Review

Meat science: From proteomics to integrated omics towards system biology

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ABSTRACT

Since the main ultimate goal of farm animal raising is the production of proteins for human consumption, research tools to investigate proteins play a major role in farm animal and meat science. Indeed, proteomics has been applied to the field of farm animal science to monitor in vivo performances of livestock animals (growth performances, fertility, milk quality etc.), but also to further our understanding of the molecular processes at the basis of meat quality, which are largely dependent on the post mortem biochemistry of the muscle, often in a species-specific way. Post mortem alterations to the muscle proteome reflect the biological complexity of the process of “muscle to meat conversion,” a process that, despite decades of advancements, is all but fully understood. This is mainly due to the enormous amounts of variables affecting meat tenderness per se, including biological factors, such as animal species, breed specific-characteristic, muscle under investigation. However, it is rapidly emerging that the tender meat phenotype is not only tied to genetics (livestock breeding selection), but also to extrinsic factors, such as the rearing environment, feeding conditions, physical activity, administration of hormonal growth promotants, pre-slaughter handling and stress, post mortem handling. From this intricate scenario, biochemical approaches and systems-wide integrated investigations (metabolomics, transcriptomics, interactomics, phosphoproteomics, mathematical modeling), which have emerged as complementary tools to proteomics, have helped establishing a few milestones in our understanding of the events leading from muscle to meat conversion. The growing integration of omics disciplines in the field of systems biology will soon contribute to take further steps forward.

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1. **Proteomics and farm animal science: a special focus on meat science**

Research tools to investigate proteins play a major role in meat science, since the ultimate end objective of farm livestock raising is the production of proteins for human consumption. Within the framework of protein-oriented investigations, proteomics has achieved a leading role over the last two decades, owing to a long series of technological innovations in the fields of protein separation (through chromatography and electrophoresis), mass spectrometry and bioinformatics and their application to farm animal science-relevant issues [1-4]. In addition, the more extensive publication of species genomes is making the proteomic approach in animal science more viable [1,2], especially as far as protein identification through mass spectrometry and database interrogation are concerned.

The flourishing of the field of farm animal proteomics is also confirmed by the constant growth and spread of international initiatives. The European Cooperation in Science and Technology (COST) farm animal proteomics (Action FA1002) [2] is an initiative that has been promoted by the EU with the goal to apply proteomics in animal science in order to reach a deeper understanding of the phenotype, physiology, pathophysiology and productivity of land and water raised farm animals.

To date, proteomics has been applied to the field of farm animal science to monitor in vivo performances of livestock animals (growth performances, fertility, milk quality etc. [1-7]), but also to further our understanding of the molecular processes at the basis of meat quality, which are largely dependent on the post mortem biochemistry of the muscle, often in a species-specific way [8]. Indeed, one of the major goals of proteomics in the field of farm animal science is to shed light on skeletal muscle biochemistry [9] and to deepen our understanding of the physiological changes taking place at the protein level following harvest. Post mortem alterations to the muscle proteome reflect the biological complexity of the process often referred to as “muscle to meat conversion” [8].

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1.1. **Proteomics and swine science and meat investigations**

The pig (Sus scrofa domesticus) is one of the most important domestic animals, with global populations estimated to be 1 billion pigs (www.thepigsite.com and www.zoosavvy.com), while pig meat represents an essential protein source to the human diet, although its consumption is often hampered by religious constraints, as it happens for example in Muslim countries. Pig has been selectively bred to fulfill different purposes, including features like growth performances, prolificacy (Chinese breeds such as the Meishan), backfat thickness for the production of lard and smoked ham production (Casertana and Iberian/Alentejano pig), meat leanness (commercial lines based on Large White and Landrace breeds), feed conversion rate, muscle growth development and size, adaptation to harsh rearing environments and stress, flavor and taste-affecting traits, like boar taint and the suitability for biomedical research (Yucatan or the Göttingen minipigs) [10].

Investigations in the field of swine farming range from insemination to slaughter and industrial pork production [10].

The control of pig reproduction and the extensive use of artificial insemination have been a key feature of pig production over the last decades. Several experimental articles and reviews address the post-fecundation events: from zygote formation and implantation, to embryo development and birth, there exists a collection of complex physiological processes, dependent on variables and conditionings both inherent to the animal and to environmental factors (the interested reader is referred to the reviews by Oestrup et al. [11], Waclawik [12] and Croy and co-workers [13]). Briefly, proteomics has been successfully applied to several aspects of both male and female reproduction as well as the interaction between the sperm and oocytes [14]. Spermatozoa’s surface proteins play a major role in the process of fecundation of the oocyte. In a recent study, sperm surface proteins were purified, identified and their changes were monitored during the different stages of maturation in the epididymis [15]. Proteomics approaches have been also applied
to investigate the modification to the porcine oocytes during in vitro maturation [16]. More recently, Powell and colleagues [17], using ExcTag™ labeling kit proteomics, have identified biomarkers of oocyte quality and developmental, reprogramming potential by comparing high and low quality oocytes.

Pork meat is the ultimate goal in pig production and muscle is the major component of meat and derived products. Many proteomic investigations have been carried out over the years to the end of gathering information relevant to improve pork meat quality in an efficient and cost-effective production system. In this view, proteomics technologies have been also applied to monitor breed-specific differences [18–22], gender differences [23], growth performances (meat leanness, backfat thickness [24–28]) in response to differential rearing conditions (e.g. intensive farming, feeding regime) and environment (outdoor vs. indoor) [23,24] and, in general, to further our understanding of pig meat quality [10,18–34] and of its derivative products, such as dry-cured [35–37] and cooked ham [38].

1.1.2. Breed and age or gender-related investigations
In 2010, Kim and colleagues [18] investigated the pig muscle proteome and transcriptome by means of 2DE and microarray analyses, respectively. The proteomes of white and red skeletal muscles (longissimus dorsi and soleus, respectively) were compared in Large White, Landrace and Duroc crossed animals. Significant breed-related differences were highlighted, including differential expression of heat shock proteins (HSPs) and metabolism-related proteins. Breed differences were also investigated by Mach and colleagues in a comparative proteomic profiling on 2 muscles from 5 different pure pig breeds [21]. From this study it emerged that there exist potential biomarkers for breed classification. These proteins can be used as suitable biomarkers to tackle the pig traceability, an economy-relevant pig farming issue.

Kwasiborski et al. [23,24] used 2DE to study proteome changes as a result of the original sire breed, rearing environment and gender. Potential gender specific markers were identified, including an actin isoform, a myosin light chain 2 isoform and cytochrome Bc1. Over the last decade, Hollung’s group played a key role in the field of proteomics application to meat science [39], also in the field of pig muscle research [25]. In like fashion to gender-related protein biomarkers, Hollung and colleagues suggested that protein-wide signatures also exist in a colleague like fashion to gender-related protein biomarkers, Hollung and colleagues also suggested that protein-wide signatures also exist in the field of pig muscle research [25]. Indeed, Cagnazzo et al. [41] showed that these differences arise yet at the 14 days embryo stage.

