Journal of Food Research; Vol. 12, No. 4; 2023
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Detection of Authentication of Meat Products by Low Cost Closed-tube Molecular Methods

George P. Laliotis[4], Anthimia Batrinou[1], Maria Giannakourou[2], E. Tsakali[1, 5], Sophia Letsiou[1], Panagiotis Halvatsiotis[3], Simen Akkermans[5], Jan Van Impe[5], Niki C. Maragou[1] & Dimitra Houhoula[1]

[1] Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece

[2] Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Greece

[3] 2nd Propaedeutic Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens, "ATTIKON" University Hospital, Chaidari, Greece

[4] Laboratory of Animal Breeding and Husbandry, Department of Animal Science, Agricultural University of Athens, Athens, Greece

[5] Department of Chemical Engineering, BioTeC+-Chemical and Biochemical Process Technology and Control, KU Leuven, Gent, Belgium

Correspondence: Dimitra Houhoula, Department of Food Science and Technology, University of West Attica, Agiou Spiridonos 28, 122 43 Egaleo, Athens, Greece. E-mail: dhouhoula@uniwa.gr

George P. Laliotis, Laboratory of Animal Breeding and Husbandry, Department of Animal Science, Agricultural University of Athens, Iera Odos 75, GR11855, Athens, Greece. E-mail: glaliotis@aua.gr

Received: June 17, 2023 Accepted: July 19, 2023 Online Published: October 25, 2023

doi:10.5539/jfr.v12n4p61 URL: https://doi.org/10.5539/jfr.v12n4p61

Abstract

Nowadays adulteration of meat products, especially of ground meat products which form an easy case scenario for implementing adulteration practices due to their structure and texture, emerges a critical issue of raised concern threatening fair trade, food quality and consumers' health and protection. Food authentication testing is the tool to address this kind of fraud. There is several analytical methodologies applied for meat authentication targeting at different biomarkers and using a variety of analytical techniques. However, the applied methodologies should exhibit suitable performance characteristics such as reliability, sensitivity, reproducibility and availability in order to be fit for purpose. During the last 20 years, amplification tests have emerged as an important diagnostic tool, not only for clinical applications, but also for food quality and safety. It was urgent to develop molecular techniques fast and sensitive. The introduction of new DNA technologies has facilitated the ease and accuracy of of methods for fraud detection. The closed-tube methods of Loop-mediated isothermal amplification (LAMP) and Gold Nanoparticles linked with oligonucleotides used as molecular probes are well known for their robust and highly sensitive and specific amplification of target DNA. Moreover, these techniques are rapid, low-cost diagnostics and available on site. This review provides a comprehensive overview of the molecular methods developed that can be applied for investigating ground meat adul-teration and focuses on the advantages of the rapid closed tube methods that can yield color results interpreted with the naked eye. The application of such time- and cost-effective molecular tools in the food market is proposed to provide a first-level filter for meat adulterated products, serving as a complementary tool to the more in-depth -omics approach.

Keywords: adulteration, food fraud, meat products, food quality, food safety, nutritional value, Gold Nano-particles, LAMP

1. Introduction

1.1 Introduce the Problem

Food consumption and in particular meat consumption is related to living standards, diet, livestock production and consumer prices, among other economic factors. Compared to other agricultural commodities, meat has high

production costs and high output prices. Consequently, meat consumption is associated with higher incomes and with a shift to food consumption which favours the intake of proteins originated from animal sources, mainly due to urbanisation (OECD, 2023). Fraud related to meat products, especially ground meat products which form an easy case scenario for implementing adulteration practices due to their structure and texture, constitute a critical issue of raised concern threatening fair trade, food quality and consumers' health and protection.

A common fraud observed in meat products towards consumers is the partial or complete replacement of the declared food component with undeclared cheaper food components. In particular, meat protein may be substituted with protein of different animal species or with proteins derived from vegetable, cereal, milk or microbial protein or with offal (Hargin, 1996). Other types of fraud and mislabeling may concern the presence of non stated ingredients, the erroneous extension of the shelf life, the absence or false declaration of processes, the over declaration of a quantitative ingredient, deceptive claims related with geographical or production origin. These practices deceive consumers, providing products of obviously lower nutritional value and different from their choice. The choice may reflect a consumer's lifestyle, religious beliefs (e.g., vegetarianism, preference for organic products, absence of pork for Jews and Muslims), or health impact (e.g., absence of peanuts, lactose or gluten for individuals with particular allergies). For these reasons incorrect description and mislabeling of a food product violates consumers' rights, deteriorates food quality and may endanger human health, especially if the ~~food~~ product has been processed eliminating, thus, the ability to distinguish the components.

In order to address the above types of food fraud, food authentication testing is required to be regularly applied, which concerns the process which confirms that a food conforms to its label description (Wang; Jun, Bittenbender, Gautz & Li, 2009; Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016). The food authenticity testing market, in terms of value, was estimated to reach USD 7.50 billion in 2022, at a compound annual growth rate (CAGR) of 7.6% from 2016 to 2022. The growing international food trade is one of the factors driving this market since manufacturers are forced to comply with the global mandates and regulations for food authenticity due to the growing economically moti-vated adulterations (EMAs). Since international trade increases the complexity in the supply chain, the chances of cross-contamination and fraud, and the demand for food authentication services is projected to remain high.

For these reasons, regular controls of meat products are in high demand, in order to protect consumers and producers from fraud, and ensure food safety and public health. There is an urgent need to develop rapid, sensitive, and inexpensive diagnostic tests, integrated within sector-specific and -generic traceability systems that will enable the determination and the objective verification of the origin of food (Wilwet & Karunanithi, 2020).

