Peptidomics on Farm Animal Research

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Abstract Although peptidomics is a discipline complementary to proteomics, since nowadays both mainly rely on analytical strategies based on mass spectrometry, there are fundamental differences. In this chapter, we discuss these differences along with the application of these technologies for the study of the different stages of meat production, from storage to processing to unravel mechanisms that will allow reaching high-quality and safer meat products. The use of peptidomics and the related high-throughput technologies, now relying on mass spectrometry but once also on N-terminal sequencing, is discussed. Clear examples are provided dealing with relevant studies on meat proteolysis and peptide generation occurring during ageing, as well as those produced during ripening of meat products by endogenous and microbial enzymes. Also the involvement of this phenomenon in the development of taste-active compounds is addressed. Finally, the application of novel omics technologies on bacterial identification in food for diagnosis and safety purposes is presented, putting emphasis on their potential advantages and future perspectives.

Keywords Peptidomics • Peptides • Sequence identification • Meat biochemistry • Meat microbiology • Mass spectrometry • Systems biology • Lactic acid bacteria • Meat contamination

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1 General Concepts

1.1 Peptidomics Versus Proteomics

The concept peptidomics is complementary to the more familiar term proteomics, which prompts us to the need to describe the two concepts. Proteomics aims the study of the expression of products of a genome (proteins) in a biological sample with the aim to have a global and integrated vision of the cellular processes at a particular time period. On the other hand, peptidomics will cover the comprehensive study of peptides that are present in a biological sample. Qualitative and quantitative features would be addressed in both cases. The term peptidomics already appeared in some scientific literature published in 2001 (Bergquist and Ekman 2001; Clynen et al. 2001; Minamino 2001; Schulz-Knappe et al. 2001; Verhaert et al. 2001). It is also important to better differentiate between these two terms to establish the difference between a peptide and a protein. It is assumed that peptides are short molecules formed from the linking of various amino acids being in the range from 2 to 50. However, this convention is rather arbitrary and flexible, not being possible in many cases to establish a clear difference, for example, between big polypeptides and small proteins. For that reason, it has also been accepted that a peptide has a less complex structure, being able to contain helices, sheets, other functional subunits or modifications of some particular residues but not reaching the more complex tertiary and quaternary structures as in the case of proteins. Peptides resulting from proteolytic cleavage of proteins will be influenced by these structural elements, which will drive the action of peptidases in the release of peptide products.

1.2 Technological Features in Peptidomics as Compared to Proteomics

Nowadays, proteomics and peptidomics are related disciplines since both rely on analytical strategies based on mass spectrometry (MS), and thus the separation between the two concepts is sometimes blurred. In proteomics, with the aim to facilitate the analysis by mass spectrometry, proteins are usually digested into peptides, which will be further analysed by single MS to obtain their peptide mass fingerprint (PMF) or by tandem mass spectrometry (MS/MS) to get the amino acid sequence of the generated peptides. This is known as the *bottom-up* approach, since the information obtained from the analysis of peptides will serve to reach up to the identity of the protein of origin. Even if the enzymatic digestion will contribute to increase sample complexity, the ease to analyse relatively short peptide sequences as compared to large polypeptide chains largely compensates for this. As a drawback of the digestion process, information about tertiary and quaternary structure of the protein will be lost. In contrast to this, peptidomics

would point at characterizing as much as possible the structure of the target peptides, getting information about the proteolytic processes responsible for their generation and the presence of post-translational modifications. This will be the case of a *top-down* approach, in which it will be necessary to manually evaluate the MS/MS spectra generated and verify that the assignment of peptides is accurate (Schrader et al. 2014). In food peptidomics, this can reveal valuable information about the proteolytic processes and final quality of food and food-derived products.

In *bottom-up* approaches, proteins are cleaved using proteolytic enzymes that have a defined substrate specificity. The most representative example is trypsin, which cleaves proteins and polypeptides having Lys or Arg at the left side (P1 position) of the peptide bond. Due to the frequency in the occurrence of Lys and Arg residues in proteins, this restricted specificity will allow for the generation of medium-size peptides, falling within the range from 7 to 25 amino acids (700–3000 Da), which are ideal for their precise mass determination using current proteomic technology based on the ionization of intact peptides by the so-called "soft" ionization techniques such as matrix-assisted laser desorption/ionization (MALDI) (Karas and Hillenkamp 1988) and electrospray ionization (ESI) (Fenn et al. 1989). Furthermore, fragmentation of peptides based on techniques such as CID (collision-induced dissociation), electron-transfer dissociation (ETD) or some others (Jones and Cooper 2011) will allow for the elucidation of the amino acid sequence in tandem mass spectrometry experiments. Protein digestion using enzymes with defined cleavage sites greatly simplifies the study of the fragmentation mechanisms, making it possible to predict the peptide fragment ions according to the type of MS instrument. This has allowed the development of several bioinformatic algorithms that, together with the expansion of protein databases by massive genome sequencing, makes it possible to analyse these peptide ion product spectra automatically by spectral matching, comparing the obtained MS/MS spectra to theoretical peptide spectra obtained by *in silico* digestion of proteins contained in previously defined protein databases. A probabilistic score derived from these comparisons will allow the identification of the most probable sequence matching our MS/MS data (Panchaud et al. 2012). Several robust and high-throughput MS/MS algorithms have been developed based on the principle of spectral matching (Hernandez et al. 2006).

Apart from making easier the study and prediction of fragment ions, tryptic peptides hold an amino group on their N-terminus, but also most of them have an additional amino group at the side chain of the C-terminal Arg or Lys. If in addition histidine residues occur in the peptide sequence, then charge states higher than 2 can also be observed. This will favour strong peptide signal intensities easily detected by the MS instruments and MS/MS fragmentation spectra of high quality, necessary for an accurate elucidation of the peptide sequence with no ambiguities (Schrader et al. 2014).

Peptides generated by protein digestion are not exclusive of *bottom-up* proteomic studies. As it will be explained below, some *top-down* peptidomic approaches are based in the identification and quantification of some of these sequences as individual entities, constituting the core of the analysis beyond the protein of origin.

Some examples are the use of peptide biomarkers in meat authentication studies (Marbaix et al. 2016; Sentandreu et al. 2010) and nutritional studies (Paolella et al. 2015; Wen et al. 2015) or the generation of bioactive peptides by the action of proteolytic enzymes on animal proteins (Fu et al. 2016; Lafarga et al. 2016; Mirdhayati et al. 2016). Other peptidomic approaches, however, deal with the characterization of the endogenous peptides naturally present in foodstuffs. These peptides, in many cases, will not follow the ideal model of those generated by digestion with known enzymes, having more difficulties for MS analyses. Special attention needs to be paid to the design of extraction protocols in order to minimize the peptide proteolysis after sample collection (Dallas et al. 2015). Because the enzymatic processes that are responsible for the formation of endogenous peptides are complex and most often imply the action of unknown peptidases and/or unspecific cleavages, size can change considerably, having sequences too large or too small for a proper identification and quantification using standard MS applications that have been developed for medium-size peptides in *bottom-up* proteomics. In the case of large peptides, elucidation of the amino acid sequence from MS/MS spectra of peptides having charge states +5 or more becomes more complicated than for peptides having charge states +2 or +3. Moreover, the non-specificity for cleavage sites makes identifications based on spectral matching considerably more complicated and time consuming in database searches. In the case of small peptides (lower than six amino acids), ambiguity in the elucidation of the sequence considerably increases, and database searches usually bring several identifications with similar scores and a confusing variety of parental proteins. For these cases, especially in very small peptides (two–four amino acids), other, less efficient bioinformatic tools not based on database search are required, together with manual verification of the MS/MS sequencing results. Another challenging aspect of small peptides is the difficulty to be retained in the reverse phase C_{18} columns that are massively used for tryptic peptides. Unless the small peptides contain some hydrophobic amino acids, they will not be retained by the reverse phase columns, and thus other separation alternatives such as ion-exchange, size-exclusion or hydrophilic interaction (HILIC) chromatography will become necessary.

1.3 Types of Peptides

According to their synthesis, three classes of peptides can be distinguished in biological samples (Zürbig and Mischak 2008):

(a) *Ribosomal peptides*, which will be those that are synthesized by translation of mRNA. These include peptides that function as hormones and signalling transmitters in superior organisms. In the case of lower organisms, these peptides can exert antibiotic action (Mahlapuu et al. 2016). In general, they are linear, though other nonlinear forms also exist (Craik et al. 2016; McIntosh et al. 2009).

(b) *Nonribosomal peptides*, which are those synthesized using a modular enzyme complex. These peptides are mainly ascribed to unicellular organisms, plants and fungi. They appear as a family of related compounds sharing a common core structure. In general they are cyclic, although linear structures also exist (Horst and Niedermeyer 2016).
(c) *Processed peptides*, which are the result of non-specific proteolysis in digestive/proteolytic processes: There are many events both inside and outside of cells by which proteins or longer peptides are cut into smaller fragments. Most of works dealing with peptidomics in food science and domestic animals revised in the present chapter are part of this group. Indeed, food scientists have mainly worked on the characterization of peptides generated from muscle tissue either by the action of digestive enzymes or by the proteolytic action occurring during the processing of meat and meat-derived products due to the action of endogenous muscle peptidases or peptidases coming from starter cultures. In the literature, apart from muscle tissue, there are also many important contributions dealing with peptides derived from milk proteins in both milk and milk-related products. Since quite recent and interesting reviews have been published, we will not focus on this part but encourage readers to also look at some of these contributions (Picariello et al. 2012; Roncada et al. 2012; Sanchez-Rivera et al. 2014). Other issues dealing with peptide research in domestic animals that have been also recently reviewed or reported and, thus out of the scope of this chapter, are researched into food authentication studies based on identification of species-specific peptide biomarkers (Ortea et al. 2016; Sentandreu and Sentandreu 2011, 2014) and characterization of bioactive peptides derived from farm animal sources (Fu et al. 2017; Lafarga and Hayes 2014; Stadnik and Keska 2015; Udenigwe and Howard 2013).

2 Peptidomics as Related to Postmortem Muscle Proteolysis

The interest of meat scientists in the study of postmortem proteolysis occurring in skeletal muscle immediately after animal slaughter has been a major issue for several decades. The reason is that this phenomenon is strongly linked to the development of the main quality attributes of meat and derived meat products. Among them, tenderness has been traditionally considered as the most important meat quality attribute by consumers (Ouali et al. 2013). Tenderness can be defined as the development of adequate toughness of meat due to the ageing process occurring after slaughter. This is because during this time, generally up to 2–3 weeks in the case of beef, there is an intense breakdown of muscle structure due to the action of several endogenous proteolytic systems, giving rise to a decrease in the mechanical resistance of meat and the generation of large polypeptides. With respect to dry-cured meat products, processing times are generally longer than

