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Proteomic Approach to Detect Meat Adulteration from Farm to Fork

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ABSTRACT

Meat has long been regarded as a significant source of protein in human life. Furthermore, with today's hectic and demanding urban lifestyle, processed meat has become the most important source of protein in one's diet. Consumers rely on food labels to determine whether or not the meat product they are purchasing is safe and reliable. To avoid consumer fraud, it is critical to verify that food labeling is completed correctly. Compared to the past, people are increasingly more concerned about food quality and safety. For food authentication, a variety of instrumental procedures have been proposed. Traditional methods are still employed, but emerging approaches like genomics, metabolomics, and proteomics are helping to supplement existing methodology for confirming claims made about specific foods. A few decades before, proteomics emerged as the most crucial technology for authentication of adulterated meat. Proteins can be employed as markers for a variety of qualities in meat and show the processes to which the meat has been treated so that they can add to the meat labeling claim.

HIGHLIGHTS

- Proteomics is playing an increasingly important role in food authenticity.
- Advances in instrumentation and bioinformatics are expected to have a significant impact on the field.
- Proteomics approach is the best way for authentication of meat and meat products.

Keywords: Meat adulteration, proteomic, thermo-stable peptides, mass spectrometry

The widespread practice of adulterating meat, usually for commercial gain, poses major threats to public health as well as ethical and religious infringements. The majority of meat adulteration is driven by economic factors, such as the inexpensive addition of duck meat to mutton (Zheng *et al*., 2019), which costs customers money. In any case, eating meat that has been adulterated poses a major danger to the public's health since it may expose consumers to toxins, infections, or allergies (Magiati *et al.*, 2019). Additionally, meat adulteration might go against religious tenets; for instance, pig or items related with pork are forbidden under Kosher and Halal dietary legislation. The adulteration of meat may be either deliberate or inadvertent. These activities are unethical in equal measure. Additionally, meat adulteration has grown to be a serious issue for the whole meat industry chain, affecting everyone from farmers to regulators to manufacturers to consumers at every stage of the manufacturing and distribution process.

Technologies for detection that are quick, precise, accurate, and dependable are essential for efficiently monitoring meat adulteration. For the majority of these reasons, accurate and trustworthy analytical methods are required to confirm that the ingredients used in a food product are of the nature or quality required by the buyer and are consistent with the seller's declaration (Li *et al*.,

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2020). As a result, the authenticity of food labelling claims must be ensured.

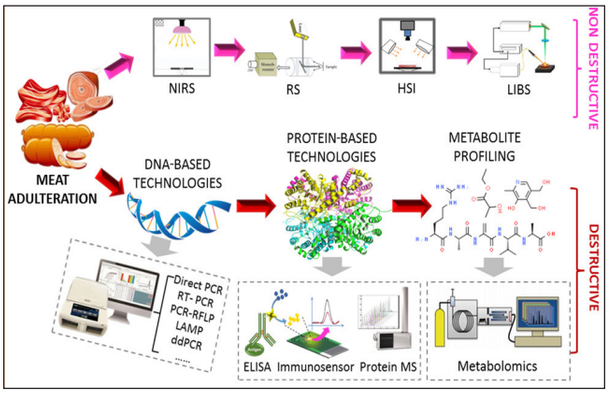

Fig. 1: Commonly used technique for detecting meat adulteration (Li *et al.,* 2020)

DNA-based techniques, particularly those based on polymerase chain reaction (PCR), are widely acknowledged as the most acceptable methods for identifying species in raw and processed meat. Meat species can be identified using PCR techniques such as species-specific PCR, duplex PCR, real-time PCR, LMAP-LFA, PCR-RAPD, PCR-RFLP, and PCR-nucleotide sequencing. PCR technique is a sensitive and accurate procedure. However, conventional PCR and fluorescent PCR are extensively utilized in the qualitative detection of meat and meat products; they cannot be used for quantitative detection. However, because DNA content varies so much between species and target tissues, DNA-based approaches aren't ideal for determining accurate percentages of meat of distinct species in a different type of meat and meat products. As a result, proteomic techniques based on identifying distinct peptide/protein biomarkers have been developed and used to provide information on dietary composition. The proteomic approach is more resilient in tackling some of the significant shortcomings of DNA-based methods, such as optimizing extraction operations according to various matrices and recovering high-quality DNA samples. In this regard, the primary amino acid sequences of significant peptide biomarkers employed in this study will be considered to be more resistant to meat processing than DNA sequences. As a result, the proteomic approach to meat speciation is a viable alternative or complement to conventional approaches. Proteome analysis can look for new marker proteins/peptides systematically, speeding up the development of assays to detect adulteration and deception. Following that, precise and reliable analytical techniques for detecting the previously discovered marker proteins/peptides can be created and verified. On the other hand, recent improvements in proteomics techniques have enabled the detection and recognition of proteins even after heat denaturation (Montowska and Pospiech, 2013). The thermal treatment may be tolerated by peptides that are unique to each species (like processing of food, cooking, etc.). These peptides could be used to identify meat species (Montowska *et al*., 2014, 2015). This short review is compiled to provide an overview for identifying species-specific peptide biomarkers by proteomic technologies as a new and appealing alternative that can overcome some of the limitations that DNA-based methods have faced, particularly when meat exposed to intense heating during processing.