Several analytical approaches, from infrared spectrum analysis up to advanced -omics techniques, have been proved a valuable proxy in the area of adulteration (Dirong, et al., 2021). However, the identification of polymorphisms at DNA level allows to trace the origin of raw and processed components accurately and indisputably, using any type of tissue or material as DNA can be isolated from various matrices (Azad, Dey, Khanam, Biswas, Akhter, 2023) rendering this approach a powerful tool for eliminating adulteration practices. Molecular methods for food authentication rely on DNA barcoding, since all foods are derived from living organisms. DNA barcoding is used to gain insight into species-level taxonomy, to define and delineate species, and to aid in the process of assigning organisms to correct species.

The aim of this review is to describe the development of simplified, inexpensive and accurate molecular methods for detecting trace amounts of meat fraud, which are not dependent of high-cost instrumentation and allow rapid and sensitive detection. Emphasis was given in rapid close-tube PCR methodologies in which the amplification and analysis of DNA take place in one tube without the need to remove the PCR products for further analysis and which provide results that can be detected with the naked eye such as the Loop-mediated isothermal amplification (LAMP) and Gold Nanoparticles linked with oligonucleotides that are used as molecular probes.

2. Analytical Approaches on Meat Adulteration Control

Meat authentication techniques are based on the identification of targeted analytes, namely biomarkers, or the identification of a chemical analyte pattern unique for the tested meat species. Classification of these techniques based on the group of the analytes to be detected includes DNA-based (classical techniques and genomics) (Dirong et al., 2021; Azad, 2023), RNA-based (transcriptomics) (Vishnuraj, Devatkal, Vaithiyanathan, Uday Kumar & Mendiratta, 2021), proteins-based (proteomics) (Stachniuk, Sumara, Montowska & Fornal, 2021; Suratno, Windarsih, Warmiko, Khasanah, Indrianingsih & Rohman, 2023), metabolites-based (metabolomics) (Zhang, Chen, Xie, Wang & Pan, 2021; Harlina, Maritha, Musfiroh, Huda, Sukri & Muchtaridi, 2022; Suratno, 2023), lipids-based (lipidomics) (Harlina et al., 2022; Jia, Di & Shi, 2023) and glycans-based (glycomics) based techniques (Shi et al., 2019; Chia et al., 2022).

Each one of the different analytical techniques has advantages and limitations. Regarding the protein-based techniques, these are limited to non heat-treated material because of the denaturation of soluble proteins during food processing (Azad et al., 2023). In general, the different omics-based approaches are able either to identify numerous analytes present in the food sample with high sensitivity or provide a large amount of data that after suitable statistical treatment can answer the authentication problem. Nonetheless for this kind of analytical approach there is a prerequisite of highly standardized laboratory with advanced analytical instruments such as liquid and gas chromatography coupled with high-resolution mass spectrometry (LC- and GC-HRMS) (Harlina et al., 2022; Suratno et al., 2023) and NMR (Kim, Ko & Jo, 2021). Moreover, a considerable amount of time and resources, such as chemical reagents, is required for the application of this approach Furthermore, these methods usually generate a great amount of data which require suitable software and laborious data processing along with high expertise in multivariate statistics and chemometrics. rendering them time-consuming, laboratory-dependent and high-cost methods.

2.1 DNA Based Approaches

The most important techniques for the authentication of species origin have been proved those of DNA thanks to the stability of DNA under production and processing techniques applied along the food-chain. According to the European Parliament Resolution of 14 January 2014, DNA testing is suggested as a standard procedure in spot checks for determining species, especially regarding meat and fish products, and it is also suggested to create a centralized DNA database (2013/2091 INI). Hence, it is shown that DNA-based techniques have a pivotal role in adulteration control of meat products in regulatory terms. Due to advances at DNA level, new methods based on molecular analysis sare developed as a “vehicle” for assuring authenticity in a more secure and precise manner. Methods based on DNA analysis enable identifications from immature life stages, or fragmentary remains, offering a powerful tool to address the validation of food authenticity and traceability of primary products entering the food chains both in fresh and processed food and therefore may be of particular use in the food and agriculture industries. DNA markers identify differences in genomic sequences that can highlight inter and intra-species variability. They potentially may be high informative due to their high degree of polymorphism and their stability. The use of polymorphic DNA markers is expand in multiple applications including the evaluation and characterization of genetic variation, molecular mapping and marker-assisted selection.

2.1.1 RFLPs

Restriction fragment Length Polymorphisms (RFLPs) were the first markers employed to study inter and intra-species genetic diversity. They belong to the methods that require the in-vitro transcription of a specific sequence of the marker in order to identify any kind of fraud and DNA variability can be noted by comparing the patterns produced by the digestion of DNA originated from different samples (i.e., species or varieties). Due to their repatability and co-dominance, RFLPs have been widely used for various purposes, including the construction of linkage maps in several species, including also the authen-tication and food traceability (Aranishi, 2005). Although RFLPs may provide a more comfortable, easy and time-efficienct method of traceability (Guan, Jin, Zhao, Xu, & Luo, 2018) there cannot be used on all the desired cases, because restriction enzyme cleavage sites are case sensitive and some restriction enzymes are still of high price rendering the method unaffordable.

2.1.2 Not Sequence-depended DNA Markers

Another category of DNA markers is those that they do not require the knowledge of a specific DNA polymorphism or the isolation and the cloning of a DNA fragment (i.e., Amplified Length Polymor-phisms, Variable Number Tandem Repeats, Random Amplified Polymorphic DNA). Amplified Length Polymorphisms (AFLPs) and Variable Number Tandem Repeats (VNTRs) methods, although they have lower cost, they also have difficulties in the interpretation of the final output and they require expertise in casting and staining the gel. In their drawbacks, apart from the latter that renders them more laborious, it is also included the need of good DNA quality and the several processes until the final result (Marmiroli, Peano & Maestri, 2003). Random Amplified Polymorphic DNA can detect loci in several areas of a genome at the same time. Although RAPDs are considered to be dominant, meaning that do not allow to distinguish whether the amplified DNA segment is heterozygous or homozygous at a particular locus, they have ~~has~~ been widely used for taxonomic and phylogenetic studies (Bartish, Rumpunen & Nybom, 2000; Mokkamul, Chaveerach, Sudmoon & Tanee, 2007; Verma, Karihaloo, Tiwari, Magotra & Koul, 2007), for species discrimination (Chaveerach, Tanomtong, Sudmoon & Tanee, 2006; Zhang et al., 2007) and for phyleogeographic studies (Wu et al., 2006). However, to achieve reproducible profiles a strictly consistent reaction conditions is needed during the run of RAPDs methodology.