1.1.3. Pig meat quality: color, water holding capacity and PSE
Pork meat quality is the ultimate goal in pig production. Changes in the organoleptic properties of the meat can be monitored through assaying of specific parameters, including water holding capacity (WHC), pH drop and Minolta values – lightness, redness and yellowness – which are related to meat color. These values are intrinsically related to muscle protein composition, if we consider for example that red muscles rely on oxygen for their metabolism and display higher concentrations of the heme group-carrying protein myoglobin. WHC is a major parameter for pork quality and, when inadequate, may lead to PSE (Pale, Soft and Exudative) or DFD (Dark Firm and Dry) meat. The PSE zones are characterized by a reduction of proteolysis rate of three proteins, troponin T, myosin light chain and α-crystallin, and the total absence of heat shock protein 27 [8]. Development of PSE zones in muscle affects negatively coherence of the meat and represents a well-known issue with texture in cooked ham production. PSE pork meat, for example, has been proposed to be primarily caused by an accelerated rate of post mortem glycolysis resulting in low muscle pH while carcass temperature remains high, thus causing protein denaturation, which is usually correlated to poor WHC [42,43].

In a comparative investigation on Casertana (fat meat) and Large White (lean meat) pigs [26,27], we could assess that breed-specific differences at the protein level were not only related to growth performances and fat accumulation tendency in vivo, but they also affected post mortem performances through a direct influence on the forcedly anaerobic behavior of pig muscles after slaughter. In particular, higher levels of the enzyme glycerol-3-phosphate dehydrogenase and glycerol 3-phosphate and glycerol metabolites were individuated in Casertana than in Large White, which could be related to the higher backfat thickness in the former and

beef. Recently, we performed a proteomics, transcriptomics and metabolomics comparison of the longissimus lumborum muscles from the high fat-depositing Casertana pig in comparison to the lean meat Large White pigs [26]. Combining proteomics to transcriptomics profiling, we evidenced that Casertana was characterized by a greater amount of proteins involved in glycolytic metabolism and thus mainly relied on energy sources that can be rapidly mobilized. On the other hand, in Large White we detected the over-expression of cell cycle and skeletal muscle growth related genes. In a second study [27], we thus combined proteomic and metabolomic techniques to understand whether higher levels of glycolytic enzymes were utterly related to actual metabolic changes (such as lactate accumulation). As a result, we observed that a slow pH drop in Casertana pigs, albeit not the rapid pH lowering in LW counterparts, significantly correlated with the alteration of enzyme levels to some extent, and this was also reflected in the relative quantities of specific metabolites, including glycolysis intermediates and end-product metabolites [26,27].

In the study by Liu et al. [28] on the longissimus dorsi from several species, it emerged that interindividual variability in intramuscular fat content might arise essentially from differences in early adipogenesis.

Indeed, Cagnazzo et al. [41] showed that these differences arise yet at the 14 days embryo stage.
lean meat in the latter. The differential individuation of glycolytic enzymes in Casertana was related to higher lactate accumulation in this breed, although it did not produce lower ultimate pH in respect to Large White. On the other hand, alterations of the levels of glycolytic enzymes, heat shock proteins (HSPB6) and anti-oxidant enzymes (SOD1, glutaredoxin and lipoygenase) were correlated to a lower chewing time value, higher proteolysis and moderately lower WHC in Casertana in comparison to Large White.

The centrifugal drip proteome was recently tested through proteomics by Di Luca and colleagues [34] in order to correlate the differential abundance of specific proteins to anomalous WHC. As a result, HSPs appear to be altered in the centrifugal drip proteome of those animals displaying poor WHC values [34].

1.2. Proteomics and bovine science and meat investigations

Cattle farming represents a leading economic sector, with global populations estimated to be 1.3 billions of cattle (www.cattletoday.com). Bovine meat quality and traceability have increasingly become two pivotal issues in the international agenda both in developing and industrialized countries. Over the years, proteomics tools have been applied in bovine research both as far as it concerns dairy cattle (milks [44–47], metabolism [48], nutrition [49], fertility [50], health [51]) and beef cattle [52–71].

As for pig meat, bovine meat quality is largely influenced by three main attributes: flavor, juiciness and tenderness [72].

1.2.1. Proteomics and bovine meat tenderness

Bovine meat tenderness biochemistry is a long-debated issue [73]. Despite decades of advancements in the field [73–76], bovine meat tenderness still remains a hot topic. A handful of key biochemical aspects underpinning bovine meat tenderness have been known since decades [73]. A role has been confirmed for the alterations of sarcoplasmic proteins, myofibrillar proteins (actomyosin complex, cleavage of disulfide linkages, depolymerization of F-actin filaments, cleavage of myosin filaments, digestion of desmin, disorganization of Z-bands and the troponin–tropomyosin complex) and sarcolemma [19,54–56,58,60,62,67–69]. Other structural alterations tackle the connective tissue elements (collagen fibrils, ground substance). In parallel, post mortem (forcedly anaerobic) metabolism triggers accumulation of lactic acid (and acidification of the muscle), both at the intra- and extracellular level [63]. Degradation enzymes are involved in the process of muscle protein fragmentation/digestion, a process in which lysosomal cathepsins and calcium-dependent calpains play a major role, while the proteasome is involved to some extent [74–76]. In the last few years, it has been recognized a substantial overlap between the process of meat conversion to muscle and apoptosis [58,76].

Most of the proteomics investigations on bovine meat tenderness have been so far performed on the longissimus dorsi, which is a valuable cut for steak (ribeye, striploin or t-bone steaks).

Over the last two decades, proteomics investigation has helped delineating a role for structural proteins, glycolytic enzymes [54,63] and heat shock proteins (HSPs)/chaperones [60] in the frame of muscle tenderization after slaughter.

Most of the above-mentioned proteomics papers on bovine meat tenderness [51–68] have been recently reviewed [1,3,8,9]. In two recent investigations on Chianina and Maremmana longissimus dorsi tenderness [70,71], we integrated tenderness-related parameters (Warner Bratzler Shear force—WBS; collagen insolubility as an indicator of intramuscular connective tissue; myofibril degradation at 48 h and 10 days after slaughter; and sarcomere length) with the results obtained from proteomics (2DE and MALDI-TOF TOF identification of differential proteins between tender and tough meat; titanium dioxide enrichment and CID–ETD determination of phosphorylation sites; protein–protein interaction modeling and gene ontology—GO—term enrichment) and results obtained through HPLC–MS metabolomics.

The puzzle resulting from the integration of the above-mentioned proteomics analyses on bovine meat tenderness, along with the ones recently reviewed by Paredi and colleagues [8], also fueled the debate about the likely benefits of low-voltage electrical stimulation to improve carcass quality and contributed to the realization of valuable studies on this topic [52].

1.2.1.1. Fat accumulation. Besides meat tenderness, juiciness and flavor constitute two main parameters affecting meat palatability. Both are largely dependent on intramuscular fat content, which influences fatty acid composition of the muscle and contributes to modifying meat flavor. Indeed, the varying fatty acid compositions of adipose tissue and muscle determine the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavor and muscle color [77,78].

