Proteomic Technologies in Pork Products

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Submitted by: Mohammed Gagaoua

Definition

This entry deals with the productive (breed, diet, stress) and technological (aging, cooking) factors that affect fresh pork and elaborates the quality of products by using proteomic tools. These technologies are a relevant approach in the meat science field to decipher the underlying mechanisms and post-mortem changes in the muscle and biofluids proteome of pigs because their study will allow better management of the outcomes such as meat quality variation and defects. In general, these new developments in molecular techniques can help researchers to control and assess this quality through biomarkers. Additionally, as food safety and pork product authentication/adulteration to avoid fraud can be evaluated with these high-throughput proteomic tools. Overall, this review describes the current and emerging proteomics studies dealing with raw pork and pork products from the farm to fork.

1. Introduction

The global demand and industrial production of pork and pork products are increasing. According to the Food and Agriculture Organization of the United Nations (FAO) statistics, worldwide swine production has increased steadily from 1961 to 2007, with Asia the continent with the highest production with around 50% of the total worldwide production. Between 2007 and 2017, production increased significantly in Asia and remained as the highest swine producers (almost 60% of the world total), since China assumed most of the production with more than 3700 million pigs produced during this time. Europe, with more than 25% of world production, is the second continent with the highest production. Germany, Poland, and Spain are the major countries with a remarkable rate in swine production [1]. Regarding pork consumption, the Asian continent is the area of the world with the greatest increase, from 2.8 million tons in 1961 to 67.4 in 2013, mainly related to China. For example, in 2013, pork demand in this country accounted for 80% of the Asian continent and around 50% worldwide [1].

From the above, it seems that the swine industry has significant interest in becoming more efficient and innovatively fitting with the consumer demand. Consumers are now demanding higher standards for the welfare of pigs and pork quality. Factors affecting the production system (breed, age/slaughter weight, sex, castration, and diet), pre-slaughter conditions (fasting, transport, lairage and stunning), and post-mortem interventions (electrical stimulation of the carcasses, aging, and storage conditions in terms of time/temperature, cooking, etc.) have an impact on pork eating quality, therefore, these factors should be carefully monitored during the whole continuum of farm-to-fork, especially at the industrial level. The relationship between animal welfare and pork quality has been detailed and rigorously researched from a proteomic point of view [2]. During the processing of meat, tenderness, color, and water holding capacity (WHC) traits are strongly impacted. Indeed, tenderness, intramuscular fat, cooking loss, and sensory traits are the main qualities of pork meat, which have been the most investigated and improved during the last decades. Currently, the meat research is focusing on a deeper understanding of the conversion of muscle into meat. This process results from myriad interconnected pathways including the enzymatic action of endogenous proteolytic enzymes of the muscle such as calpains; afterward, the impact of pH decline and that of lysosomal enzymes (cathepsins) that break the supramolecular structure of sarcomere as well as weakening the Z-discs, hence reducing the strength of the anchoring of the actomyosin complex and myofibrillar proteins [3]. In these stages, protein degradation plays an important role in the development of the pork quality traits (pH, color, WHC, and tenderness), causing when uncontrolled, serious technological defects and economic losses to occur [4].

One of the main problems associated with poor pork quality is the PSE (pale, soft, exudative) meat defect. This defect can be, for example, exacerbated by inadequate mixing with animals from different pens or batches raising fights and aggressions, increasing their stress pre-slaughter, hence causing PSE meat [5]. Stressed pigs are associated with a higher concentration of lactate and rapid pH decline during rigor mortis, provoking PSE meat [6]. Moreover, long journeys could result in lower carcass pH at 45 min, leading to a high incidence of PSE meat [7]. Along the same line, an inadequate carcass cooling process could modify the metabolic processes and the extent of pH decline, hence leading to PSE meat [5]. In the case of PSE meat, changes in the sarcoplasmic and myofibrillar proteins are observed, and specifically, a lower solubility and higher denaturation of the proteins are evidenced [8]. This phenomenon could be the result of several biological pathways such as fast post-mortem glycolysis in the muscle, which are specifically targeted by activation of glycogen phosphorylase and phosphofructokinase, reducing the pH and protein denaturation and therefore inducing the PSE defect [9][10]. Consequently, pig stress has adverse consequences on fresh pork due to the impact on the transformation of muscle into meat. Considering the above, this is a major problem to the industry due to the economic losses, as this type of meat cannot be used for the elaboration of high-value cured meat products and other products. In this sense, a prediction of PSE through protein modifications would be a powerful strategy [11], among other emerging chemometric methods [10].