2.1.3 Single Nucleotide Polymorphisms (SNPs)

SNPs (Single Nucleotide Polymorphisms) detection has recently offer technological advances in the investigation of DNA polymorphisms. These polymorphisms are evenly distributed throughout the genome offering an exceptional discrimination power. SNPs have been firstly used as an approach for parentage verification (Tortereau, Moreno, Tosser-Klopp, Servin & Raoul, 2017), breeds assignment, and population genetic variability (Grasso et al., 2014; Edea, Dessie, Dadi, Do & Kim, 2017) and for phylogenetic and biodiversity purposes (Kawęcka, Gurgul & Miksza-Cybulska, 2016; Leaché & Oaks, 2017). Subsequently this information can be applied to the genetic identification of breed and furthermore to find possible adulteration practices. SNP are very promising in food traceability since their polymorphism detection is dependent on amplification of very small fragments. Therefore, SNPs can be adapted to highly fragmented DNA allowing for quick and efficient genotyping of of many samples while still being adaptable to high throughput automation. Finally, the use of SNPs, as well as all of polymorphic markers, should be assessed for each matrix or food product, considering any chemical changes that industrial processing or storage conditions may cause in DNA sequence. Many applications of SNP technology have been reported in animal products with most of them to focus on traceability aspects of cattle/ beef meat (Karniol et al., 2009; Orrù et al., 2009; Lasagna et al., 2015; Xu et al., 2012; Li et al., 2020).

2.1.4 Mitochodrial DNA (mt DNA)

Another category of DNA markers proposed for DNA barcode are those developed from mitochondrial genome that is a standard region of the genome, which is usually characterized by a high inter-specific, and low intra-specific variability (Hebert, Cywinska, Ball & de Waard, 2003; Woolfe & Primrose, 2004). For example, mitochondrial DNA markers CO1 (the gene coding for cytochrome c oxidase 1). has been demonstrated to be effective for identifying animal species in food products. This DNA barcode was used to differentiate between a large number of cattle breeds, to analyze fresh and deteriorated meat substrates, and to identify mislabeled fishing goods (Teletchea, Bernillon, Duffraisse, Laudet & Hänni, 2008; Filonzi, Chiesa, Vaghi & Marzano, 2010; Barbuto, et al, 2010; Cai et al., 2011). Plant mitochondrial DNA, on the other hand, did not exhibit similar power in species discrimination, probably due to intra-molecule recombination that characterizes plant mitochondrial DNA (Chase, 2007). As a result, the attention in plants focused on plastid genes, which were initially proposed as candidate barcoding markers (Kress & Erickson, 2007).

It should be noted that it possible to confront the drawbacks of the DNA-based approach, wherein extreme food processing conditions such as heat and chemical treatment can potentially result in DNA degradation yielding non-specific DNA fragments (Dirong, 2021). In any case many many variations of DNA-based techniques have been developed in an attempt to easily, quickly and reliably detect adulteration in meat products as well as to overcaome limitations and difficulties related to previous developed methods, especially based on biochemistry level. Table 1 depicts a short brief of advantages and limitations of the current available methods for species and adulteration determination.

Table 1. Advantages and disadvantages of the contemporary available methods for adulteration detection on meat products

Method	Characteristics
Biochemical assays	**Sample**: blood, tissue, other fluids. **Advantages**: rapid, economic, straightforward method. **Limitations**: labor intensive, not based on genetic material, based on the product of gene expression, vulnerable to environmental effects.
ELISA	**Sample**: same as above. **Advantages**: simple, high specificity and sensitivity, cost effective, **Limitations**: labor intensive, need of specific antibody, Sophisticated technique, high possibility of false positive/negative, antibody instability, no signal amplification.
Chromatographic assays	**Sample**: same as above. **Advantages**: sensitive, selective, accuracy, high resolution, automation. **Limitations**: time-consuming, multi-stage sample preparation, calibration with standards, high-cost, high expertise.
RFLPs	**Sample**: DNA. **Advantages**: high reliability, co-dominant, selective neutral, low automation, cost effective. **Limitations**: labor intensive, time consuming, low multiplexing level, medium level of polymorphism, restriction-enzyme dependent.
AFLPs	**Sample**: DNA. **Advantages**: stability, rapid, low-cost, multiplex detection, high level of polymorphism. **Limitations**: dominant, medium automation, specific sequence based.
RAPDs	**Sample**: DNA. **Advantages**: low-cost, simple, quick, no prior sequence knowledge, high level of polymorphism. **Limitations**: intermediate repeatability and reliability, non-specific products, dominant.
VNTRs	**Sample**: DNA. **Advantages**: high reproducibility, fast, no prior sequence knowledge, high level of polymorphism, intermediate cost, co-dominant. **Limitations**: laborious, large quantities of sample, low sensitivity.
STRs	**Sample**: DNA. **Advantages**: repeatability, stability, co-dominant, high-polymorphism, high accuracy and reproducibility, high multiplex level, low sample quantity, ease of automation. **Limitations**: time consuming, high cost, stutter bands, specific sequence required, misclassification of heterozygotes as homozygotes when null allele occur.
SNPs	**Sample**: DNA. **Advantages**: stability, high repeatability and accuracy, high throughput genotyping, abundant variation, automation. **Limitations**: low level of genetic information, high cost, labor and time consuming, sequence specific.
mt-DNA	**Sample**: DNA. **Advantages**: small size, mediate cost, ease isolation and annotation, small sample amount, less subjected to degradation. **Limitations**: low discrimination power (only maternally inherited), low polymorphism, time-consuming, labor intensive.
AuNPs (Gold-nanoparticle)	**Sample**: DNA. **Advantages**: stability, high efficiency, sensitivity, and specificity, intermediate labor consuming, low-cost equipment. **Limitations**: high cost, time-consuming, sequence specific, probe specific design, calibration for each case.
LAMP	**Sample**: DNA. **Advantages**: ease, low-cost infrastructure (instrument), rapid, highly selective and sensitive, facilitates multiplexing approach. **Limitations**: sequence specific, complex of primer design.