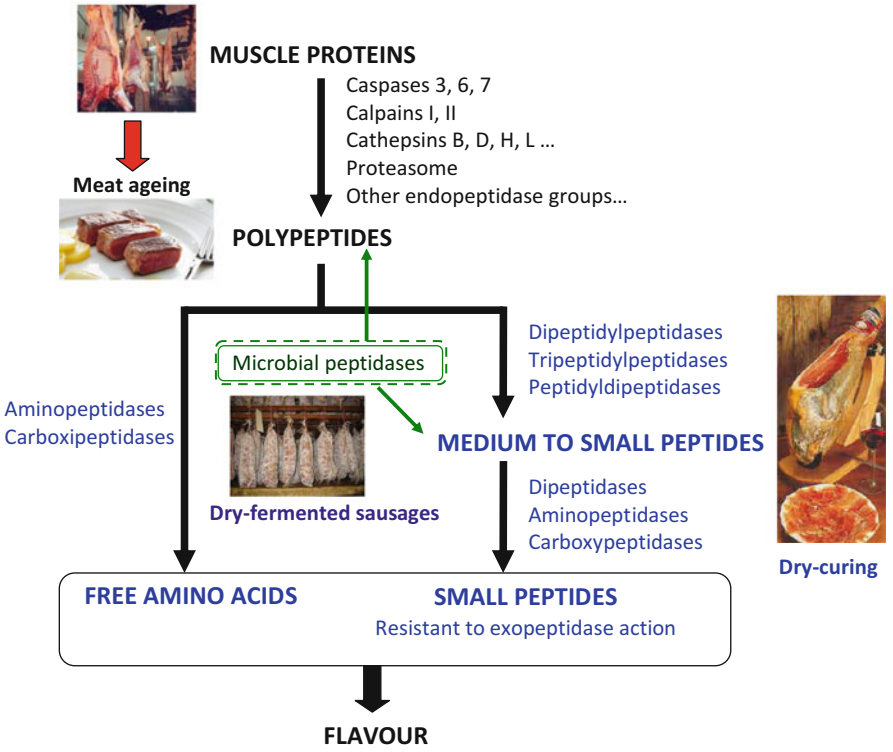


Fig. 1 Overview of the proteolytic systems implicated in the degradation of muscle proteins during both postmortem meat ageing and dry-curing

meat ageing. In the case of dry-cured ham, for example, processing times of 12 months and even more are usual. As a consequence, degradation of muscle proteins will be much more intense, and large polypeptides will be further degraded to generate medium-to-small peptides, together with free amino acids, that will contribute to reach their appreciated final flavour and texture characteristics (Fig. 1). Postmortem proteolysis is not a single process but a complex set of enzymatic actions driven by a wide variety of endo- and exopeptidase groups endogenous of muscle tissue that will be active far beyond animal slaughter. In the case of meat products in which starter cultures have been used during processing, proteolytic reactions will be even more complex due to the additional implication of bacterial proteolytic systems. This complexity of the proteolytic events occurring in postmortem muscle is summarized in Fig. 1. With respect to the proteolytic events occurring early postmortem, triggering of apoptosis is most probably the first step responsible for the degradation of protein cell constituents, with the activation of both initiator and executor caspases (Ouali et al. 2013). After this, other endopeptidase groups such as calpains, cathepsins, proteasome, etc. will continue the degradation of the myofibrillar structure. In that respect, additional

research is needed to clearly understand the role and importance of each peptidase group in the initial steps of muscle degradation (Sentandreu et al. 2002).

During the processing of meat products, the proteolytic action extends far beyond the initial days after slaughter; as a result, large polypeptides generated during the initial steps will be further degraded to smaller peptides and free amino acids due to the action of exopeptidase groups such as di- and tri-peptidilpeptidases, peptidyl dipeptidases, dipeptidases, carboxypeptidases and aminopeptidases (Fig. 1). Recent research works based on mass spectrometry applied to peptidomics have notably contributed to gain knowledge about the main protein substrates and peptides generated during postmortem muscle proteolysis, but it is also true that much work is still needed to explain the whole proteolytic processes as a way to predict and standardize the final quality of meat and processed meat products.

2.1 Meat Storage and Development of Its Final Quality

There have been notable efforts during the last two decades in the development of state-of-the-art peptidomic approaches focused on the study of peptides generated during the storage of meat in relation to product quality. Table 1 summarizes the main contributions in this field, highlighting the topics of research and the different strategies that have been employed to achieve the identification of peptides derived from muscle proteins. With respect to the identification of peptides generated during meat storage, one of the pioneer contributions is the work carried out by Nishimura and colleagues. In 1995, this group carried out the identification of a peptide produced during beef meat storage up to 10 days postmortem. To achieve this, trichloroacetic acid (TCA) muscle-soluble extracts were fractionated by HPLC, allowing isolation and collection of the target peptide. Determination of the peptide sequence was achieved by N-terminal sequencing using automated Edman degradation, identifying a 15-amino acid peptide derived from bovine muscle troponin T (Nakai et al. 1995). A similar sequence, found in peptides generated during pig meat ageing, was shown to be able to suppress the sour taste (Okumura et al. 2004). All these results contributed to establish troponin T as one of the key proteins involved in meat tenderization. Following a similar approach, Stoeva et al. (2000) were able to identify small peptide fragments derived from glyceraldehyde-3-phosphate dehydrogenase, troponin T and creatine kinase during bovine meat storage up to 15 days postmortem. In addition to N-terminal sequencing, these authors made use of MALDI-TOF MS for determining the exact molecular mass of the isolated peptides. They proposed the identified peptides as potential good indicators of beef meat quality, suggesting that rapid analytical procedures such as HPLC, capillary electrophoresis or immunoassays could be developed for routine analysis of these quality markers (Voelter et al. 2000).

A different strategy was carried out by Muroya et al. (2004) at the time to reveal the identity of large peptide fragments (25–32 kDa) generated during postmortem ageing of bovine muscle. Western blot analysis revealed that these polypeptides

Table 1 Peptidomics as related to meat storage

Topic	Focus	Peptide separation	Peptide identification	Peptides derived from	Animal model (References)
(1) Peptides generated during meat storage/ageing	(1.A) Small peptides	HPLC	N-terminal sequencing	Troponin T	Beef (Nakai et al. 1995)
				Troponin T, GAPDH	Pork (Okumura et al. 2003)
			MALDI-TOF/N-terminal sequencing	GAPDH, troponin T, CK	Beef (Stoeva et al. 2000)
			ESI-tripleQ/N-terminal sequencing	Troponin T	Pork (Okumura et al. 2004)
		1D-PAGE/HPLC/capillary electrophoresis	ELISA/N-terminal sequencing	Troponin T, GAPDH	Beef (Voelter et al. 2000)
	(1.B) Polypeptides	1D-PAGE	Western blot/N-terminal sequencing	Troponin T	Beef (Muroya et al. 2004)
			MALDI-TOF/LC-ESI-QTOF	MHC	Beef (Sawdy et al. 2004)
				GAPDH, troponin I, CK, AK	Pork (Park et al. 2007)
			MALDI-TOF/TOF	GAPDH, troponin T, Troponin I, MHC, PK	Beef (Sierra et al. 2012)
		2-D-PAGE	2-D-PAGE patterns comparison	Muscle proteins	Pork (Lametsch and Bendixen 2001)
			MALDI-TOF	Actin, myosin, troponin T, GP, CK, PPH, myokinase, PK, DHLST	Pork (Lametsch et al. 2002, 2003)
				Actin, myokinase, CK, α-crystallin	Pork (Morzel et al. 2004)
				Actin, GPDH, CK, PGK, HSC 71, GAPDH, β-enolase	Beef (Laville et al. 2009)
				Actin, MyBPH, HSP27, α-crystallin	Beef (Morzel et al. 2008)
			MALDI-TOF/Western blot	Troponin T	Beef (Muroya et al. 2007)
			LC-ESI-ion trap MS/MS	Actin, CK, GAPDH, MLC, MHC, titin, troponin I, troponin T, UCE	Beef (Polati et al. 2012)

(2) Specificity of enzymes implicated in muscle proteolysis	(2.A) Calpains	1-D/2-D-PAGE	MALDI-TOF	Myofibrillar proteins	Pork (Lametsch et al. 2004)
		1-D-PAGE/HPLC	MALDI-TOF/N-terminal sequencing	Troponin T	Rabbit (Hughes et al. 2001)
			Western blot/N-terminal sequencing	Troponin T	Pork (Kitamura et al. 2005)
		1-D-PAGE	Western blot/N-terminal sequencing	Desmin	Pork (Baron et al. 2004)
	(2.B) Cathepsins	1-D-PAGE/HPLC	[a]PDMS-TOF/N-terminal sequencing	F-actin	Beef (Hughes et al. 1999, 2000)
		1-D-PAGE	Western blot/N-terminal sequencing	Desmin	Pork (Baron et al. 2004)
	(2.C) Caspases	1-D-PAGE	MALDI-TOF/ Western blot	Desmin, troponin I, MLC, actin, troponin T	Pork (Kemp and Parr 2008)
			LC-ESI-ion trap MS/MS	Actin, MHC	Pork (Rodriguez-Frometa et al. 2013)

GAPDH glyceraldehyde-3-P dehydrogenase, *GPDH* glycerol-3-P dehydrogenase, *CK* creatine kinase, *AK* adenylate kinase, *GP* glycogen phosphorylase, *PPH* phosphopyruvate hydratase, *PK* pyruvate kinase, *PGK* phosphoglycerate kinase, *DHLST* dihydrolipoamide succinyltransferase, *HSC 71* heat-shock cognate 71 kDa, *MLC* myosin light chain, *MHC* myosin heavy chain, *UCE* ubiquitin-conjugating enzyme E2N-like

[a]*PDMS-TOF* plasma desorption time-of-flight mass spectrometry

were fragments of troponin T. N-terminal sequencing corresponding to the first amino acid residues of these fragments confirmed troponin T as the protein of origin and allowed to define the N-terminal cleavage sites responsible for the generation of these fragments.

With the aim to study changes in muscle proteome during storage by pointing at a larger set of protein coverage, Bendixen and co-workers introduced two-dimensional gel electrophoresis (2-DE) for characterizing changes occurring in pig muscle during postmortem storage. This technique has proved to be particularly suitable for the study of large polypeptides; however, gel electrophoresis approaches are limited in the fractionation of peptides lower than 3–5 kDa since they will be eluted from the gel unless special protocols are applied. Protein changes were initially reported by comparing 2-DE patterns of muscle samples taken at different postmortem times (Lametsch and Bendixen 2001), whereas identification of differently abundant spots was accomplished by trypsin digestion followed by MALDI-TOF MS (Lametsch et al. 2002). This way, they were able to identify 18 polypeptides generated from postmortem storage that could serve as possible markers of pork quality. Furthermore, 2-DE proteome analysis established interesting correlations between tenderness development and postmortem degradation of actin and myosin (Lametsch et al. 2003). As seen in Table 1, the use of 2-DE as a high-throughput resolving technique coupled with different MS approaches for spot identification has been widely used in subsequent works to characterize the generation of considerable amounts of polypeptides during meat storage in relation to tenderness. There are also some works reporting the use of one-dimensional electrophoresis (1-DE) as the fractionation step to separate and further identify, by MS approaches, some proteolytic fragments that were found to correlate with ultimate beef tenderness (Sawdy et al. 2004; Sierra et al. 2012). Compared to automated Edman degradation, current high-throughput peptide sequencing based on tandem mass spectrometry (MS/MS) has made affordable the generation of massive sequencing data and the analysis of meat peptidome at great scale. As a drawback, the standard identification of polypeptides based on *bottom-up* MS approaches requires a prior trypsin digestion step that most often does not give information about the identity of neither N- nor C-terminal ends, thus being difficult to elucidate the cleavage sites responsible for the generation of these polypeptides.

Peptidomic research has been decisive in gaining knowledge about the role that the different endopeptidase groups can have in the development of meat tenderness through the cleavage of key proteins. Works carried out by Hughes and collaborators showed the ability of cathepsins B and D to hydrolyse bovine F-actin. Cathepsin B was able to generate both large (29–35 kDa) and small peptides of around 1 kDa (Hughes et al. 1999), whereas cathepsin D caused an extensive degradation of actin, generating small peptides (0.8–2.2 kDa) that could be mainly detected by HPLC (Hughes et al. 2000). The combined use of N-terminal sequencing and peptide mass determination using mass spectrometry allowed authors characterizing, for the small peptides, both the N- and C-terminal cleavage sites of the cathepsin endopeptidase action. On the contrary, incubation of desmin with

cathepsin B caused hydrolysis of this protein but through its peptidyl dipeptidase action, liberating successive dipeptides from the C-terminal end (Baron et al. 2004).

The action of calpains on myofibrillar proteins has been also a major topic of research since this enzyme group is considered to play also an important role in postmortem myofibril disruption (Sentandreu et al. 2002). Using the aforementioned strategy of combining N-terminal sequencing and mass spectrometry for peptide mass determination, Hughes et al. (2001) were able to characterize the proteolytic action of μ-calpain on rabbit troponin T. These authors were able to identify some peptides, as well as to characterize cleavage sites of the calpain action at both N- and C-terminal sides, for both large polypeptides (18–22 kDa) and medium-to-small peptides (2–5 kDa). A similar work was carried out by Kitamura et al. (2005); however, the molecular mass of generated troponin T peptides was not determined; thus, only the nature of N-terminal cleavage site was elucidated. In a different approach, using 2-DE and spot identification by in-gel digestion and MALDI-TOF MS, Lametsch et al. (2004) confirmed the hydrolysis of troponin T by μ-calpain together with eight additional myofibrillar proteins. They identified polypeptide fragments coming from actin, tropomyosin α4, myosin heavy chain and myosin light chain 1. They also reported that the degree of hydrolysis of both actin and myosin by the action of μ-calpain was rather low compared to other protein substrates. Considering the hypothesis that postmortem muscle proteolysis is a multienzymatic process, involving different peptidase groups in addition to cathepsins and calpains (Sentandreu et al. 2002), further studies were driven to elucidate the ability of other peptidases to hydrolyse actin and myosin more efficiently.