In Korean cattle steers, triosephosphate isomerase and succinate dehydrogenase are highly expressed in the early fattening stage and qualify as potential candidate biomarkers for intramuscular fat content of beef [79].

In the study by Zhao et al. [80], the differences in protein expression detected in subcutaneous adipose tissues between two breeds showing different backfat thickness accumulation tendencies (Hereford and Angus breeds versus Continental Charolais), indicated that annexin 1 expression was responsible for variation in adipogenesis in different breeds.

In a recent study [53], Zhang and colleagues concluded that carbonic anhydrase 2 and myosin light chain 3, which expressed down during adipogenic differentiation could be indicative markers for negative regulation of IMF development.

In a similar investigation, Aldai and colleagues observed that Tudanca beef had a better fatty acid profile than Limousin counterparts, especially in terms of the content of polyunsaturated (P < 0.05), long-chain polyunsaturated fatty acids (P < 0.05) [81].

1.2.1.2. Beef color. Meat color is an economy-relevant parameter, since it affects meat quality and consumers’ appraisal, in likewise fashion to the PSE and DFD meat discussed above for pig meat.

Changes in the sarcoplasmic proteomes may lie at the basis of beef color-stable (longissimus lumbrorum) and color-labile (psoas major) muscles [82]. In particular, aldose reductase, creatine kinase, and β-enolase positively correlated with redness values, while peroxiredoxin-2, dihydropteridine reductase, and heat
1.3. Proteome mapping of poultry and rabbit

Despite the fact that chicken meat is a food commonly and intensively consumed worldwide, very few investigations have been aimed at characterizing chicken muscle and meat proteomics and, in most of these studies, chickens are mainly regarded as “animal models” [83–89]. Notably, the chicken meat tryptic peptidome is characterized by some unique entries, which eases detection of chicken traces in meat mixtures containing as low as 1% chicken in pork meat [90,91].

Generally, protein profiles in chicken are strongly influenced by growth and diet. In a recent investigation [88], Doherty et al. reported that the weight of chicken pectoralis muscle increased approximately 44-fold from day 1 to 27 days. Since substantial energy derived from the glycolytic enzymes is required to maintain this mass of tissue, chickens showed a dramatic change in the relative expression levels of glycolytic enzyme, in particular of two enolase isoforms, triosophosphate isoforms, creatine kinase isoforms and tubulin isoforms and their post-translational modifications [88]. Upon comparison of Thai chicken meat to commercial broiler chicken meat, all the species showed a specific protein profile that changed during growth [85]. Also investigated was the higher quality of Thai chicken meat with respect to commercial broiler chicken meat, the former being characterized by a firmer texture and improved flavor, although it grows slowly and contains less fat. Higher meat quality of Thai chicken was largely attributed to the expression and activity of glycolytic enzymes [85].

Proteomic tools also helped evaluating the effects of dietary methionine on breast-meat accretion and protein expression in the skeletal muscles of broiler chickens [92]. A total of 190 individual proteins were identified in the pectoralis major muscle tissue, out of which three resulted to correlate with methionine deficiency in the diet.

Chicken breed proteomics produced encouraging results, as it was possible to discriminate three different chicken breeds (Pépoi, Padovana and Erminellata di Rovigo) on the basis of breed-specific protein profiles [84]. Such an application might pave the way for new scenarios in the field of chicken meat traceability and authenticity certification, still a compelling issue just a few years later than the avian flu international alarm.

Fewer reports are present in the literature about turkey meat proteomics [93], although this investigation represents only a preliminary breakthrough in turkey muscle proteomics profiles during early development.

Analogously, rabbit (Oryctolagus cuniculus) meat has been largely underinvestigated through proteomics [94–96], although the beneficial properties of rabbit meat are widely recognized, as it has been recently reviewed [97]. Again, most of the proteomics investigation on rabbit muscles in the literature address this species in the perspective of a “model” for human diseases (especially at the cardiocirculatory level), and thus only cover but marginal aspects of food science-relevant aspects of rabbit muscles/meat.

2. Technological innovation in omics disciplines and farm animal science

In this section, we will highlight how the fields of farm animal, and in particular, of meat science are increasingly taking advantage of other non-proteomics “omics” disciplines or, as in the case of post translational modifications, of newly introduced omics that stemmed from the main proteomics workflow. This brief discussion is a mandatory step to be taken before introducing the concept of the integration of multiple omics, envisaged by systems biology (see Section 3).

Recent trends in farm animal research involve the application of high-throughput technologies to alternative omics disciplines, such as the study of miRNAs [98–100], protein post-translational modification—PTM (PTMomics [101]), mass spectrometry and NMR-based metabolomics [102,103], quantitative proteomics [104] and lipidomics [105].

2.1. miRNAomics

Questions still arise and persist about the reasons underpinning the scarce overlap among transcriptomics and proteomics datasets [106]. It is still a matter of debate whether these inconsistencies only result from intrinsic technical bias of both approaches, such as in the case of déjà vu proteins in proteomics, which might either result from technical bias [107] or rather reflect an actual biological phenomenon (as in the case of “balancer proteins” [108]).

On the other hand, it is often easily forgotten that many levels of control do exist in between gene transcription regulation and actual translation of miRNAs into functional proteins. Micro RNAs (miRNA) are noncoding small RNA, 18 to 26 nucleotides long, that regulate gene expression by altering translation of protein-encoding transcripts, on the basis of multiple mechanisms that involve i) degradation of target mRNA, ii) blocking of initiation of mRNA translation and iii) blocking of translocation of mRNA to processing bodies [99]. Micro RNAs represent one key intermediate actor in the genome-to-proteome cascade, and almost a thousands of miRNAs have been resolved over the last few years which display 1165 either direct or indirect relationships between 270 miRNAs and 581 genes [109]. The miRNAome (the whole miRNA complement of a given cell/tissue) is now one of those fields attracting the bulk of attention of researchers worldwide [98–100,109–111], also in the field of farm animal science [112,113]. Indeed, miRNAs have been demonstrated to play a role in porcine pre- and post-natal development [112], intestinal development [113], growth performances and fat accumulation [114]. In 2006, Clop et al. reported that a muscle-specific miRNA regulated a gene that directly affects economic traits in livestock animals, myostatin [115]. In detail, the authors reported that a mutation in the myostatin gene of heavily muscled Belgian Texel sheep creates a target site for miR-1 and miR-206 containing RISC complexes in the exon encoding the 3′ UTR of the transcript. As a result, the Clop et al. evidenced a decreased translation of the myostatin protein and a consequent increase in muscle mass.

However, miRNAs seem not only to be involved in biological phenomena of living farm animal, but they also play a role after slaughter, as they have been related to meat tenderness and acute stress responses in Angus cattle [116], mainly by
modulating the expression of metabolism-related proteins, thereby expanding transcriptomics observations on the same breed [116].

