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Review

Recent Proteomics, Metabolomics and Lipidomics Approaches in Meat Safety, Processing and Quality Analysis

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Abstract: With a view to understand and resolve the complexity of the food matrix, omic technologies alone or in combination are extensively employed. In this sense, the newest developments and advances of proteomics, metabolomics and lipidomics with their unique benefits could simplify and help to understand the link between physiological and pathological activities in biology, physiology, pathology and food science and processing. This review aims to briefly introduce the basis of proteomics, metabolomics and lipidomics, then expansively review their impact on the assessment of meat quality and safety. Here, also, we discuss the application of proteomics, metabolomics and lipidomics for the authentication and adulteration of meat and meat derivatives from different sources and provide some perspectives regarding the use of emerging techniques such as rapid mass spectrometry (MS) and non-invasive measurements for the analysis of meat quality and safety. This paper summarizes all significant investigations into these matters and underlines the advances in analytical chemistry technologies and meat science areas. By emphasizing the requirement for additional examinations, this paper attempts a comprehensive knowledge of "foodomics" and the potential to improve its employment in meat science.

Keywords: lipidomics; meat quality; safety and processing; proteomics; lipidomics

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1. Introduction

For many years, the complexity of the food matrix has been a matter of concern for the scientific community. As a result, foodomic technologies are used widely and advanced methodologies are applied to food science. Foodomics, a high-throughput approach for the exploration of food science, comprises the transcriptomic, genomic, proteomic and/or metabolomic investigation of foods for compound profile, authenticity and/or biomarker detection associated with food quality/safety, food contaminants, toxicity tests, etc. Proteomics, metabolomics and lipidomics and other subdisciplines, *viz*. epigenomics, interactomics, metallomics, diseasomics, etc., have started to develop, each with their own set of instruments, techniques, reagents and software. In contrast to previous techniques based on hypothesis-driven research, in foodomics technologies the experimental approach is data-driven.

Proteomics focuses on characterization of proteins that are expressed in a cell or tissue type [1]. It is a high-throughput tool in elucidating the responses, functions, modifications and abundance of all proteins and their isoforms as well as the interactions between them [2,3]. It should be noted that through proteomics it is possible to overcome false positive results or limitations in DNA-based and immunoassay techniques. For example, the degradation of DNA after exposure of meat to high temperature or nutrient loss and excess pathogens can be easily discriminated using mass spectrometry technologies based on protein analysis [4]. In the literature, several techniques are reported aiming to obtain the proteome information

and its relationship with the specific product characteristics [5] and have been classified as targeted or untargeted. Untargeted proteomic approaches are suitable for early stages of biomarker discovery, while targeted approaches are selected for validation, implementation and detection of proteins of interest with high sensitivity [6]. Consequently, the use of proteomics in meat science enables us to deeply explore, understand and predict meat quality through studying and analyzing difficult to detect effects or interactions among a series of complex events [7].

Metabolomics is the study of the metabolite composition of a tissue or biological fluid [8] and focused on small molecules of relative molecular weights < 1500 Da [9] and approaches have been classified as targeted or untargeted. More specifically, in targeted metabolomics a specific group of metabolites is examined with identification and quantification of many metabolites within the group. According to the reviewed literature, targeted metabolomics can be used in order to interpret the behavior of specific group of metabolites in connection with determined conditions and analyze quantitatively preselected metabolites [10–12] as compared with untargeted metabolomics which can be used in order to detect groups of metabolites, without necessarily identifying nor quantifying a specific metabolite [13,14]. Furthermore, untargeted metabolomics can be further divided into two types: fingerprinting and profiling [15].

Lipidomics is extensively employed for lipid composition analysis and the quality identification of lipids in foods. Lipidomics forms a system-level analysis of lipids on a large scale and is employed to detect food adulteration and labeling, along with quantification of individual lipid molecular species. Various analytical methods based on lipidomics have been used to quantify trace lipid molecules in foods and hence obtain a comprehensive lipid profile [16,17]. For instance, by the quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) technique, Li et al. [16] analyzed lipid composition and investigated the lipid differences in Xinjiang Bactrian camel meat, hump, beef and fatty tails.

A bibliometric analysis on Lens (lens.org) displays that between 2014 and 2024 (May), a total of 1603 peer-reviewed scholarly works were published on [Metabolomics and meat OR proteomics and meat OR lipidomics and meat] (Figure 1).

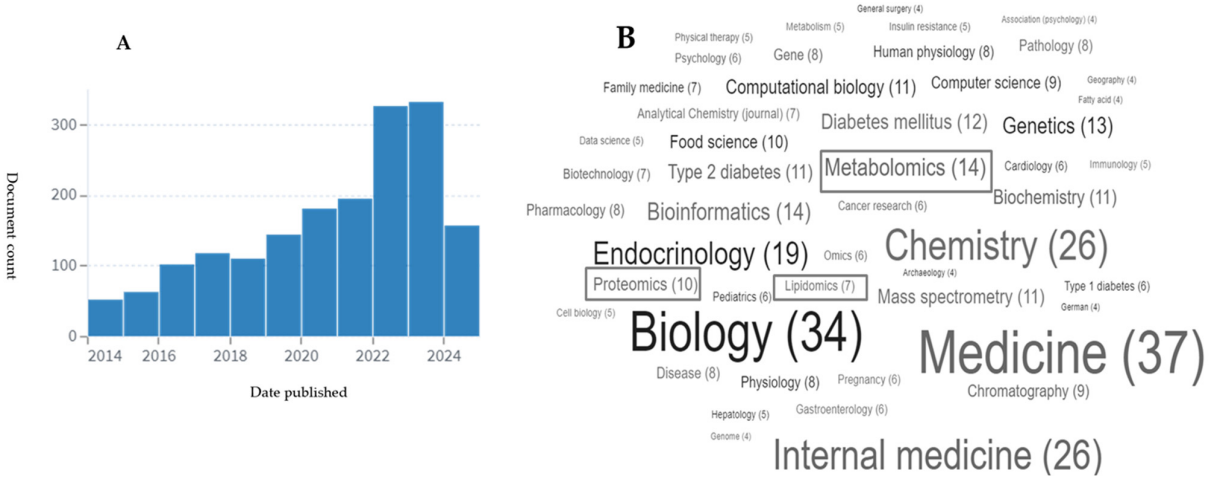

Figure 1. Number of publications on metabolomics, proteomics, lipidomics and meat quality/safety between 2014 and 2024 grouped by (**A**) year of publication and (**B**) publication subject (data were obtained from www.lens.org (accessed on 6 June 2024)).

Over the last few years, there has been an increasing interest in foodomics and its use in meat quality and safety, as can be seen from the number of publications on this topic that has increased continuously according to lens.org (Figure 1). This ever-growing interest could be explained by the increased scientific attention on foodomics, as well as the rapid technological advances.

The aim of the present review is to cover the recent proteomics, metabolomics as well as lipidomics studies on meat in the areas of meat quality, safety and processing. Our search was carried out in Scopus and used the following combination of keywords: metabolomics and meat quality, metabolomics and meat safety, metabolomics and meat. Proteomics and meat quality, proteomics and meat safety, proteomics and meat. Lipidomics and meat quality, lipidomics and meat safety, lipidomics and meat. Here, we summarize the latest research advances regarding the application of advanced foodomics in muscle origin and meat processing.