The way to look at the conversion of muscle into meat experienced a step forward from the conceptual point of view when the group of Ouali and co-workers introduced in 2006 the hypothesis of programmed cell death or apoptosis as one of the first events occurring in postmortem muscle after animal bleeding, providing answers to observations that remained unclear by that time. As depicted in Fig. 2, the onset of apoptosis in the muscle would probably imply the activation of caspases as the first peptidase group initiating the cell dismantling process and facilitating the action of the rest of peptidase groups, with important consequences on the development of the final quality of meat (Ouali et al. 2006). Following this idea, Kemp and Parr (2008) investigated the ability of caspase 3 to disrupt myofibrillar proteins by means of SDS-PAGE and MALDI-TOF MS. They found that caspase 3 was able to efficiently degrade desmin and troponin I, observing also the appearance of degradation products at around 32, 28 and 18 kDa, which were identified as fragments of actin, troponin T and myosin light chain 3, respectively. These findings confirmed the hypothesis of caspase action on postmortem muscle proteolysis and development of meat tenderness. This was also supported by the results obtained by Rodriguez-Frometa et al. (2013), who studied the action of caspases 3 and 7 on both bovine and porcine myofibrillar proteins based on SDS-PAGE followed by identification of fragments using LC-ESI-MS/MS. According to their results, caspase 7, but not caspase 3, efficiently hydrolysed myosin heavy chain of both animal species, generating a polypeptide of around

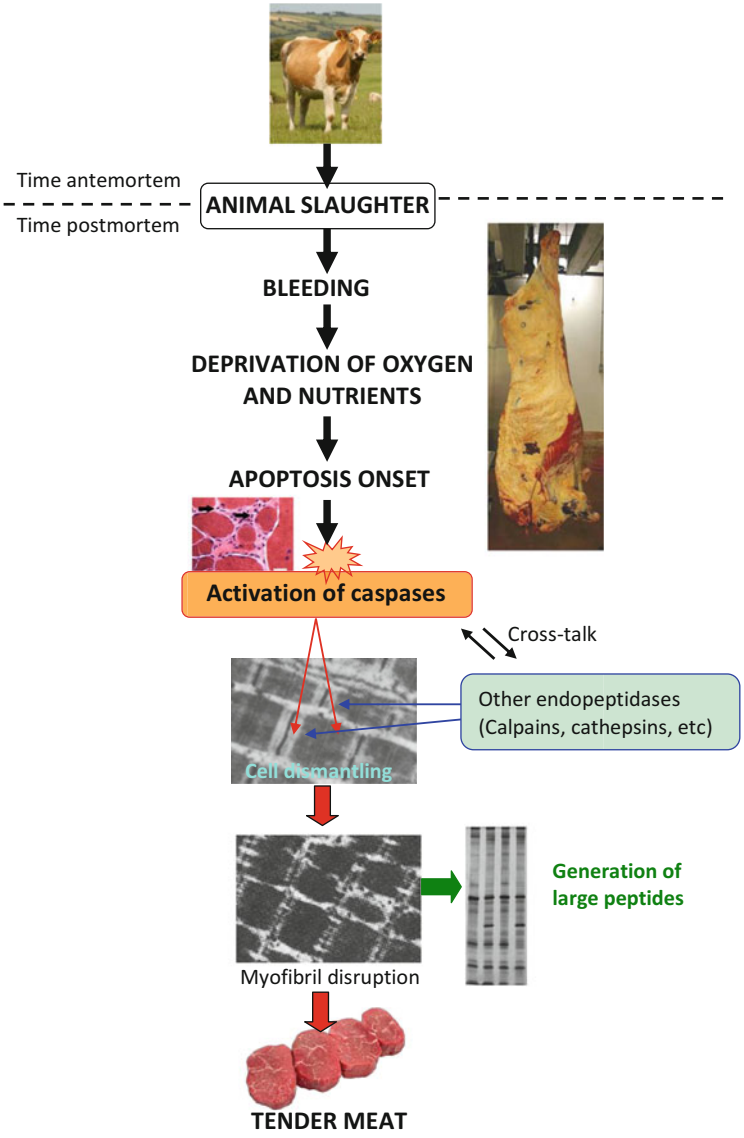


Fig. 2 Schematic view illustrating the conversion of muscle into meat after animal slaughtering. Triggering of apoptosis by caspase activation would be the first step in the dismantling of muscle myofibrillar structure, following the concerted and complementary action of other endogenous endopeptidase groups

200 kDa. On the other hand, porcine and bovine myosin light chain 1 was extensively hydrolysed by caspase 3 but not by caspase 7, suggesting a complementary and coordinated action between these two effector caspases during cell dismantling. But probably the main hallmark of caspase-mediated proteolysis on both porcine

and bovine myofibrillar proteins was the generation of a polypeptide of around 30 kDa of remarkable intensity, indistinctive of either caspase 3 or caspase 7 action. MS/MS data of tryptic peptides generated from these polypeptides revealed that it was a fragment of actin in all cases, supporting the idea that caspases would be probably the main peptidase group, through triggering of apoptosis, implicated in the early postmortem degradation of the actomyosin complex during the development of meat tenderness (Ouali et al. 2013). Despite the commented advances on peptidomic research in relation to the conversion of muscle into meat, there is still a long way to do if we want to fully characterize the main proteolytic products of muscle peptidases to really assess the real contribution of each group to the final tenderness and overall meat quality.

2.2 *Proteolysis During the Processing of Meat Products*

Studies on Non-Fermented Meats The main contributions of peptidomic research dealing with the quality of processed meat product are summarized in Table 2. First works were focused on the characterization of taste-related peptides present in different types of dry-cured ham. Sforza et al. (2001) attempted to characterize small peptides present in Parma ham in relation to the occurrence of a bitter taste. Since peptide analysis was carried out by HPLC coupled with single MS detection, the exact peptide sequence was difficult to achieve for each full scan spectrum; however, they were able to determine the presence of lipophilic amino acids in peptides, establishing a correlation to bitter taste. Following a similar approach but using N-terminal sequencing, these authors were able to elucidate the sequence of a 25-amino acid peptide having a correlation to ageing time but not to bitter taste, supporting the assumption that the main contribution to bitter taste may be due to free amino acids and small peptides (Sforza et al. 2003). This was in agreement to the results obtained by Sentandreu et al. (2003), who determined the sequence of peptides contained in the size-exclusion savoury fractions of Serrano dry-cured ham, most of them being dipeptides. More recently, taste-active peptides associated to umami taste from Jinhua and Parma hams were elucidated by Dang et al. (2015) using MALDI-TOF/TOF MS.

Research has also been applied to characterize the great variety of peptides generated during the ripening of dry-cured meat products, as can be seen in Table 2. Usual approaches include fractionation of deproteinized ham extracts by classical size-exclusion chromatography and HPLC followed by peptide sequence identification using tandem mass spectrometry. In the work carried out by Sentandreu et al. (2007), they identified four oligopeptides derived from the degradation of actin in Serrano dry-cured ham using both MALDI-TOF MS and ESI-MS/MS. Some of the cleavage sites associated to the generation of these fragments were shown to be produced by the action of cathepsin D on bovine F-actin (Hughes et al. 2000), thus supporting a relevant action of this enzyme during the processing of dry-cured ham. Peptides identified in Serrano dry-cured ham also include many others derived from

Table 2 Peptidomics in processed meat products

Topic	Peptide fractionation/ separation	Peptide identification	Product	References
(1) Study of peptide generation during the processing period	SDS-PAGE + HPLC	ESI-MS/MS + Western blot	San Daniele, Parma and Toscano ham	Fabbro et al. (2016)
		MALDI-TOF/TOF	Argentinean fermented sausages	López et al. (2015a)
	2-DE	MALDI-TOF	Parma and San Daniele ham	Di Luccia et al. (2005), Picariello et al. (2006)
	2-DE + HPLC	MALDI-TOF+ ESI-MS/MS	Bayonne ham	Theron et al. (2011)
	Size-exclusion + HPLC	MALDI-TOF/TOF	Serrano ham	Mora et al. (2010)
		MALDI-TOF + ESI-MS/MS	Serrano ham	Sentandreu et al. (2007), Mora et al. (2009a, b), Gallego et al. (2015b)
		ESI-MS/MS	Serrano ham	Mora et al. (2011a, b, 2015b), Gallego et al. (2014, 2016)
			Meat-fermented models	López et al. (2015b, c)
			Serrano ham + dry-fermented sausages	Mora et al. (2015c)
	Solid phase extraction + HPLC	ESI-MS/MS	Serrano ham	Gallego et al. (2015a)
	Deproteinized extract	MALDI-TOF/TOF	Dry-fermented sausages	Mora et al. (2015a)
(2) Characterization of taste peptides	Size-exclusion + HPLC	N-terminal sequencing	Serrano ham	Sentandreu et al. (2003)
		MALDI-TOF/TOF	Jinhua and Parma ham	Dang et al. (2015)
		ESI-MS	Parma ham	Sforza et al. (2001)
		N-terminal sequencing + ESI-MS	Parma ham	Sforza et al. (2003)
(3) Characterization of raw and/or final product according to animal characteristics	2-DE + HPLC	MALDI-TOF+ ESI-MS/MS	Kraski prsut ham	Skrlep et al. (2011)
	HPLC	ESI-MS/MS	Serrano ham	Mora et al. (2016)

myofibrillar proteins such as troponin T (Mora et al. 2010), titin (Gallego et al. 2015b) or myosin light chains (Mora et al. 2009b, 2011a), for example, which will be directly related to the final texture and taste characteristics of this product. In the same line, an intense proteolysis has also been reported for glycolytic enzymes at the end of dry-curing with the identification of small and medium peptides (Mora et al. 2009a, 2011b, 2015b). Under the same approach, Gallego et al. (2016) studied the evolution of peptide formation along the dry-curing process with the aim to identify potential biomarkers of the curing time and final product quality.

In the case of Parma and San Daniele hams, the use of 2-DE, trypsin hydrolysis and MALDI-TOF MS allowed reporting the presence of large polypeptides of myosin heavy chain still at 14 months of curing, whereas no such polypeptides were found for actin and myosin light chain, indicating an extensive proteolysis, thus as proof of a ripening time of at least 12 months (Di Luccia et al. 2005). The same authors reported a separation approach, alternative to conventional 2-DE, which combined acetic acid, urea, and Triton X-100 polyacrylamide gel electrophoresis, with standard SDS-PAGE. This would allow achieving a higher resolution in the separation of complex mixtures of basic proteins having also similar molecular weights, which is the case of water-soluble extracts of meat and meat products. Concomitant to the disappearance of several sarcoplasmic proteins, they reported the generation of several polypeptides ranging from 14 to 38 kDa in several Italian dry-cured meat products (Picariello et al. 2006). 2-DE coupled with MS identification of spots of interest has also been used in the study of proteome changes and polypeptide formation in other dry-cured ham types. In the case of Kraski prsut ham, pig genotype influenced the quantity of an actin polypeptide, whereas the salt level had a marked effect on the quantity of several protein fragments, indicating its important effect on proteolysis during dry-curing (Skrlep et al. 2011). This is in accordance to the results obtained in Bayonne dry-cured ham, where a more intense formation of protein fragments was reported in the *biceps femoris* compared to *semimembranosus* muscle as consequence of a higher proteolysis due to the fact that the enzyme inhibitory effect of salt occurs later in this muscle, which is protected by rind and fat (Theron et al. 2011).