Recently, McDanel reviewed the role of miRNA in gene regulation of livestock animals [99]. Also miRNAs play a role in cell cycle regulation and apoptosis [111], a biological process underpinning post mortem muscle biochemistry. Specific miRNAs affect protein expression of glycolytic enzymes and result in suppression of their activity, a phenomenon which has significant pitfalls in the frame of cancer cells [110], and it might also be relevant in post mortem muscles, which rely on glycolysis for energy production.

2.2. Post translational modification “omics”—PTMomics in farm animal science

Whether post-transcriptional control utterly modulates mRNA translation into proteins, post-translational regulation is a key process which influences protein functionality. Among post-translational modifications, phosphorylation [117–120], glycosylation/glycation [121], oxidation [122,123] and ubiquitinylation/sumoylation [124] seem to play the major roles in animal and meat science.

Phosphorylation of muscle proteins has also been suggested to play a role in the post-mortem process and hence in meat quality, both in cattle [117–119] and pigs [33]. During the last few years, protein phosphorylation has been related to intramuscular fat content [137] and meat tenderness [119] in cattle.

Phosphorylation of the myosin regulatory light chain during rigor mortis has been demonstrated to be involved in muscle contraction and thus meat tenderness in the bovine longissimus muscle [118].

Recently, through TiO2 preliminary enrichment of phosphopeptides, followed by mass spectrometry analysis via collision induced dissociation (CID) and electron transfer dissociation (ETD), we observed that, in the longissimus dorsi of Chianina and Maremmana cattle, higher levels of phosphorylation of structural proteins were related to a lower myofibrillar degradation index, while higher phosphorylation of glycolytic enzymes resulted in a reduction in their enzymatic activity [70,71]. The “phosphorylation-induced enzyme inhibition” hypothesis in muscle conversion to tender meat is further underpinned by recent observation that the process of meat tenderization induced through electrical stimulation appears to be related to phosphorylation levels [119], which is consistent with the activation of calcium-sensitive kinases resulting from Ca2+-release from the sarcoplasmic reticulum during muscle conversion to meat [5,75], in an apoptosis-like fashion [76].

2.3. Metabolomics and lipidomics

One of the most widely investigated aspects of the process of “muscle to meat” conversion is post mortem metabolism. As the animal dies and the heartbeat stops, blood flow arrests and muscle oxygenation drops. Therefore, post mortem muscle metabolism is forcibly anaerobic and thus mainly lies upon glycolysis and creatine-phosphocreatine shuttle to provide energy before rigor occurs [124]. Glycolysis is probably the best structurally characterized pathway in enzymology and it is considered the archetype of a universal metabolic pathway [125]. Under anaerobic conditions, pyruvate is converted into lactate in muscle tissues, which results in pH drop and acidification of the muscle [126].

Metabolomics represents one founding pillar of Systems Biology [102,103]. However, despite decades of biochemical investigations, it is but in recent years that actual metabolome and lipidome-wide technologies have become available and readily applicable to the field of muscle/meat research. Nuclear magnetic resonance (NMR) [127–130] and mass spectrometry-based metabolomics have been applied to meat-relevant issues, such as fat accumulation and tenderness and WHC [127], though only in recent years. This is mainly due to the rather recent advancements in both technological platforms (such as the introduction and broader diffusion of triple quadrupoles, quadrupole-time of flight, Fourier transform ion cyclotron resonance and orbitrap MS instruments) and, most importantly, to the growing completeness of accurate metabolite spectra databases and availability of bioinformatic tools to interrogate them, such as XCMS, MetLine, MetaboSearch, the Human Metabolome Database and KEGG [131–134]. Co-accumulation of Omics data and integrated interpretation has also been simplified by the diffusion of ad hoc browsing tools, such as KaPPA-View4, which is based upon the KEGG pathway repository [135].

In parallel, meat science metabolomics and lipidomics still take advantage from the application of classic biochemical, spectrophotometry and fluorescence-based assays, which allow monitoring oxygen consumption and mitochondrial respiration rate [136,137].

Metabolism is often considered to be “one-step closer to the phenotype” in comparison to other omics, in that protein expression is not necessarily tied to enzymatic activity, owing to the tuning effect of PTMs and, above all, phosphorylation [136]. Glycolytic enzymes are amongst the most abundant proteins in the muscle [124] and, although phosphorylation is long known to play a role in modulating their activity [137], it is but in recent times that mass spectrometry-based proteomics has allowed identifying phosphorylated aminoacid residues and, more recently, to relate them to actual metabolic modulation in post mortem muscles and meat tenderness.

Modern lipidomics almost relies on the same technological approaches which have contributed to the rapid growth of the field of metabolomics [138,139]. Implications of fatty acids and lipid metabolism in the determination of meat organoleptic properties (fat accumulation, flavor and taste) have been mentioned above and recently reviewed [77].

3. From “Omics” to system biology in farm animal and meat science

Recent trends in the scientific community have prompted reconsidering proteomics as one of the essential components of systems biology, rather than a separated science. Systems biology is a methodological and holistic approach that integrates, as a whole, the results gathered through multiple “omics” disciplines [140–145]. Indeed, systems biology is a multi-faceted approach that tackles the biological complexity of the sample under investigation from several perspectives, from the genome to the
transcriptome, the proteome and the metabolome, and then introduces mathematics and statistical modeling in order to interpret biological phenomena in the light of high-throughput results [140]. An interesting definition of systems biology is provided by Woelders and colleagues, according to which “systems biology can be described as the study of the emergence of functional properties that are present in a biological system but not in its individual components” [140], and these properties are investigated through the “analysis, filtering, combining and integration of mass “omics” data” [140]. For, as Lucretius was aware of, almost two thousand years ago, “rerum summa novatur semper, et inter se mortales mutua vivunt” (Lucretius – De rerum naturae; Book II, line 54 – “Thus the sum of things is ever being renewed, and mortals live dependent one upon another”). In more simplistic terms, biological systems are more than a mere collection of molecules, cells or organs (the parts of the system), as we need to investigate how these parts work together in order to understand the “emerging properties” of the system. “Emerging properties” imply those characteristics that are not present in the independent components but they emerge from their proper integration. To put it simply, you can have all the pieces to build a bike, but you cannot ride it unless you have put all the pieces in the right place. In biological terms, you have to look at the system as a whole in order to understand how the orchestra of genetics and regulatory interactions and the environmental factors end up affecting animal development, aging, response to diseases and, utterly, higher quality meat [140–145].

The main goal of systems biology is to produce information to make biology predictive (through mathematical models [144]) at the whole organism level. Such prediction at the animal level would result in many practical applications, including the improvement of phenotypic traits, especially those with low heritability, among which there are meat quality traits [143].