In the framework of high-throughput omics technologies, proteomics offers insights about the complex network of proteins and pathways underlying variation in pork quality. The study of the post-mortem muscle proteome together with protein–protein interactions, and post-translational modifications of proteins become a challenging task to deliver high-quality meat products [12]. In particular, the proteome is the result of a gene expression influenced by environmental and processing conditions related to the functional quality characteristics of the meat [13]. Gel-based proteomics and analytical approaches based on mass spectrometry are increasingly being used. In the proteomic workflow, often the initial step is a fraction of protein extracts separated using gel electrophoresis [one dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) or two-dimensional gel electrophoresis (2-DE)] coupled to liquid chromatography tools for identification. The large capacity and power of these technologies to study plant and animal products has been demonstrated [14][15][16][17]. Afterward, the bottom-up approach is the most common strategy resulting from the extraction of proteins and digestion by sequence-specific enzymes for later analysis by mass spectrometry [18]. It should be highlighted that those post-translational modifications of the proteins and their interactions with other proteins or macromolecules have a strong impact on pork tissues because of the change in the three-dimensional structure and consequently of the cellular functions [19]. Indeed, solubility, thermal stability, gelation, emulsifying, foaming, fat binging, and water-binding are only a few of parameters that directly depend on the protein structure, resulting in its great importance in food science [20]. For all these reasons, food proteomics, also known as foodomics [21], provide a great opportunity in the quality and safety controls of pork and pork products (Figure 1).

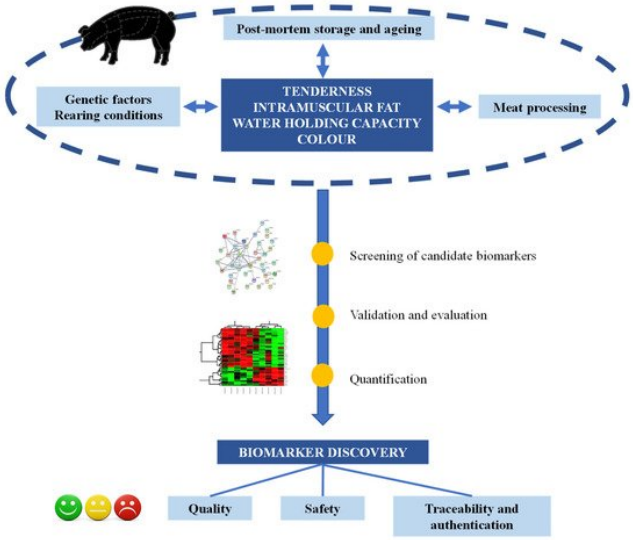

Figure 1. Proteomic workflow in the search for protein biomarkers regarding pork quality.

2. Proteomic Perspective of Breeding and Rearing Practices

For consumers, the perception of muscle color, texture, and marbling are the main aspects that drive their choice. The consumer's first impression is strongly associated with the pork color at the point of sale whereas muscle firmness/texture and marbling are used to predict the final eating quality. This complex network of sensory attributes defined by color, pH, texture, water holding capacity, and marbling can be, for example, impacted by breed selection and rearing practices [22]. In general, meat quality traits are the result of genetic and environmental interactions. Therefore, livestock production systems including feeding, housing conditions, genotypes, behavioral, and physiological responses are widely recognized as the key factors driving pork quality. In recent years, different strategies aiming to improve the pork quality such as breed selection, animal management, and feeding, and other aspects of pork processing have been considered. Some recent studies in this context are briefly summarized in Table 1. Proteomics has mainly been employed to evaluate the meat quality by searching protein biomarkers and defining the molecular pathways as reviewed by Schilling et al. [4]. The great majority of proteomic experimental designs have been aimed to understand the underlying biological processes and identify biomarkers for meat processing [23].

