# Chapter 9
# Dry-Cured Ham

**Leticia Mora and Fidel Toldrá**

## 9.1 Introduction

Dry-cured ham has been consumed for centuries in many countries and is a typical high-quality product with characteristic texture and flavor. The processing of dry-cured ham is very long, lasting several months or even a few years, including the stages of salting, post-salting, and ripening/drying. The water activity of the ham is progressively reduced by the use of sodium chloride and drying. Many biochemical reactions take place during the process and are responsible for its final characteristic texture and flavor (Toldrá 1998, 2004).

One of the most important biochemical phenomena is the intense proteolysis that takes place as a result of the action of endogenous muscle peptidases. The main enzymes responsible for the degradation of muscle proteins are endopeptidases and certain groups of exopeptidases that have been widely reviewed (Toldrá 2002). Endopeptidases, mainly cathepsins and calpains, are able to degrade a large portion of the muscle protein structure by cleaving myofibrillar proteins, affecting the texture, and also giving rise to large polypeptides that are further degraded by exopeptidases into small peptides and free amino acids (Toldrá and Flores 1998; Toldrá 2006).

This chapter describes the latest advances in the application of proteomic tools to dry-cured ham in order to better understand the intense proteolysis phenomena that occur during its processing.

L. Mora • F. Toldrá (✉)
Department of Food Science, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avenue Agustín Escardino 7, Paterna, Valencia, 46980, Spain
e-mail: ftoldra@iata.csic.es

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## 9.2 Muscle Proteins

Myofibrillar and sarcoplasmic proteins, together with the stroma proteins such as collagen, constitute the main groups of proteins in skeletal muscle. Sarcoplasmic proteins are water soluble or soluble in diluted salt solutions, whereas myofibrillar proteins are soluble in highly concentrated salt solutions. The structure of the skeletal muscle is mainly constituted of muscle fibers, which are essential for the generation and transmission of the contractile force. In fact, the muscle fiber is the basic machinery necessary for muscle contraction (Yamada et al. 2004). Each muscle fiber contains hundreds of myofibrils, which are long bundled tubes of cytoskeleton, made of long chains of a repeating unit called the sarcomere, the basic functional unit of the muscle fiber. These units are composed of actin and myosin proteins that act as thin and thick filaments, respectively, and are responsible for skeletal muscle's striated appearance (Lawrie and Ledward 2006). Other relevant myofibrillar proteins in skeletal muscle are titin, nebulin, actinins (α and β forms), tropomyosin, and troponins C, I, and T (MacIntosh et al. 1996).

Sarcoplasmic proteins are those proteins found in the sarcoplasm, or the fluid surrounding the myofibrils. Myoglobin is considered the most important protein of sarcoplasm as it is the most abundant water-soluble protein and gives the typical red color to the sarcoplasm (Toldrá 2006). However, the sarcoplasmic fraction also contains lysosomal enzymes including cathepsins, which participate in hydrolytic degradation of waste material, and nucleoproteins, that regulate protein synthesis and deposition (Lawrie and Ledward 2006), as well as oxidative enzymes, including cytochromes, flavin nucleotides, various heme pigments, mitochondrial oxidative enzymes, and glycolytic enzymes, which control both aerobic and anaerobic glycolysis (Toldrá 2006).

## 9.3 Muscle Enzymes and Their Role in Ham Processing

Cathepsins and calpains have been the object of research in the last decades regarding post-mortem proteolysis. Thus, calpains have been considered the major contributors to meat tenderization (Lawrie and Ledward 2006) even though in recent years it has been also attributed to the action of other muscle proteolytic enzymes such as caspases and the proteasome (Ouali et al. 2006; Kemp et al. 2010). On the other hand, cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), and L (EC 3.4.22.15) have been reported to be stable during dry-cured ham processing, showing activity even after 15 months of processing (Toldrá et al. 1993a; Toldrá 1998), whereas cathepsin D activity disappears after 6–10 months of processing (Toldrá et al. 1993a; Rico et al. 1991). Calpains (EC 3.4.22.17) can participate in the post-mortem muscle proteolysis only during the first weeks of the curing process, especially due to their relatively poor stability (Toldrá and Flores 1998). All these endopeptidases contribute to the release of numerous polypeptides and peptides during the processing of dry-cured ham.