The integration of omics disciplines (see previous sections) envisages a close future of new discoveries in the areas of animal growth, development, and production and in related infectious diseases. In order to fulfill this ambitious agenda, it is essential to bridge the gap from genome-centric and transcriptome-centric investigations, by complementing results with information about the production of proteins in a cell, tissue or fluid with the metabolic processes and functions [2]. This integration is achieved by means of mathematical descriptions of the interactions (either chemical or physical) among molecules in living systems [144]. Amongst the various possible mathematical descriptions, the simplest one is based upon graph theory [144]. A graph is a set of objects called nodes or vertices that are connected by links, called lines or edges. Nodes can represent different biomolecular species (either proteins, metabolites, exogenous compounds), while edges are representative of the interactions between these molecules [144]. Graphs can be either directed or undirected: in an undirected graph, a line (edge) from a node A to node B is considered equal to a line from B to A, which is not true in directed graphs, where the two directions are counted as distinct arcs or directed edges [144].

While graph representations are often merely qualitative, quantitative mechanics models imply that the descriptions of the interactions among molecules are represented in terms of mass action or Michaelis–Menten kinetics [144]. Whether real thermodynamics are included in the equation describing the reaction rates in a kinetic model, the model is also called a (nonequilibrium) thermodynamic model [141]. Such a representation is more informative, though most of the researchers in the field tend to limit quantitative representations to subsets of the whole organism as a system, such as single organs or compound classes. Depending on the extent of variables considered, a model can be described as simplified or detailed (please, refer to Ref. [144] for further details).

Finally, in systems biological models there are three strategies to build the link between the layer of interacting biomolecules (e.g. genes, enzymes, metabolites) and the systemic functioning of the organism emerging from these interactions [144]:

i) bottom-up strategies, that describe the mechanisms in terms of mathematical equations on the basis of the properties of the components, in order to deduce systems functions as a consequence; the model undergoes testing and verification through the comparison of the simulated behavior upon changing a variable, against the behavior of the real system;

ii) top-down approaches, where the systemic behavior is the starting point and, only subsequently, on the basis of data-driven hypotheses, the system is perturbed and investigated as a whole to understand how the single components react on a genome, proteome or metabolome-scale.

iii) middle-out approaches, which imply that a sub-system is complex enough to exhibit its own emergent properties (metabolic networks, for example), which in turn integrate with other sub-systems in order to form more complex systems (for example, cells can be seen as small albeit complex systems, which are integrated in a tissue and, utterly, in more complex systems such as organs). In this approach, fragmentary knowledge of smaller systems can be further integrated in order to understand, in a step by step process, the system as a whole.

As anticipated above, the final goal of these models is to deliver a robust in silico platform that eases biological data interpretation and supports experimental design by anticipating the likely effect of a perturbation on a whole system or, conversely, by suggesting the most likely working solution for the improvement of a peculiar trait. A paradigmatic example is E-Cell, a modeling and simulation environment for biochemical and genetic processes that allows defining functions of proteins, protein–protein interactions, protein–DNA interactions, regulation of gene expression and other features of cellular metabolism, as a set of reaction rules [146].

Despite a significant theoretical background, systems biology is a young field [145], especially its application to livestock science [140–144]. Although some genetic quantitative traits have been successfully related to organoleptic properties (for example, DNAJA1 and PRKAG1 in the case of meat tenderness [147–152]), heritability estimate for carcass traits is often poor [153]. Therefore, many authors have indicated that other parameters, such as ante mortem stress [154] and birth and rearing environment and conditions [155] end up influencing not only growth performances, but also meat quality, and can be possibly used to predict the latter more effectively than
genome-wide signatures. While it is inopportune to indicate this as a failure of genomics, it is to be considered that genes (encrypted in breed specificities and animal sex) mainly affect in vivo characteristics in the long term (growth rate, food conversion) other than post mortem events. The processes leading to muscle conversion to meat are thus both tied to genome fingerprints, but also (and more closely) to post mortem specific transcripts [156] and utterly translated proteins. This is even more intriguing when considering that transcriptomics and proteomics results on the same biological matrix often scarcely overlap [157,158]. On the other hand, where poor or no direct overlap among transcriptomics and proteomics datasets is found, integration through pathway analysis often results in highlighting the same biological pathways being altered (either over-activated or down-regulated) both at the transcript and the protein level, a phenomenon described as “indirect overlap” [106,158]. From this standpoint, the integration of huge datasets from multiple “omics” approaches should be preferred to independent single-omic investigations. However, it should be also pointed out that the integration of multiple omics strategies can be a double-edged sword, in that results from a single approach might be either confirmed or confuted by integrated assays. In other terms, a jigsaw puzzle gets more complicated proportionally to the number of pieces it is made up of; thus, analogously, pages and pages of high-throughput data from integrated platforms become more difficult to interpret, although they better help elucidating the scheme behind and unraveling the whole picture (or, in other terms, the complexity of biological phenomena).

One major advantage of farm animal research is that, owing to centuries of livestock breeding selection, farm animal genetic diversity is narrower than in the human population. This is a non-secondary aspect when performing “omics” approaches in human research, where statistically significant and biologically meaningful results are often achievable only when screening large cohorts of patients [159], which help coping with the presence of outliers and the biological variability issue. On the other hand, species diversity further complicates the analysis of high-throughput data from proteomics and other “omics” approaches, which results in delays in the uptake of relevant applications. Nevertheless, characterization of species-specific muscle protein compositions enables compositional analysis of meat mixtures, which is pivotal to detect fraud where either the less expensive meat is mixed in bovine and pig meat or mechanical methods are applied to recover cheap meat versus the more costly hand-made recovery upon deboning [90,91]. Indeed, peptide sequences are resistant to food processing, and high sensitivity mass spectrometry or targeted quantitative approaches for peptide analysis, such as multiple reaction monitoring (MRM), also overcome the issue of heat-induced epitope modification, hampering reliable antibody-based recognition approaches.

Oomics technologies have also paved the way for new strategies in the identification of illegal growth promoters in biological fluids of farm animals [160]. To understand the importance of this issue, it is worth mentioning that, in 2007, the official monitoring of residues in cattle throughout the EU found <0.2% non-compliance for the use of illegal growth-promoters (GPs), including sex steroids, corticosteroids and β-agonists [160].

4. Muscle to meat conversion: an overview through integrated omics

Muscle to meat conversion is a multi-factorial process, which has been largely reviewed by expert zootechnicians and biochemists over the last decades [8,29,73–76]. What we are hereby proposing is a brief summary, which attempts to introduce the main novelties in the field, as they emerged from proteomics and recent multiple omics integrated studies (see previous paragraphs).

In this section, we will not discuss into details the effects of pre-slaughter stress for simplicity, despite its critical relevance in the field of meat quality. Indeed, proper “quality assurance” (QA) programs should be designed as to aim for a “whole of chain” approach, in order to implement the system “from the paddock to the plate,” each step in the process corresponding to a critical control point in the production chain [161]. As it has been recently reviewed, animal management, transportation and the slaughter process itself at the abattoir level significantly influence the conversion of muscle to meat, its tenderness and ultimately meat quality [162,163]. Pre-slaughter variables affect meat quality of cattle [163], pigs [164–169] and ovines [170–172]. Pre-slaughter stresses range from physical such as high ambient temperature, vibration and changes in acceleration, confinement, noise, and crowding; to psychological such as the breakdown of social groupings and mixing with unfamiliar animals, unfamiliar or noxious smells and novel environment [162]. The effects of these stresses on meat quality range from weight loss to impaired tenderness or WHC, reduced carcass yield or carcass contamination, as it has been extensively reviewed [162]. The interested reader is referred to these reviews for further details [162,163].