Studied Factors		Protein Source	Proteomic Approach	Main Findings	Ref
Genotypes	Genotypes: Tibetan and Duroc × (Landrace × Yorkshire) (DLY). Feasibility of using differential proteomic analysis to discriminate among pig breeds and cuts	Different muscle cuts	LC-MS/MS	The differential proteins belong to two major categories: meat quality-associated proteins (calcium ion binding protein, Calsarcin 1, CA1 and MYBPH), and energy metabolism-associated proteins (CSRP3, GSTK1, COX6A, AMPD, and TXNL1). Regardless of pork cuts, comparative proteome analysis between Tibetan and DLY pork identified 102 differentially abundant proteins.	[24]
	Genotypes: Chinese indigenous Shaziling and the Yorkshire breeds	LD	2-DE and MALDI-TOF/TOF	23 differentially expressed proteins were identified and associated with fatty acid metabolism, glycolytic pathway, and skeletal muscle growth. These differentially expressed genes and proteins are candidate genes for improving meat quality in Shaziling pigs.	[25]
	Mechanisms of Halothane (HAL) and Rendement Napole (RN) Genes	LD	iTRAQ, TiO2 enrichment and LC-MS/MS	The HAL mutation contributes to the upregulation of phosphorylation in proteins related to calcium signaling, muscle contraction, glycogen, glucose and energy metabolism and cellular stress.	[26]
	Feed efficiency: high-FE and low-FE pigs were compared.	LD	iTRAQ LC-MS/MS	124 proteins were differentially expressed between the high- and low-FE pigs. The glucose–pyruvate–tricarboxylic acid–oxidative phosphorylation energy metabolism signaling pathway was an important regulated pathway. Enzymes involved in the conversion of glucose to pyruvate were upregulated in the high-FE pigs.	[27]

Feeding	Feed efficiency: Pigs with low-RFI (“efficient”) and high-RFI (“inefficient”)	LD	2-DE and LC-MS/MS	11 proteins showed a differential abundance between RFI lines. However, the differentially expressed proteins were not affected by feed restriction.	[28]
	Dietary ractopamine supplementation to improve pork leanness.	LD	2-DE and MALDI-TOF/TOF	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglucomutase-1 (PGM1) were over-abundant in control pigs, whereas serum albumin (ALB), carbonic anhydrase 3 (CA3), L-lactate dehydrogenase A chain (LDHA), fructose-bisphosphate aldolase A (ALDOA), and myosin light chain 1/3 (MYL1) were over-abundant in pigs ingested with ractopamine. Ractopamine suggested to influence the abundance of enzymes involved in glycolysis.	[29]
	Dietary L-arginine supplementation to reduce backfat thickness and increase intramuscular fat (IMF).	LD	2-DE and LC-MS/MS	The proteome changes in LD muscle between the control and supplemented pigs showed that L-arginine significantly influenced the abundance of proteins involved in energy metabolism, fiber type, and muscle structure.	[30]
Castration	Immune and surgical castrated pigs	LD	LC–MS/MS and Western blot validation	Fifty proteins were differentially abundant between the two groups. Proteins involved in cytoskeleton and immunity were more abundant in the immune castrated group. Several heat shock proteins (HSPs) and laminins were abundant in the surgical castrated group.	[31]
	Surgical castration against entire male pigs	LD	2-DE and MALDI-TOF/TOF	Entire male pigs have a more oxidative metabolic profile than surgically castrated pigs. More abundance of structural protein fragments suggests a higher degree of proteolysis in entire male pigs.	[32]
Growth	Muscle growth and lipid deposition. Comparison between two Chinese mini-type breeds and two western breeds.	LD	iTRAQ, LC-MS/MS	288 differentially abundant proteins of which 169 were upregulated and 119 were downregulated between the two types of genotypes. Among them, 28 were related to muscle growth and 15 to lipid deposition.	[33]
Animal welfare	Animal stress and welfare changes in the housing system	Blood	2D-DIGE and MALDI-TOF/TOF iTRAQ	Changes in two main homeostatic mechanisms: the innate immune and redox systems. The acute phase proteins haptoglobin, apolipoprotein A-I and α1-antichymotrypsin 3 (SERPINA3), and the antioxidant enzyme peroxiredoxin 2 were differentially expressed.	[34]
	Heat stress. Effect of chronic heat stress, thermal neutral and restricted feed intake conditions on hepatic proteomes	Liver	2-DE and LC-MS/MS	Forty-five hepatic proteins were differentially abundant among groups. The proteins were involved in response to stress and immune defense, oxidative stress response, cellular apoptosis, energy metabolism, signal transduction, and cytoskeleton.	[35]
	Heat stress. Effect of chronic heat stress against thermal neutral on meat quality	LD	2-DE and LC-MS/MS	Changes in the expression of myofibrillar proteins, glucose and energy metabolism-related proteins, heat shock proteins, and antioxidant enzymes might be affecting tenderness.	[36]
	Animal welfare (heat stress) Effect of acute heat stress for 2, 4 and 6 h against thermal neutral on meat quality	ST (red and white areas)	2-DE and Western Blot	Several proteins were altered affecting metabolism, cell structure, chaperone, antioxidant, and proteolytic activity. The proteome data showed that the muscle proteome was altered from 2 h of heat stress.	[37]