Exopeptidases degrade the large polypeptides and peptides generated by endopeptidases, giving rise to small peptides and free amino acids that, together with volatile compounds, are responsible for the characteristic flavor of dry-cured ham (Toldrá et al. 1997). Dipeptidyl peptidases (DPP I, II, III, and IV) are exopeptidases able to release different dipeptides from the N-terminal site of polypeptides. The action and stability of these enzymes during the processing of Spanish dry-cured ham are variable but DPP II and DPP IV contribute even though at slow rates during the process (Sentandreu and Toldrá 2001).

On the other hand, a remarkable increase in the concentrations of free amino acids has been reported during dry-cured ham processing. Glutamic and aspartic acids, alanine, leucine, lysine, valine, and threonine appear to be some of the amino acids experiencing larger increases (Toldrá et al. 2000). This accumulation of free amino acids, which is relevant for the development of the characteristic dry-cured flavor, has been attributed to muscle aminopeptidases, responsible for the release of amino acids from the N-terminus of peptides and proteins. Aminopeptidase activity has been detected in meat products even after more than 12 months of processing, suggesting that these enzymes are involved in the later stages of protein degradation. Many factors, such as curing agent or the presence of other peptides, can modulate the activity of these enzymes (Toldrá et al. 1993b; Gianelli et al. 2000).

Carboxypeptidases and peptidyl dipeptidases constitute other groups of exopeptidases responsible for the hydrolysis of amino acids and dipeptides, respectively, from the C-terminal side of the protein fragments (Bodwell and Meyer 1981) although their action and contribution to the process remain quite unknown.

## 9.4 Proteolysis of Myofibrillar and Sarcoplasmic Proteins During the Processing of Dry-Cured Ham

Techniques such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DGE), free solution conjugate electrophoresis (FSCE), size-exclusion chromatography (SEC) also known as gel filtration chromatography (GFC), and reversed-phase high-performance liquid chromatography (RP-HPLC) have been used during the last decades to study proteolysis and to separate and identify the proteins from complex mixtures.

In this sense, SDS-PAGE has been the method of choice in the last decades for the separation and quantitation of protein changes in meat and processed meats. This method is simple, reproducible, and reliable, and also easier to interpret in comparison to 2-D separations as they are mainly based on separation according to molecular mass, and do not require expensive material and instrumentation. Many authors have reported the structural alteration and progressive disappearance of muscle proteins as well as the generation of polypeptides during tenderization and processing of dry-cured ham. Muscle cathepsins

play a relevant role in protein breakdown during dry-curing (Toldrá and Etherington 1988). In this way, a progressive disappearance of myofibrillar proteins including myosin heavy chain, myosin light chains 1 and 2 (MLC 1 and MLC 2), and troponins C and I, as well as the appearance of numerous smaller fragments in the 50–100 and 20–45 kDa regions have been reported (Toldrá et al. 1993b; Buscailhon et al. 1994). The generation of polypeptides (Rodríguez-Núñez et al. 1995) and small peptides of 2–4 amino acid residues (Sentandreu and Toldrá 2007a) was also reported. More recently, an intense proteolysis of actin, tropomyosin, and myosin light chains extracted in the myofibrillar fraction of dry-cured hams with different ripening times was also reported (Di Luccia et al. 2005). These authors concluded that after 12 months of ripening, most myofibrillar proteins were completely hydrolyzed.