2. An Outline of Proteomics

In recent years, the study of proteomes has been applied in food technology in order to correlate the quality and safety of foods with health issues and welfare of the public. Microbial metabolism and responses to stress can be described more precisely by using proteomics [2]. Proteomics is an important method because multiple species detection and unknown target screening can be performed by using mass spectrometry technologies, gas chromatography–mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography–MS (UHPLC-MS). For example, UHPLCMS-MS and liquid extraction surface analysis mass spectrometry (LESA-MS) methods can identify species-specific markers for meat adulteration detection and heat-stable peptide markers in processed meat. In addition, two-dimensional gel electrophoresis mass spectrometry (2DE-MS) and OFFGEL-MS technologies successfully determined undeclared species in commercial processed meat products [4] and tandem mass spectrometry (LC-MS/MS) determined the relationship between proteomes and beef exudate with the color and oxidative quality [18]. Additionally, two-dimensional gel electrophoresis together with MS and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technologies were used not only to identify specific protein but also to understand proteome modifications as a consequence of growth, development, postmortem metabolism and tenderness [5,19]. Last, but not least, LC–high-resolution mass spectrometry (HRMS) and LC-MS/MS multiple reaction monitoring acquisition mode (MRM) methods are considered complementary tools for the detection, identification and confirmation of species-specific heat-stable peptides in processed meat products [20]. In this line, several MS approaches have been developed recently, and along with chromatographic and nuclear magnetic resonance (NMR) techniques they have become some of the most commonly applied approaches for metabolomic fingerprinting [20]. Conventionally, MS methods are joined with chromatographic separation techniques, such as LC-MS [20].

Briefly, proteomics has been classified as targeted or untargeted, and targeted methods can be further classified into four categories (Figure 2). The first category performs targeted data acquisition for the molecule of interest, the second category is based on targeted data analysis acquiring data for all molecular species, the third category is based on peptide ion data and relies on the mass of the entire molecule, while the last category is based on peptide fragments and relies on the fragments of a molecule [21].

Regarding quantitation using the mass spectrometry, proteomics can be classified into two broad categories: the label-based method and label-free method. In the label-based method, samples are first differentially labeled and pooled before being subjected to LC-MS/MS analysis. As a consequence of using the label-based method, the expected disparities are minimized. On the other hand, in the label-free quantitative proteomics method the samples are labeled, processed and analyzed independently by LC-MS/MS. The quantification is performed by the measurement of the peak area and/or consideration of the number of MS/MS spectra from each peptide using various software due to the large amount of data [22].

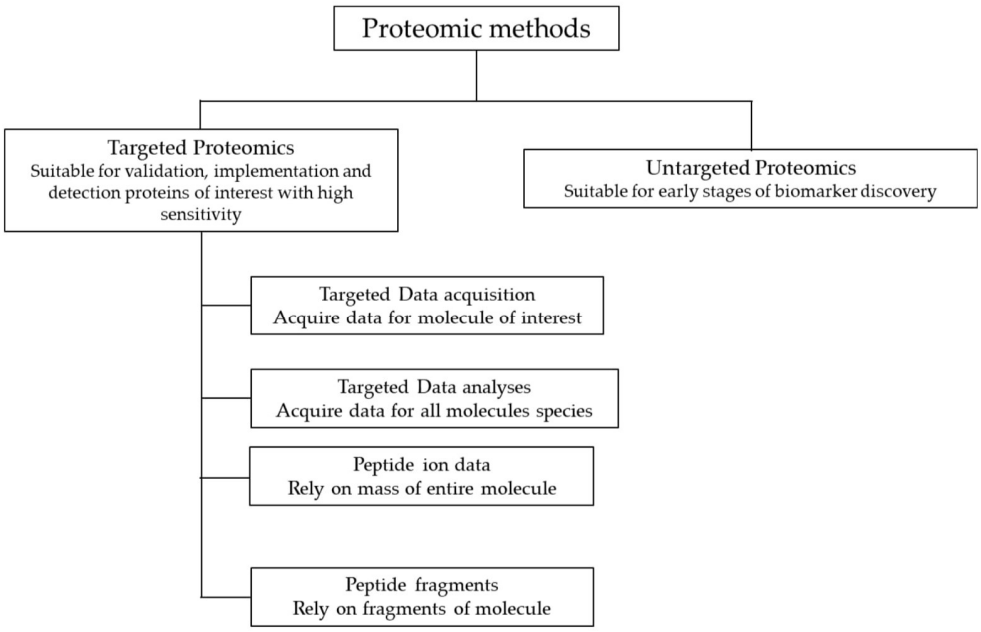

Figure 2. Classification of proteomics methodologies [6,21].

2.1. Proteomic Analyses in Meat Quality Control

The general plan of proteomics tools in meat quality monitoring involves (i) animals, treatments or muscles with diverse quality features, (ii) protein extraction, 2-DE, image analysis and statistical investigation, (iii) data assessment and extract of significantly different proteins and (iv) protein identification via MS and interpretation.

According to Setyabrata et al. [18], untargeted protein profiling identified 737 proteins in beef exudate using LC-MS/MS and demonstrated a distinct proteome profile primarily affected by the muscle source and slightly impacted by aging. The forty-nine significantly affected proteins have been classified into five groups based on their potential function. Five of the significantly affected proteins were affected by aging, more specifically in the 23rd day postmortem with structural proteins, and forty-four proteins were affected by muscle type.

Moreover, through untargeted proteomics the major proteomes in beef exudate were characterized in relation to meat color and oxidative stability of meat. In beef loin muscle exudate, five proteins were correlated to meat color stability while no proteins in tenderloin muscle exudate were found to be correlated. The majority of the identified proteins from tenderloin and beef loin muscles revealed a correlation to meat oxidative stability [18].

Proteomic analyses on chicken breast fillets with white striping myopathy were carried out with the use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) [23]. In particular, 148 differentially abundant proteins were identified in the white striping meats compared to normal non-affected meat. Of those, 43 more and 105 less abundant proteins were identified in the white striping meat compared with normal non-affected meat. Vimentin, which is known as marker of white striping myopathy as well as wooden breast abnormality, was one of the identified upregulated proteins. Several proteomic analyses have been reporting regarding white striping and wooden breast meat in order to understand the protein expression as well as the mechanisms and biochemical pathways behind breast myopathies [24–28].

Similarly, image analysis of whole muscle proteome gels was used and eight differentially abundant sarcoplasmic and myofibrillar proteins were identified in wooden breast (WB) chicken meat including serum albumin, creatine kinase and others. Proteomic differences in moderate WB meat from the commercial broiler industry in the United States pertaining to oxidation and glycolysis were detected.