	Animal stress (lysine acetylation)	LD	Acetylpeptide enrichment and LC-MS/MS	The acetylation of proteins was enriched in muscle contraction, carbohydrate metabolism, cell apoptosis and calcium signaling.	[38]

LD = Longissimus dorsi, ST = Semitendinosus.

3. Application of Proteomics to Assess Pork Quality and Authentication

3.1. Influence of Different Post-Mortem Traits on Pork Quality

Meat quality is generally determined by farm production factors as well as by post-mortem interventions techniques applied at the industrial scale. Recent studies regarding the effect of different post-mortem traits aiming to assess and improve pork quality are shown in Table 2. Within pork quality, tenderness is the main important sensory quality trait that is mainly affected by connective tissue, intramuscular fat content, and myofibrillar structure, which may interact with storage time [39]. In the early stages of rigor mortis, meat tenderization is widely studied from a proteomic point of view. In this process, protein degradation due to the muscle enzymes, pH decline, and tissue oxidation are key factors in developing tenderness, flavor, color, and juiciness. In the case of protein oxidation, relevant biomarkers for the oxidative process are the group of peroxiredoxins that control the hydrogen peroxide concentration to protect cells. Indeed, peroxiredoxin-2 (PRDX2) was found to be more abundant in tenderer pork loin during the aging [40]. Furthermore, PRDX3 and PRDX6 were found to be significantly correlated with the lightness (L*) and redness (a*) color traits, respectively [41][42]. Other oxidative stress proteins were found to be related with pork color such as Parkinson disease protein 7 (PARK7), playing a pivotal role in cell protection against oxidative stress and cell death [42]. During the processing and storage of products, protein oxidation contributes to reduce pork quality [43]. For this reason, the storage technologies of pork were assessed to avoid protein oxidation. For instance, in the case of high-oxygen atmospheres, the disulfide bonds among myosins are formed as a result of cysteine and methionine oxidation. Additionally, the oxidation of both amino acids produces different oxidation products at different sites, representing a high complexity [44].

In the case of pork quality, proteomic technologies have been demonstrated to be very effective in the prediction of drip loss and ultimate pH from 50% to 80% of protein biomarkers in the total proteome [45]. This trend of prediction was further confirmed by Kwasiborski and co-workers on these quality traits as well others such as color parameters [42]. This means that proteomics is a promising approach to efficiently assess pork quality. An intense change of proteome was observed and associated with pH, color, and drip loss traits, resulting in 140 differentially expressed proteins. Functional analysis showed a decreased release of Ca^{2+}, lower contents of type II fibers and those of glycogen, which decreased the extent of glycogenolysis in high-quality meats from the longissimus dorsi muscle [21]. Considering only drip loss, an enrichment analysis resulted in sphingolipid metabolism and glycolysis/gluconeogenesis as key pathways significantly influencing drip loss [46]. However, water holding capacity trait over post-mortem aging is not yet fully understood by the preliminary proteomic studies and further work is needed [47]. On the other hand, intramuscular fat content positively influences the taste and is both regulated by adipogenesis and myogenesis, which balances the number and the size of adipocytes and myocytes [48]. A strategy to reduce the intramuscular fat is by lowering the use protein diets, which seems to be at the origin of the enhancement of glycolysis and the Krebs cycle pathways as well as modifications in mitochondria, contractile proteins, and calcium signaling, therefore, impacting the extent of the glycolytic to oxidative properties of fibers [49]. It is well-known that in pork quality, the fiber distribution of oxidative and glycolytic types (red and white muscles) is a key factor in post-mortem metabolism [50]. Myosin-1, myosin-4, troponin complex (fast), myosin light chains, and metabolic enzymes are overexpressed in glycolytic fibers and myosin-2, myosin-7, and myoglobin; meanwhile, mitochondrial oxidative metabolic enzymes were especially abundant in oxidative fibers [51]. The high amount of oxidative fibers suggests, at first glance, an increased quality due to the link with a higher amount of intramuscular fat.