The hydrolysis of sarcoplasmic proteins during dry-curing has also been widely described. Myoglobin protein has been described as degraded during the post-mortem storage of pig meat (Lametch et al. 2006) as well as during the processing of dry-cured ham. In this respect, electrophoretic bands for the sarcoplasmic proteins at different stages of the dry-curing process of Iberian ham were reported up to 17.5 months of curing, showing that the levels of myoglobin protein were undetectable after this period of time (Córdoba et al. 1994). A similar study was done on Bayonne hams cured for 8 months (Monin et al. 1997) and Serrano hams cured for 15 months (Toldrá et al. 1993a), and also a gradual decrease in the intensity of the electrophoretic band corresponding to myoglobin was detected until the end of curing. These results agree with those reported by other authors (Soriano-Pérez et al. 2003) who identified a 16 kDa electrophoretic band obtained after SDS-PAGE separation as myoglobin. Other authors (Picariello et al. 2006) have also described the partial hydrolysis of myoglobin protein in dry-cured ham after 12 months and after the analysis of the bands using two-dimensional gel electrophoresis. The same authors also reported the total disappearance of phosphoglycerate kinase (PGK), lactate dehydrogenase (LDH), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glycogen phosphorylase (PYGM), and creatine kinase (CK) during the ripening period of dry-cured ham aged up to 12 months. However, enolase and phosphoglycerate mutase (PGAM) enzymes remained visible until the end of curing in Bayonne hams (Monin et al. 1997), whereas pyruvate kinase (PK) was the only enzyme that remained constant after 11 months of curing (Soriano-Pérez et al. 2003).

Size-exclusion chromatography (SEC), performed under low-pressure, has been also used as an initial step for the analysis of dry-cured ham peptides. Peptides are fractionated according to their molecular size and the gel should have an exclusion limit smaller than the molecule of interest in order to obtain the desired separation. Thus, peptides of different molecular weight are separated within the gel matrix within the fractionation range of the gel (see Chap. 4) and then further separated through reversed-phase HPLC (see Fig. 9.1).

Figure 9.2 shows the most frequently used workflow for the identification of the complete sequence of naturally generated peptides in dry-cured ham muscle.

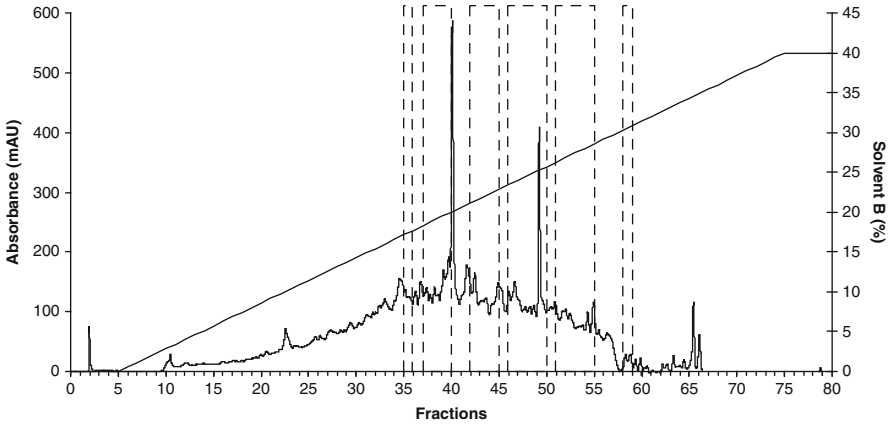



**Fig. 9.1** Reversed-phase chromatographic separation of the selected pooled fractions obtained from size-exclusion chromatography. *Dotted* fractions indicate the fractions where creatine kinase fragments have been detected. *Bold line* indicates the solvent gradient of the chromatographic separation (Reprinted with permission from Mora et al., 2009a (*J. Agric. Food Chem. 57*, 8982–8988). Copyright (2009) American Chemical Society)

## 9.5 Mass Spectrometry Techniques for the Identification of Dry-Cured Ham Naturally Generated Peptides

### *9.5.1 Mass Fingerprinting (MFP)*

The most frequently used strategy to study protein changes and characterize the proteomic profile of a complex mixture starts with SDS-PAGE separation. In one dimension (1D) electrophoresis proteins are isolated only according to molecular weight whereas in two dimensions (2-D) electrophoresis proteins are isolated according to both the isoelectric point and the molecular weight. Once the proteins are separated, they can be stained by using either Coomassie blue or silver staining compatible with MS, and usually digested in-gel with specific proteases such as trypsin that specifically cleave the protein on the C-terminal side of the basic amino acids arginine and lysine.

