptidases, like dipeptidyl peptidases, aminopeptidases, and
46 carboxipeptidases, release small peptides and free amino acids (Toldrá, 1998).

47 Peptides can experience chemical modifications and matrix interactions during processing
48 that can alter their functionality. They are known as post-translational modifications or
49 (PTMs). Some examples of these modifications can be oxidation, deamidation,
50 carbamilation, amidation, aminoacidic addition and loss or formilations. In this sense,
51 previous studies on dry-cured ham have reported the oxidation of methionine in peptide
52 sequences derived from the myofibrillar proteins titin, nebulin, α -actin, troponin I, myosin
53 light chain 1, myosin light regulatory chain 2, myosin-1 and myosin-2 (Gallego, Mora,
54 Aristoy & Toldrá, 2015). Deamidation is a post-translational modification in which
55 ammonia is removed from the peptide chain by hydrolysis of the amide groups where a
56 glutamine or asparagine residue is transformed into an acidic carboxylate group, glutamic
57 acid and aspartic acid, respectively. This modification exerts an important role in food
58 products and is a widely extended methodology used, for example, to alter cereal protein
59 structures and properties by converting glutamine into glutamic acid (Zhao, Tian & Chen,
60 2011; Lei, Zhao, Selomulya & Xiong, 2015).

The present study is focused on the identification of naturally generated deamidated peptides 12 months dry-cured ham using a peptidomic approach based on ESI-Q-TOF mass spectrometry.

2. Materials and methods

2.1. Materials

For sample extraction, absolute ethanol and hydrochloric acid (37%) of analytical grade have been purchased from Scharlab (Barcelona, Spain). For LC-MS analysis, all reagents were of mass spectrometry grade. Acetonitrile, methanol and pure H₂O were purchased from Scharlab (Barcelona, Spain) and formic acid from Sigma (St Louis, USA).

This study was carried out with 12 months dry-cured hams (n=3) from white pigs (Landrace x Large White and Duroc) prepared. *Semimembranosus* and *Biceps femoris* muscles were sampled for the analysis.

2.2. Dry-cured ham extraction and deproteinisation

20 g of both *Semimembranosus* and *Biceps femoris* muscles (n=3) were minced and homogenised with 80 ml of 0.01 N HCl for 8 min at 4°C in a stomacher after removing the free O₂ of sample with N₂. The homogenate was centrifuged at 4°C and 10,000 rpm (12,000 g) for 20 min, filtered with glass wool, and 250 µl of supernatant were deproteinised by adding 3 volumes of ethanol (750 µl) and the mix was maintained at -20°C for 2 h. Then, samples were centrifuged for 20 min at 10,000 rpm (12,000 g) and 4°C, and ethanol was removed in a rotatory evaporator. Finally, the extracts were lyophilized and stored at -80°C until use.

2.3. Determination of salt content

Sample extraction and analytical ion chromatography for determination of salt content were performed as described by Armenteros, Aristoy & Toldrá (2012).

2.4. Peptide identification

Peptides were identified using nanoliquid chromatography–tandem mass spectrometry with an Eksigent Nano-LC Ultra 1D Plus system (Eksigent of AB Sciex, CA, USA), and quadrupole/time-of-flight TripleTOF_5600+ system (Q-ToF) (AB Sciex Instruments, MA, USA) with nanoelectrospray ionization source (nESI). Once salt was removed and the rest of content was lyophilised, the samples were resuspended to a concentration of 10 mg/ml with 0.1% of TFA and centrifuged at 200 g for 3 min under refrigeration. After that, 15 µl of the resuspension were wiped and concentrated using a Zip-Tip C18 with standard bed format (Millipore Corporation, Bedford, MA). Once eluted, peptides were lyophilised and resuspended in 20 µl of 0.1% TFA.

Mass spectrometry analysis was carried out following the methodology described by Gallego, Mora, Aristoy & Toldrá (2015). 15 µl of each sample were injected into the nESI-LC–MS/MS system, and preconcentrated on a C18 trap column (3 µm, 350 µm x 0.5 µm; Eksigent of AB Sciex, CA, USA), with a flow rate of 3 µl/min for 5 min, using 0.1% v/v TFA such as mobile phase. The trap column was automatically switched inline onto a nano-HPLC capillary column (3 µm, 75 µm x 12.3 cm, C18; Nikkoy Technos Co, Ltd. Japan). Mobile phases were composed of a solvent A (0.1% (v/v) FA in water), and a solvent B, (0.1% (v/v) FA in 100% acetonitrile). In HPLC generated a gradient from 5 to 35% of solvent B for 90 min, and another gradient from 35% to 65% of solvent B for 10 min, with a flow rate of 0.3 µl/min and 30°C. A nanoelectrospray ionization system (nESI) was coupled directly to the column leak.