More specifically, twenty-two proteins were detected only in chicken breasts with severe wooden breast myopathy. It should be noted that these proteins involved in redox homeostasis repaired the respective oxidative damage in tissue as well as maintained the muscle structure and combatted the inflammatory process [28]. A label-free proteomic analysis was also conducted in order to identify the differences in protein profile between young and older duck breast muscles (60, 300 and 900 days old). To discriminate differences in the meat between young and older ducks, Gu et al. [29] combined metabolomics/proteomics analyses. Three groups were found, 616 differentially expressed proteins were identified and 61 proteins were screened. Among the pathways examined, purine metabolism was uniquely enriched, which regulated flavor improvement. In addition, NME3, RRM2B, AMPD1 and AMPD3 may also be potential targets to distinguish young and older ducks. The results indicated that the oldest meat had a unique biochemical signature providing biomarkers for distinguishing young from older ducks.

Furthermore, the impact on the quality of meat and the proteomic profiling of duck breast muscle by riboflavin supplementation was determined [30]. As revealed by the results, riboflavin supplementation activated mitochondrial aerobic respiration. Therefore, supplementing duck diets with riboflavin improved breast meat quality.

2.2. Proteomic Analyses in Meat Safety and Authenticity

Proteomic analyses have been widely used in the identification of new markers that are suitable to distinguish accidental contamination from intentional adulteration using LC-MS/MS methods, based on a detection limit of less than 1% (*w*/*w*). Generally, they identify species-specific markers which are also stable during food processing [20].

Moreover, proteomic analyses have been used in order to detect fraud and adulteration of different animal species and issues in production systems, postmortem processing and storage [31].

Intentional modifications of product composition can be associated with food proteins. Proteomics are able to provide quick information regarding the food proteins in this regard. As a result, the relevant information can be effectively used for its correlation with food allergies in consumers because of undeclared compounds. Safety and authenticity issues are successfully addressed [5]. According to Prandi et al. [32], marker peptides were detected in a complex food matrix such as Bolognese sauce samples. Undoubtedly, these processed meat products are highly associated with adulteration. Prandi et al.'s validated method demonstrated a good specificity (the limit of detection (LOD): 0.2–0.8% in the finished product) and sensitivity in authentication of eight meat species (duck, rabbit, chicken, turkey, buffalo, equine, deer and sheep).

The rapid parallel reaction monitoring (PRM) method was developed using high-resolution Orbitrap MS in order to detect pork meat. When the most sensitive peptide was selected, the LOD in mixed meat can reach 0.5%. The relative standard deviation (RSD) values between detected and designated pork levels (1%, 5% and 50%) were 4–15%. A total of 125 peptides were identified; however, only 5 peptide markers were selected, derived from myosin-1 and myosin-4, which could be used for authenticating pork meat in mixtures of sheep, beef and chicken. Nevertheless, the respective peptide markers were heat-sensitive and, as a result, this method can only be applied for authentication of raw meat samples rather than cooked [33].

Notwithstanding the above method, Naveena et al. [34] developed another accurate OFFGEL electrophoresis method together with a label-free mass spectromic-based proteomics approach, which could be used in both raw and cooked meat mixes containing cattle, water buffalo, sheep and goat meat. In this investigation, species-specific peptides derived from myosin light chain-1 and -2 were recognized for authenticating buffalo meat spiked at a minimum 0.5% level in sheep meat with high confidence. Relative quantification of buffalo meat mixed with sheep meat was carried out by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) and the PLGS search engine to substantiate the confidence level of the data.

In the DNA-based method, PCR amplification of the mitochondrial D loop gene using species-specific primers found 226 bp and 126 bp product amplicons for buffalo and cattle meat, respectively. These authors reported that this method was effective in detecting a minimum of 0.5–1.0% when buffalo meat was spiked with cattle meat in raw and cooked meat combinations. The same trends were reported by Naveena et al. [35] who developed a 2DE and OFFGEL-based proteomic approach for authenticating raw and cooked water buffalo, sheep and goat meat and their blends. In-gel and OFFGEL-based proteomic approaches are effectual in validating meat mixes spiked at minimum 1.0% and 0.1% levels, respectively, in triple meat mixes for both raw and cooked samples.

Electrophoretic approaches for meat authentication display certain limitations, for example, in the investigation of hydrophobic and poorly solvable proteins, the limited dynamic series of the obtainable detection methods, great complexity and the dynamic range of the proteins in the sample. Moreover, other limitations involve the degradation of proteins caused by meat processing, the poor determination of closely related species, misidentification when analyzing samples with a combination of numerous species or the need for reference samples.

2.3. Proteomic Analyses in Meat Processing

Identification of proteins in chicken breast after various thermal treatments was conducted using a label-free proteomics strategy. According to Yang et al. [36], a total of 638 proteins were identified, and 84 of them were differentially abundant proteins after steaming, 89 after boiling, 50 after roasting and 43 after microwaving between processed pale, soft, exudative and normal chicken breast muscles. Through the statistical analysis, 20 proteins with significant contributions to color and texture were screened. Regarding the texture of processed meat samples, deterioration is associated with changes in myofibrillar and connective tissue structural proteins as well as sarcoplasmic proteins relevant to heat-induced oxidation. Possible indicators for the stability or variation of color of the above chicken breast muscles (pale, soft, exudative and normal) could be collagen, tropomyosin, myoglobin, as well as hemoglobin.

According to Wang et al. [37], 20 heat-stable peptides were identified by LC-MS in cooked pork, chicken, duck, beef and sheep and confirmed by NMR. Furthermore, 24 peptides were identified for the raw meats. This study showed that processing such as grilling and boiling did not affect the structure of peptides and as a result a species identification could be obtained. Moreover, 26 heat-stable peptides have been identified for chicken, 1 for turkey and 1 for rabbit [20]. In Table 1 proteomics for quality control of meat are described.

Table 1. Proteomics for quality control of meat products.