Studies on Fermented Meats In the case of meat products in which bacterial fermentation occurred and led mainly by lactic acid bacteria (LAB), meat protein degradation will be a quite more complex process. As mentioned before, the proteolytic system of the microorganisms growing spontaneously or added as starter cultures will complement meat endogenous proteolytic machinery to achieve protein hydrolysis during ripening. Prediction and/or characterization of the final quality of meat-fermented products is a difficult task since a high number of factors such as the type of raw materials, technologies and starter cultures are involved in the process. The identification of the protein fragments naturally generated during sausage fermentation and ripening would be beneficial in order to better understand proteolysis and flavour development mechanisms that occur during the processing of fermented products. Recently, López et al. (2015a, b, c) have focused their studies on fermented meat proteolysis applying both proteomic and peptidomic

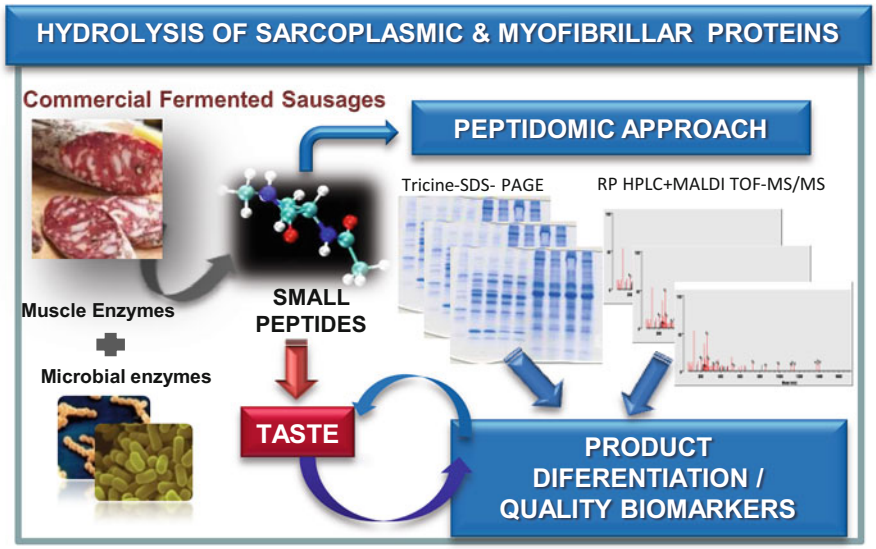


Fig. 3 Schematic summary of proteolysis that occurred in Argentinean fermented sausages carried out by López et al. (2015a) from a peptidomic approach

approaches. In the first work, low molecular weight (LMW) peptides (< 3 kDa) and protein profiles from commercial Argentinean fermented sausages obtained by tricine-SDS-PAGE and RP-HPLC-MS, respectively, allowed to distinguish two different types of fermented sausages, although no specific biomarkers relating to commercial brands or quality were recognized (Fig. 3). Thirty-six LMW peptides arising from sarcoplasmic (28) and myofibrillar (8) proteins were identified. These peptides had been originated from α-actin, myoglobin and creatine kinase M-type but also from the hydrolysis of other proteins not previously reported. Results showed that although muscle enzymes exerted a major role on peptidogenesis, microbial contribution cannot be excluded. This work represents the first peptidomic approach for fermented sausages, thereby providing a baseline to define key peptides acting as potential biomarkers (López et al. 2015a). Subsequently, the study of the degradation of proteins was approached in a beaker model system inoculated with a selected autochthonous starter culture to evaluate the role of the microorganisms in the proteolytic process. Sausage models inoculated with *Lactobacillus curvatus* CRL705 and *Staphylococcus vitulinus* GV318 were incubated 10 days at 22 °C. Low molecular weight peptides (<3 kDa) derived from sarcoplasmic and myofibrillar proteins were analysed by 2-DE and LC-MS/MS and complemented with amino acid profiles, in order to provide a whole map of proteolysis results (Fig. 4). A diverse number of protein fragments were identified. Results indicated that peptides mainly arose from myoglobin, creatine kinase, glyceraldehyde-3-phosphate dehydrogenase and fructose-biphosphate aldolase (ALDOA). Also the hydrolysis of actin, myosin light chain 1/3 (MLC 1/3), myosin

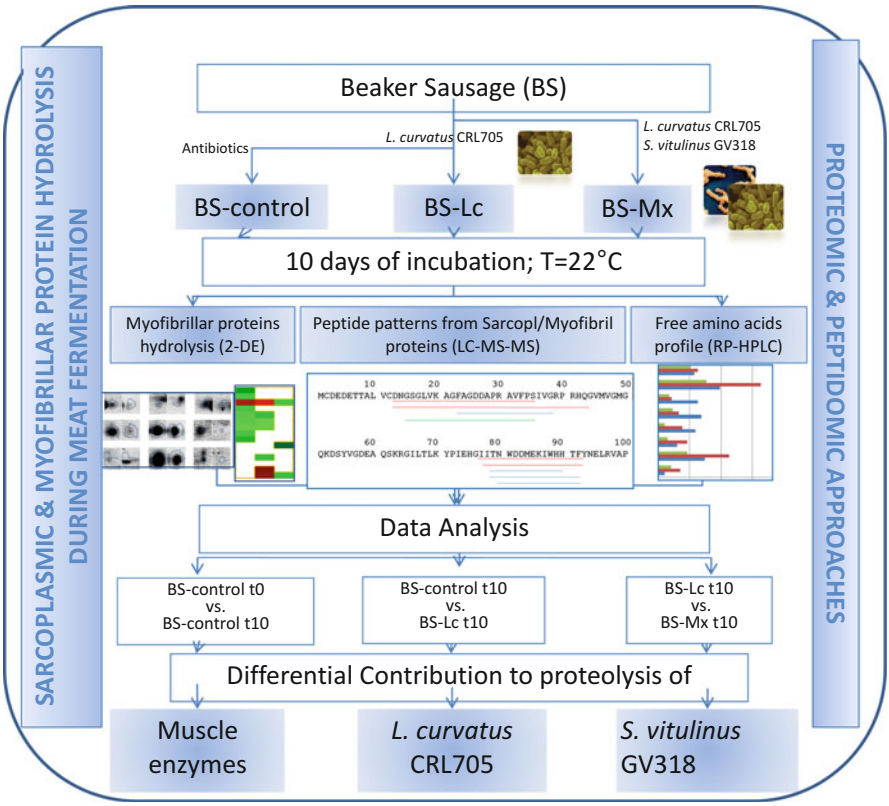


Fig. 4 Graphical summary of López et al.'s (2015b, c) research in which proteolysis of sarcoplasmic and myofibrillar protein hydrolysis by the action of an autochthonous starter culture was studied in a beaker sausage model using a proteomic and peptidomic approach

regulatory light chain 2 (MRLC-2) and myosin heavy chain (MHC) were evidenced by 2-DE. Thirty-three peptides arisen from troponin T, MRLC-2 and particularly from actin were identified. These results showed that, in addition to the endogenous enzymes, the starter culture enhanced the hydrolysis of the above-mentioned proteins. *L. curvatus* CRL705 highly enriched both peptide pattern and amino acid concentrations. The primary structure of actin was highly susceptible to degradation by the starter culture especially in three different regions. Additionally, the essential role of exopeptidases—from meat and bacteria—was evidenced by the diversity of actin-derived peptides during fermentation. These studies improved the knowledge of the proteolysis of sarcoplasmic and myofibrillar proteins, as well as the role of the studied autochthonous starter culture. In fact, the use of a specific autochthonous starter culture guarantees hygiene and tipicity of fermented sausages. The identification of new peptides as well as new target proteins by means of peptidomics represents a significant step towards the elucidation of the role of microorganisms in meat proteolysis. Moreover, these peptides may be further used

as biomarkers capable to certify the use of the applied autochthonous starter culture described here (López et al. 2015b, c).

3 Meat Microbiology: Introduction to Meat Ecosystems

In animals under good condition, tissues that eventually develop into meat are usually considered to be germ-free. However, if not properly handled, processed and preserved, meat will support the growth of microorganisms, thereby creating a significant health risk. Usually contamination arises during the slaughter, dressing, cutting and/or packaging process (Koutsoumanis and Sofos 2004; Sharma and Chattopadhyay 2015). Sources of contamination are diverse: faeces, ingestion, hides, lymph nodes and/or intestines of the animals. Although some differences exist between animal species regarding the type and frequency of microorganisms isolated from carcasses, the same bacterial species from beef, pork, sheep and even chicken carcasses are generally isolated (Zhao et al. 2001).

3.1 Meat Contamination: Pathogenic Microorganisms

In recent years, the major short-term health risks associated with the consumption of meat have been from infections with enteric pathogens. *Campylobacter*, *Salmonella* and pathogenic *E. coli* all colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. *Salmonella* is the main causative agent of food-borne outbreaks reported in Europe in 2014 (EFSA European Food Safety Authority 2015). High levels of *Salmonella* in the meat may arise from animal production practices at the rearing stage as well as cross-contamination after slaughter (McEvoy et al. 2006). This pathogen is most frequently detected in poultry meat followed by turkey and pork meat (Carraturo et al. 2016). Food-poisoning staphylococci are also widely distributed; meat contamination is often connected to poor hygienic practices during slaughtering, transportation, chopping, storage and points of sale by the personnel implicated in the manufacture process. Moreover contaminated meat can move staphylococci to different processing surfaces and packaging materials (Karmi 2013). The etiologic agent is *Staphylococcus aureus*, and its related heat-stable enterotoxins are a major cause of food-poisoning cases and outbreaks worldwide (Liu et al. 2006). For instance, 50 isolates of methicillin-resistant *Staphylococcus* spp. were detected recently in raw meat samples in Nigeria (52% from pork, 28% from beef and 20% from chicken samples) (Igbinosa et al. 2016).

On the other hand, Shiga toxin-producing *Escherichia coli* (STEC) emerged as a food-borne pathogen more significant than other well-known ones because of the severe consequences of infection, its low infection dose, its acid tolerance and its association with ruminants used for food (Hussein and Bollinger 2005). STEC is of

major concern for the sustainability of the meat industry and a serious threat for public health. As such, STEC is still a very serious menace that can jeopardize the sustainability of beef- and pork-meat chain. In fact pigs are also reservoirs of STEC. The entrance of these strains into the food chain implies a risk for consumers because of the severity of haemolytic uremic syndrome. Colello et al. (2016) have recently reported the prevalence and characterization of STEC during pork production.

Listeria monocytogenes has continued to raise food safety concerns, especially with respect to ready-to-eat (RTE) products. Listeriosis, caused by this pathogen, is a significant public health concern as a result of its clinical severity and high mortality rates (Vaillant et al. 2005).

Species within the genus *Campylobacter* and *Yersinia* have also emerged as pathogens of human public health concerns (Buncic et al. 2014). Campylobacteriosis was the most commonly reported zoonosis in the European Union (EU) since 2008. In food, the incidence of *Campylobacter* remained high in broiler meat. Positive findings for *Yersinia* were mainly reported in pork meat and products thereof (EFSA 2015).

As mentioned before, food contamination with these pathogens occurs generally along the food chain (production, processing, distribution, retail marketing and handling). In consequence, microbial food safety is an increasing public health worry worldwide. Many epidemiological studies have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these pathogens (Zhao et al. 2001). To prevent food-borne outbreaks related to harmful microorganisms, the official control of meat is critical in order to verify and ensure the safety of products and, therefore, guarantee public health (Carraturo et al. 2016). As proposed by many authors, effective control of meat-borne pathogens requires an integrated approach involving all the actors of meat chain, the use of Good Manufacturing/Good Hygienic Practice (GMP/GHP) and Hazard Analysis and Critical Control Point (HACCP) principles (Buncic 2006; Nørrung et al. 2009).

3.2 Meat Contamination: Spoilage Microorganisms

Some of the microorganisms that grow in food can cause unacceptable sensory alterations, through the production of metabolites, such as off-flavours or changes in texture or appearance (Ellis and Goodacre 2001). These are the microorganisms defined as specific spoilage organisms for food, as other microorganisms may also grow in food but without causing any sensory changes.

Cold storage of meat will decrease bacterial growth, only 10% of the bacteria initially present being able to grow at refrigeration temperatures. As meat is a selective agent for aerobic microbiota, a consortium of bacteria commonly dominated by *Pseudomonas* spp. is responsible for the spoilage of meat stored

aerobically at temperatures between −1 and 25 °C. Cold-tolerant *Enterobacteriaceae*, *Brochothrix thermosphacta* and LAB also occur in chilled meat stored aerobically, but in terms of numbers, they do not contribute to dominate microbial associations (Nychas et al. 2008). Oxygen restriction by the use of vacuum or modified atmospheres will drastically reduce the presence of *Pseudomonas*, and bacterial microbiota will be gradually selected towards CO_2-tolerant organisms. Under these conditions, the dominating microorganisms involve *Brochothrix thermosphacta*, *Enterobacteriaceae* and LAB.