The Q/ToF worked in positive polarity and information-dependent acquisition mode, in withn a scan of 0.25s MS from 100 to 1250 m/z valor, followed of scans of 0.05s of product ion from 100 to 1500 m/z values.

2.5.Data analysis

Parameters by default were used in ProteinPilot v4.5 (ABSCIEX, CA, USA) to get a peak list directly from wiff files generated in the 5600 TripleTof instrument. The ProteinPilot algorithm used to search in NCBI protein database was the Paragon algorithm. This search was carried out with the following parameters: non-enzyme specificity, without cyst-alkylation, and with False Discovery Rate (FDR) correction. Taxonomy was set to *Sus scrofa*. All possible post-transductional modifications were considered in the identification. The identified proteins were grouped in base on MS/MS spectra through the algorithm of ProteinPilot called Progroup to avoid taking more than one protein for the same spectral evidence.

3. Results and discussion

The peptide extracts from dry-cured ham were lyophilized and prepared to be analyzed using nESI-Q-TOF mass spectrometry in order to identify the peptides and any PTM that might be present.

The diffusion of salt during early stages of dry-curing is not homogeneous and differences in salt content between muscles have been reported (Grau, Albarracín, Toldrá, Antequera & Barat, 2007). This is quite relevant because salt exerts an inhibitory effect on muscle enzymes like cathepsins (Rico, Toldrá & Flores, 1991), dipeptidylpeptidases (Sentandreu & Toldrá, 2001) and aminopeptidases (Toldrá; Cerveró & Part, 1993a). This is the main reason why two different muscles, an external (*Semimembranosus*) and an internal (*Biceps*

femoris) with different salt content were analysed. This can be also reflected in different proteolysis products by the end of the dry-curing process (Toldrá, 2002). Endopeptidases (calpains I and II, cathepsins B, B+L and H) generate protein fragments that act as substrate for exopeptidases (dipeptidylpeptidases or DPP I, II, III and IV, aminopeptidases and carboxypeptidases) (Armenteros, Aristoy and Toldrá, 2009).

In summary, salt diffusion together with other process parameters such as water activity, pH and temperature have been reported to strongly affect muscle enzymes activity (Petrova, Tolstorebrov, Mora, Toldrá & Eikevik, 2016; Toldrá, Rico & Flores, 1993; Flores, Aristoy & Toldrá, 1997). So, *Semimembranosus* is the external muscle accumulating more salt during the salting step. For this reason, a higher enzyme inhibition should be expected in *Semimembranosus* muscle due to the increased salt content (medium value of 18.8 mg/g of sample) as compared to the internal muscle (medium value of 16.5 mg/g of salt content). This should affect the proteolysis resulting in the generation of different peptide sequences between muscles.

In this study, a total of 1922 and 2118 peptides from *Biceps femoris* and *Semimembranosus* muscles, respectively, have been identified. This constitutes a massive peptide generation resulting from proteins degradation during the dry-curing process. Main identified proteins were myosin-light chains (myosin regulatory chain 2 (MLRS) and myosin light chain-1 (MYL1)), myosin-heavy chains (myosin-1 (MYH1), myosin-4 (MYH4) and myosin-7 (MYH7)), creatine kinase (CK), actin (ACT), aerobic glycolytic enzymes (such as glucose-6-phosphate-isomerase, glyceraldehyde-3-phosphate hydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, β -enolase and pyruvate kinase), troponins (type T or TTNT, and I or TTNI) and hemoglobin (HBB). From the identified peptides, PTMs were detected in 52% and 48% in *Biceps femoris* and *Semimembranosus* muscles, respectively.