Meat Substrate	Extraction Method	Protein Identification Methodology	Data Analysis	Results	Reference
Meat exudate	Kim et al. method, Mohallem and Aryal et al. method	LC-MS/MS	Analysis of variance (ANOVA), principal component analysis (PCA), hierarchical cluster analysis (HCA), Kyoto Encyclopedia of Genes and Genomes (KEGG)	In total, 737 proteins were detected: 222 affected by muscles, aging or their interaction. The samples clustered based on muscle type	[18]
Small-tailed Han sheep, Simmental cattle, Sanyuan hybrid pig, Pekin duck, broiler chicken	Sarah et al. method	UPLC-TripleTOF-MS, NMR	Analyst 1.6.2 software	In total, 53 biomarkers were identified in total: 20 heat-stable peptides were identified for cooked meat and 24 peptides for the raw meats	[37]
Chicken breast fillets	Kong et al. and Kuttappan et al. method with modifications	Orbitrap Lumos, tandem mass tag (TMT) analysis, LC-MS/MS	*t*-test, IP analysis	In total, 148 differentially abundant proteins were identified in the white striping meats compared with normal non-affected meat	[23]
Chicken	Montowska and Fornal et al. mehod	LC-HRMS LC-MS/MS MRM		In total, 26 heat-stable peptides	[20]
Normal and woody broiler breast muscles	Zhang et al. method	2DE, LC-MS/MS	SAS 9.4 general linear model, Fisher's test	In total, 20 differentially abundant proteins were identified at 0 min, 15 min, 4 h and 24 h postmortem time points in either normal broiler or woody broiler breast muscles	[27]
Normal and wooden breast chicken meat	Zhu et al. method	SDS-PAGE, Q-Exactive Plus MS, coupled to a Dionex Ultimate 3000 RSLCnano	*t*-test, Bonferroni, ANOVA, Tukey's test, XLSTAT	In total, 127 differentially relatively abundant proteins, 22 of them detected only in wooden breast meat and 2 in N breast	[28]
Duck breast muscle		UHPLC, Orbitrap, LC-MS/MS	UniProt-GOA, KEGG, Fisher's test, one-way ANOVA, GraphPad Prism 8.0 software	In total, 616 differentially expressed proteins were identified; 61 proteins were screened	[29]
Pale, soft, exudative and normal chicken breasts (pectoralis major muscle)	Yang et al. method	Q-Exactive HF-X MS/MS, HPLC-MS/MS	UniProt-gallus, MaxQuant 1.6.1.0., Fisher's test, ANOVA, PCA, partial least square discriminant analysis (PLS-DA)	In total, 638 proteins were identified, 84, 89, 50 and 43 differentially abundant proteins were identified in steaming, boiling, roasting and microwaving, respectively	[36]
Duck breast muscle	Tang et al. method	Isobaric tags for relative and absolute quantitation (iTRAQ)	ANONA, Student's *t*-test KEGG	In total, 1641 proteins were identified, 23 selected differentially expressed proteins were involved in energy metabolism	[30]

Table 1. *Cont.*

Meat Substrate	Extraction Method	Protein Identification Methodology	Data Analysis	Results	Reference
Bolognese sauce		UHPLC/ESI-MS/MS, μHPLC-LTQ-Orbitrap	Peaks Studio, SRM	Good specificity (LOD: 0.2–0.8% in finished product) and sensitivity in authentication of duck, rabbit, chicken, turkey, buffalo, equine, deer and sheep	[32]
Shitou and Wuzong geese		UHPLC- MS/MS, 4D-DIA	ANOVA, PCA, KEGG	In total, 63.436 peptides were identified, which covered 5.183 proteins; 163 differentially expressed proteins were identified in the comparison between the leg muscles of Shitou goose and Wuzhong goose. Metabolic pathway played a major role in determining the quality differences in two breeds	[38]

3. An Outline of Metabolomics

Considering that metabolites are able to give information of the biochemical activity of an organism such as the intermediates or end products of enzymatic reactions, it is consequential to use metabolomic technologies. As mentioned above, metabolomics focuses on small molecules of relative molecular weights < 1500 Da [9] in a biological system and became known by the usage of high-resolution analytical technologies such as NMR as well as MS. MS can identify relevant metabolites by comparing database information, for example, the Fiehn library, Golm Metabolome Database and Wiley database [39], while NMR can provide structural information about metabolites [40]. Moreover, liquid chromatography–mass spectrometry (LC-MS/MS) is widely used because of its high sensitivity and wide detection range and respective analyses were performed using a UHPLC system with a Q-Exactive HF-X mass spectrometer under the control of the Xcalibur acquisition software [41]. Generally, target analysis is commonly performed with UPLC-ESI-MS/MS, LC-MS/MS and NMR [9,42,43] while in untargeted analysis UHPLC-QTOF-MS/MS and LC-Orbitrap-MS/MS [9,42,44] are commonly used, as referred to in Figure 3.

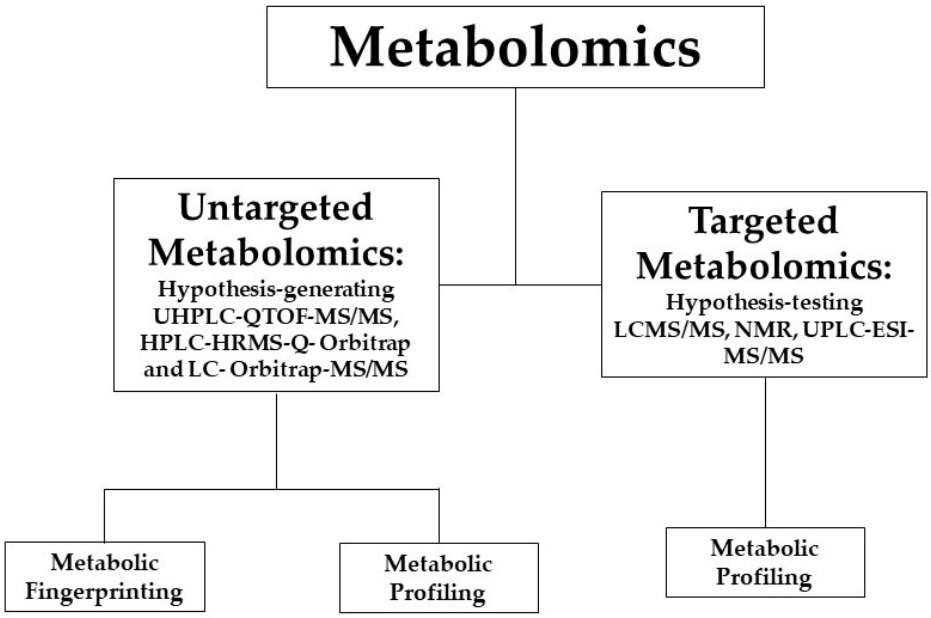

Figure 3. Classification of metabolomics and analytical approaches [9,42–46].

It should be noted that targeted and untargeted metabolomics are highly complementary and both omic methods are used to identify metabolites that change in abundance between two or more conditions [42].

Untargeted metabolomics has better coverage of metabolites, though it possesses limited reproducibility and poor sensitivity for metabolites with low abundance [47]. Targeted metabolomics is typical for metabolite quantification as a consequence of its high sensitivity, high dynamic extent and faithful quantification accuracy [48,49]. On the other hand, it should be noted that targeted metabolomics covers restricted existing recognized metabolic data [50]. In this sense, dynamic MRM-based pseudo-targeted metabolomics quantification and PRM-based larger-scale targeted metabolomics quantification are two emerging tools which measure a huge number of metabolites with dependable quantitative arrays and are currently endorsed as potential strategies for meat metabolomics investigations [50].

3.1. Metabolomic Analyses in Meat Quality Control

In meat quality studies, metabolomics could enable drawing maps of the metabolic network in postmortem muscle aging and flavor development during cooking. Despite the impact on meat quality, the metabolic changes have not been easily predicted because

coordinated metabolism in live muscle is no longer maintained due to a lack of energy supply and arrest of de novo gene expression after animal death. Postmortem metabolic maps could result in the identification of the metabolic factors responsible for meat quality traits and thereby contribute to the exploration of biomarkers in quality monitoring, processing and authentication of meat products [51]. In the last decade, extensive research has been conducted to determine and evaluate the volatile organic compounds in meat products such as aldehydes, ketones, hydrocarbons, alcohols, as well as sulfur-containing compounds [51], and identified the most important compounds in sausages by the SPME-GC–MS technique [52–54].