3. Analytical Approaches on Ground Meat Adulteration Control

Due to the lack of external diagnostic characteristics for species identification, ground beef products and precooked, ready-to-eat meat items appear to be more prone to adulteration. Several factors, including economical gain, increase in diversity of products, the complexity of international food trading networks, deficiencies in traceability systems for ethnic foodstuffs, and a lack of effective methods to identify meat sources, particularly in thermally processed products appear to contribute to this growing phenomenon (Özbay Dogu, 2016). To combat mislabelling, sensitive, accurate and inexpensive diagnostic tests for quickly detecting meat species should be developed and integrated into sector-specific and generic traceability systems. The majority of existing approaches for determining food authenticity are based on the of identification of species-specific proteins and DNA analysis. DNA-hybridization approaches for identifying DNA sequences are considerably more effective in authentication tests (Asensio, Gonz ález, Garc á & Martin, 2008). Therefore, a duplex PCR assay for identifying horse, donkey and mule species in raw and heat-processed meat products has been developed (Chen, Wei, Chen, Zhao & Yang, 2015), which is based on the simultaneous amplification of mitochondrial ATP synthase subunit 8/6 gene and ND2 (gene coding for NADH dehy-drogenase 2) fragments. The authors reported that the target meat species could be detected at a 1% level. Similarly, Ali et al. (2014) used multiplex PCR assays based on species-specific fragments of the mitochondrial and cytochrome b genes. to determine the origin of different meat species (beef, sheep, pork, goat, horse, cat, dog, monkey, and rat), using amplified species-specific fragments of the mitochondrial and cytochrome b genes.

Although Single Nucleotide Polymorphism panels have been developed for many species providing a powerful tool both for genetic diversity and traceability purposes of certain breed/species, the same method is rather unexplored in the case of origin's discrimination in the situation of product adulteration or mixed final products (Laliotis, Koutsouli & Bizelis, 2018). Due to their unique optical features, gold nanoparticle chemistry, and particularly gold nanoparticle chemistry, gives unprecedented prospects for the rapid and simple diagnosis of authenticity, being able to detect minuscule quantities of fraud. The general workflow of the experimental

procedure for the detection of meat adulteration with a closed-tube method is shown in Figure 1.

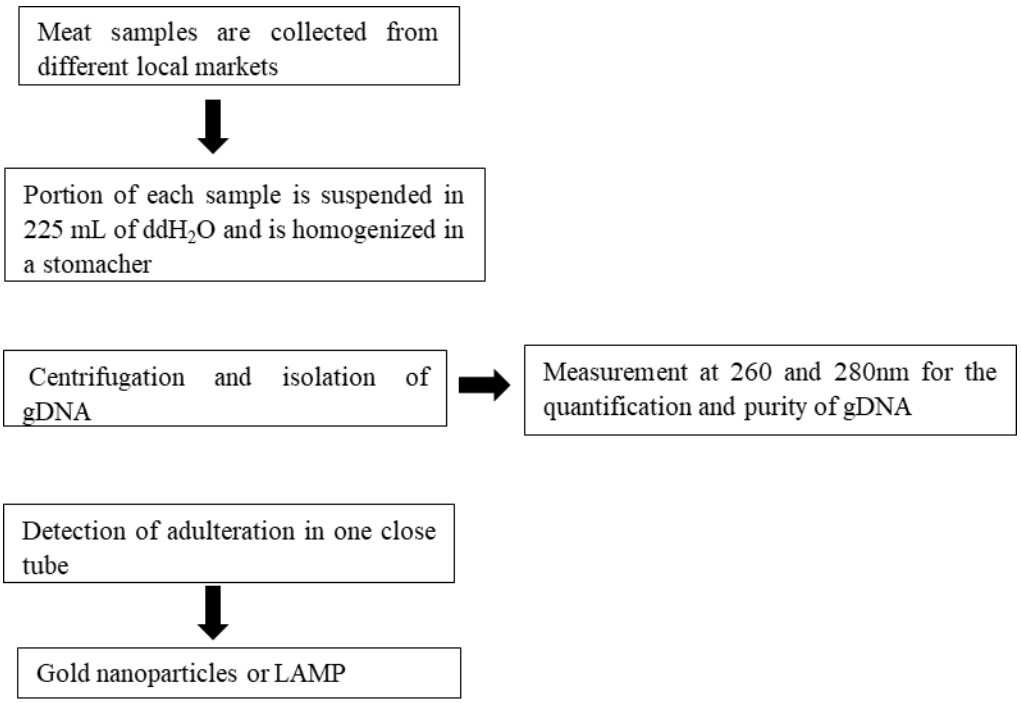

Figure 1. General workflow for the detection of the adulteration of meat with a closed-tube method

3.1 Gold Nanoparticles Assay

Gold nanoparticles have been successfully used as colorimetric sensors for visually detecting pork adulteration in beef and chicken meatball preparations (Ali et al., 2014). Also, Houhoula et al. (2017), had developed a methodology fast, easy, and economical to detect low concentration of adulterated meat with horse. Nanotechnology and especially, gold nanoparticles (AuNPs) chemistry offers innovative opportunities for quick and easy analysis of authenticity, being capable to evidence small amounts of adulteration, thanks to their particular optical properties. The team of Subara and Jaswir (2018), worked on Halal verification in pork-adulterated meat using AuNPs coupled with thin layer chromatography. The AuNPs were produced using citrate reduction of HAuCl4. They explained that as low as 0.1μ mol/l of analyte was successfully measured using the naked eye, and more sen-sitive semi-quantification of 0.001-1μ mol/l of DNA could be achieved using a camera to measure the intensity of the analyte. Also, Magiati et al. (2019) proposed a new lateral flow device (strip) that was constructed for the visual detection, by the naked eye, of DNA sequences specific to four meat species: horse, pork, beef, and sheep. The detection was completed within 25-30 min after amplification. The assay offered high detectability and good selectivity and reproducibility. As low as 0.01% of horse and 0.02% of pork DNA were detectable in binary mixtures by the reported lateral flow device. The AuNPs methodology, which has been successfully employed to colorimetric sensors for visual identification, is a less expensive and simpler alternative to other molecular approach, and it has been proven to work with various food matrices.