The modifications of some peptide residues are very common during the processing of food especially when conditions of temperature, pH, salt content etc., are altered. In fact, cheesemaking PTMs are described to complete the role of the intense lipolysis, glycolysis and proteolysis giving its characteristic flavor and taste properties to the final product (Kindstedt, 2010). Also during wine production the PTMs phosphorylation or acetylation has been described to influence the wine-yeast fermentation (Chambers & Pretorius, 2010; Albertín et al, 2013). In this study, the identified PTMs include the carbamilation of lysine residues, formylation (addition of formyl functional group, a carbonyl bonded to hydrogen), oxidations of susceptible amino acids, or deamidations of asparagine and glutamine residues. The deamidation of asparagine and glutamine was the most abundant reaching 27% of the total modifications in both types of muscles. This is the first time that the specific sequences of deamidated peptides have been described for dry-cured ham. Deamidation can be an enzymatic or non-enzymatic reaction. Transglutaminases, peptidases and peptidoglutaminase are the only enzymes reported in literature as protein deamidation enzymes. Main factors influencing the non-enzymatic or chemical deamidation are heating, acid or basic conditions, (Ramahan, Vasiljevic & Ramchandran, 2016; Wu, Nakai & Powrie et al, 1976) or changes in ionic strength (Robinson & Ruud, 1974). So, Izzo, Lincoln and Ho (1993) reported a positive correlation between high temperature and the increase of deamidation amount whereas Ramahan, Vasiljevic and Ramchandran (2016) described that at low pH near 3 gliadins suffered acidic deamidation with hydrolysis into very small fractions. Finally, Robinson and Robinson (2001), showed that the rate of deamidation of asparagine residues have a positive dependence on ionic strength. Thus, temperature and salt conditions used during the processing of dry-cured

ham could be the main reason for the large amount of observed post-translational modifications.

The deamidation rate depends on the residues preceding and following the carboxamide group and the protein tertiary conformation. Also, the ratio of asparagine to glutamine can affect deamidation rate because asparagine is deamidated at higher rates than glutamine, due to the distance of the side chain amide group from the peptide nitrogen (Robinson & Ruud, 1974). In this respect, a total of 112 of the 277 deamidated peptides in *Biceps femoris* and 104 of 282 in *Semimembranosus* are glutamine deamidations whereas the rest (60% and 64%, respectively) were in asparagine residues. **Table 1** shows the number of peptides affected by deamidation that have been identified in both muscles of dry-cured ham including the protein of origin. The ratio values indicate the prevalence of deamidation in the identified peptides. The highest amount of identified peptides with a 95% of confidence were from MYH, MLRS, CK and ACT proteins whereas MHC and HBB proteins were described to show the highest ratio value of deamidation. The deamidated peptides identified in myosin heavy chains (MYH-1, MYH-4 and MYH-7) are shown in **Table 2** where most of the deamidated peptides have a residue of asparagine (N) or glutamine (Q) in an extreme of the sequence, that could make them more susceptible to be deamidated than peptides with N or Q residues in central positions. For instance, creatine kinase protein (Accession Number KCRM_PIG) has only 362 residues in comparison with the macrostructure of other identified proteins. However, more than 200 peptides have been identified with CK as protein of origin in both muscles. The possible reason is that CK is a molecule located between fibers, more available and accessible to the action of endogenous enzymes. **Figure 1** shows an example of MS/MS spectrum for a deamidated and non-deamidated creatine kinase-derived peptide. Finally, **Table 1 of Supplementary material**

shows the sequences of the deamidated peptides in each muscle, including protein of origin, molecular mass, charge state and position of deamidation.

Conclusions

This study highlights the importance of deamidation because this PTM is affecting major proteins of dry-cured ham. This is the first time that this modification has been described in a meat matrix and affected the described sequences. In fact, a total of 1922 and 2118 peptides have been identified in *Semimembranosus* and *Biceps femoris* muscles, respectively, including the deamidation of 277 and 282 residues, respectively. The results of this study show that deamidation does not seem to be influenced by the type of muscle but probably occurs due to the characteristic conditions of temperature, pH, and salting during the dry-cured ham processing. The knowledge of the intense deamidation together with the affected peptide sequences provides novel information about the changes occurred and opens new lines of study to detect the potential influence of this PTM on the final nutritional or quality characteristics of dry-cured ham.

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292 **FIGURE CAPTIONS**

293 **Figure 1.** MS/MS spectrum of the peptide NADRLGSSEVEQ from creatine kinase M-type
294 (KCRM_PIG). A) Peptide with no modifications and B) Same peptide showing a
295 deamidation in Q residue.