The peptides obtained after the digestion of the protein are analyzed by mass spectrometry achieving a list of peak masses. The set of measured peptide masses is the peptide mass fingerprint. This experimental mass profile is matched against the theoretical masses obtained from the *in silico* digestion at the same enzyme cleavage sites of all protein sequences in the database. The proteins in the database are then ranked according to the number of peptide masses matching their sequence within a given mass error tolerance. In order to successfully identify a peptide or protein, it is required that the masses of a certain number of fragments match with the theoretical peptide masses contained in the protein databases (Cañas et al. 2006).

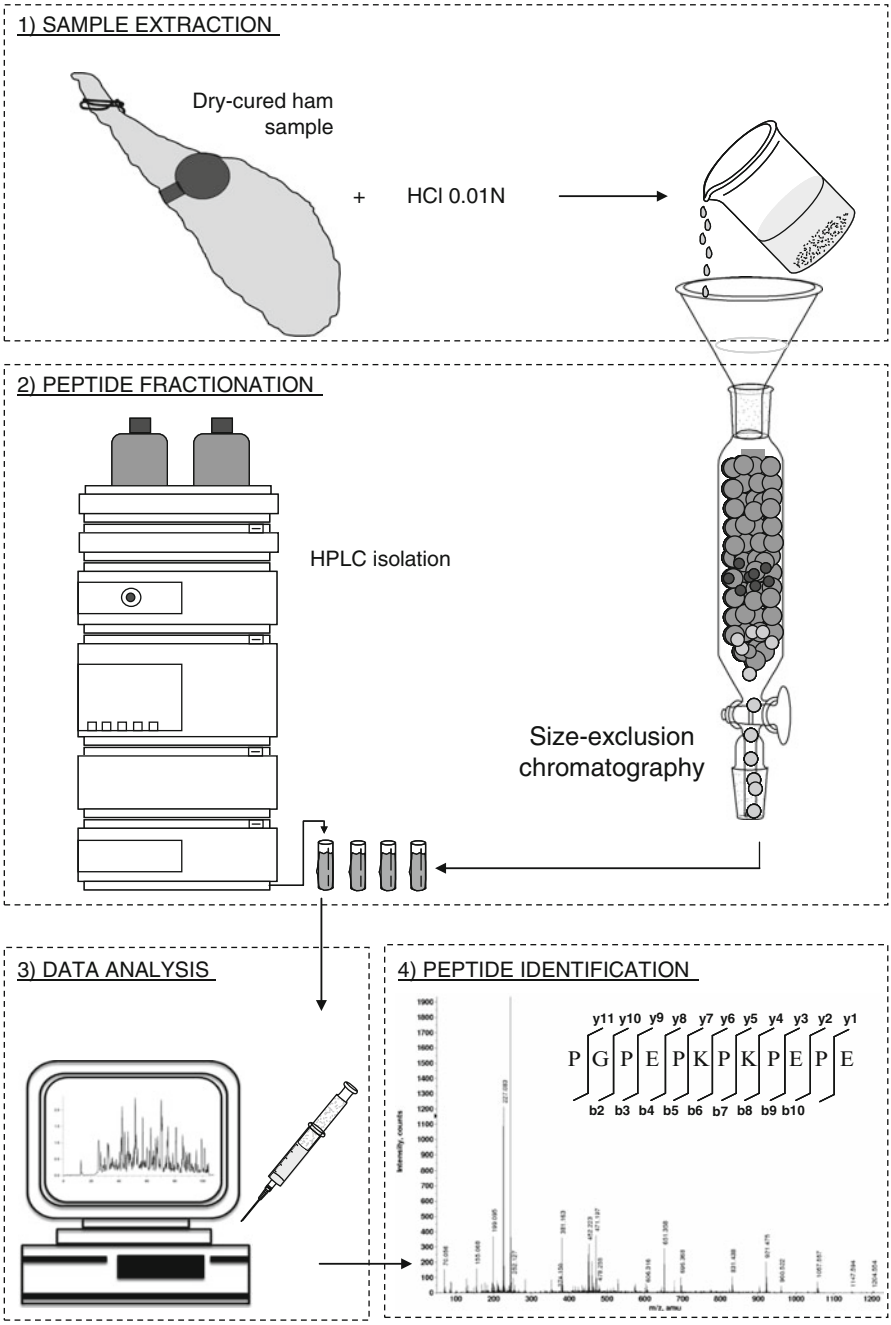



**Fig. 9.2** Typical experimental workflow for the identification of the complete sequence of naturally generated peptides in dry-cured ham

This result is accepted if the data pass a statistical probability threshold of being a correct match. Proteins can be identified in this way with high-throughput compatibility and a high sensitivity even below the *f*mol range.

Two-dimensional SDS-PAGE and MALDI-TOF mass spectrometry analysis were used to study the myofibrillar and water-soluble fraction of raw ham muscles and dry-cured hams with different ripening times (Di Luccia et al. 2005). The two-dimensional maps showed the progressive disappearance of actin, tropomyosin, and myosin light chains during ripening. Some of the sarcoplasmic proteins in water extracts from pork meat markedly decreased in amount or disappeared totally during ripening. Some of the bands were digested using trypsin enzyme and subsequently analyzed by MALDI-TOF MS. Two fragments of myosin heavy chain and a new form of actin were identified in the myofibrillar fraction whereas tropomyosin α- and β-chains were detected in the water soluble protein fraction.

Sarcoplasmic proteins undergoing proteolysis during the ripening of products were also identified by MALDI-TOF mass spectrometry peptide mass fingerprinting after 2-D AUT-PAGE/SDS electrophoresis (acetic acid–urea–triton polyacrylamide gel in the first dimension and sodium dodecyl sulphate polyacrylamide gel in the second dimension). This separation technique has resulted in the identification of 13 sarcoplasmic proteins and shows higher resolution in their separation in comparison to standard 2-D IPG/SGS-PAGE as well as an easier interpretation of the 2-D maps (Picariello et al. 2006).