Generally, the principle of the method is based on gold colloid nanoparticles (AuNPs) in different nanosizes 10-100 nm, that are conjugated with specific oligonucleotides. All OLG (oligonucleotides) are thiolated (modified with 10xdATP in the 5'-end of the primer). The AuNP merging with the OLG was performed by adding 1 ml of an aqueous solution of AuNPs to 4 nmol of the thiolated OLG pair (forward and reverse primer) after an incubation with the addition of salting buffer. When the AuNP-OLG solutions are prepared, are stored in the dark at room temperature in glass vials. The obtained functionalized AuNPs solution are measured with a Spectrophotometer and the maximum absorbance is between 520 and 530 nm. This implies that the Gold NP was properly conjugated with the oligonucleotides.

This method is based on the specific target hybridization of the AuNPs with a specific DNA sequence. Later on, it is possible to induce the aggregation on the NPs with the addition of acid, which leads to a colorimetric discrimination. In general, colloidal solutions of AuNPs with diameters ranging between 5-20 nm show a pink color (red when very concentrated), due to its optical absorption peak at around 520-525 nm, caused by the

collective excitation of the free conduction band electrons of the dispersed particles, known as the surface plasmon resonance. Hybridization on the extracted DNA from the isolates with the AuNP thiolated with the oligonucleotide's solution is performed by adding 20 µl of eluted DNA, followed by, five minutes at 95ºC as the denaturation step and by five minutes at 55ºC after the addition of 20 µl Au-NPs-oligonucleotides solution and 10 pl of phosphate buffer as the hybridization step. The aggregation of the AuNPs displaces the absorption peak to a longer wavelength (>570nm) and the color of the colloidal solution turns purple with the addition of hydrochloric acid (HCl), as a result of coupling in the surface plasmons of the aggregated particles (Figure 2). Therefore, the color of the gold colloid is determined by the degree of aggregation of AuNPs in suspension. The results can be confirmed by UV-V spectroscopic analysis. AuNP sensors are able to detect very small quantities of analyte i.e. as small as µmol/l and have the potential to replace conventional techniques for meat authentication such as gel electrophoresis (Özbay, 2016). AuNP sensing is rapid and does not require any equipment during the analysis. Unlike PCR, AuNP analysis employs small and portable kits that can be carried everywhere for rapid and effective testing. However, AuNP systems need to be calibrated for each analyte before use in detection. Moreover, specific probes for different animal meats of interest need to be designed.

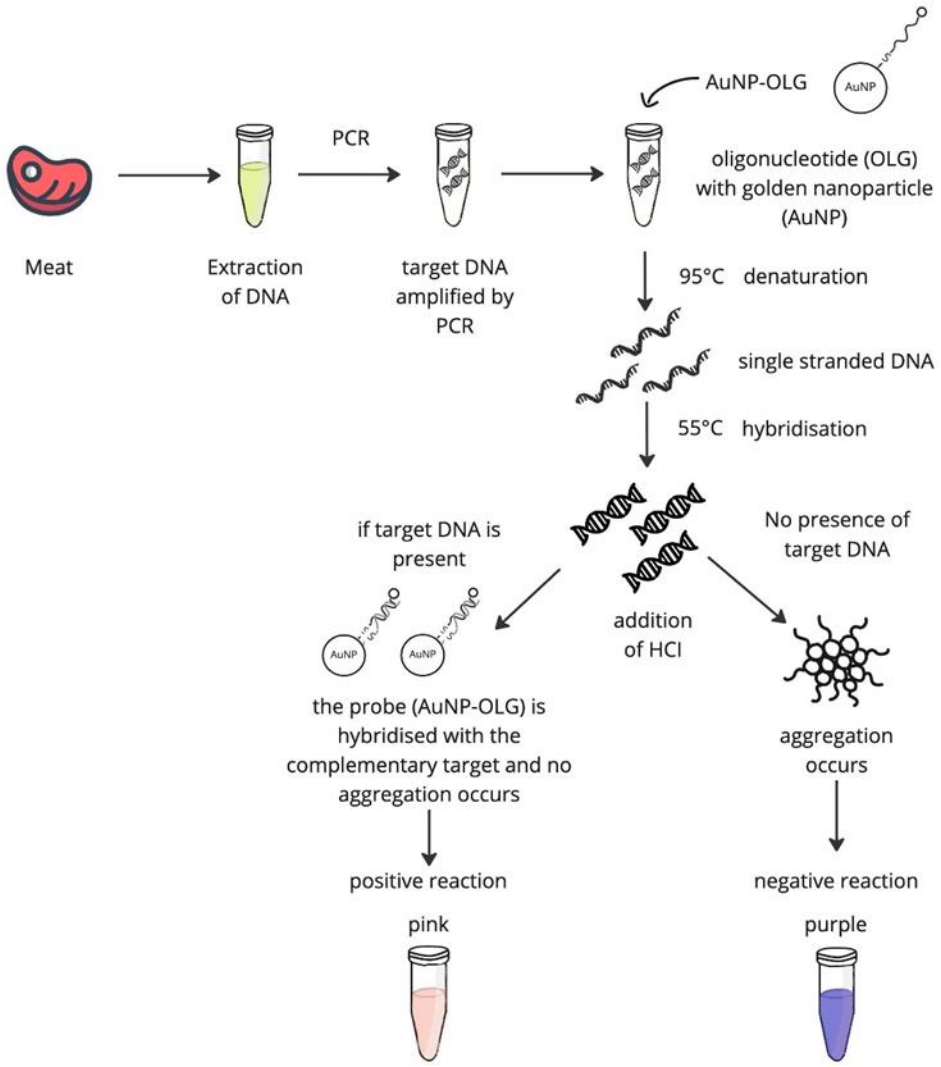

Figure 2. Simplified schematic representation of the detection of specific meat genes by using golden nanoparticles (AuNP) with oligonucleotides (OLG) as probes. Positive and negative reactions can be identified by different colors which can be visualized with the naked eye