Proteomic technique used to meat authentication

During the preceding decade, remarkable developments in mass spectrometry applied to the analysis of peptides and proteins. Proteomic technology is gaining traction as an alternative to existing approaches for species identification and meat verification due to significant breakthroughs in the application of mass spectrometry to analyze peptides and proteins. Mass spectrometry has also been used to investigate the protein maps of muscles with diverse fiber compositions and identify muscles originating from distinct genetic sources (Hollung *et al*., 2009) using a discriminating activity similar to DNA-based analytical approaches. The discriminatory capacity of this technology is equivalent to approaches based on DNA analysis because it is based on the detection of peptide biomarkers specific to one type of meat or ingredient. However, the proteomic approach is more resilient when it comes to some of the primary shortcomings of DNA-based techniques, such as the examination of highly processed meats and quantitative measures. When compared to DNA extraction, protein and peptide extraction would be easier. This, combined with the fact that primary amino acid sequences in peptides are significantly sturdier to meat processing than DNA sequences, would allow the proteomic technique to generate more reliable quantitative measurements (Ortea *et al*., 2016; Montowska and Pospiech, 2011b; Montowska and Pospiech, 2013). In

addition to actin and myosin, muscle contains myoglobin and significantly less quantity of haemoglobin from the bloodstream. Although the functions of these proteins are very similar in many species but differ in the amino acid sequences that are species-specific. This change in primary structure contributes to the unique immune responses of the molecules and gives each protein a unique molecular weight. It is now possible to discriminate proteins and estimate their molecular weights to 0.01 percent using electrospray mass spectrometry; as long as the molecular weights differ by 0.1 percent. ESI-MS of intact proteins has been touted as a viable approach for meat speciation since 1993. The mass discrepancies between myoglobins and haemoglobins of different meat species could aid in the authentication of meat of distinct species using the mass spectrometry approach. This approach proved effective in detecting horse hemoglobin in a mixture with beef (Taylor *et al*., 1993).

ESI-MS/MS technique on unbroken myoglobin isolated from pork and beef, as well as sheep and horse protein used as commercial proteins, to distinguish beef and sheep from one another and horse and pig. Still, the resolution power of this instrument was insufficient to distinguish horse and pork. This problem is likely to be solved with the current array of high-resolution mass spectrometers available (Ponce-Alquicira and Taylor, 2000). 2-DE (2-dimensional gel electrophoresis) was use by (Timperio *et al*., 2009) to identify differentially expressed proteins in the livers of the two breeds and link them to various liver functions. They discovered that, although being genetically closely related, the variance in the proteomic and transcriptome profiles of these two breeds allows us to do way analysis and pinpoint proteins whose expression would render the latter capable of more significant milk production. With the help of similar technique (Montowska and Pospiec, 2012) discovered interspecies variances in 2-DE protein patterns between turkey, chicken, duck, cow, and pig in both raw and processed meat products. Some proteins remained stable after meat ageing and thermal processing and some proteins could even be identified in highly processed foods like fermented sausages, suggesting that they may be used as markers. However, to build a high-throughput targeted MS/MS-based approach for species differentiation, such as SRM, a validation study concentrating on amino acid sequence information from these stable proteins would be required. This requirement is emphasized because there is still modest protein sequence information for goose and duck in current databases. The identification of marker proteins for the recognition of soybean protein addition to processed meat products has been investigated by Leitner *et al*. (2006) with using of MS/MS technique and find out that all the commercial heat-processed meat products (from beef, pork, turkey and chicken) were contain unique peptides from a glycinin G4 subunit A4. This peptide is unique peptide in soya protein isolates. Species-specific peptides marker, fibrinopeptides, released from the blood protein fibrinogen during thrombin gelling of the blood protein was used by Grundy *et al.* (2007). They found that Fibrinopeptide-A was an effective marker in all types of food matrix-like cooked, processed and fresh spiked with 5% (v/w) bovine binding agent. A successful approach for evaluating genus-specific collagen peptides isolated from the fragmentary bone of 32 distinct mammal species, resulting in the identification of 92 peptide markers that might be utilized for species identification in processed food and animal feed (Buckley *et al*., 2009). They have also analyzed the processed bone meal and meat of cattle, sheep, pig, and chicken with the help of mass spectrometry using solid-phase extraction instead of liquid chromatography/mass spectrometry (LC/MS).