It is also worthwhile to stress that muscle glycogen content not only varies in a species-specific fashion (higher in pigs than in cattle, for example), but also in a breed-specific and muscle-specific (fast twitch, slow twitch) fashion, vanishing any attempt to cursorily over-generalize the process of “muscle to meat conversion” [173–175]. Nevertheless, decades of research in this field have helped individuating a few cornerstones in the post mortem biology of animal muscles.

Previously, Ouali and colleagues had schematized this process by considering three main phases: the pre-rigor step, the rigor step and the tenderizing step [74]. For the sake of clarity, we will dissect the process of “muscle to meat conversion” in seven steps and briefly discuss what is known from basic biochemistry and recent omics investigations. However, omics investigations have suggested that the boundaries among these steps might be more labile than previously thought.

4.1. Step I: Blood supply loss: oxygen and nutrient levels decline

After slaughter, animals are dressed, deboned and muscles are stored at refrigerated temperature for one week or more depending on the current national practice and/or regulations before selling. This period largely affects the organoleptic qualities of the final product, meat, and largely depends on species and breed characteristics.
Post mortem changes involve several biochemical pathways and protein metabolism, because muscle remains functional and metabolically active for several days after slaughter although depleted of the circulating blood that supplies oxygen and removes metabolic end products (i.e. lactate) [125-127].

Residual oxygen in the muscle is related to the concentrations of hemoglobin and myoglobin molecules. Myoglobin levels also affect meat color, since oxygenated myoglobin gives an appealing light red colored meat and a dark red color meat in the absence of oxygen. In Large White pigs, residual myoglobin levels positively correlated with the redness Minolta value a[27]. Absence of oxygen. In Large White pigs, residual myoglobin appears as a synonymous to bad hygienic quality[72].

Blood flow also exerts a key role in nutrient delivery and end-product removal. The interruption of this process triggers consumption of the local energy stores and accumulation of metabolic end products.

4.2. Step II: Glycolysis ensues, pH drops

Under aerobic conditions, the enzymatic conversion of glucose to pyruvate is followed by the reactions of the citric acid cycle and oxidative phosphorylation [125,127]. In parallel, a minor role in skeletal muscles is also played by the creatine/ phosphocreatine shuttle, which provides an eligible substrate for rapid reconstitution of ATP reservoir through dephosphorylation of phosphocreatine [125,127]. Within the framework of post mortem metabolism, when phosphocreatine stores rapidly tend towards exhaustion, energy is mainly produced through degradation of glycogen by glycolysis. Therefore, initial phosphocreatine stores only affect to a lesser extent the continuation of the “muscle to meat” conversion process.

Muscle glycogen content is the subsequent parameter, affecting the theoretical extent of glycolysis, of which it represents the main fuel in the muscle after slaughter [29].

Fast twitch and slow twitch muscles have their peculiar glycolytic enzyme levels [173,177], although most of the proteomics papers published so far indicated a positive correlation between glycolytic enzyme levels (especially of phosphoglucomutase 1, aldolase, glyceraldehyde 3-phosphate dehydrogenase, and triose-phosphate isomerase, enolase, pyruvate kinase and lactate dehydrogenase) and meat tenderness [58] (Fig. 1). On the other hand, it has been also proposed that this might reflect a technical bias of the 2DE approach in the way to apoptosis[74], and also supports the alteration of WHC polarity of the plasmatic membrane probably takes place between the myofibrillar proteins, which decreases the repulsion between the filaments and contributes to lateral shrinkage of the muscle fibers, sarcomere extension and, utterly, tender meat [34,42,127]. Altered ion homeostasis in post mortem muscles might be further exacerbated by differential expression or post-translational control over specific ion transporters and channels, such as carbonic anhydrase 3 and ATP sensitive inward rectifier potassium channel 15 [70,71].

A transient pH stability occurs between 1 and 8 h post-mortem (and apoptosis is thought to ensue within few minutes to 1 h after animal slaughter [74,76]), inversion of polarity of the plasmatic membrane probably takes place during the first 8 h postmortem when pH ranged between 6.4 and approximately 6.8 [74]. Another tentative explanation involves protein PTMs and, in particular, phosphorylation of glycolytic enzymes, a phenomenon that we reported to ensue in post mortem muscles yielding altered glycolytic enzyme activity and, in the end, tough meat. Indeed, tough meat muscles displayed lower levels of glycolytic enzymes, and these proteins were more phosphorylated than in tender meat counterparts [70,71]. Meat tenderness correlated with
phosphorylation of many metabolic enzymes that regulate anaerobic metabolism (including glycogen phosphorylase, pyruvate kinase and phosphofructokinase—Fig. 1) and, therefore, it may be directly linked to post-mortem pH decline. However, it remains unclear whether low pH leads to differential phosphorylation of sarcoplasmic proteins, or whether differential phosphorylation leads to an extended pH decline.

Furthermore, it has been recently reported that electrical stimulation, which promotes meat tenderization, also alters the phosphorylation pattern of metabolic enzymes [119].

4.3. Step III: Apoptosis ensues: caspases and heat shock proteins

In the previous paragraph, we have mentioned the phenomenon of phosphatidylserine externalization in tenderizing meat (Fig. 2). This phenomenon is also linked to prothrombin release in the extracellular space (Fig. 3), where the enzyme is cleaved in its activated form, thrombin, and it is thought to contribute to neural plaque degeneration [74]. This holds relevant consequences in the frame of post slaughter handling of carcasses, since a compromised neuromuscular synapsis translates into electrical stimulation being less effective if performed later during storage [74].

Most of the studies about meat proteomics indicated a strict correlation between tenderness and a series of heat shock proteins (HSPs), including DNAJA1 (HSP40) [104], HSPB1 (HSP27) [60], HSP70, HSPA8, α-crystallin (CRYAB) and other chaperone proteins, as it has been recently reviewed by Guillem and colleagues [178]. HSPs are known to play a pivotal role in apoptosis, in that they protect HSP-interacting proteins (including transcription factors, for example) from denaturation/digestion through direct binding [74] (Figs. 2–3). HSPs might contribute to tenderness-related phenomena through i) the modulation of caspase activities (initiators or effectors) through direct interaction; ii) the direct binding of protease cleavable substrate, thus preventing their degradation; iii) a chaperone role in refolding those proteins which unfold/denaturate in the harsh environment of post mortem muscles, where pH drop and protease activities end up dramatically altering protein integrity and native conformation; iv) the promotion of apoptosis when stress levels become unbearable for muscle cells [70,74].

Other than glycolytic enzymes, we could report higher levels of phosphorylated HSPs in tender meat, thereby modulating their multimerization state and thus their functions, through a mechanisms that might involve changing of steric interactors of HSPs (an example is reported for HSPB1 [186]) (Figs. 2–3).