Despite knowledge regarding the disappearance of proteins and the generation of novel peptides, the sequences corresponding to the hydrolysis products were not analyzed in any of these studies. The major difficulty in the study of naturally generated peptides resides is the small size of these fragments that cannot be trypsin-digested because they have been hydrolyzed previously by endogenous muscle enzymes. This fact gives double difficulty: the small size of the generated peptides that are sometimes in the limit of some mass spectrometry techniques, and the impossibility of controlling the hydrolysis, having as a consequence a complex mixture of peptides from different proteins with unspecific cleavage sites. In this sense, the use of advanced proteomic techniques such as tandem mass spectrometry is essential to elucidate the sequence of these small peptides.

### *9.5.2 Tandem Mass Spectrometry (MS/MS)*

Tandem mass spectrometry is the method of choice for protein identification in complex mixtures as well as for elucidation of the sequences of the naturally generated peptides through proteolysis.

Tandem mass spectrometry can also be performed on peptides derived by trypsin digestion and involves their fragmentation using collisionally induced dissociation (CID) to produce the characteristic spectrum that is specific for each peptide ion. The identification of the proteins is facilitated by using the generated spectra to interrogate the protein sequence databases. To make separations possible in mass

spectrometry instruments, peptides have to be converted into ions and transferred into the gas phase in an ionization source. The ionization with MALDI and ESI are the most commonly employed in the analysis of peptides derived from meat and meat products. MALDI has the advantage over ESI that it is more tolerant of low concentrations of sample contaminants such as buffers and salts. One of the advantages of ESI is that ions, depending on their molecular mass and structure, may acquire multiple charges. So, the identification of peptides with higher molecular masses may be achieved using a smaller *m/z* ratio interval; also multiple charges fragment with less activation energy in the mass spectrometer.