Table 1

Protein of origin	<i>Biceps femoris</i>			<i>Semimembranosus</i>		
	Peptides					
	Total	Deamidated	Ratio	Total	Deamidated	Ratio
Myosin heavy chains	252	69	0.274	210	73	0.347
Haemoglobin	38	9	0.237	76	27	0.355
Creatine kinase	213	46	0.216	242	32	0.132
Myosine light chains	541	111	0.205	540	69	0.128
Actin	218	14	0.064	222	15	0.068
Myosine-binding protein	117	7	0.060	129	12	0.093
Glycolitic enzymes	102	5	0.049	86	9	0.105
Troponin	56	1	0.018	58	9	0.155
Total	1537	262	0.170	1563	246	0.157

Table 2

<i>Semimembranosus</i>				
Names	Sequence	Obs m/z	Theor MW	Theor z
myosin-1	EDQLSELKTKEEEQQLIND	816.062	2445.18701	3
myosin-1	QQIEELKRQLEEEIK	479.2721	1913.01062	4
myosin-1	EDQLSELKTKEEEQQLIND	816.062	2445.18701	3
myosin-1	EDQLSELKTKEEEQQLIND	816.062	2445.18701	3
myosin-1	QQIEELKRQLEEEIK	479.2721	1913.01062	4
myosin-4	NAV GALAKAVYDK	440.924	1319.7085	3
myosin-4	QREEQAEPDGTEVAD	837.8491	1673.70166	2
myosin-4	QREEQAEPDGTEVADKA	625.2897	1872.83374	3
myosin-4	NITGWLDKNKDPLNETVVG	705.364	2113.06909	3
myosin-4	QREEQAEPDGTEV	744.8235	1487.63757	2
myosin-4	NITGWLDKNKDPLNE	586.6346	1756.86316	3
myosin-4	QGTLEDQIISANP	693.843	1385.66748	2
myosin-4	NASAIPEGQFIDSKKASEKL	534.2903	2133.09546	4
myosin-4	NITGWLDK	474.258	946.476013	2
myosin-4	QAEPDGTEVADKA	666.3024	1330.58887	2
myosin-4	NITGWLDKNKDPLNETVVGL	743.0571	2226.15332	3
myosin-4	NASAIPEGQFIDSKK	535.9518	1604.80457	3
myosin-4	NASAIPEGQFIDSKKASEK	506.0216	2020.01135	4
myosin-4	SDQEMAIFGEAAPYL RKSEKER	671.8327	2683.32764	4
myosin-4	SDQEMAIFGEAAPYL RKSEKERIEA	750.1186	2996.49146	4
myosin-4	SDQEMAIFGEAAPYL RKSEKERIEAQN	810.6417	3238.59277	4
myosin-4	QKQREEQAEPDGTEVADKA	533.2635	2128.9873	4
myosin-4	SDQEMAIFGEAAPYL RKSEK	800.3843	2398.18384	3