3.2 LAMP (Loop-Mediated Isothermal Amplification)

LAMP is a well-established nucleic acid amplification method termed loop-mediated isothermal amplification which was first developed by Notomi (2000). A pair of outer primers and a pair of inner primers, are used to

specifically recognize six distinct DNA sequences on the target gene. DNA polymerase (Bst) is used, and the LAMP reaction is performed under isothermal conditions without a thermal cycler. The DNA denaturation at a high temperature is not required due to the activity of Bst DNA polymerase on DNA strand displacement (Figure 3). At present, LAMP is extensively applied to amplify DNA for many pathogens such as viruses, fungi, bacteria, and parasites (Wong, Othman, Lau, Radu & Chee, 2018). A standard method for the detection of LAMP is the measurement of the turbidity caused by pre-cipitated magnesium pyrophosphate, as well as endpoint detection by naked eye ob-servation (Ali et al., 2014; Houhoula et al., 2017; Subara & Jaswir, 2018). Other target sequence-independent detection methods rely on gel electrophoresis, metal indicators for calcium, colorimetric LAMP, fluorescent dyes such as SYBR green, bioluminescence through pyrophosphate conversion or electrochemilu-minescence (Tomita, Mori, Kanda & Notomi, 2008; Tanner & Evans, 2014).

A new method based on loop-mediated isothermal amplification combined with a lateral flow device was developed for mammalian DNA, offering a detection limit of 10 pg of isolated DNA and 0.01% of mammalian DNA in mixtures of processed meat (Tomita et al., 2008). A real-time loop-mediated isothermal DNA amplification method was also performed in compact disc micro-reactors detecting 10 μg/g of bovine DNA (Santiago-Felipe, Tortajada-Genaro, Carrascosa, Puchades & Maquieira, 2016). Another approach involves the rapid visual detection of eight meat species using optical thin-film biosensor chips with a detectability of as low as 0.001% of deer and beef meat powder in pork powder (Tanner & Evans, 2014). LAMP primers, were designed to specifically identify garlic, Chinese leek, Chinese onion, green onion and onion, respectively, based on the ITS1-5.8S-ITS2 nuclear ribosomal DNA sequences available in GenBank (Santiago-Felipe et al., 2016)]. Cho, Dong & Cho (2014) designed a LAMP assay targeting a mitochondrial DNA which was respectively designed in combination to be specific for identifying and discriminating eight animal species simultaneously namely cattle, pig, horse, goat, sheep, chicken, duck, and turkey. The limits of detection of the LAMP assays in raw and cooked meat were 10 pg/μL to 100 fg/μL levels in 30 min reaction time. This shows the ability of multiplex LAMP assays for discriminating multiple meat species simultaneously. The detection limits of the LAMP assays in raw meat, cooked meat, raw admixtures, and cooked admixtures were determined in 30 min and revealed greater sensitivity than PCR assays. Meanwhile, Roy, Rahman, & Ahmed (2016), developed a sequence-specific and simple method using isothermal loop mediated amplification (LAMP) and MB-mediated aggregation. Following LAMP amplification, the MBs were added to the LAMP products. These produced aggregates showed up as dark spots on filter paper. In contrast, stable aggregates did not form in the absence of LAMP products. Optical images of the aggregates could be used for the simple detection of the DNA. This method could detect genomic DNA at low picogram levels (chicken 1 pg μL−1 and pork 100 pg μL−1).

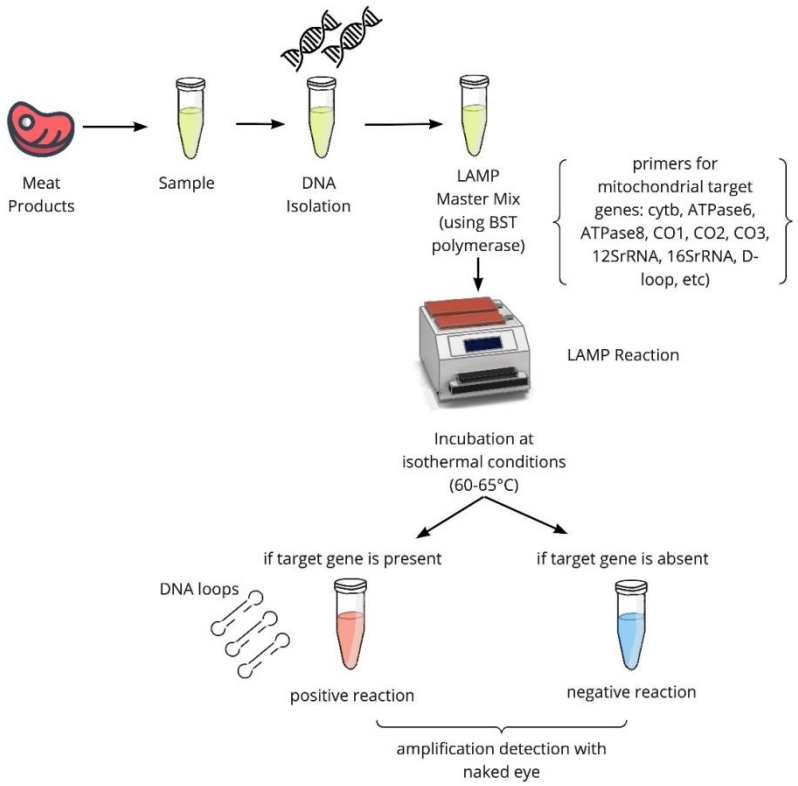

Figure 3. Simplified schematic diagram of the LAMP method for the detection of specific meat genes to identify adulteration