Systems for retail meat distribution and commercialization are mainly based on vacuum packaging (VP) or modified atmosphere packaging (MAP) of meat cuts using low gas permeability films and refrigeration. These methods using gas mixtures containing variable O_2 and CO_2 concentrations and low temperatures proved to be very effective in extending the shelf life of perishable foods such as muscle foods preventing the growth of pathogens such as pseudomonads and *Enterobacteriaceae* so that spoilage typically occurs associated with the growth of psychrotolerant LAB (Jones 1999; McMillin 2008). Both LAB and *B. thermosphacta*, the most important cause of spoilage commonly associated with meat packaged under VP and MAP conditions, result from the competition between facultative anaerobic Gram (+) biota (Russo et al. 2006). The LAB most often isolated from meat belong to the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc* and *Weissella*. They show varying potential to cause spoilage, e.g. by souring, discoloration, swelling and slime or off-odour compound production (Nieminen et al. 2011). The metabolites produced by LAB do not tend to cause spoilage of packaged meat until LAB species have reached maximum numbers and their metabolic by-products accumulated to sensorial detectable levels (Borch et al. 1996).

3.3 Bacteria of Technological Interest in Meat: The Focus on Lactic Acid Bacteria

Lactic acid bacteria (LAB) are cocci, coccobacilli or rods Gram (+), non-spore forming with a low G+C content (less than 53%). They involve a heterogeneous group of microorganisms that produce lactic acid from the fermentation of sugars as the main metabolic product. They are generally nonrespiratory organisms and lack catalase, fermenting glucose primarily to lactic acid, CO_2 and ethanol. LAB grow anaerobically, but they are aerotolerant and possess superoxide dismutase and peroxidase enzymes to detoxify peroxide radicals (Carr et al. 2002). The genera included in the LAB group are *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Weissella*, *Carnobacterium*, *Leuconostoc*, *Enterococcus*, *Tetragenococcus*, *Oenococcus* and *Vagococcus* (Holzapfel et al. 2001). Although most of LAB members are non-pathogenic being generally recognized as safe (GRAS) organisms, only a few of them are pathogenic, such as some members of

the genus *Streptococcus*. In addition, LAB are heterotrophic and generally have complex nutritional requirements because they lack many biosynthetic capabilities; most species have multiple requirements for amino acids and vitamins. Because of these nutritional exigencies, they can be found in environments where these requirements can be provided such as meat and meat products, milk and dairy products and cereals. They are often associated with animal and human oral cavities and intestines (e.g. *Streptococcus mutans*, *Enterococcus faecalis*), plants (*Lactobacillus*, *Leuconostoc*) and decaying plant materials.

LAB are the microorganisms of major technological importance in the food industry. Due to their characteristics and metabolic properties, they are essential to carry out the fermentation in fermented food, such as yogurt, cheese and cured sausages. Specifically in fermented meat products, LAB contribute to the development of the texture and flavour of the final product and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Fadda et al. 2010). LAB are either present as contaminants or deliberately added as starter cultures to guarantee the optimal fermentation process. They are also considered the most important microorganisms responsible for the health-promoting effects of fermented foods, such as immunomodulation, intestinal integrity and pathogen resistance (Venema and do Carmo 2015). Indeed, strains of some species have traditionally been used as probiotics and added as functional bacteria in various food commodities (Florou-Paneri et al. 2013). Due to the huge economic significance of industrial application of LAB as starters, biopreservatives and probiotics, a research emphasis on their metabolism, genetics and applications has been placed in the last 30 years (Das and Goyal 2012).

On the other hand, in response to consumer's demands related to food free from pathogens, with minimal processing and less preservatives and additives, but keeping its sensorial quality, present trends in the meat technology include the research of alternative inhibitors of pathogen and spoilage microorganisms. Especially, biopreservation has gained increasing attention as a means of naturally controlling the shelf life and safety of foods. Certainly the use of bioprotective cultures to ensure the hygienic quality of food is a promising tool, and LAB stand as the best candidates to be used because of their prevalence during storage, their GRAS status and its inhibitory potential towards certain pathogen and spoilage microorganisms. In fact, the inhibitory properties of LAB are ascribed mainly to the production of organic acids, especially lactic and acetic, and production of hydrogen peroxide or bacteriocins. Bacteriocins are peptides ribosomally synthesized by bacteria that have antibacterial activity towards closely related strains, including food pathogens (*L. monocytogenes* and *Clostridium*) and other meat spoilage bacteria such as *B. thermosphacta*, which play an important role in food preservation (Pérez et al. 2014). New insights into LAB metabolism present perspectives for the application of a novel generation of functional cultures which contribute to the safety or offering technological, nutritional and sensorial advantages. However, the application of biopreservation technology in meat and meat products, by means of LAB, constitutes only an additional hurdle that complements good manufacturing and processing practices (Vignolo et al. 2015).

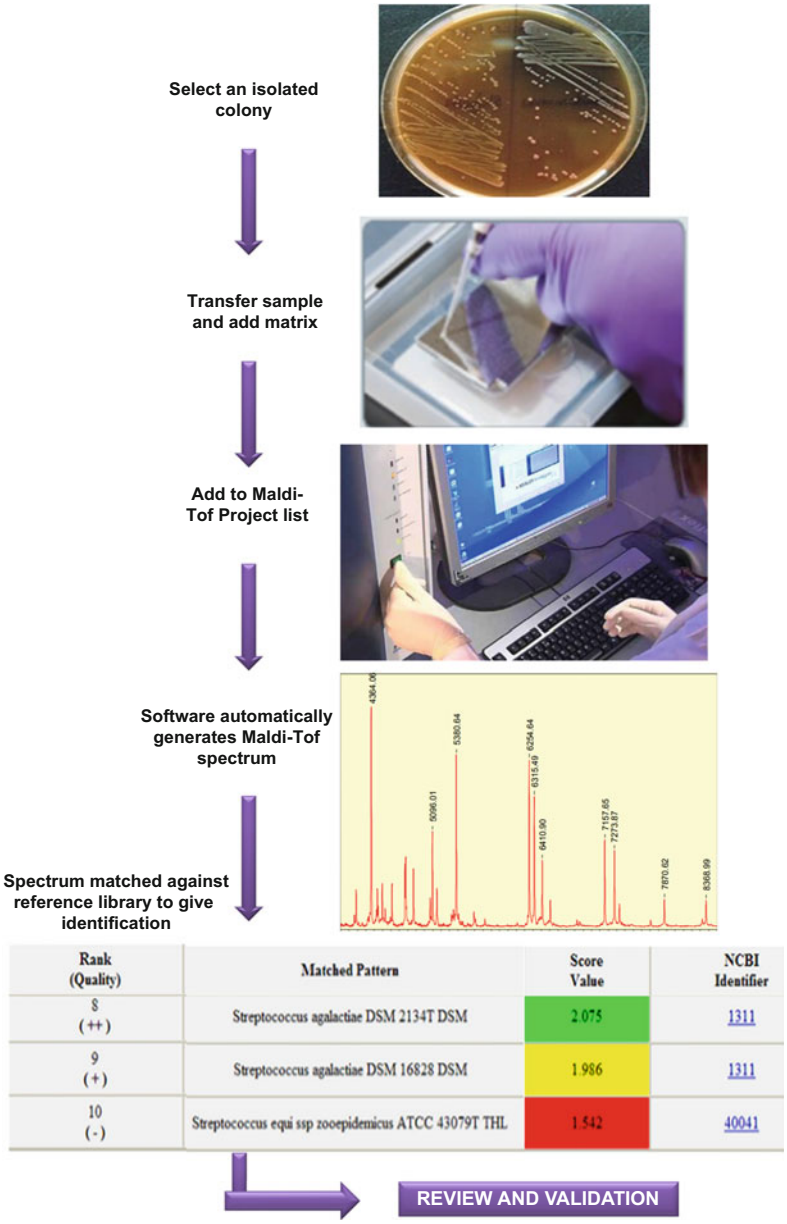
3.4 Omics for Bacterial Quantification and Identification

Microbial contamination of food from animal origin such as milk and meat has been a constant nuisance and an unavoidable problem throughout history. The role of the analytical tools for food products, involving the detection of spoilage and pathogenic organisms through quantitative and qualitative processes, is of utmost importance for food product appraisal and in the promotion of public health. In an attempt to preserve food quality manufacturing and production levels while safeguarding the public health, HACCP system has been implemented worldwide.

In an effort to achieve the necessities of food testing, research is being directed towards rapid methods of detection that could be implemented in highly automated processing facilities. Numerous different techniques have been explored and utilized over the years for the detection and quantification of microbiological contamination in food products, such as microscopy, polymerase chain reaction, ATP bioluminescence, nucleic acid probing, immunoassay methods and electronic nose techniques with positive and negative results into their application (Jay 2000; Commas-Riu and Rius 2009; Flint et al. 2006; Luo et al. 2009). Major drawbacks of these techniques include the high demand on operator skills, time consuming and relatively slow sample turnaround times, which limit them for daily use in the food industry.

The emergence of MALDI-TOF MS as a simple, rapid and economical technique for characterization of bacteria, largely at the species level and occasionally at the strain level, has greatly revolutionized microbial diagnostics (Nomura 2015; Cheng et al. 2016). Already in 1996, two reports indicated that MALDI-TOF mass spectral "fingerprints" could be simply and rapidly obtained from whole bacterial cells without any pretreatment before the MS analysis (Holland et al. 1996; Claydon et al. 1996). The use of small sample quantities and simple analysis techniques and the applicability on heterogeneous food samples are some of the potential advantages of the technique. Although other MS techniques, such as LC-MS, have also been used for microbial identification (Everley et al. 2008), MALDI-TOF MS has been adopted for routine diagnostics primarily because of the simple sample preparation during analysis that enables greater automation and high throughput.

MALDI-TOF MS-Based Identification: How Does It Work? A small number of cells from a colony cultured onto the agar plate are directly smeared onto the MALDI target plate and overlaid with the matrix solution. A solution of alpha-cyano-4-hydroxycinnamic acid in a mixture of organic solvents and water is used as the matrix for routine identification. The spotted mixture is air-dried and then inserted into the mass spectrometer for automated measurement. The final mass spectral signature is composed of peaks ranging from 1,000 to 30,000 m/z. MALDI usually produces singly charged ions; thus, the m/z of an analyte corresponds to its mass. The spectra obtained are compared with a library of known spectra, and a result is generated (Fig. 5). The characteristic spectrum pattern of this proteomic fingerprint is used to reliably and accurately identify a particular microorganism by



Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
8 (++)	Streptococcus agalactiae DSM 2134T DSM	2.075	1311
9 (+)	Streptococcus agalactiae DSM 16828 DSM	1.986	1311
10 (-)	Streptococcus equi ssp zooepidemicus ATCC 43079T THL	1.542	40041

Fig. 5 MALDI-TOF mass spectrometry workflow in the microbiology laboratory with the Bruker MALDI Biotyper® system

matching thousands of reference spectra from microorganisms. The characterization of bacteria by MALDI-TOF MS involves the analysis of the whole bacterial proteomes. The profile obtained is known to include mainly ribosomal proteins that are expected to be minimally affected by changes in culture conditions (Wieser et al. 2012; Teramoto et al. 2007). In addition to ribosomal proteins, nucleic acid-binding proteins and cold shock proteins were assigned (Ryzhov and Fenselau 2001). It is noteworthy, however, that the identity of each peak is not necessarily relevant for bacterial identification by MALDI-TOF MS which is based on the following facts: (1) spectral fingerprints vary between microorganisms; (2) among the compounds detected in the spectra, some peaks (molecular masses) are specific to the genus, species and sometimes subspecies levels; (3) spectra are reproducible as long as the bacteria are grown under the same conditions. Currently, two systems for applications of MALDI-TOF MS-based bacterial identification are widely used (including their specific databases): the Bruker Biotyper® (Bruker Daltonics; https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html) and the VITEK® MS (bioMerieux; http://www.biomerieux-diagnostics.com/sites/clinic/files/9300819-002-gb-a_vitek-ms.pdf). The analytical principles of the two systems are similar, although there are differences in database construction and also in the algorithms used to identify microorganisms.