Flavor substance formation is directly related to metabolic mechanisms and LC-MS/MS is widely used in food flavor metabolomic research. According to a recent study [41], metabolomics was employed to characterize key aroma substances and their water-soluble markers affecting chicken meat. Metabolomics analyses showed the presence of L-glutamine, a key metabolite of nonanal, hexanal, heptanal, octanal and 1-octen-3-ol affecting and improving chicken flavor through the Maillard reaction.

Moreover, physiological and metabolic differences in meat quality between two local breeds of Tibetan sheep were investigated by analyzing differential metabolites through untargeted metabolomic analysis. White Tibetan sheep were clearly distinguished from Black Tibetan sheep as 49 differential metabolites were successfully identified, including carbohydrates, amino acids and derivatives, fatty acids and derivatives and other organic compounds [9].

Through metabolomic profiling, more information regarding quality of beef muscles can be reported using the exudate as an analytical sample and indicator due to the presence of sarcoplasmic proteins, amino acids, sugars, lipids and enzymes [55]. According to Setyabrata et al. [18], metabolomic analysis of meat exudate used aging periods for clustering of the metabolites by muscle type (beef loin muscles and tenderloin muscles) and PCA of exudate metabolites was employed.

Another metabolomic study, according to Zhang et al. [56], revealed significant differences between the meat extract from Liancheng white duck breast meat and Cherry Valley duck meat. The differentiated metabolites were categorized into 28 classes. Of these, there were four main ones: carbohydrates, amino acids, fatty acids and eikosanoids. The results revealed that breed has a great impact on meat quality and metabolomic profiling of duck meat and quality metabolites (correlated with meat) may function as markers for the above breeds. Similarly, metabolomic profile was employed for the determination of the metabolites of volatile compounds in slow-growing Liancheng white duck and fast-growing Cherry Valley duck meat. Targeted metabolomics showed a lower carnitine content in Liancheng white duck meat. This might promote lipid deposition for production of additional octanal and nonanal. Conversely, the higher carnitine content in Cherry Valley duck meat may be attributed to fewer lipid oxidation products. In addition, the results showed that the sweet and meaty aroma in slow-growing Liancheng ducks was derived from higher sugar and amino acid contents [57].

In a recent study by Weng et al. [43], the effect of age (70, 120 and 300 days old) on the nutritive profile of goose meat was investigated. In detail, using widely targeted metabolomics analysis, a total of 776 metabolites were detected in goose meat. In particular, carnitine, anserine as well as nicotinamide riboside increased with age and played a key role in achieving a greater nutritional value. Conversely, hypoxanthine, 2-methylsuccinic acid and glutaric acid decreased with age. In short, Weng et al. [43] concluded that the older geese meat (300 days old) was more nutritious and healthy for humans.

Ge et al. [58], using a high-performance liquid chromatography coupled with triple-quadrupole linear ion trap tandem mass spectrometry (HPLC-QTRAP-MS) metabolomic approach, found that age affected the metabolites of Beijing You chicken, a local Chinese breed with superior meat quality [59]. More specifically, the metabolites in the breast muscle were significantly changed during their aging (56, 98 and 120 days old). A total of 544 metabolites were detected, of which 60 differential metabolites were detected from

56–98 days old as well as 55 differential metabolites from 98–120 days old. L-carnitine, L-methionine and 3-hydroxybutyrate increased with age. L-methionine is responsible for the flavor of food, as it produces dimethyl sulfide after heating. L-carnitine is equally important as it promotes growth and improves antioxidant capacity.

Furthermore, during the aging of Wuding chickens, significant changes in metabolic profile were also demonstrated [60]. Using the ^{1}H-NMR-based metabolomic approach it was found that the metabolic compositional profile of lactate, creatine, IMP, glucose, carnosine, anserine, taurine and glutamine was significantly different in 230-day-old chicken meat compared to the younger ones.

Interestingly, NMR spectroscopy could (i) unmistakably detect unknown metabolites, (ii) differentiate isomers and (iii) be used for structure elucidation of unknown compounds [61]. An additional applicable advantage of NMR is that it can carry out non-destructive data acquisition. For example, NMR methods such as ^{1}H high-resolution magic-angle spinning NMR (^{1}H HR-MAS NMR) can be performed on complete animal tissues [62].

3.2. Metabolomic Analyses in Meat Safety Control

In order to reduce the incidence of foodborne diseases caused by microorganisms, the WHO encourages the use of the irradiation process only if the irradiation dose is up to 10 kGy. Even though irradiation is reported as a safe and effective method to extend the shelf-life of meat and protect its hygienic quality, in the European Union only a few products are permitted to be irradiated in some Member States in substantial amounts. Especially for meat products, only chicken meat, poultry, poultry preparations, mechanically recovered chicken meat and chicken offal are proposed for irradiation in the Netherlands and France [63].

Metabolomic analyses are expected to be a tool to monitor the impact of irradiation and could be a useful food inspection tool. Qualitative untargeted metabolomics using HPLC–high-resolution magic angle spinning (HRMS)–Q-Orbitrap analysis was used by Panseri et al. [46] to evaluate the changes in metabolome profiling of irradiated meat, such as chicken, turkey and mixed ground meat for sausages, regarding food safety issues relating to metabolome alteration. Four hundred and two metabolites were detected, and all three matrices exhibited a specific metabolome profile that was not affected by the application of irradiation intensities. The three meat groups exhibited the following similarities: (i) the free amino acid pool was unaffected by irradiation, (ii) taurine was the most important differentiator for all three meat groups, (iii) reduction of the glutathione level and (iv) an increase in adenosine nucleotide degradation. More specifically, changes in amino acids, monosaccharides, nucleotides and free fatty acid profile as well as the potential presence of any oxidative products could have been due to irradiation. According to this study, the irradiation did not cause changes in the main food ingredients such as the free amino acid pool. Metabolomic analysis did not determine any relevant negative impact of irradiation on meat (only alterations in a few metabolic pathways) and as a result the original quality of the meat was maintained.

Additionally, the effect of electron beam irradiation treatment on spicy yak jerky, a typical snack meat product and local specialty in China, was investigated. According to this study, a low irradiation dose did not result in obvious changes in the protein biological value of the spicy yak jerky, but high-dose irradiation (9 kGy) decreased the protein nutrition value of spicy yak jerky. More specifically, when the spicy yak jerky was irradiated with 0, 2, 5 and 7 kGy, no significant change was observed in amino acid values, while when it was irradiated with 9 kGy a significant decrease in total amino acids was observed [64].

3.3. Metabolomic Analyses in Meat Processing

According to Trithavisup et al. [44], the metabolomic profiles of cooked wooden breast chicken meat and chicken breast without the wooden breast abnormality were compared in

order to identify the differences in metabolic compositions of pepsin-hydrolyzed samples. Untargeted metabolomic analysis did not show any toxic metabolites in either of the samples, with no apparent compounds that could cause acute adverse health effects due to consumption of cooked wooden breast chicken meat. Metabolomic changes between chicken breast with or without the abnormality also remained in the cooked product. More specifically, 322 differential metabolites were identified between the cooked samples. Metabolites associated with taurine, hypotaurine metabolism, phenylalanine, tyrosine, tryptophan biosynthesis, D-glutamine and D-glutamate metabolism were mostly affected because of the wooden breast abnormality. Moreover, amino acids together with short peptides as well as carboxamides were also identified.