4. Discussion

Many tools have been developed towards adulteration practices ranging from chemical and enzymatic approaches to molecular level searching for DNA bases' differentiations. Table 2 sumarises the existent methodologies for detecting adulteration in food matrixes. Analyzing all the existent does not fall in the purposes of the current review, as the aim was to give emphasis on rapid close-tube PCR methodologies which permits the amplification and analysis of DNA in one tube without the need to remove the PCR products for further analysis and the results that can be detected with naked eye. However, the reader could retrieve further information for the existed methodologies via the highlighted literature (Table 2). According to the presented information, Gold nanoparticles and LAMP are gaining attention, becoming more popular techniques and user-friendlier molecular tools. This work reviews, also, a comprehensive evaluation of these molecular methods regarding different user requirements, ranging from the overall performance of the method to the related instrumental platforms. In addition, depicts advantages and limitations of the mentioned methodologies (Table 1). Ultimately, it may serve as a guideline for the selection of the most appropriate fast and sensitive molecular methods for the detection of adulteration in ground meat and as a first-level filter for meat adulterated products in the food market.

Table 2. Indicative literature referred to methods used for traceability and fraud detection in meat products

Author(s)	Referred Methods	Further information	doi
Barai et al. (1992)	Electrophoretic techniques (PAGE, IEF); Immunological techniques (ELISA); other chemical based (acid phosphatase activity; pentoses and pentosans concentration; atty acid profile; amino acid composition).	Meat adulteration.	https://doi.org/10.1016/0924-2244(92)90133-H
Schwägele (2005)	Protein based methods (electrophoretic; isoelectric based; Western-blotting; ELISA); lipid-based methods (GC, GC-MS); DNA methods (PCR based; mtDNA), NMR, MS, infrared spectroscopy.	Traceability approaches in EU.	https://doi.org/10.1016/j.meatsci.2005.03.002
Ballin etal. (2009)	Protein based; DNA hybridization with DNA probes; PCR based; RFLPs; Fluorescence sensor capillary	Species determination as a tool for detection and	http://dx.doi.org/10.1016/j.meatsci.2009.06.00

	electrophoresis; RAPD; Real time PCR.	quantification of meat adulteration.	
Ballin (2010)	Methods based on the identification of meat origin, meat substitution, meat processing treatment and ingredient additions.	Approaches for authentication of meat and meat products.	http://dx.doi.org/10.1016/j.meatsci.2010.06.001
M. Sentandreu & E. Sentandreu (2014)	Chemometrics; Magnetic resonance; Histology and image analysis; Metabolomics; Electrophoretic and chromatographic methods; Immunoassays; DNA analysis (PCR-based); mass spectrometry.	Focused on authenticity of meat products.	http://dx.doi.org/10.1016/j.foodres.2014.03.030
Doosti et al. (2014)	PCR-RFLPs.	Case study application.	https://doi.org/10.1007%2Fs13197-011-0456-3
Houhoula et al (2017)	Gold nanoparticle (AuNP) method.	Nanoprobe sensor for horse adulteration .	https://doi.org/10.5539/jfr.v6n4p34
Laliotis et al. (2019)	DNA based techniques.	traceability for small ruminant species and products.	https://doi.org/10.5455/javar.2018.e274
Zhao et al. (2019)	RFID tag; DNA fingerprinting (SSR; SNPs); Stable isotope ratios fingerprinting; Mineral element fingerprinting; Organic component fingerprinting.	Animal identification and meat product traceability.	https://doi.org/10.1080/13102818.2019.1711185
Hassoun et al. (2020)	Vibrational Spectroscopy; Nuclear Magnetic Resonance; Fluorescence Spectroscopy; laser-induced breakdown spectroscopy, terahertz spectroscopy, and hyperspectral imaging; DNA-Based Techniques (PCR-RFLPs, Real-Time PCR, NGS); Protein-Based Techniques; Isotopic Technique; Elemental Technique.	Focused on spectroscopic detection methods.	https://doi.org/10.3390/foods10020448
Edwards et al. (2020)	Near-Infrared- and Mid-Infrared Spectroscopy; Fourier Transform Spectroscopy; Raman Spectroscopy; Colour Imaging; Hyperspectral Imaging; X-Ray Imaging and Computed Tomography.	Non-Destructive Spectroscopic and Imaging Techniques.	https://doi.org/10.3390/foods9081069
Qian et al. (2020)	Lot differentiation; batch association; isotope analysis; DNA tracking; block-chain approaches.	Traceability in food processing.	https://doi.org/10.1080/10408398.2020.1825925
Li et al (2020)	DNA technologies (mtDNA, PCR RFLPs, Real-time PCR, LAMP; Droplet-digital PCR; CytB, SNPs, NGS); protein-based technologies (ELISA, immunosensors, protein mass spectrometry); metabolite profile (GC-MS, UHPLC-MS); infrared spectroscopy; Raman spectroscopy; Hyperspectral imaging (IRS, RS); Laser-induced breakdown spectroscopy (LIBS)	Detection technologies for meat product adulteration.	https://doi.org/10.1111/1541-4337.12579
Kamruzzaman M (2021)	Hyperspectral Imaging	Use of spectral and image analysis.	https://doi.org/10.22175/mmb.12946
Kumar & Narsaiah (2021)	Lateral flow assay; dna based assays (PCR; LAMP; AuNPs; Cytb, CPA)	Point of Care assays and devices.	https://doi.org/10.1111/1541-4337.12674
Grundy et al (2023)	Mass spectrometry, Genomic technologies (DNA, miRNA), Electronic Spin Resonance; Total Reflection X-ray Fluorescence; atomic spectrometry	Offal adulteration in meta products.	https://doi.org/10.1016/j.foodchem.2022.133818

Acknowledgments

This work was supported by the European Commission within the framework of the Erasmus+ FOOD4S Programme (Erasmus Mundus Joint Master Degree in Food Systems Engineering, Technology and Business 619864-EPP-1-2020-1-BE-EPPKA1-JMD-MOB).