MALDI-TOF MS-Based Identification: Current Applications and Future Perspectives Numerous studies already have explored the subtyping ability of MALDI-TOF MS for several pathogens such as *Streptococcus pneumoniae* (Williamson et al. 2008), *L. monocytogenes* (Hsueh et al. 2014), *Streptococcus agalactiae* (Lartigue et al. 2009), *Staphylococcus aureus* (Wang et al. 2013) and *Enterococcus* species (Quintela-Qunitela-Baluja et al. 2013). Most of the above-mentioned studies have mainly focused on subtypification of pathogens during epidemiological or clinical research. In contrast, Siegrist et al. (2007) successfully used MALDI-TOF MS to classify environmental isolates of *E. coli* according to their origin.

This technology has been used extensively in clinical diagnostics; however, its use for food pathogen detection is comparatively less explored (Jadhav et al. 2014; Pavlovic et al. 2013). A few reports show MALDI-TOF MS for the identification of isolates from food and beverage sources. These reports describe different aspects of the usefulness of MALDI-TOF to food microbiology and spanned from the classification of lactic acid bacteria in fermented meat and the inspection of probiotics to strain identification and characterization of biogenic amine-producing bacteria (Nguyen et al. 2012; Angelakis et al. 2011; Ruiz-Moyano et al. 2012; Fernández-No et al. 2010). The analysis of microbes in food is a challenging task because food-borne pathogens may be dispersed in low concentrations in food and high concentrations of harmless background microbiota. Then, a pre-concentration step is necessary, which separates the target bacteria from other bacteria with the complex background of food products. In this sense, Ochoa and Harrington (2005) successfully evaluated a rapid method for the isolation and identification of the enterohaemorrhagic *E. coli* O157:H7 in ground beef by using microscopic

magnetic beads coated with specific O157 antibodies covalently bonded to the surface. The immunomagnetic separation increased the sensitivity of the method and permitted the detection and identification of bacteria in meat by using MALDI-TOF MS. On the other hand, Jadhav et al. (2014) reported a proof-of-concept study using a proteomics-based MALDI-TOF MS approach that proposes a detection scheme that is more rapid and simple compared to conventional methods of *Listeria* detection. Very low levels of the pathogen could be identified from different food samples (milk, cheese or chicken paté) post-enrichment in selective broths. Also another standardized MALDI-TOF MS scheme can provide an inexpensive, rapid and simple solution for detecting and source tracking *L. monocytogenes* isolates obtained from different food processing environments (Jadhav et al. 2015). Biomarker peaks specific to isolates obtained from different sources were identified. However, standardization of culture conditions may play an important role in assessing or testing the robustness of the technique.

These rapid and accurate merits of MALDI-TOF MS (only 4 min per sample) in relation to bacterial detection and enumeration (up to 2 days) make this approach a very good candidate for potential use in the dairy and meat industries. Such methods are urgently needed within HACCP, and such a general spoilage detection technique will be important for improving consumer safety and product quality. Particularly in the case of pathogen detection in foods, MALDI-TOF MS analysis represents an attractive tool for detection and subtyping of multiple pathogens, considering its rapid turnaround time according to the timely information required by industry to avoid product recalls or delays in product release. The bottleneck for successful identification is related to there being no or insufficient information on the organism in the database. This is indicating that an up-to-date and comprehensive database is a critical requirement for accurate identification of isolates using MALDI-TOF MS. As mentioned before, also culture conditions such as culture media, time and temperature can affect MALDI-TOF MS efficiency. Following this, there are reports showing difficulties of MALDI-TOF MS for differentiating closely related species (van Veen et al. 2010). This topic leads to some controversial results as some studies report excellent differentiation up to the subspecies level and some studies report that the MALDI-TOF MS was unable to identify particular isolates at all. Finally, the discussion thus far has centred on the identification of bacteria from cultured colonies. Direct analysis of clinical or food samples without the need for prior culturing might shorten the time required for identifying microorganisms and would therefore further increase the usefulness of the MALDI-TOF MS-based approach, this point remaining as a pending issue in the next future.

4 Conclusion and Future Prospects

Despite the terms “peptidomics” and “proteomics” referring to different concepts, it is also true that the difference between them is somewhat blurred. Among the reason for that, one is the fact that, nowadays, both are mainly based on mass

spectrometry analyses of peptides of different nature. Many proteomic workflows usually follow a *bottom-up* approach, which implies the prior digestion of proteins with enzymes of known specificity into peptides, to be further analysed by mass spectrometry as a way to get information about the protein of origin. Peptidomics, on the contrary, would aim at analysing peptides from its intact structure in a *top-down approach* with the objective to get the maximum information about the proteolytic processes responsible for their generation in the matrix of study. High-throughput analysis of peptides has become possible with the advent of the so-called soft ionization techniques (MALDI and ESI) together with massive genome sequencing linked to the expansion of protein databases and the development of powerful bioinformatic algorithms, capable to automatically elucidate the sequence of thousands of peptides from MS/MS spectra generated by modern MS equipment.

Peptidomics has become a relevant subject in meat science since it is directly related to postmortem muscle proteolysis, contributing to better understand the role of the different endopeptidase groups in myofibrillar protein disruption and development of meat tenderness. In the case of processed meat products, peptidomics has notably contributed to identify those protein fragments present at the different stages of the ripening period, reporting the intense proteolysis occurring during the whole period by the action of both endo- and exopeptidases and also, in the case of fermented meats, by the activity of microbial peptidases. Peptide characterization is important since they contribute, directly or indirectly, to the typical flavour characteristics of these products. On the other hand, MALDI-TOF MS has emerged as a promising technique to achieve a rapid, simple and economical characterization of the bacterial population that can be present in meat and meat-derived products. This would allow the detection of both pathogenic and non-pathogenic microorganisms in short times, something of outmost importance to assure safety of food and product appraisal. Future prospects in this approach will have to face the current limitations of the technique such as the need for standardized culture conditions prior to species identification and the difficulty to differentiate between closely related species. In addition to this, peptidomics needs to face the challenge to develop new and powerful bioinformatic tools enabling high-throughput and confident analysis of peptides that do not follow the ideal chemical structure of peptides obtained by cleavage with enzymes of known specificity, as in the case of trypsin in *bottom-up* proteomic approaches. This is even more remarkable in the case of peptides originated from proteins whose sequence is hosted in protein databases, for which peptide identification algorithms based on spectral matching and comparison with theoretical spectra cannot be applied. For those cases, improvements should address the development of tailored high-throughput peptide identification tools based on *de novo* sequencing from MS/MS spectral information independent of protein databases.

References

Angelakis E, Million M, Henry M, Raoult D (2011) Rapid and accurate bacterial identification in probiotics and yoghurts by MALDI-TOF mass spectrometry. J Food Sci 76:M568–M571

Baron CP, Jacobsen S, Purslow PP (2004) Cleavage of desmin by cysteine proteases: Calpains and cathepsin B. Meat Sci 68:447–456

Bergquist J, Ekman R (2001) Future aspects of psychoneuroimmunology - lymphocyte peptides reflecting psychiatric disorders studied by mass spectrometry. Arch Physiol Biochem 109:369–371

Buncic S (2006) Integrated food safety and veterinary public health. CABI International Publishing, Wallingford

Borch E, Kant-Muemansb ML, Blixt Y (1996) Bacterial spoilage of meat products and cured meat. Int J Food Microbiol 33:103–120

Buncic S, Nychas GI, Lee MRF, Koutsoumanis K, Hébraud M, Desvaux M, Chorianopoulos N, Bolton D, Blagojevic B, Antic D (2014) Microbial pathogen control in the beef chain: recent research advances. Meat Sci 97:288–297

Carr FJ, Hill D, Maida N (2002) The lactic acid bacteria: a literature survey. Crit Rev Microbiol 28:281–370

Carraturo F, Gargiulo G, Giorgio A, Aliberti F, Guida M (2016) Prevalence, distribution, and diversity of *Salmonella* spp. in meat samples collected from Italian slaughterhouses. J Food Sci 81(10):M2545–M2551. https://doi.org/10.1111/1750-3841.13430.

Cheng K, Chui H, Domish L, Hernandez D, Wang G (2016) Recent development of mass spectrometry and proteomics applications in identification and typing of bacteria. Proteomics Clin Appl 10:346–357. https://doi.org/10.1002/prca.201500086

Claydon MA, Davey SN, Edwards-Jones VD, Gordon B (1996) The rapid identification of intact microorganisms using mass spectrometry. Nat Biotechnol 14:1584–1586

Clynen E, Baggerman G, Veelaert D, Cerstiaens A, Van der Horst D, Harthoorn L, Derua R, Waelkens E, De Loof A, Schoofs L (2001) Peptidomics of the pars intercerebralis-corpus cardiacum complex of the migratory locust, Locusta migratoria. Eur J Biochem 268:1929–1939

Colello R, Cáceres ME, Ruiz MJ, Sanz M, Etcheverría A, Padola NL (2016) From farm to table: follow-up of Shiga toxin-producing *Escherichia coli* throughout the pork production chain in Argentina. Front Microbiol 7:93. https://doi.org/10.3389/fmicb.2016.00093

Commas-Riu J, Rius NJ (2009) Flow cytometry applications in the food industry. J Ind Microbiol Biotechnol 36:999–1011

Craik DJ, Young Shim Y, Goransson U, Moss GP, Tan N, Jadhav PD, Shen J, Reaney MJ (2016) Nomenclature of homodetic cyclic peptides produced from ribosomal precursors: an IUPAC task group interim report. Biopolymers 106:917–924

Dallas DC, Guerrero A, Parker EA, Robinson RC, Gan JN, German JB, Barile D, Lebrilla CB (2015) Current peptidomics: applications, purification, identification, quantification, and functional analysis. Proteomics 15:1026–1038

Dang YL, Gao XC, Ma FM, Wu XQ (2015) Comparison of umami taste peptides in water-soluble extractions of Jinhua and Parma hams. Lwt-Food Sci Technol 60:1179–1186

Das D, Goyal A (2012) Lactic Acid Bacteria in Food Industry. In: Satyanarayana T, Johri B, Prakash A (eds) Microorganisms in sustainable agriculture and biotechnology. Springer, Dordrecht

Di Luccia A, Picariello G, Cacace G, Scaloni A, Faccia M, Liuzzi V, Alviti G, Musso SS (2005) Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. Meat Sci 69:479–491

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2015) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J 2015 13:191. https://doi.org/10.2903/j.efsa.2015.4329

Ellis DI, Goodacre R (2001) Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. Trends Food Sci Technol 12:414–424

Everley RA, Mott TM, Wyatt SA, Toney DM, Croley TR (2008) Liquid chromatography mass spectrometry characterization of *Escherichia coli* and *Shigella* species. J Am Soc Mass Spectrom 19:1621–1628

Fabbro A, Bencivenni M, Piasentier E, Sforza S, Stecchini ML, Lippe G (2016) Proteolytic resistance of actin but not of myosin heavy chain during processing of Italian PDO (protected designation of origin) dry-cured hams. Eur Food Res Technol 242:881–889

Fadda S, López MC, Vignolo G (2010) Role of lactic acid bacteria during meat conditioning and fermentation: peptides generated as sensorial and hygienic biomarkers. Meat Sci 86:66–79

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64–71

Fernández-No IC, Böhme K, Gallardo JM, Barros-Velázquez J, Cañas B, Calo-Mata P (2010) Differential characterization of biogenic amine-producing bacteria involved in food poisoning using MALDI-TOF mass fingerprinting. Electroph 31:1116–1127

Flint S, Drocourt J-L, Walker K, Stevenson B, Dwyer M, Clarke I, McGill D (2006) A rapid, two-hour method for the enumeration of total viable bacteria in samples from commercial milk powder and whey protein concentrate powder manufacturing plants. Int Dairy J 16:379–384