3.4. Metabolomic Analyses of Meat Authenticity

According to Wang et al. [41], the basic characteristic aroma substances of chicken are nonanal, octanal and dimethyl tetrasulfide, whereas breed-specific aroma compounds found only in native Chinese chickens contain hexanal, 1-octen-3-ol, (E)-2-nonenal, heptanal and (E,E)-2.4-decadienal. Through this study, using non-targeted metabolomes, metabolic differences between white-feathered broilers and native Chinese breeds were identified. More specifically, 821 metabolites were detected and divided into 16 substance classes of which the largest (314 metabolites) was amino acids and their metabolites followed by organic acids and their derivatives (102 metabolites).

Based on Ma et al. [9], identification of local breeding varieties of Tibetan sheep (White Tibetan sheep and the Black Tibetan sheep in Qinghai) was investigated. The results showed that Black Tibetan sheep were superior to the White Tibetan sheep. In targeted metabolomic profiling, significant differences in terms of amino acid composition as well as amino acid content were observed. Total amino acids and non-essential amino acids for the Black Tibetan sheep were higher compared to the White Tibetan sheep while no differences were found in the saturated and monosaturated fatty acids.

Using NMR-based metabolomics fingerprinting, differential metabolites were identified from beef extracts originating from Australia, Korea, New Zealand and the United States. The major metabolites were succinate and various amino acids including isoleucine, leucine, methionine, tyrosine and valine, making them usable biomarkers in order to distinguish the geographical origin of beef [65].

According to Weng et al. [53], differential metabolites were identified between two indigenous poultry breeds, the Liancheng white duck and Cherry Valley duck. In particular, carnitines were among the top upregulated metabolites in Cherry Valley duck meat compared to Liancheng white duck meat. Moreover, according to Zhou et al. [66], the metabolome data were different between the Liancheng white duck and Mianyang shelduck. In particular, L-arginine, L-ornithine and L-lysine were found in considerably higher concentrations in Liancheng white duck meat than in Mianyang shelduck meat.

Furthermore, widely targeted metabolomics and statistical analysis were performed in order to identify potential biomarkers for authentication of older goose meat [43]. Carnitine, anserine as well as nicotinamide riboside could be considered as good biomarkers in order to guarantee authenticity.

3.5. Metabolomic Analyses of Meat and Impact on Human Health

Untargeted ^{1}H NMR has been used to identify the associations of metabolites–inflammation and diet–inflammation. According to Wood et al. [67], higher intake of processed red meat is associated with lower levels of two anti-inflammatory amino acids. Similarly, red meat intake and cardiovascular disease risk involve higher levels of inflammation according to the same authors in other research [68]. Untargeted ^{1}H NMR metabolomic analysis shows an association of glutamine, an anti-inflammatory metabolite, with red meat intake when controlling for body mass index. Meanwhile, glutamine was also associated with lower C-reactive protein levels. In Table 2 metabolomics for quality control of meat are described.

Table 2. Metabolomics for quality control of meat products.

Meat Substrate	Extraction Method	Metabolite Identification Methodology	Data Analysis	Results	Reference
Beijing You chicken		HPLC-QTRAP-MS	SPSS 22.0, one-way ANOVA and Ducan's test, PCA, orthogonal projection to latent structures (OPLS-DA)	In total, 544 metabolites were sorted into 32 categories. L-carnitine, L-methionine and 3-hydroxybutyrate increased with age.	[58]
Cooked wooden breast chicken and chicken breast without wooden breast abnormality	Solid phase extraction	LC-MS/MS, Orbitrap HF MS	Student's *t*-test	In total, 1155 metabolites were identified; 322 differential metabolites were identified between the cooked samples. Taurine, hypotaurine metabolism, phenylalanine, tyrosine, tryptophan biosynthesis, D-glutamine and D-glutamate metabolism were most affected because of the wooden breast abnormality	[44]
Chicken, turkey, mixed ground meat for sausages		HPLC-HRMS–Q-Orbitrap	Hierarchical clustering analysis for BWC and VP, one-way ANOVA with Tukey post hoc test, multivariate paired *t*-test.	Irradiation did not cause changes in main food ingredients such as free amino acid pool, only alteration in a few metabolic pathways	[46]
Goose meat	Chen et al. method	UPLC-ESI-MS/MS	OPLS-DA, K-means cluster, KEGG	In total, 776 metabolites were sorted into 16 classes. Carnitine, anserine, nicotinamide riboside increased with age. Conversely, hypoxanthine, 2-methylsuccinic acid and glutaric acid decreased with age.	[43]
Red meat		^{1}H NMR	Bonferroni correction	Glutamine, an anti-inflammatory metabolite, was associated with red meat intake when controlling for body mass index and lower CRP levels.	[68]
Liancheng white duck breast meat and Cherry Valley duck meat		UHPLC-QTOF-MS	SPSS 17.0, one-way ANOVA and Mann–Whitney test, PCA, OPLS-DA	Significant differences between the two breeds; 28 differentiated metabolites were classified. Of these, carbohydrates, amino acids, fatty acids and eikosanoids were the main ones	[56]
Meat exudate	Bligh & Dyer et al. method	UPLC-MS	ANOVA, PCA, HCA, KEGG	In total, 518 metabolites were detected; 159 were affected by muscles, aging or their interaction. The samples clustered based on aging periods	[18]
White and Black Tibetan sheep		UPLC-QTOF-MS, NMR for targeted, UHPLC-QTOF-MS/MS for untargeted	SPSS 20.0, PCC	Black Tibetan sheep were superior to the White Tibetan sheep; 49 differential metabolites were identified, including carbohydrates, amino acids and derivatives, fatty acids and derivatives and other organic compounds	[9]
Chicken		UHPLC-Orbitrap MS	PCA, OPLS-DA	In total, 821 metabolites were detected and divided into 16 classes. The amino acids and their metabolites class was the largest (314 metabolites) followed by organic acids and their derivatives (102 metabolites)	[41]

4. An Outline of Lipidomics

Lipidomics and quality

Lipidomics is responsible for the identification of key lipid biomarkers in metabolic regulation. This is achieved mainly by comparison of lipid metabolic changes under different conditions. The final aim is revealing the mechanisms of lipid action [69].

Differences in lipid-metabolizing capacity arise from different salt substitutions affecting microorganisms' activity associated with lipolytic enzymes. Hence, the overall flavor might be affected by lipid changes in meat products [70]. Moreover, there might be an accumulation of certain fat-soluble volatile flavor compounds in lipid molecules, leading to a slow release over time [71].

Lipidomics can use the identification information derived from large amounts of data for lipid identification from different sources. In this direction, gas chromatography tandem ion mobility spectrometry (GC-IMS) technology can be employed to identify and characterize compounds with ham flavor (Yang, [72]).