3.2. Proteomics and Authentication/Adulteration of Pork Products

In addition to the above-mentioned, other objective of the pork industry is food authentication and the detection of adulterations. A non-extensive summary of pork product authentication/adulteration is shown in Table 2. The replacement of ingredients with undeclared species, animal tissues, and geographic origin or the distinction of non-ecological meat and freeze/thawed meat is a technical challenge for proteomics. This is a complex issue because proteins undergo chemical modifications such as Maillard reactions in highly processed food products, hampering efficient authentication. In animal species identification, a set of heat-stable peptide markers from myosin, myoglobin, hemoglobin, L-lactase dehydrogenase A, and β-enolase allows for the preliminary authentication of eleven types of white and red meats from chicken, duck, goose, turkey, pork, beef, lamb, rabbit, buffalo, deer, and horse [52]. Despite the fact that immunoassay techniques (ELISA) are often used to quantify target compounds, some disadvantages are likely to be the low efficiency and cross-reactivity between species, making more difficulties and hindering identifications. For example, the skeletal muscle protein troponin I detection was quantifiable from 8.7 to 52 ng/mL by the ELISA method, and similar results were achieved by 2-DE in combination with Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF/TOF) [53]. In this regard, mass spectrometry becomes a relevant method to quantify specific peptides of different species that could be proposed as biomarkers. For example, five peptides from myosin were used to identify and quantify trace pork (up to 0.5%) in meat mixtures by parallel reaction monitoring (PRM) [54]. On the other hand, pork products and unprocessed meat should be packed, distributed, and stored appropriately. Fresh meat is more appreciated than frozen meat because of the formation of ice crystals than can destroy the ultrastructure of the matrix and consequently denature the proteins, hence reducing the potential meat quality. By using meat exudates to search for protein biomarkers, it was possible to discriminate between fresh and frozen meat [55]. From this study, a total of 22 proteins were found as candidate discriminatory biomarkers using gel-based techniques.

Table 2. Recent studies regarding the effect of different post-mortem traits aiming to assess and improve pork quality as well as studies related to food safety and pork product authentication/adulteration.

Studied Factors		Protein Source	Proteomic Approach	Main Findings	Ref
Ageing	Ageing pork. Effect of protein lysine acetylation in post-mortem muscle changes	LD	Acetilpeptide enrichment and LC-MS/MS	Acetylproteins involved in apoptosis, calcium signaling, and IMP synthesis were identified in post-mortem porcine muscle. The lysine acetylation of proteins regulate the conversion of muscle into meat.	[56]
	Tenderness and aging	LD	2D-DIGE and mass spectrometry	Soluble desmin and peroxiredoxin-2 could be used as biomarkers of tenderness in aged pork products.	[40]