Florou-Paneri P, Christaki E, Bonos E (2013) Chapter 25: Lactic acid bacteria as source of functional ingredients. In: Marcelino Kongo (ed) Lactic acid bacteria - R & D for food, health and livestock purposes. Biochemistry, genetics and molecular biology. https://doi.org/10.5772/47766

Fu Y, Young JF, Lokke MM, Lametsch R, Aluko RE, Therkildsen M (2016) Revalorisation of bovine collagen as a potential precursor of angiotensin 1-converting enzyme (ACE) inhibitory peptides based on in silico and in vitro protein digestions. J Funct Foods 24:196–206

Fu Y, Young JF, Therkildsen M (2017) Bioactive peptides in beef: endogenous generation through postmortem aging. Meat Sci 123:134–142

Gallego M, Mora L, Fraser PD, Aristoy MC, Toldra F (2014) Degradation of LIM domain-binding protein three during processing of Spanish dry-cured ham. Food Chem 149:121–128

Gallego M, Mora L, Aristoy MC, Toldra F (2015a) Evidence of peptide oxidation from major myofibrillar proteins in dry-cured ham. Food Chem 187:230–235

Gallego M, Mora L, Aristoy MC, Toldra F (2015b) Titin-derived peptides as processing time markers in dry-cured ham. Food Chem 167:326–339

Gallego M, Mora L, Toldra F (2016) Peptidomics as a tool for quality control in dry-cured ham processing. J Proteomics 147:98–107

Hernandez P, Muller M, Appel RD (2006) Automated protein identification by tandem mass spectrometry: issues and strategies. Mass Spectrom Rev 25:235–254

Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, Lay JO Jr (1996) Rapid identification of intact whole bacteria on spectral patterns using matrix assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 10:1227–1232

Holzapfel WH, Haberer P, Geisen R, Björkroth J, Schillinger U (2001) Taxonomy and important features of probiotic microorganisms in food nutrition. Am J Clin Nutr 73:365S–373S

Horst T, Niedermeyer J (2016) Annotating and interpreting linear and cyclic peptide tandem mass spectra. In: Evans BS (ed) Nonribosomal peptide and polyketide biosynthesis: methods and protocols, methods in molecular biology. Springer, New York, pp 199–207

Hsueh P-R, Lee T-F, Du S-H, Teng SH, Liao CH, Sheng WH, Teng LJ (2014) Bruker biotypermatrix-assisted laser desorption ionization-time of flightmass spectrometry system for identification of *Nocardia*, *Rhodococcus*, *Kocuria*, *Gordonia*, *Tsukamurella*, and *Listeria* Species. J Clin Microbiol 52:2371–2379

Hughes MC, O'Neill EE, McSweeney PLH, Healy A (1999) Proteolysis of bovine F-actin by cathepsin B. Food Chem 64:525–530

Hughes MC, Healy A, McSweeney PLH, O'Neill EE (2000) Proteolytic specificity of cathepsin D on bovine F-actin. Meat Sci 56:165–172

Hughes MC, Geary S, Dransfield E, McSweeney PLH, O'Neill EE (2001) Characterization of peptides released from rabbit skeletal muscle troponin-T by mu-calpain under conditions of low temperature and high ionic strength. Meat Sci 59:61–69

Hussein HS, Bollinger LM (2005) Prevalence of Shiga toxin-producing Escherichia coli in beef. Meat Sci 71:676–689. https://doi.org/10.1016/j.meatsci.2005.05.012

Igbinosa EO, Beshiru A, Akporehe LU, Oviasogie FE, Igbinosa OO (2016) Prevalence of methicillin-resistant *Staphylococcus aureus* and other *Staphylococcus* species in raw meat samples intended for human consumption in Benin city, Nigeria: implications for public health. Int J Environ Res Public Health 13(10):949. https://doi.org/10.3390/ijerph13100949

Jadhav S, Sevior D, Bhave M, Palombo E (2014) Detection of *Listeria monocytogenes* from selective enrichment broth using MALDI-TOF mass spectrometry. J Proteomics 97 (31):100–106

Jadhav S, Gulati V, Fox E, Karpe A, Bealec D, Sevior D, Bhave M, Palombo E (2015) Rapid identification and source-tracking of *Listeria monocytogenes* using MALDI-TOF mass spectrometry. Int J Food Microbiol 202:1–9

Jay JM (2000) Modern food microbiology. Chapman and Hall, London

Jones RJ (1999) Immunisation against lactic-acid bacteria as a technique to extend the chilled storage life of vacuum-packed lamb. Food Agric Immunol 11:75–81

Jones AW, Cooper HJ (2011) Dissociation techniques in mass spectrometry-based proteomics. Analyst 136:3419–3429

Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 60:2299–2301

Karmi M (2013) Prevalence of methicillin-resistant *Staphylococcus aureus* in poultry meat in Qena, Egypt. Vet World 6:711–715

Kemp CM, Parr T (2008) The effect of recombinant caspase 3 on myofibrillar proteins in porcine skeletal muscle. Animal 2:1254–1264

Kitamura SI, Muroya S, Tanabe S, Okumura T, Chikuni K, Nishimura T (2005) Mechanism of production of troponin T fragments during postmortem aging of porcine muscle. J Agric Food Chem 53:4178–4181

Koutsoumanis K, Sofos J (2004) Microbial contamination of carcasses and cuts. In: Jensens W (ed) Encyclopedia of meat sciences. Elsevier Academic Press, Amsterdam, pp 727–737

Lafarga T, Hayes M (2014) Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Sci 98:227–239

Lafarga T, Wilm M, Wynne K, Hayes M (2016) Bioactive hydrolysates from bovine blood globulins: generation, characterisation, and in silico prediction of toxicity and allergenicity. J Funct Foods 24:142–155

Lametsch R, Bendixen E (2001) Proteome analysis applied to meat science: characterizing post mortem changes in porcine muscle. J Agric Food Chem 49:4531–4537

Lametsch R, Roepstorff P, Bendixen E (2002) Identification of protein degradation during post-mortem storage of pig meat. J Agric Food Chem 50:5508–5512

Lametsch R, Karlsson A, Rosenvold K, Andersen HJ, Roepstorff P, Bendixen E (2003) Postmortem proteome changes of porcine muscle related to tenderness. J Agric Food Chem 51:6992–6997

Lametsch R, Roepstorff P, Moller HS, Bendixen E (2004) Identification of myofibrillar substrates for mu-calpain. Meat Sci 68:515–521

Lartigue M-F, Héry-Arnaud G, Haguenoer E, Domelier A-S, Schmit P-O, van der Mee-Marquet N, Lanotte P, Mereghetti L, Kostrzewa M, Quentin R (2009) Identification of *Streptococcus agalactiae* isolates from various phylogenetic lineages by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 47:2284–2287

Laville E, Sayd T, Morzel M, Blinet S, Chambon C, Lepetit J, Renand G, Hocquette JF (2009) Proteome changes during meat aging in tough and tender beef suggest the importance of

apoptosis and protein solubility for beef aging and tenderization. J Agric Food Chem 57:10755–10764
Liu X, Chen Y, Fan Y, Wang M (2006) Foodborne diseases occurred in 2003 - report of the national foodborne diseases surveillance system, China. Chin J Hyg Res 35:201–204
López CM, Sentandreu MA, Vignolo GM, Fadda SG (2015a) Proteomic and peptidomic insights on myofibrillar protein hydrolysis in a sausage model during fermentation with autochthonous starter cultures. Food Res Int 78:41–49
López CM, Bru E, Vignolo GM, Fadda SG (2015b) Identification of small peptides arising from hydrolysis of meat proteins in dry fermented sausages. Meat Sci 104:20–29
López CM, Sentandreu MA, Vignolo GM, Fadda SG (2015c) Low molecular weight peptides derived from sarcoplasmic proteins produced by an autochthonous starter culture in a beaker sausage model. EuPA Open Proteomics 7:54–63
Luo J, Liu X, Tian Q, Yue W, Zeng J, Chen G, Cai X (2009) Disposable bioluminescence-based biosensor for detection of bacterial count in food. Anal Biochem 394:1–6. https://doi.org/10.1016/j.ab.2009.05.021.
Mahlapuu M, Hakansson J, Ringstad L, Bjorn C (2016) Antimicrobial peptides: an emerging category of therapeutic agents. Front Cell Infect Microbiol 6:194
Marbaix H, Budinger D, Dieu M, Fumiere O, Gillard N, Delahaut P, Mauro S, Raes M (2016) Identification of proteins and peptide biomarkers for detecting banned Processed Animal Proteins (PAPs) in meat and bone meal by mass spectrometry. J Agric Food Chem 64:2405–2414
McEvoy, J. M., A. M. Doherty, M. Finnerty, J. J. Sheridan, L., McGuire, I. S. Blair, D. A. McDowell, and D. Harrington. (2000) The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. Lett Appl Microbiol 30, 390–395.
McIntosh JA, Donia MS, Schmidt EW (2009) Ribosomal peptide natural products: bridging the ribosomal and nonribosomal worlds. Nat Prod Rep 26:537–559
McMillin K (2008) Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Sci 80:43–65
Minamino N (2001) Peptidome: the fact-database for endogenous peptides. Tanpakushitsu kakusan koso. Protein Nucleic Acid Enzyme 46:1510–1517
Mirdhayati I, Hermanianto J, Wijaya CH, Sajuthi D, Arihara K (2016) Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of Kacang goat (Capra aegagrus hircus). J Sci Food Agric 96:3536–3542
Mora L, Sentandreu MA, Fraser PD, Toldra F, Bramley PM (2009a) Oligopeptides arising from the degradation of creatine kinase in Spanish dry-cured ham. J Agric Food Chem 57:8982–8988
Mora L, Sentandreu MA, Koistinen KM, Fraser PD, Toldra F, Bramley PM (2009b) Naturally generated small peptides derived from myofibrillar proteins in serrano dry-cured ham. J Agric Food Chem 57:3228–3234
Mora L, Sentandreu MA, Toldra F (2010) Identification of small troponin T peptides generated in dry-cured ham. Food Chem 123:691–697
Mora L, Sentandreu MA, Toldra F (2011a) Intense degradation of myosin light chain isoforms in Spanish dry-cured ham. J Agric Food Chem 59:3884–3892
Mora L, Valero ML, del Pino MMS, Sentandreu MA, Toldra F (2011b) Small peptides released from muscle glycolytic enzymes during dry-cured ham processing. J Proteomics 74:442–450
Mora L, Escudero E, Toldra F, Aristoy M-C (2015a) A peptidomic approach to study the contribution of added casein proteins to the peptide profile in Spanish dry-fermented sausages. Int J Food Microbiol 212:41–48
Mora L, Gallego M, Aristoy MC, Fraser PD, Toldra F (2015b) Peptides naturally generated from ubiquitin-60S ribosomal protein as potential biomarkers of dry-cured ham processing time. Food Control 48:102–107
Mora L, Gallego M, Escudero E, Reig M, Toldra F, Aristoy M-C (2015c) Small peptides hydrolysis in dry-cured meats. Int J Food Microbiol 212:9–15

Mora L, Calvo L, Escudero E, Toldra F (2016) Differences in pig genotypes influence the generation of peptides in dry-cured ham processing. Food Res Int 86:74–82

Morzel M, Chambon C, Hamelin M, Sante-Lhoutellier V, Sayd T, Monin G (2004) Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. Meat Sci 67:689–696

Morzel M, Terlouw C, Chambon C, Micol D, Picard B (2008) Muscle proteome and meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: a central role of HSP27 isoforms. Meat Sci 78:297–304

Muroya S, Kitamura S, Tanabe S, Nishimura T, Nakajima I, Chikuni K (2004) N-terminal amino acid sequences of troponin T fragments, including 30 kDa one, produced during postmortem aging of bovine longissimus muscle. Meat Sci 67:19–24

Muroya S, Ohnishi-Kameyama M, Oe M, Nakajima I, Chikuni K (2007) Postmortem changes in bovine troponin T isoforms on two-dimensional electrophoretic gel analyzed using mass spectrometry and western blotting: the limited fragmentation into basic polypeptides. Meat Sci 75:506–514

Nakai Y, Nishimura T, Shimizu M, Arai S (1995) Effects of freezing on the proteolysis of beef during storage at 4 degrees C. Biosci Biotech Bioch 59:2255–2258