The ions obtained in the ion source are isolated in the analyzer. The most commonly used analyzers are quadrupoles, time-of-flight instruments, and ion traps. From the point of view of resolution and reproducibility, the TOF analyzers are not as satisfactory as quadrupoles. However, certain advantages compensate for these limitations; TOF instruments are robust, fast in data acquisition, and show very good mass accuracy as well as a virtually unlimited mass range. Specialized personnel are not required, and access to the ion source is very easy. However, if the peptides of interest present molecular masses smaller than 800 Da, it is important to consider that the ions of the matrix used in MALDI ionization, commonly DBA (dihydroxy benzoic acid) or HCCA (α-Cyano-4-hydroxycinnamic acid) to ionize meat peptides, also appear as contaminants between 200 and 800 Da. They can inhibit and make the identification of peptides at that range more difficult if not impossible.

These analyzers can be combined arranging three quadrupoles, two time-of-flight, or a quadrupole and time-of-flight in tandem. There are other possible combinations of analyzers but TOF/TOT and Q/TOF have been the most commonly used in the analysis of complex mixtures of naturally generated peptides.

The first studies that focused on the elucidation of the complete sequence of meat and meat product peptides were performed using automatic Edman degradation. This is a good technique for the analysis of small peptides but it becomes tedious when the objective is to sequence longer peptides. The main disadvantage of Edman degradation is that it only can be used with pure peptides because mixtures of peptides or contaminants present in the sample could mislead the sequencing. In this sense, one peptide from glyceraldehyde-3-phosphate dehydrogenase protein, one peptide from troponin T protein, and three peptides from creatine kinase protein were isolated and identified during post-mortem aging in bovine *longissimus dorsi* muscle. Peptides generated during the aging of beef meat were isolated by HPLC and subsequently analyzed by MALDI-TOF to see the purity of the peaks. Amino acid sequencing was done by automatic Edman degradation (Stoeva et al. 2000).

Regarding meat products, a water-soluble extract of dry-cured ham was fractionated by gel filtration chromatography and fractions with the highest concentration in peptides were separated by reverse-phase and cation-exchange high-performance liquid chromatography. Small peptides of two, three, and four amino acids were sequenced from the N-terminal by automated Edman degradation (Sentandreu and Toldrá 2007b). Five peptides with structures between 151 and 381 amino acids and generated from sarcoplasmic (myoglobin and creatine kinase) and myofibrillar (troponin-I, troponin-T, and myosin light chain-2) proteins were isolated from semi-dry

fermented sausages using SDS-PAGE and RP-HPLC as qualitative methodologies, and subsequently sequenced by automatic Edman degradation (Hughes et al. 2001). A study in dry-cured Parma hams resulted in the identification of a peptide with a very high degree of homology with the N-terminal part of different mammalian pyruvate kinases. The peptide was purified by means of RP-HPLC and identified by its molecular mass and amino acid sequence analysis using automatic Edman degradation (Sforza et al. 2003).

The use of modern mass spectrometry techniques with the analyzers in tandem allows a faster and more reliable identification of the peptide sequences because the obtained MS/MS spectra are matched with the theoretical sequences contained in the databases. Specific peptide sequences of the myofibrillar proteins actin (Sentandreu et al. 2007a), titin (Mora et al. 2009b), myosin light chains (Mora et al. 2011a), and troponin T (Mora et al. 2010), as well as some sarcoplasmic proteins such as creatine kinase (Mora et al. 2009a) and a group of glycolytic enzymes (Mora et al. 2011b; Sforza et al. 2003) have already been identified in dry-cured ham using ESI-Q/TOF and MALDI-TOF/TOF mass spectrometers. In this sense, Table 9.1 shows the number of peptides that have been sequenced in dry-cured ham also including their respective protein of origin.