	Protein oxidation (oxidation of cysteine and methionine residues) Effect of hydroxyl radicals on the myosin	LD	SDS-PAGE, cysteine and methionine labelling and LC-MS/MS	The cysteine at the head of the myosin and that at the coiled tail of myosin easily generated disulfide. Further, the methionine at the coiled tail of myosin was more easily oxidized than that of the head.	[44]
Pork quality	Effect of proteome profiles on meat quality (high-quality samples against low-quality)	LD	Tandem mass tag labelling and mass spectrometry	Lower degree of glycolysis in high-quality compared to low-quality meat. The levels of oxidative stress and apoptosis were low in high-quality meat.	[21]
	Meat quality: drip loss Identification of candidate genes	LD	Isotope coded protein labelling followed by selected reaction monitoring analysis	The enrichment analysis resulted in 10 pathways. The most relevant pathways were sphingolipid metabolism and glycolysis/gluconeogenesis in relation to drip loss. It allowed proposing genetic markers and candidate genes for drip loss.	[46]
	Meat quality: pH, color traits, drip loss, water holding capacity	LD	2DE + MALDI-TOF	Proteins associated with ultimate pH, lightness, drip, thawing and cooking loss were related to the glycolytic pathway, phosphate transfer, or fiber type composition. In the case of thawing loss, the proteins were related to denaturation of myofibrils or lipid content. Redness involved proteins were enriched in post-mortem oxidative activity.	[42]
	Intramuscular variation, neat quality (color, drip loss and tenderness) and their relation to proteome	LD	Label-free quantification + LC-MS/MS	Glycolysis enzymes (enolase 3, ALDOA, LDHA, PGM1, and TPI1) were highly abundant in the medial and posterior region. GAPDH and myoglobin were overexpressed in the medial region	[57]
	Water holding capacity measured as centrifugal exudate (High drip vs. Low drip) across post-mortem aging on different phenotypes	LD	2-D DIGE followed by MALDI-TOF/TOF and nano-ESI LC-MS/MS.	Discriminatory proteins identified include metabolic enzymes, stress response, transport and structural proteins. Twenty-five proteins were used to discriminate between high drips and lower drips with accuracy higher than 72%.	[47]
	Intramuscular fat content	LD	Tandem mass tag labelling and parallel reaction monitoring analysis	ALDH1B1, OTX2, ANXA6 and Zfp512 were proposed as candidate biomarkers associated with intramuscular fat deposition and fat biosynthesis in Laiwu pigs.	[58]
	Muscle fiber type distribution in semimembranosus and semitendinosus muscles	SM, ST separated into dark and light portion	LC-MS/MS	According to fiber type (oxidative vs. glycolytic) distribution, differentially expressed muscle proteins was detected resulting in intramuscular variations of pork quality.	[51]
	Effect of feeding regime on intramuscular fat increase. Comparison between normal protein diet vs. reduced protein diet.	LD	iTRAQ and LC-MS/MS	The categories “muscle contraction” and “structural constituents of cytoskeleton” were the most significantly up-regulated proteins in muscle from reduced protein diets and up-regulated proteins involved in the regulation of energy metabolism.	[49]
	Authentication of pork in meat mixtures (chicken, sheep and beef)	Meat mixtures	Parallel reaction monitoring mass spectrometry	Five peptides from myosin were screened and then used for pork detection by PRM of Orbitrap MS. The LOD in mixed meat can be up to 0.5%.	[54]
Mislabeling	Adulteration. Search for species-specific biomarker of mammalian muscle tissues in raw meat and meat products.	Meat mixtures	2-DE and MALDI-TOF/TOF	Troponin I (TnI) has been characterized as a potential thermally stable and species-specific biomarker of mammalian muscle tissues in raw meat (beef, pork, lamb, and horse) and meat products.	[53]
	To discriminate fresh and freeze-thawed pork	LD	2-DE and MALDI-TOF/TOF	Twenty-two proteins were discrimination markers for fresh or and freeze-thawed pork.	[55]

Food safety	Prevention and control of *Salmonella typhimurium* in pigs along a time course of 1, 2, and 6 days post infection	Intestinal sections (ileum and colon)	iTRAQ	The expression changes in colon were found in proteins involved in cell death and survival, tissue morphology or molecular transport at the early stages and tissue regeneration at 6 days post-infection. A higher number of changes in protein expression was quantified in ileum at 2 days post-infection	[59]

SM = semimembranosus muscle ST = semitendinosus muscle.

4. Advances in Proteomics for Pork Products

Nowadays, pork is an important part of the diet of many cultures because of its great versatility and thee abundant foods that can be manufactured from sausage to a dry-cured ham. Indeed, in recent years, most pork meat is sold as ham, bacon, and sausages than fresh pork. Further processing of pork should be considered in great detail to achieve high quality and palatability of products. The quality is associated, as evidenced above, with protein structure and lipid and protein oxidative reactions occurring during industrial processing and storage. Therefore, proteomics emerges as a relevant field, giving rise to new knowledge and understanding of the mechanisms. Table 3 displays the recent studies regarding the use of proteomics/peptidomics to evaluate the quality of pork products.

Table 3. Recent studies regarding the use of proteomics/peptidomics to evaluate the quality of pork products.