Nguyen DT, Van Hoorde K, Cnockaert M, De Brandt E, Aerts M, Le Thanh B, Vandamme P (2012) Validation of MALDI-TOF MS for rapid classification and identification of lactic acid bacteria, with a focus on isolates from traditional fermented foods in Northern Vietnam. Lett Appl Microbiol 55:265–273

Nieminen TT, Vihavainen E, Paloranta A, Lehto J, Paulin L, Auvinen P, Solismaa M, Björkroth KJ (2011) Characterization of psychrotrophic bacterial communities in modified atmosphere-packed meat with terminal restriction fragment length polymorphism. Int J Food Microbiol 144:360–366

Nomura F (2015) Proteome-based bacterial identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS): a revolutionary shift in clinical diagnostic microbiology. Biochim Biophys Acta 1854:528–537

Nørrung B, Andersen JK, Buncic S (2009) Main concerns of pathogenic microorganisms in meat. In: Todra F (ed) Safety of meat and processed meat. Springer, New York, pp 3–29

Nychas G-J, Skandamis P, Tassou C, Koutsoumanis K (2008) Meat spoilage during distribution. Meat Sci 78:77–89

Ochoa ML, Harrington PB (2005) Immunomagnetic isolation of enterohemorrhagic *Escherichia coli* O157:H7 from ground beef and identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and database searches. Anal Chem 77:5258–5267

Okumura T, Yamada R, Nishimura T (2003) Survey of conditioning indicators for pork loins: changes in myofibrils, proteins and peptides during postmortem conditioning of vacuum-packed pork loins for 30 days. Meat Sci 64:467–473

Okumura T, Yamada R, Nishimura T (2004) Sourness-suppressing peptides in cooked pork loins. Biosci Biotech Bioch 68:1657–1662

Ortea I, O'Connor G, Maquet A (2016) Review on proteomics for food authentication. J Proteomics 147:212–225

Ouali A, Herrera-Mendez CH, Coulis G, Becila S, Boudjellal A, Aubry L, Sentandreu MA (2006) Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Sci 74:44–58

Ouali A, Gagaoua M, Boudida Y, Becila S, Boudjellal A, Herrera-Mendez CH, Sentandreu MA (2013) Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. Meat Sci 95:854–870

Panchaud A, Affolter M, Kussmann M (2012) Mass spectrometry for nutritional peptidomics: how to analyze food bioactives and their health effects. J Proteomics 75:3546–3559

Paolella S, Falavigna C, Faccini A, Virgili R, Sforza S, Dall'Asta C, Dossena A, Galaverna G (2015) Effect of dry-cured ham maturation time on simulated gastrointestinal digestion: characterization of the released peptide fraction. Food Res Int 67:136–144

Park BY, Kim NK, Lee CS, Hwang IH (2007) Effect of fiber type on postmortem proteolysis in longissimus muscle of landrace and Korean native black pigs. Meat Sci 77:482–491

Pavlovic M, Huber I, Konrad R, Busch U (2013) Application of MALDI-TOF MS for the identification of food borne bacteria. Open Microbiol J 7:135–141

Pérez RH, Zendo T, Sonomoto K (2014) Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Factories 13(Suppl 1):S3. http://www.microbialcellfactories.com/content/13/S1/S3

Picariello G, De Martino A, Mamone G, Ferranti P, Addeo F, Faccia M, SpagnaMusso S, Di Luccia A (2006) Proteomic study of muscle sarcoplasmic proteins using AUT-PAGE/SDS-PAGE as two-dimensional gel electrophoresis. J Chromatogr B 833:101–108

Picariello G, Mamone G, Addeo F, Ferranti P (2012) Novel mass spectrometry-based applications of the 'omic' sciences in food technology and biotechnology. Food Technol Biotech 50:286–305

Polati R, Menini M, Robotti E, Millioni R, Marengo E, Novelli E, Balzan S, Cecconi D (2012) Proteomic changes involved in tenderization of bovine Longissimus dorsi muscle during prolonged ageing. Food Chem 135:2052–2069

Quintela-Qunitela-Baluja M, Böhme K, Fernández-No IC, Morandi S, Alnakip ME, Caamaño-Antelo S, Velázquez JB, Calo-Mata P (2013) Characterization of different food-isolated *Enterococcus* strains by MALDI-TOFmass fingerprinting. Electrophoresis 34:2240–2250

Rodriguez-Frometa RA, Rodriguez-Diaz J, Sentandreu E, Ouali A, Sentandreu MA (2013) Proteolytic action of caspases 3 and 7 on the hydrolysis of bovine and porcine muscle myofibrillar proteins. In: de Almeida A, Eckersall D, Bencurova E, Dolinska S, Mlynarcik P, Vincova M, Bhide M (eds) Farm animal proteomics 2013. Wageningen Academic Publishers, Wageningen, pp 278–281

Roncada P, Piras C, Soggiu A, Turk R, Urbani A, Bonizzi L (2012) Farm animal milk proteomics. J Proteomics 75:4259–4274

Ruiz-Moyano S, Nannan T, Underwood MA, Millls DA (2012) Rapid discrimination of *Bifidobacterium animalis* subspecies by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Food Microbiol 30:432–437

Russo F, Ercolini D, Mauriello G, Villani F (2006) Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups. Food Microbiol 23:797–802

Ryzhov V, Fenselau C (2001) Characterization of the protein subset desorbed by MALDI from whole bacterial cells. Anal Chem 73:746–750

Sanchez-Rivera L, Martinez-Maqueda D, Cruz-Huerta E, Miralles B, Recio I (2014) Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides. Food Res Int 63:170–181

Sawdy JC, Kaiser SA, St-Pierre NR, Wick MP (2004) Myofibrillar 1-D fingerprints and myosin heavy chain MS analyses of beef loin at 36 h postmortem correlate with tenderness at 7 days. Meat Sci 67:421–426

Schrader M, Schulz-Knappe P, Fricker LD (2014) Historical perspective of peptidomics. EuPA Open Proteomics 3:171–182

Schulz-Knappe P, Zucht HD, Heine C, Jurgens M, Hess R, Schrader M (2001) Peptidomics: the comprehensive analysis of peptides in complex biological mixtures. Comb Chem High Throughput Screen 4:207–217

Sentandreu MA, Sentandreu E (2011) Peptide biomarkers as a way to determine meat authenticity. Meat Sci 89:280–285

Sentandreu MA, Sentandreu E (2014) Authenticity of meat products: tools against fraud. Food Res Int 60:19–29

Sentandreu MA, Coulis G, Ouali A (2002) Role of muscle endopeptidases and their inhibitors in meat tenderness. Trends Food Sci Tech 13:400–421

Sentandreu MA, Stoeva S, Aristoy MC, Laib K, Voelter W, Toldra F (2003) Identification of small peptides generated in Spanish dry-cured ham. J Food Sci 68:64–69

Sentandreu MA, Armenteros M, Calvete JJ, Ouali A, Aristoy MC, Toldra F (2007) Proteomic identification of actin-derived oligopeptides in dry-cured ham. J Agric Food Chem 55:3613–3619

Sentandreu MA, Fraser PD, Halket J, Patel R, Bramley PM (2010) A proteomic-based approach for detection of chicken in meat mixes. J Proteome Res 9:3374–3383

Sforza S, Pigazzani A, Motti M, Porta C, Virgili R, Galaverna G, Dossena A, Marchelli R (2001) Oligopeptides and free amino acids in Parma hams of known cathepsin B activity. Food Chem 75:267–273

Sforza S, Boni M, Ruozi R, Virgili R, Marchelli R (2003) Identification and significance of the N-terminal part of swine pyruvate kinase in aged Parma hams. Meat Sci 63:57–61

Sharma KP, Chattopadhyay UK (2015) Assessment of microbial load of raw meat samples sold in the open markets of city of Kolkata. J Agric Vet Sci 8:24–27

Siegrist TJ, Anderson PD, Huen WH, Kleinheinz GT, McDermott CM, Sandrin TR (2007) Discrimination and characterization of environmental strains of *Escherichia coli* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). J Microbiol Methods 68:554–562

Sierra V, Fernandez-Suarez V, Castro P, Osoro K, Vega-Naredo I, Garcia-Macia M, Rodriguez-Colunga P, Coto-Montes A, Olivan M (2012) Identification of biomarkers of meat tenderisation and its use for early classification of Asturian beef into fast and late tenderising meat. J Sci Food Agric 92:2727–2740

Skrlep M, Candek-Potokar M, Mandelc S, Javornik B, Gou P, Chambon C, Sante-Lhoutellier V (2011) Proteomic profile of dry-cured ham relative to PRKAG3 or CAST genotype, level of salt and pastiness. Meat Sci 88:657–667

Stadnik J, Keska P (2015) Meat and fermented meat products as a source of bioactive peptides. Acta Sci Pol Technol Aliment 14:181–190

Stoeva S, Byrne CE, Mullen AM, Troy DJ, Voelter W (2000) Isolation and identification of proteolytic fragments from TCA soluble extracts of bovine M-longissimus dorsi. Food Chem 69:365–370

Teramoto K, Sato H, Sun L, Torimura M, Tao H, Yoshikawa H, Hotta Y, Hosoda A, Tamura H (2007) Phylogenetic classification of *Pseudomonas putida* strains by MALDI-MS using ribosomal subunit proteins as biomarkers. Anal Chem 79:8712–8719

Theron L, Sayd T, Pinguet J, Chambon C, Robert N, Sante-Lhoutellier V (2011) Proteomic analysis of semimembranosus and biceps femoris muscles from Bayonne dry-cured ham. Meat Sci 88:82–90

Udenigwe CC, Howard A (2013) Meat proteome as source of functional biopeptides. Food Res Int 54:1021–1032

Vaillant V, De Valk H, Baron E, Ancelle T, Colin P, Delmas MC, Dufour B, Pouillot R, Le Strat Y, Weinbreck P, Jougla E, Desenclos JC (2005) Foodborne infections in France. Foodborne Pathog Dis 2:221–232

van Veen SQ, Claas EC, Kuijper EJ (2010) High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 48:900–907

Venema K, do Carmo AP (eds) (2015) Probiotic and prebiotics: current status and future trends. Caister Academic Press, Norfolk, pp 1–3

Verhaert P, Uttenweiler-Joseph S, de Vries M, Loboda A, Ens W, Standing KG (2001) Matrix-assisted laser desorption/ionization quadrupole time-of-flight mass spectrometry: an elegant tool for peptidomics. Proteomics 1:118–131

Vignolo, G., Castellano, P., and Fadda S. (2015). Part III, Chapter 15: Starter cultures: bioprotective cultures. In: Toldrá F, Talon R (eds) Handbook of fermented meat and poultry, 2nd edn. Blackwell, Malden, MA, pp 129–137. isbn: 978-1-118-52269-1

Voelter W, Stoeva S, Echner H, Beck A, Schutz J, Lehmann R, Haring HU, Schleicher E, Mullen AM, Casserly U et al (2000) Analytical tools for rapid, sensitive, quantitative identification of potential meat quality markers. J Prakt Chem Chem Ztg 342:179–191

Wang YR, Chen Q, Cui SH, Li FQ (2013) Characterization of *Staphylococcus aureus* isolated from clinical specimens by matrix assisted laser desorption/ionization time-of-flight mass spectrometry. Biomed Environ Sci 26:430–436

Wen SY, Zhou GH, Song SX, Xu XL, Voglmeir J, Liu L, Zhao F, Li MJ, Li L, Yu XB et al (2015) Discrimination of in vitro and in vivo digestion products of meat proteins from pork, beef, chicken, and fish. Proteomics 15:3688–3698

Wieser A, Schneider L, Jung J, Schubert S (2012) MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl Microbiol Biotechnol 93:965–974. https://doi.org/10.1007/s00253-011-3783-4

Williamson YM, Moura H, Woolfitt AR, Pirkle JL, Barr JR, Carvalho MDG, Ades ED, Carlone GM, Sampson JS (2008) Differentiation of *Streptococcus pneumonia* conjunctivitis outbreak isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 74:5891–5897

Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J (2001) Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C. area. Appl Environ Microbiol 67:5431–5436

Zürbig P, Mischak H (2008) Peptidomics approach to proteomics. In: Soloviev M, Andrén P, Shaw C (eds) Peptidomics methods and applications. Wiley, Hoboken, NJ, pp 155–175