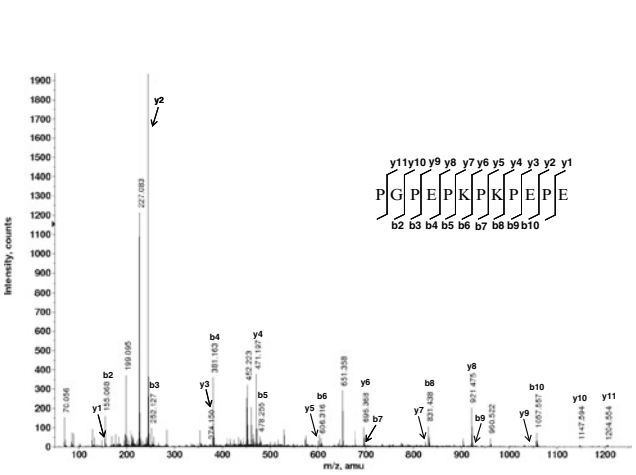
## 9.6 Data Analysis

As has been previously mentioned, the identification of peptides is mostly carried out using database search approaches. This type of data analysis can also be used for the direct analysis of peptides naturally generated by endogenous enzymes (Fricker et al. 2006; Geho et al. 2006; Hardt et al. 2005a, b; Villanueva et al. 2006) although the appropriate choice of the search parameters and sequence database are crucial for successful application of this methodology. In fact, database searching of non-tryptic peptides is less effective due to the lack of charge localization at the N and C termini of the naturally generated peptides. Some of the most popular search engines used in protein identification are Mascot, Sequest, and X! TANDEM, but there are more software packages designed to identify the most likely peptide sequence to match a MS/MS spectrum. Mascot from matrixscience (http://www.matrixscience.com/) is the most widely used software but the choice of its search parameters requires some user expertise, and it has to be previously evaluated with a control sample that in the case of meat peptides used to be bovine serum albumin (BSA). The use of no specific enzyme as well as the error-tolerant option to search the unmatched spectra considering possible modifications are some of the parameters to take into account in the Mascot MS/MS Ions Search when naturally generated peptides are studied.

There are many available databases, but SwissProt and NCBInr are the most commonly used in the identification of meat-derived peptides. Swiss-Prot has approximately 500,000 entries and is considered the best annotated database although it is important to consider that it is nonredundant so it is ideal for PMF

**Table 9.1** Naturally generated protein fragments identified in dry-cured ham

| Protein name | Accession number NCBInr | Number peptides | Sequence coverage (%) | Instrument (MS, MS/MS) | Search engine | Database | References |
|---|---|---|---|---|---|---|---|
| Small peptides (2, 3, and 4 amino acids) | – | – | – | Edman degradation | – | – | Sentandreu and Toldrá 2007b |
| Actin | NP_001161267 | 4 | 8 | MALDI-ToF Qtrap | Mascot | UniProt | Sentandreu et al. 2007a |
| Myosin light chain 1 (MLC 1) | ABK55642 | 9 | 8 | MALDI-ToF | Mascot | NCBInr | Mora et al. 2009b and 2011a |
| | | 137 | 68 | nESI-LC-Q/ToF | Mascot | NCBInr | |
| Titin | AAD00528 | 5 | 10 | MALDI-ToF nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2009b |
| Creatine kinase (CK) | NP_001123421 | 58 | 44 | MALDI-ToF nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2009b |
| Slow troponin T (TnT) | Q75ZZ6 | 2 | 9 | MALDI-ToF/ToF | Mascot | NCBInr | Mora et al. 2010 |
| Fast troponin T (TnT) | BAD15381 | 25 | 18 | MALDI-ToF/ToF | Mascot | NCBInr | Mora et al. 2010 |
| Myosin light chain 2 (MLC 2) | NP_001006592 | 88 | 47 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011a |
| Glycogen phosphorylase (PYGM) | ABF81977 | 2 | 4 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) | ABI29187 | 8 | 8 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| Phosphoglycerate kinase 1 (PGK) | NP_001093402 | 4 | 7 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| Phosphoglycerate mutase 2 (PGAM) | CAD89670 | 7 | 47 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| Enolase (ENO) | NP_001037992 | 18 | 14 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| Piruvate kinase 3 isoform 2 (PK) | XP_001929120 | 2 | 2 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |
| | | 1 | – | Edman degradation | – | – | |
| Lactate dehydrogenase (LDH) | P0039 | 4 | 5 | nESI-LC-Q/ToF | Mascot | NCBInr | Mora et al. 2011b |
| | | | | | Paragon | UniProt | |