Fig. 1 – After slaughter, blood flow stops and the muscle becomes anoxic. Therefore, energy is produced through glycolysis by mobilizing glycogen and consuming local sugars, while the creatine/phosphocreatine represents an alternative pathway to provide ATP for rapid consumption. Metabolites are enlisted in the figure along with the abbreviated UniProt names for each specific enzyme catalyzing the production of the subsequent metabolite in the column. Enzymes highlighted in red have been associated to tender meat in proteomics investigations in the literature. Red and green ATP circles are indicated next to those reaction characterized by the consumption or production of ATP, respectively. Finally, in the left panel we briefly summarize the main effects of ATP deprivation in muscle cells. Abbreviations (in alphabetical order): ALDO (aldolase), CKM (creatine kinase M), ENO (enolase), GAPDH (glyceraldehyde 3-phosphate dehydrogenase), HEX (hexokinase), LDH (lactate dehydrogenase), PCr (phosphocreatine), PFK (phosphofructokinase), PGAM (phosphoglycerate mutase), PGM (phosphoglucone mutase), PGK (phosphoglycerokinase), PYGM (glycogen phosphorylase), TPI (triose phosphate isomerase).
In relation to DNAJA1 zinc finger domain, it is worthwhile to stress that proteomics investigation highlighted a significant correlation of certain zinc finger protein (zinc finger protein 197, for example [71]) to bovine meat tenderness (Fig. 3).

Calcium dysregulation, low pH and altered levels/PTM-modulation of HSP activity end up triggering apoptotic cascades [74,76]. However, apoptosis in muscle cells might also follow alternative pathways, as the one promoted by the caspase-triggered poly ADP ribose polymerase (PARP) fragmentation [71], a mechanism which is known to trigger caspase 3 activation and skeletal muscle apoptosis, other than being indirectly related to calpain over-expression via PARP-mediated transcriptional control [187] (Fig. 3).

Another mechanism through which apoptosis might unfold in muscle cells could involve the protein 14-3-3 gamma (YWHAG) (overexpressed in tender meat in Chianina [71]), which is a mediator of signal transduction playing a role in apoptosis, through binding to MDM and thus affecting the activity of p53 (Fig. 2).

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**Fig. 2** – An overview of the intricate pathways leading to tenderization of the muscle through apoptosis. The interplay between blood flow arrest (causing anoxia, accumulation of oxidized iron and of byproducts of metabolism, such as lactate and nutrient deprivation) is linked to the increase in reactive oxygen species (ROS) within the cell. Altered metabolism leads to the arrest of mitochondrial metabolic activity, while the cells end up relying on glycolysis and the phosphocreatine shuttle. However, the negative feedback on glycolysis triggered by pH lowering and lactate accumulation result in ATP consumption and, subsequently, accumulation of AMP (activation of AMPK and autophagic pathways), alteration of ion homeostasis modulation (blockade of ion pumps and calcium release from mitochondria and sarcoplasmic reticulum). Calcium intracellular accumulation activates calpains, which activate Bax and promote apoptosis through the mitochondrial intrinsic pathway (release of cytochrome C, activation of caspases). In turn, activated caspases cleave PARP, which relocates in the nucleus and promotes transcription of calpains. A role for zinc finger proteins and alternative histone 2 proteins (H2AFX and Y) is suggested, as discussed in the text. Kinase activation triggers downstream phosphorylation which affects Heat shock protein binding-partners (hampering their protective role against proteases), enzymatic activity of glycolytic enzymes and structural protein resistance to degradation (please, refer to the main body of the article for further details). Finally, miRNA appears to contribute to tenderness through modulation of glycolyte enzyme expression.
Finally, as predicted through bioinformatic by Guillemin and colleagues [178] and subsequently confirmed elsewhere [70], specific histone protein isoforms might take part in the process of programmed muscle cell death, such as histone H2AF isoforms (e.g. H2AFY and H2AFX) (Fig. 2).

4.4. Step IV: Energyless muscle and onset of rigor: control is lost over Calcium reservoirs, kinases are activated and proteins are phosphorylated

As a consequence of the slaughter, glycogen levels in the muscle decrease, and so does the energy available to keep the muscle in a relaxed state. Indeed, ATP is a fundamental energy token to trigger dissociation of the actin–tropomyosin complex [188]. This process is also influenced by free Ca2+ concentrations. Muscle acidification triggers dysregulation in calcium release and, along with ATP exhaustion, it leads to the formation of cross-bridges between myosin and actin filaments and thus to the onset of rigor [74,185] (Fig. 2). Being a secondary intracellular messenger, calcium release results in downstream cascades leading to the activation of calcium-dependent kinases (such as PKC) and phosphorylation of target proteins [120]. Energy consumption and an increased AMP/ATP ratio might in turn activate the kinase AMPK in an apoptotic-like fashion, triggering downstream cascades leading to protein phosphorylation and utterly to apoptosis [120] (Fig. 2). This is also consistent with the role of AMPK in PSE meat [120]. Phosphorylation of glycolytic enzymes might be modulated by this mechanism [189]. On the other hand, protein phosphorylation events might occur early after death (1 h post mortem, for example [119]), when high energy phosphate-rich metabolites (such as ATP) are still abundant, and phosphorylation of proteins could parallel or participate in apoptotic cascades. The drop of pH can cause the development of PSE zones in muscle, which produces zones that are characterized by alteration in texture, color and exudation, related to a reduction of proteolysis rate of three proteins, troponin T, myosin light chain and α-crystallin, and the total absence of heat shock protein 27 [8,34].

4.5. Step V: Calcium-dependent and independent proteases cleave myofibrils at the Z-disk

Although it has been long thought that cathepsins and calpains were the only proteases responsible for meat tenderization, it is now clearly emerging that the tenderization process is a multienzymatic phenomenon implying the activation of

Fig. 3 – An overview of the interplay among muscle proteases (in bold font) in the frame of meat tenderization. While activation of calpains is triggered by calcium ions and sustained at the transcriptional level by PARP-mediated pro-apoptotic signaling, calpains themselves trigger release of cathepsins from the lysosome. In the frame of the apoptotic process, caspase are activated via the intrinsic mitochondrial pathway of apoptosis. In addition, a role is proposed for the proteasome (inhibited by acidic pH of longer stored meat), metalloproteinases (activated by extracellular serin peptidases) and matrix metalloproteinases. Each one of these proteases ends up attacking the structure of myofibrils, especially at the Z-disk level, promoting tenderization.
cathepsin and calpains, along with other proteolytic systems (proteasomes, caspases, metalloproteases, metallopeptidases, serine proteases—Fig. 3) [74].

In most unsupervised proteomics studies, calpains and cathepsins (at the protein level) do not usually emerge as major players in the tenderization process (no statistically significant differential protein concentrations are usually observed in proteomics studies), though targeted quantitative approaches (multiple reaction monitoring—MRM; western blot) might have bypassed the probable technical bias of certain experimental tools such as 2DE, which are more likely to reveal major fluctuations or differences in most abundant species (i.e. glycolytic enzymes, structural proteins and HSPs in muscles). Also, it is worthwhile to stress that fluctuations of protein activities are not necessarily tied to over-expression of those specific enzymes, since activity might be influenced by the alteration of the biochemical milieu (e.g. higher free calcium level, lower pH) and post translational modifications.