Conventional LC-ions trap–MS/MS techniques based on OFFGEL enrichment fractionation steps were developed by (Sentandreu *et al*., 2010) and found that with the help of this technique, it was possible to detect as low as 0.5% w/v contaminating chicken in pork meat due to the acquisition of discriminating sequence information. The discriminating power of this method is based on the detection of chicken-specific peptides derived from trypsin digestion of previously enriched myosin light chain 3, which is comparable to DNA-based approaches. Using heat-stable species-specific peptides and a DDA technique, processed and raw horse meats were detected in meat mixes at 0.5 percent levels (Claydon *et al*., 2015). The reverse phase-high performance liquid chromatography followed by o-phthalaldehyde (OPA) derivatization and ultraviolet (UV) techniques was used by Jorfi *et al.* (2012) for identification of pork meat from the distinct species meat viz; beef, mutton, chevon, and chicken. Histidine, valine, alanine, serine, and arginine are the most critical discriminatory amino acids between pork and other meat species. They also said that the amino acids content of meat is used as a marker for Halal meat validation. To

detect horse and pork meat in beef, von Bargen *et al*. (2013) devised a sensitive mass spectrometric method. After using a shotgun MS/MS methodology to identify the biomarker peptides, peptides specific to horse and pig were incorporated in an SRM assay capable of detecting as little as 0.55 percent horse or pork contamination in a beef matrix or 0.13 percent pork contamination in beef when using an MRM method. They reported MRM (Multiple Reaction Monitoring Mass) as first rapid and sensitive mass spectrometric method for detecting horse and pork. Raman spectroscopy allows for high-accuracy determination of adulteration of beef with horsemeat in seconds with no sample pretreatment (Boyaci *et al*., 2014). The mid-infrared ATR spectroscopy was use by Zhao *et al*. (2014), under fresh and frozen circumstances; they were able to distinguish between genuine greater and lesser quality beef burger samples from other samples contaminated with beef offal. Porcine-specific peptide markers in cooked meat were discovered by Sarah *et al*. (2016) that might be used to distinguish pork from chevon, beef, and chicken. LC-QTOF-MS was used to study seven porcine-specific peptides. Four were obtained from serum albumin protein, two from lactate dehydrogenase, and one was derived from creatine kinase. On the other hand, four thermostable peptides have been proposed as markers for detecting pig in cooked meat products using SRM and they suggested that tandem mass spectrometry is a perfect platform for Halal authentication. MRM has also been used to reveal beef, lamb, pork, and horse myoglobin in adulterated meat mixes and told that the detection limit of this technique is 1% (w/w) in one meat added to other meat (Watson *et al*., 2015). SRM and pSRM techniques were also employed by Ruiz Orduna *et al*. (2015) to detect beef, hog, horse, and lamb myoglobin in meat mixtures. Label-free quantification combined with high-resolution infusion-based mass spectrometry (MS) were used to validate 'horse sausages' manufactured from horse meat and pork.