myosin-4	SDQEMAIFGEAAPYLKSEKE	843.3953	2527.22656	3
myosin-4	SDQEMAIFGEAAPYLKSEKERIEAQNKPF	932.4435	3725.83594	4
myosin-4	QGTLEDQIISANP	871.4355	1740.87817	2
myosin-4	NITGWLDKNKDPLNET	620.3159	1857.91089	3
myosin-4	NITGWLDKNKDPLN	543.6233	1627.82056	3
myosin-4	SDQEMAIFGEAAPYLKSEKERIE	732.3608	2925.4541	4
myosin-4	NASAIPEGQFIDSKKASEKLL	562.5598	2246.17944	4
myosin-4	QVFPMNPPKFDKIED	602.6367	1804.87061	3
myosin-4	SDQEMAIFGEAAPYL	963.9309	1925.91931	2
myosin-4	QGTLEDQIISANP	693.843	1385.66748	2
myosin-4	QKQREEQAEPDGTEVAD	644.2968	1929.85522	3
myosin-4	QKQREEQAEPDGTEVAD	644.2968	1929.85522	3
myosin-4	QKQREEQAEPDGTEVAD	644.2962	1929.85522	3
myosin-4	QKQREEQAEPDGTEVADKA	533.2635	2128.9873	4
myosin-4	QREEQAEPDGTEVADK	601.6125	1801.79663	3
myosin-4	QREEQAEPDGTEVADKA	625.2897	1872.83374	3
myosin-4	SDQEMAIFGEAAPYLRS	714.6761	2141.04639	3
myosin-4	TVKEDQVFPMNPPKFD	631.6529	1891.90259	3
myosin-4	TVKEDQVFPMNPPKFD	631.6529	1891.90259	3
myosin-4	VAEPKESFVKGTQSREG	650.34	1947.99011	3
myosin-7	NYAGADTPVEKGKGKAK	434.4951	1733.89478	4
myosin-7	QREEQAEPDGTEE	759.8094	1517.61182	2
myosin-7	QREEQAEPDGTEEADK	611.6029	1831.77075	3
myosin-7	QREEQAEPDGTEEADKS	640.613	1918.80286	3
myosin-7	QREEQAEPDGTEEAD	852.8353	1703.67578	2
myosin-7	NIIGWLQKNKDPLNE	594.9926	1781.93115	3
myosin-7	NYAGADTPVEK	583.2787	1164.52991	2
myosin-7	NYAGADTPVEKGKGKAKK	466.5173	1861.98975	4
myosin-7	NIIGWLQKNKDPLNETVVD	733.0528	2196.14258	3
myosin-7	NIIGWLQKNKDPLNETVVDL	770.7459	2309.22681	3
myosin-7	NYAGADTPVEKGKGKAKKG	480.7723	1919.01123	4
myosin-7	NIIGWLQKNKDPLN	551.9796	1652.88855	3
myosin-7	NIIGWLQKNKDP	713.887	1425.7616	2
myosin-7	NIIGWLQKNKDPLNET	628.6713	1882.97888	3
myosin-7	NKDPLNETVVD	622.8087	1243.59326	2
myosin-7	NYAGADTPVEKGKGK	512.6057	1534.7627	3
myosin-7	QTRPFDLKKDVVPDDKEEFVK	540.2902	2696.36963	5
myosin-7	QNPPKFDKIEDM	488.247	1461.68103	3
myosin-7	QTPGKGTLEDQ	587.7899	1173.55139	2
myosin-7	DHNQYKFGHTK	459.2432	1374.63171	3
myosin-7	LDIDHNQYKFGHTK	429.9877	1715.82678	4

myosin-7	NYAGADTPVEKGKGKA	536.2836	1605.7998	3
myosin-7	QTRPFDLKKDVYPDDKE	556.2972	2221.09033	4
myosin-7	QKNKDPLNETVVD	500.9343	1499.7467	3
myosin-7	DHNQYKFGHTK	459.2432	1374.63171	3
myosin-7	LDIDHNQYKFGHTK	429.9877	1715.82678	4
myosin-7	QNPPKFDKIEDM	488.247	1461.68103	3
myosin-7	QREEQAEPDGTE	695.2913	1388.56921	2
myosin-7	QREEQAEPDGTEEAD	852.8353	1703.67578	2
myosin-7	QTRPFDLKKDVYPDDKE	556.2972	2221.09033	4

<i>Biceps femoris</i>				
Names	Sequence	Obs m/z	Theor MW	Theor z
myosin-4	NASAIPEGQFIDSKKASEKLL	562.5608	2246.17944	4
myosin-4	QREEQAEPDGTEVADKA	625.2897	1872.83374	3
myosin-4	QKQREEQAEPDGTEVADKA	533.2634	2128.9873	4
myosin-4	QREEQAEPDGTEVA	780.3397	1558.67468	2
myosin-4	QREEQAEPDGTEVAD	837.8489	1673.70166	2
myosin-4	NASAIPEGQFIDSKK	535.9509	1604.80457	3
myosin-4	NASAIPEGQFIDSKKASEKL	534.2903	2133.09546	4
myosin-4	QGEITVPSIDQEE	780.8444	1559.68384	2
myosin-4	QREEQAEPDGTE	695.2903	1388.56921	2
myosin-4	QKQREEQAEPDGTEVAD	644.2963	1929.85522	3
myosin-4	QREEQAEPDGTEV	744.8225	1487.63757	2
myosin-4	QKQREEQAEPDGTEVADK	686.9922	2057.9502	3
myosin-4	QAEPDGTEVADKA	666.3033	1330.58887	2
myosin-4	NAV GALAKAVYDK	660.8628	1319.7085	2
myosin-4	QKQREEQAEPDGTEV	582.277	1743.79114	3
myosin-4	QKQREEQAEPDGTEVA	605.9556	1814.82825	3
myosin-4	QQIEELKRQLEEETK	634.6686	1900.97424	3
myosin-4	QKQREEQAEPDGTE	549.2569	1644.72278	3
myosin-4	NASAIPEGQFIDSKKASEK	506.0209	2020.01135	4
myosin-4	SDQEMAIFGEAAPYLKSEKERIEAQN	810.6408	3238.59277	4
myosin-4	SDQEMAIFGEAAPYLKSEK	800.3835	2398.18384	3
myosin-4	NITGWLDK	474.2589	946.476013	2
myosin-4	NITGWLDKNKDPLNETVVG	705.3618	2113.06909	3
myosin-4	QAEPDGTEVAD	566.7428	1131.45679	2
myosin-4	RQEAPPHIFSISDNAYQ	658.704	1972.92786	3
myosin-4	QVFPMNPPKFDKIED	602.6372	1804.87061	3
myosin-4	QGEITVPSIDQEEL	837.3811	1672.76794	2
myosin-4	QREEQAEPDGTEVADK	837.8489	1673.70166	2
myosin-4	QGTLEDQIISANP	693.844	1385.66748	2