Free fatty acids (FFAs), glycerolipids (GLs), glycerophospholipids (GPs), sphingolipids (SLs), sterol lipids, prenol lipids, saccharolipids and polyketides are considered the main lipid categories of meat [73]. Triacylglycerols (TAGs) and phospholipids (PLs) constitute two of the most common and abundant categories of lipids rich in ω3 polyunsaturated fatty acids (PUFAs). They have health- and nutrition-related roles in the body. Lipidomics is the analytical strategy for investigation lipids in various food matrices. Fingerprinting and dynamic alterations related to lipids continue to be unexplored and are ambiguous [74,75]. Lipidomics based on HPLC-HRMS can be employed for characterization of lipids in pork [76], duck [77] and lamb [78].

Lipid metabolism changes and their compositions for discrimination of lipid species have been detected by mass spectrometry (MS)-based lipidomics [79].

iKnife and rapid evaporative ionization mass spectrometry (REIMS)-based lipidomics have been employed by Song et al. [80] for identification of salmon and rainbow trout in real time [81]. In this direction, lipid composition changes of hams from different origins have been studied extensively [78].

Raw pork meat has been discriminated by lipidomic fingerprints [76] and the mechanism of lipid fragmentation in refrigerated Tan sheep has been well documented [82]. Many previous studies have been reported on ham lipids, focusing on the famous dry-cured pork ham produced in the Mediterranean region and China [83–87]. There is a significant variation in lipid composition among different raw materials [78].

Chen et al. [69] used non-targeted lipidomics, correlation analysis and KEGG pathway analysis and investigated microbial and lipid metabolism changes during fermentation of restructured duck ham with different salt substitutions.

Different techniques have been employed in the classification of hams based on the lipid profile. For example, gas chromatography with flame ionization detector (GC-FID) of the lipidic fraction after transmethylation in acid medium [88].

Lipid analysis of different types of food has been accomplished by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy [89–91] and analysis of the lipid profile of Iberian dry-cured hams using high-field ^{1}H NMR spectroscopy [92,93].

Figure 4 depicts an MS-based lipidomics approach, which can be divided into: lipid extraction, lipid identification by either gas chromatography (GC)/liquid chromatography (LC)-MS and data processing with lipid classification using a chemometric approach by PCA, PLS-DA or other bioinformatics data analysis.

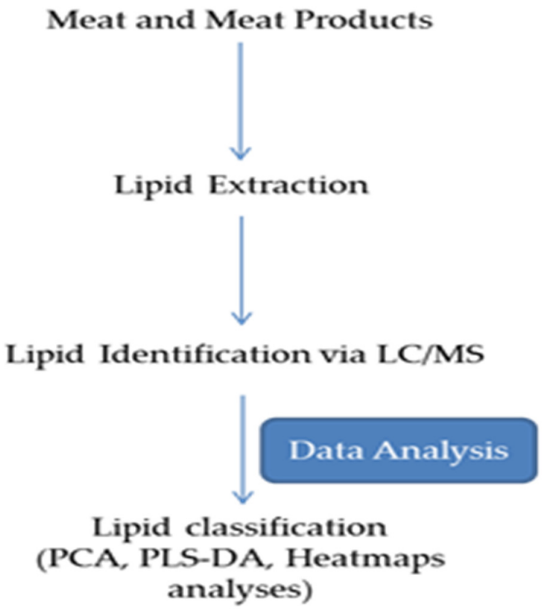

Figure 4. Lipidomic Approach for determination of the Functional Quality of meat Products (Adapted from Harlina et al. [94]).

Shotgun lipidomics and LC-MS-based lipidomics [80,95–97] differ in whether the lipid (phospholipids, triacylglycerols, fatty acids, etc.) in the sample is separated before injection into the mass spectrometer [77,98].

Fatty acid characterization between breeds was the main area of analysis of lipidomics in chickens [17,99].

One of the advantages of LC-MS in metabolomics and lipidomics is the ease with which samples can be organized. Mass spectrometry could authenticate the different kinds of metabolites and lipids existing in the sample. Another benefit of LC-MS in metabolomic and lipidomic research is that it can classify all types of metabolites/lipids in a single sample run. This makes both analyses very efficient while using LC-MS [100].

Table 3 describes lipidomics for quality control of meat products as adapted from Jia et al. [101].

Table 3. Lipidomics for quality control of meat products (Harlina et al. [94]).

Meat Substrate	Extraction Method	Lipid Identification Methodology	Data Analysis	Results	Reference
Chicken meat	Folch et al. method	UPLC-ESI-MS	PCA, PLS-DA, OPLS-DA	Significant phospholipid decrease, lysophospholipid increase	[102]
Chicken, turkey and mixed ground meat for sausage preparation	Bligh and Dyer method	GC analysis of fatty acid methyl esters, HPLC Q-Exactive Orbitrap high-resolution mass spectrometry for lipidomics analysis	PCA, volcano plot	Identification of 345 lipids categorized into 14 subclasses. Identification of oxidized glycerophosphoethanolamines and oxidized glycerophosphoserines in irradiated turkey meat	[103]
Pork	Folch et al. method (from Ulmer et al. [104])	Ultra-high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry	PCA and OPLS-DA analysis	Ether-linked phosphatidylethanolamine and phosphatidylcholine containing more than one unsaturated bond were greatly influenced by frozen storage	[105]
Grass-fed and grain-fed beef	-	-	-	Variations in the fatty acid composition between grass-fed and grain-fed beef. Grass-based diets have been shown to enhance total conjugated linoleic acid (CLA) (C18:2) isomers, trans vaccenic acid (TVA) (C18:1 t11), a precursor to CLA, and omega-3 (n-3) FAs	[106]
Dry-cured mutton ham	Lipid extraction buffer (MTBE:methanol = 3:1, v/v)	Lipid metabolomics based on UPLC-MS-MS	PCA and OPLS-DA	Most abundant lipids were glycerolipids (GLs) followed by glycerophospholipids. Quality of mutton ham changed during the P3 fermenting stage	[71]
Chicken breast	Soxhlet extraction	Ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS)	Volcano plot analysis	Triacylglycerol (TAG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) significantly decreased	[107]

Table 3. *Cont.*

Meat Substrate	Extraction Method	Lipid Identification Methodology	Data Analysis	Results	Reference
Hengshan goat meat sausages		LC-ESI –MS (Q-Orbitrap)		Lipid variables related to glycerophospholipid and sphingolipid metabolism	[108]
Chicken	Soxhlet extraction	UPLC-Q-Exactive Orbitrap/MS	PCA, PLS-DA, PCA of E-tongue	Significant differences between Cobb chicken and Taihe silky chicken lipids at the taxonomic and molecular levels	[109]
Duck	Phospholipid extraction according to previous methodology	DI -ESI –MS (Q-Trap)	PCA, PLS-DA	The spices had a significant effect on individual phospholipid molecules during processing	[110]
Donkey meat	FAME by GC, muscle lipids were extracted with $CHCl_3$:CH_3OH (2:1, *v*/*v*)	LC –MS (triple TOF)	OPLS-DA, heatmap analysis	In total, 1143 lipids belonging to 14 subclasses were identified in donkey meat, of which 73 lipids showed changes (23 upregulated and 50 downregulated), including glycerolipids (GLs), glycerophospholipids (GPs) and sphingolipids (SPs)	[111]
Camel meat	Lipid fraction was extracted with MTBE	UPLC-Q-TOF/MS	PCA, OPLS-DA, volcano plot	In total, 342 lipid species were detected, and 192, 64 and 79 distinguishing lipids were found in camel hump compared to camel meat, camel meat compared to beef and camel hump compared to fatty tails, respectively	[16]
Irradiated goat meat	Dual-phase extraction with methanol and MTBE	UHPLC–Q-Orbitrap	PCA, PLS-DA	In total, 12 subclasses of 174 lipids were identified with significant differences	[82]

5. Discussion

Meat and meat products are complex food matrices with several challenging difficulties. Their biochemical mechanism and composition are affected by breed, processing and storage. In addition to this, consumer needs for quality food with potential health effects as well as challenges in the food production industry regarding distribution and several health and quality risks lead to the need for improving the regulatory system and analytical methods used.