Product	Objective	Proteomic Technology	Main Findings	Ref
Cooked pork products (cooked ham and emulsion sausages)	Effect of cooking process on protein modifications	2-DE and MALDI-TOF/TOF	The protein aggregation systems of cooked hams and emulsion sausages reflect the heat processing conditions. The disulfide bridges and additional covalent interprotein links determine the final product.	[60][61]
Parma dry-cured ham	Effect of pressure treatment before salting stage	2D-PAGE and LC–ESI-MS/MS	Specific proteins were found differentially abundant in exudates from pressed versus unpressed hams. The pressure caused a faster loosening of the myofibrillar structure with the release of specific groups of proteins	[62]
Dry-Cured Ham	Effect of Proteolysis indices and adhesiveness on proteins degradation Use of high pressure and ultrasound to correct textural defect in dry-cured ham	2-DE and MALDI-TOF/TOF	Myosin-1, α-actin and myosin-4 proteins were the main changing due to proteolysis. The high-pressure conditions caused a greater level of proteolysis displaying that actin was differentially degraded, unlike myosin. Fragments of the major myofibrillar protein were abundantly caused by ultrasound heating.	[63][64][65]
Dry-cured ham (Jinhua)	Sensory attributes (formation mechanisms of bitterness and adhesiveness) in raw, normal and defective hams	LC-MS/MS	Defective hams showed more proteolytic index that normal ham. Creatine kinase, myosin, α-actinin and troponin-T showed the most intense response to bitterness and adhesiveness of dry-cured ham. Myosin was proposed as a suitable biomarker to monitor bitterness and adhesiveness	[66]
Dry-cured ham	Peptide oxidation in PDO Teruel dry-cured ham	nESI-LC–MS/MS	KDEAAKPKGPIKGVAKK, KKLRPGSGGEK, KNTDKWSECAR and ISIDEGKVL were proposed as peptide biomarkers of processing conditions.	[67]
Cooked pork	Effect of cooking on peptidomic profile and digestibility	SDS-PAGE and MALDI-TOF/TOF	The cooking process led to a reduction in digestibility. Peptides sequenced from pepsin-digested samples under lower degrees of doneness disappeared as the temperature increased. The trypsin cleavages appeared more consistent among different degrees of cooking	[68]
Pork soup	Protein modifications in presence of salt (treated 2%) and without salt (control)	i-TRAQ	Proteolytic index of salted samples was 5% higher than the control and 112 differentially abundant proteins were detected.	[69]

Dry-cured ham	Antioxidant peptides from Xuanwei (XHP) and Jinhua (JHP) ham	nano-LC-MS/MS and quadrupole ion trap Orbitrap spectrometer	XHP showed higher antioxidant ability than JHP. The oligopeptides with less than 1000 Da and high antioxidant activity were detected.	[70]
Dry-Cured Ham	Degradation of sarcoplasmic proteins	nLC–MS/MS and SDS-PAGE	Twenty proteins were identified and quantified suggesting intense degradation during processing.	[71]

5. Conclusions and Future Prospects

Proteomics is an emerging technology for the rapid and sensitive identification of biomarkers aiming to assess the potential quality of pork products and the impact of food processing technologies. Genetic and rearing conditions influencing technological and sensory meat quality provoke different biochemical and molecular reactions that are regulated by several proteins and pathways including metabolic enzymes. Furthermore, pork quality determined by tenderness, color, drip loss, and intramuscular fat is conducted by structural and sarcoplasmic proteins. In this regard, proteins play a key role in the textural and sensory quality of pork fresh, showing the importance of the study of muscle proteome in pork. The most relevant quality traits were assessed by gel and mass spectrometry analysis. Gel-based proteomics are widely used for the search of protein biomarkers of these quality traits. Even the most sensitive gel-based methods such as protein labeling with fluorescent dyes as fluorescence difference gel electrophoresis (DIGE) were considered. However, gel-free alternatives such as Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS), Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS), or Isobaric tags for relative and absolute quantitation (iTRAQ) should be employed to enhance the efficiency of our quest for protein biomarkers and further validate previous results. Other technical improvements in pork processing were assessed from a proteomic perspective, providing an insight into protein modifications. Peptidomic profiles could further offer an overall overview of the protein digestibility and bioavailability that determine the effect of protein fraction on human health. In the framework of data analysis, we expect that statistics will play a great role in future proteomics, especially in handling the huge data produced by different proteomics methods. Regarding this, we expect that there is great interest in combining different omics techniques in the framework of multi-omics to study the interplay between different macromolecules in relation to the pork phenome. Indeed, phenomics or high-throughput phenotyping is becoming a reality in livestock production systems including pork, and we expect that this global approach will play an important role in the next years and decades.

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Keywords

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