Ultra-high performance liquid chromatography coupled with mass spectrometry was developed by Jiru *et al*. (2019). It is an efficient approach for assessing meat provenance and determining net muscle protein (NMP; based on amino acids and β-alanylhistidine dipeptides) in meat products and found that the detection of undeclared addition of 2% chicken meat to pork was made possible with the use of specific ratios of 1-methylhisitidine/3-methylhistidine. A rapid and susceptible PRM technique for screening and identification of surrogate peptides to detect pork from mixed meat based on high-resolution Orbitrap MS was developed by Pan *et al*. (2018). They discovered that the limit of detection of this technique in mixed meat was 0.5 percent. The heat-stable species-specific peptides marker was identify by LESA-MA technique from different types of cooked meats (beef, horse, pork, chicken, and turkey) and found that cooked meat samples were discriminated using principal component analysis and orthogonal partial least-squares discriminate analysis (Montowska *et al*., 2014). LC-MS/MS technique was used to establish a method for quantitative measurement of pork meat in various types of meat products (Li *et al*., 2021) and discovered that three peptides from Carbonic anhydrase 3 have high quantitative capacity. The approach was found to have reasonable specificity, sensitivity, repeatability, and the detection limit was also very low, allowing it to be used to a variety of meat products with varying contents. A liquid chromatography-tandem mass (LC-MS/ MS) was developed by Zhang *et al*. (2022) its reliable method for identification and quantitative analysis of fox meat products and discovered that this method's detection limit for distinguishing fox meat from other meat (dog, duck) is exceptionally high. It can detect meat adulteration of up to 1%.

Proteomic technique used to fish authentication

Directly differentiating fish species based on fish appearance or morphological data, such as skeletal, muscular, bifurcation taxonomy, and even fin traits, scales, and life history, is the most fundamental form of fish species identification. However, when it comes to identifying fish species, the appearance features of whole fish might be difficult to use, and processed fish commodities are far more difficult to recognize. Gene analytical techniques have been increasingly popular in recent years because to their ease of use, although they are not ideal for some types of samples, such as heat or acid-treated materials. Heat or acid treatment can degrade DNA, affecting the ability to identify species. Until now, mitochondrial DNA has been preferred for species identification over nuclear DNA because it can withstand processing-induced degradations better. Despite the fact that mtDNA may be used to identify species, there is still a risk that the high processing approaches will destroy

mtDNA in all species. In recent era, peptide biomarker based technology emerging out to solve the problem that were arise use of DNA for detection of fish and fish product adulteration. Fish authentication has been solved with remarkable success using proteomic technology. Proteomic technology may successfully recognize protein specific to different fish species in processed and fresh fish products, using MALDI-TOF MS and MALDI-TOF and LC-ESI-MS/MS as a quick screening strategy (Ortea *et al*., 2016; Mazzeo *et al*., 2008; Carrera *et al*., 2007). The parvalbumins were identified by Mazzeo *et al*. (2008); it is a protein biomarker that could be used to distinguish distinct fish species without ambiguity. These authors stressed the need to select appropriate protein biomarkers capable of certifying product authenticity in both processed and fresh fish products. Using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) with Sequential window acquisition of all theoretical fragment ion spectra, (Chien *et al*., 2022) propose a proteomic-based technique for fish and fisheries product verification (SWATH). Protein biomarkers from Alaska pollock, Atlantic cod, and Greenland halibut meat were found and confirmed for species authentication of cod and related fishery products, potentially preventing consumer replacements and mislabeling. For the detection of species-specific peptide signatures in rainbow trout and Atlantic salmon, (Gu *et al*., 2020) use both targeted and untargeted proteomics techniques. Relevant peptide signatures were identified by comparing HRMS data to the UniProt database, screening with BLAST, and then confirming with samples. With rainbow trout and Atlantic salmon, five peptide signatures were discovered. The MRM method was developed for quantifying the adulteration of Atlantic salmon with rainbow trout, and it demonstrated great specificity and repeatability. For quantification, the signature peptide GDPGPGGPQGEQGVVGPAGISGDK was employed. Adulteration of rainbow trout has a detection limit of 0.19 percent and a limit of quantification of 0.62 percent.

CONCLUSION

Identifying animal species of origin in meat and meat products is fraught with religious, economic, legal, and medical implications. As a result, numerous analytical procedures for identifying meat species in individual or mixed samples have been proposed to safeguard customers from fraudulent and poor marketing practices. Methods such as chromatography, immunology, electrophoresis, and genetics can be used to verify the species composition of meat products. Although, when compared to other well-established methodologies such as immunoassays or DNA-based analysis, the use of protein/peptide biomarkers via proteomic technologies is still limited in terms of determining food authenticity, it is a promising alternative due to its robustness, sensitivity, multiplexing capacity, high-throughput, and discriminating power. Furthermore, peptide-based approaches can circumvent one of the most significant limitations of DNA technologies: DNA degradation in highly processed samples. Still, peptides marker can be quite stable against processing, amino acids modifications during processing monitored, and heat stable proteins can be selected as targets.

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