Without a doubt, foodomic technologies are able to resolve quality, safety, processing and authenticity issues. Numerous studies have been conducted using proteomics, metabolomics and lipidomics technologies in meat and meat products. Based on the reviewed data, the implementation of such advanced methodologies together with statistical approaches can predict critical issues relating to quality, adulteration, processing and authenticity.

First and foremost, it should be noted that by using omic technologies many problems can be overcome, like false positive results or limitations that take place in DNA-based and immunoassay techniques [4]. We should also consider that proteomics has been classified as untargeted or targeted, detecting biomarkers at the beginning of the research or proteins of interest for validation and implementation, respectively, with high sensitivity [6]. In addition to this, metabolomics has been also classified as targeted or untargeted, interpreting the behavior of specific groups of metabolites in connection with determined conditions and quantitatively analyzing preselected metabolites or detecting groups of metabolites, respectively, without necessarily identifying or quantifying specific metabolites [10–14]. So, it is clear that foodomic technologies in meat science enable us to deeply explore, understand and predict meat quality.

In proteomics, different mass spectrometry technologies like GC-MS, UHPLC-MS, LESA-MS, 2DE-MS, MALDI-TOF/TOF-MS and OFFGEL-MS have been successfully used in order to determine not only specific proteins or undeclared species in meat products and the relationship between proteomes and meat quality but also to understand proteome modifications as a consequence of meat aging [4,5,18,19].

The great advantage of MALDI-TOF-MS is the rapidity of analysis due to the fast sample preparation step that avoids protein digestion, but the main disadvantage of this type of analysis due to only one stage of mass spectrometry analysis (MS1 or MS) is that only the mass of the analyte is acquired. Consequently, since the peptide or protein is not isolated and no product ion spectra are produced, no structural information is found. The comparison in this approach is made purely by pattern recognition, and since no unique biomarker is reported, an extensive validation of such an approach would be essential.

Last, but not least, LC-HRMS and LC-MS/MS MRM methods are used for the detection, identification and confirmation of species-specific heat-stable peptides in processed meat products, giving us an excellent tool for distinguishing accidental contamination from intentional adulteration [20].

In metabolomics, high-resolution analytical technologies like NMR as well as MS have been successfully used in order to identify relevant metabolites by comparing database information, while NMR can provide structural information about metabolites [39,40]. In particular, LC-MS/MS, UPLC-ESI-MS/MS, UHPLC-QTOF-MS/MS and LC-Orbitrap-MS/MS methods are used because of their high sensitivity and wide detection range [9,41–44].

The elucidation of unidentified metabolites is one of the main challenges. While significant advances have been achieved in the last decade, the databases of metabolites are still restricted and incomplete; the current metabolic findings may be only the "tip of the iceberg" of the whole picture of omic technologies for meat quality. Another important issue is the diverse nature of individuals; differences in genotype are likely to affect the metabolome profiling of meat, hence affecting meat quality and safety. Additionally, the reported works usually used diverse analytical techniques and different sample preparation methods based on different designs, thus controversial deductions are not surprising. In this direction, further research is needed in order to explore new aspects in foodomic

methodologies, with the possible application of them in industry and providing valuable insights into meat safety and quality.

The techniques of proteomics, metabolomics and lipidomics could be employed to identify meat products at different stages. In this regard, the combination of these tools will give a clearer picture of the meat profile [112]. The complete protein, lipid and metabolite profiles efficiently differentiate between meat varieties [113]. The combination of these three approaches could be used in the authentication of meat products by considering the proteins, metabolites and lipids in meat products. Advantages and disadvantages of proteomics, metabolomics and lipidomics for authentication in meat product can be seen in Table 4.

Table 4. Advantages and disadvantages of proteomics, metabolomics and lipidomics in the authentication of meat products.

Method	Advantages	Drawbacks (Limitations)	References
Proteomics	• Proper tool for the assessment of the meat quality • Traceability and authenticity • Suitable strategy to authenticate processed meat • MALDI-MS was the main technique which was well suited for peptide mass fingerprinting	• Lack of available protein sequences from different animal species in the databases • Need for information on the relevance of some detected proteins • Database availability or access to proteomics platforms and funding	[114,115]
Metabolomics	• ↗accuracy value • Complete investigation of the whole metabolome • Exploration of all kinds of molecules • Investigation of organic components • MS can detect and measure metabolites at very low concentrations • ↗dynamic range and resolution	• Needs suitable tools for analytical processes such as LC-MS and metabolomic analysis • Costly equipment • MS only detects metabolites that can punctually ionize	[116,117]
Lipidomics	• Properly explored in meat adulteration • Quick detection of meat quality and safety, processing and authenticity	• Attained data are restricted to lipid compounds and sublipids	[118,119]

6. Conclusions

Though traditional methods for evaluating intrinsic meat quality and safety characteristics comprising instrumental texture and color studies, pH and sensory traits deliver evidence on meat quality, they are not appropriate for estimating the biochemical mechanisms, linking meat quality modifications or inspecting meat quality. Unquestionably, proteomic, metabolomic and lipidomic methodologies or combinations of them are considered complementary tools. In this sense, these attractive measurement tools are becoming progressively more popular for researchers intending to clarify quality differences of meat products caused by many features from farm to fork.

While reliability of the discussed proteomic, metabolomic and lipidomic tools is shown in practical applications, mostly in the scope of data investigation, chemometrics should assist towards a comprehensive evaluation of experimental data and a profound understanding of how internal metabolic pathways or biological processes change in meat and meat derivatives. Accordingly, to expand the metabolite range and increase data quality with the grouping of diverse analytical approaches and platforms, it is indispensable to improve the precision and the sensitivity of instruments. As an illustration, the new technology of ion mobility separation (IMS) was able to grant a novel dimension for chromatography and MS, permitting the tracking of quality traits through meat processing

with in situ automatic sampling. Additionally, potent statistical program tools need to be developed to manage and control huge amounts of experimental data for efficient determination of the security of new or traditional processing technologies in meat systems. Similarly, metabolite libraries of MS/NMR software and web servers require frequent additions of more metabolites, forming and recording more reliable standards, mining data from the literature and explaining structures of new metabolites.

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