| Peptide 14 (651.352+) | | | | |
|---|---|---|---|---|
| | b | Seq. | y | |
| 1 | 98.06 | P | | 12 |
| 2 | **155.08** | G | **1204.62** | 11 |
| 3 | **252.13** | P | **1147.60** | 10 |
| 4 | **381.18** | E | **1050.55** | 9 |
| 5 | **478.23** | P | **921.50** | 8 |
| 6 | **606.32** | K | **824.45** | 7 |
| 7 | **703.38** | P | **696.36** | 6 |
| 8 | **831.47** | K | **599.30** | 5 |
| 9 | **928.52** | P | **471.21** | 4 |
| 10 | **1057.57** | E | 374.16 | 3 |
| 11 | 1154.62 | P | **245.11** | 2 |
| 12 | | E | **148.06** | 1 |

**Fig. 9.3** MS/MS spectrum of ion $651.35^{2+}$ of the main peak obtained after the second reversed-phase chromatography. Peptide sequence matching the product ion spectrum is shown in capital letters, together with observed *b* and *y* ions. The spectrum is presented with its corresponding table showing the ions matched by MASCOT in *bold letters* (Reprinted with permission from Mora et al., 2009b (*J. Agric. Food Chem. 57*, 3228–3234). Copyright (2009) American Chemical Society)

searches, where the loss of one or two peptides is not very important. For MS/MS searches, the NCBInr database is a better choice. This database is large (10,000,000 entries approx.), comprehensive, and nonidentical. In the data analysis of meat-derived peptides, results obtained from both databases used to be very similar although a comparison between SwissProt and NCBInr databases revealed that the NCBInr database provided the highest number of identifications (Mora et al. 2011b). Figure 9.3 shows a MS/MS spectrum obtained with a quadrupole/time-of-flight mass spectrometer after RP-HPLC separation of a size-exclusion chromatography fraction of dry-cured ham.

## 9.7 Future Trends

The proteomic analysis of naturally generated peptides during the dry-cured processing of ham improves knowledge of the post-mortem proteolysis occurring during dry-cured ham processing and the assessment of the peptidases responsible for this action. The peptides generated during the proteolysis in dry-cured ham could be biomarkers of the process and, what is more interesting, they could be acting as bioactive peptides exerting either antimicrobial, antihypertensive, or any other bioactivity in the final product. In recent times, several studies have described the

generation of bioactive peptides, often with cardiovascular promoting properties (usually ACE-I-inhibition or antioxidant effects) following hydrolysis of sarcoplasmic and/or myofibrillar proteins or whole meat muscle with proteolytic enzymes (Di Bernardini et al. 2011, 2012). In this sense, Sentandreu and Toldrá (2007a, b) suggested that the proteolytic action of porcine muscle dipeptidyl peptidases during the ripening period of dry-cured ham could contribute to the generation of ACE-I inhibitory peptides. The angiotensin-converting enzyme inhibitory peptides generated during the curing of meat products have been studied extensively. For example, dipeptidyl peptidases (DPP) could contribute to the generation of antihypertensive peptides among which Arg–Pro showed the strongest angiotensin-converting enzyme inhibitory activity (Jang and Lee 2005; Sentandreu and Toldrá 2007b). The use of these peptides in the development of novel meat products and their potential as ingredients of healthier food requires a better knowledge of the mechanisms that control the generation of these peptides during proteolysis and their possible biological activity, and advanced techniques of proteomics such as the mass spectrometry in tandem play a crucial role in reaching this understanding.

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