myosin-4	SDQEMAIFGEAAPYLKSEKER	671.8318	2683.32764	4
myosin-4	SDQEMAIFGEAAPYLKSEKERIEAQNKPF	932.4435	3725.83594	4
myosin-4	AEKQRSDLSRELEEI	451.7432	1802.901	4
myosin-4	SDQEMAIFGEAAPYLKSEKERIEA	750.1172	2996.49146	4
myosin-4	KNLTEEMAGLDENIA	840.8878	1679.75598	2
myosin-4	QKQREEQAEPDGTEV	582.277	1743.79114	3
myosin-4	QKQREEQAEPDGTEVA	605.9556	1814.82825	3
myosin-4	QKQREEQAEPDGTEVAD	644.2963	1929.85522	3
myosin-4	QKQREEQAEPDGTEVAD	644.2965	1929.85522	3
myosin-4	QKQREEQAEPDGTEVAD	638.2919	1911.8446	3
myosin-4	QKQREEQAEPDGTEVADKA	533.2634	2128.9873	4
myosin-4	QKQREEQAEPDGTEVADKA	710.6688	2128.9873	3
myosin-4	QQIEELKRQLEETK	634.6686	1900.97424	3
myosin-4	QREEQAEPDGTEVAD	837.8486	1673.70166	2
myosin-4	QREEQAEPDGTEVAD	828.8428	1655.69104	2
myosin-4	QREEQAEPDGTEVADK	601.6125	1801.79663	3
myosin-7	NIIGWLQKNKDPLNETVVD	733.0519	2196.14258	3
myosin-7	NIIGWLQKNKDPLNETVVDL	770.7431	2309.22681	3
myosin-7	NYAGADTPVEK	583.2785	1164.52991	2
myosin-7	QREEQAEPDGTEE	759.8093	1517.61182	2
myosin-7	QREEQAEPDGTEEADK	611.6026	1831.77075	3
myosin-7	QREEQAEPDGTEEADKS	640.6115	1918.80286	3
myosin-7	QREEQAEPDGTEEAD	852.8359	1703.67578	2
myosin-7	NYAGADTPVEKGKGKAKKG	480.7716	1919.01123	4
myosin-7	NYAGADTPVEKGKGKAK	434.4948	1733.89478	4
myosin-7	NYAGADTPVEKGKGKAKK	466.5172	1861.98975	4
myosin-7	NYAGADTPVEKGKGK	512.605	1534.7627	3
myosin-7	GSLDIDHNQYKFGHTK	465.9989	1859.88025	4
myosin-7	NYAGADTPVEKG	611.787	1221.55139	2
myosin-7	NKDPLNETVVD	622.8082	1243.59326	2
myosin-7	QTPGKGTLEDQ	587.7904	1173.55139	2
myosin-7	QNPPKFDKIEDM	488.2462	1461.68103	3
myosin-7	NYAGADTPVE	519.2361	1036.43494	2
myosin-7	DHNNQYKFGHTK	459.2419	1374.63171	3
myosin-7	GSLDIDHNNQ	500.2529	998.430481	2
myosin-7	LDIDHNNQYKFGHTK	429.9877	1715.82678	4
myosin-7	DHNNQYKFGHTK	459.2419	1374.63171	3
myosin-7	GSLDIDHNNQYKFGHTK	465.9989	1859.88025	4
myosin-7	QNPPKFDKIEDM	488.2462	1461.68103	3
myosin-7	QREEQAEPDGTEEADK	611.6026	1831.77075	3