Despite technical difficulties encountered by proteomics investigation in elucidating the role of calpains, cathepsin and the proteosome in meat tenderness, biochemical evidences have been accumulated over the years about their central role in meat protein digestion after slaughter [190–193] (Fig. 3). Protease activity is either boosted by pH lowering or calcium accumulation (such as in the case of μ-calpains) [29,74,193]. Calcium-binding proteins, such as calmodulin and calsequerin, which are involved in calcium signaling modulation in the living animal [194], after slaughter contribute to lowering the levels of free Ca²⁺ and thus in reduced calpain activity. Calpastatin exerts an inhibitory action on calpains by directly binding to multiple calpain proteins through specific subdomains [193]. Calpain inhibition utterly influences meat tenderness and WHC.

Another factor contributing to reducing the extent of protease-mediated degradation of myofibrillary proteins is phosphorylation of structural proteins, especially of Z-disc-related proteins, thereby preventing proteases from attacking fibers and thus resulting in tough meat. Z-disc proteins regulate muscle functions through reciprocal interaction. Disruption of these interactions results in muscle disorders. Phosphorylations of Z-disc proteins increase interactions of myotilin, myozinin 1 and troponin at the Z-disc line [195], and modulate actomyosin complex formation [196,197], increasing sarcomere cohesion and reducing accessibility to proteases. Phosphorylation of synaptopodin also affects its compartmentalization, mediating its release from the Z-line and nuclear import [198].

While proteomics is probably not suited to directly investigate the correlation of protease levels to myofibrillar fragmentation, indirect hints of the extent of protease activity (the utterly relevant biological phenomenon, rather than differential protease expression) appear to emerge from fragmentation levels and the overall number of protein spots that could be discriminated through electrophoretic approaches. Studies over the years have indicated how specific protein fragments arise after slaughter and how the levels of specific proteins (including glycolytic enzymes, HSPs and antioxidant enzymes) do correlate with myofibrillar degradation indexes, either at 24 h and 48 h post mortem [19,70,71].

4.6. Step VI: the controversial role of oxidative stress in the promotion of myofibrillar degradation

Antioxidant enzymes are perhaps the most interesting categories of proteins, though their correlation to bovine meat tenderness is controversial. Indeed, myofibril protein fragmentation is not only a protease-mediated process, since oxidative stress might play a role as well (Fig. 4). The extent of protein oxidation in muscle food has been recently reviewed by Lund and colleagues [142]. Both in pigs [27] and cattle [70,71], meat quality parameters such as WHC and tenderness significantly correlated with oxidative stress accumulation (GSH/GSSG ratios, altered levels of anti-oxidant stress enzymes, such as superoxide dismutase [199], glutaredoxin, lipoxygenase and ascorbate carrier [26] (Fig. 4). Residual myoglobin and non-heme iron [176], along with altered mitochondrial activity in the anaerobic environment of post mortem muscles lead to the formation of reactive oxygen species (ROS) that cause oxidative damages to proteins (Fig. 4). During the first 4 days a sharp increase (57%) in oxygenation levels in sarcoplasmic proteins takes place [200].

According to Rowe and colleagues [200], protein carbonyl content positively correlated with WBS values in beef, which is suggestive that increased oxidation of muscle proteins early post mortem could have negative effects on fresh meat color and tenderness. SOD1 (free radical scavenger) positively correlated with meat tenderness also in the report by Guillemin and colleagues [178], although opposite correlation trends were obtained for peroxiredoxin 6 (tackling hydrogen peroxide) [57].

A tentative explanation of the process is provided by Rowe and colleagues [201], which indicates that antioxidant enzymes might protect proteases (such as μ-calpain) from oxidation and inactivation, thus indirectly favoring meat tenderization. Often, overexpression of antioxidant enzymes is more frequent in glycolytic muscles (such as in the semitendinosus, as in Ref. [178]) or in more “glycolytic-breeds” [26,27] (Fig. 4). However, we should also report that controversial results have been obtained when studying cattle beef tenderness [70], although in this case oxidative stress was related to promotion of apoptosis and thus improvement of meat tenderness. Cursory summarizing the role of oxidative stress in the process of muscle to meat conversion, it could be concluded that the presence of higher levels of anti-oxidant enzymes (and vitamin E [202]) protects the muscle from early oxidative stress and thus reduces the extent of protein carbonylation; it protects proteases from oxidative stress-induced inactivation and thus prevents yielding of tougher and darker meat (Fig. 4).

4.7. Step VII: Muscle structure is altered by proteolysis and ion homeostasis dysregulation

Myofibrillar degradation is one fundamental phenomenon during meat tenderization [19,177,189].

Degradation of troponin T isoforms during postmortem muscles aging is known to progress simultaneously with the post-mortem tenderization of beef meat [203]. Despite rather complex and multi-factorial processes at the basis of meat tenderization events, all the nine fast-type and the two slow-type isoforms present in the bovine muscle are cleaved during post-mortem aging primarily in the glutamic acid-rich amino-terminal region to generate basic fragments that are
likely good predictive markers for beef aging and development of tenderness [204]. In this view, Bauchart and colleagues [204] performed a characterization of low molecular weight peptides (lower than 5 kDa) generated in bovine pectoralis profundus muscle during meat aging and cooking in order to reveal post mortem proteolytic degradation of muscle which occurs as part of cellular death and meat aging process.

However, the disruption of myofibrillar structure integrity results both in improved tenderness and impaired WHC. This is the main reason why increasing storage length will be profitable for tenderness and flavor, although it will have a rather deleterious effect on juiciness and color [74].

5. Conclusion

Despite decades of investigations, the mechanisms underpinning meat tenderness are only partially understood. This is mainly due to the enormous amounts of variables affecting meat tenderness per se, including biological factors, such as animal species, breed-specific-characteristic, muscle under investigation. However, it is rapidly emerging that the tender meat phenotype is not only tied to genetics (livestock breeding selection), but also to extrinsic factors, such as the rearing environment, feeding conditions, physical activity, administration of hormonal growth promotants, pre-slaughter handling and stress. Finally, post mortem handling plays a role as well, including storage temperature and duration, hanging method, delays in transfer to cold tunnel, electrical stimulation and, last but not the least, cooking methods.

From this intricate scenario, biochemical approaches and omics investigations have helped establishing a few milestones in our understanding of the events leading from muscle to meat conversion. While some groups are already at that point when integration and mathematical modeling are being optimized, most of proteomics investigators still rely on single omics approaches. Therefore, the growing need for integration of omics disciplines in the field of systems biology will soon contribute to take further step forward also within the context of meat science. Soon enough, we could end up realizing that, by looking at each independent pathway separately in the framework of meat tenderization, we could not see the forest for the trees.

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