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

Open access

Copyrights

References

Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., Adam, T., & Humayun, Q. (2012). Nanobiosensor for the detection and quantification of pork adulteration in meatball formulation. *Journal of Experimental Nanoscience, 9*(2), 152-160. https://doi.org/10.1080/17458080.2011.640946

Ali, Md. Eaqub, Razzak, Md. A., Hamid, S. B., Rahman, Md. M., Amin, Md. A., & Rashid, N. R. (2015). Multiplex PCR assay for the detection of five meat species forbidden in Islamic Foods. *Food Chemistry, 177*, 214-224. https://doi.org/10.1016/j.foodchem.2014.12.098

Aranishi, F. (2005). PCR-RFLP analysis of nuclear nontranscribed spacer for mackerel species identification. *Journal of Agricultural and Food Chemistry, 53*(3), 508-511. https://doi.org/10.1021/jf0484881

Asensio, L., Gonz ález, I., Garc á, T., & Mart ín, R. (2008). Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). *Food Control, 19*(1), 1-8. https://doi.org/10.1016/j.foodcont.2007.02.010

Azad, M. A., Dey, M., Khanam, F., Biswas, B., & Akhter, S. (2023). Authentication of meat and meat products using molecular assays: A Review. *Journal of Agriculture and Food Research, 12*, 100586. https://doi.org/10.1016/j.jafr.2023.100586

Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P., & Casiraghi, M. (2010). DNA barcoding reveals fraudulent substitutions in shark seafood products: The Italian case of "Palombo" (Mustelus spp..). *Food Research International, 43*(1), 376-381. https://doi.org/10.1016/j.foodres.2009.10.009

Bartish, I., Rumpunen, K., & Nybom, H. (2000). Combined analyses of RAPDs, cpdna and morphology demonstrate spontaneous hybridization in the plant genus Chaenomeles. *Heredity, 85*(4), 383-392. https://doi.org/10.1046/j.1365-2540.2000.00773.x

Cai, Y., Zhang, L., Shen, F., Zhang, W., Hou, R., Yue, B., … Zhang, Z. (2011). DNA barcoding of 18 species of Bovidae. *Chinese Science Bulletin, 56*(2), 164-168. https://doi.org/10.1007/s11434-010-4302-1

Chase, M. W., Cowan, R. S., Hollingsworth, P. M., van den Berg, C., Madriñán, S., Petersen, G., … Wilkinson, M. (2007). A proposal for a standardised protocol to barcode all land plants. *TAXON, 56*(2), 295-299. https://doi.org/10.1002/tax.562004

Chaveerach, A., Tanomtong, A., Sudmoon, R., & Tanee, T. (2006). Genetic diversity among geographically separated populations of Nepenthes mirabilis. *Biologia, 61*(3), 295-298. https://doi.org/10.2478/s11756-006-0054-4

Chen, A., Wei, C., Chen, G., Zhao, Y., & Yang, S. (2014). Duplex PCR approach for the detection and quantification of Donkey, horse and mule in raw and heat-processed meat products. *International Journal of Food Science & Technology, 50*(3), 834-839. https://doi.org/10.1111/ijfs.12720

Chia, S., Teo, G., Tay, S. J., Loo, L. S. W., Wan, C., Sim, L. C., Yu, H., Walsh, I. & Pang, K. T. (2022). An Integrative Glycomic Approach for Quantitative Meat Species Profiling. *Foods, 11*, 1952. https://doi.org/10.3390/foods11131952

Cho, A.-R., Dong, H.-J., & Cho, S. (2014). Meat species identification using loop-mediated isothermal amplification assay targeting species-specific mitochondrial DNA. *Korean Journal for Food Science of Animal Resources, 34*(6), 799-807. https://doi.org/10.5851/kosfa.2014.34.6.799

Choi, J.-W., Chung, W.-H., Lee, K.-T., Cho, E.-S., Lee, S.-W., Choi, B.-H., … Kim, T.-H. (2015). Whole-genome resequencing analyses of five pig breeds, including Korean wild and native, and three European origin breeds. *DNA Research, 22*(4), 259-267. https://doi.org/10.1093/dnares/dsv011

Danezis, G. P., Tsagkaris, A. S., Camin, F., Brusic, V., & Georgiou, C. A. (2016). Food authentication: Techniques, trends & emerging approaches. *TrAC Trends in Analytical Chemistry, 85*, 123-132. https://doi.org/10.1016/j.trac.2016.02.026

Dirong, G., Nematbakhsh, S., Selamat, J., Chong, P. P., Idris, L. H., Nordin, N., … Abdull Razis, A. F. (2021). OMICS-based analytical approaches for assessing chicken species and breeds in food authentication. *Molecules, 26*(21), 6502. https://doi.org/10.3390/molecules26216502

Edea, Z., Dessie, T., Dadi, H., Do, K.-T., & Kim, K.-S. (2017). Genetic diversity and population structure of Ethiopian sheep populations revealed by high-density SNP markers. *Frontiers in Genetics, 8*. https://doi.org/10.3389/fgene.2017.00218

Filonzi, L., Chiesa, S., Vaghi, M., & Nonnis Marzano, F. (2010). Molecular barcoding reveals mislabelling of commercial fish products in Italy. *Food Research International, 43*(5), 1383-1388. https://doi.org/10.1016/j.foodres.2010.04.016

Grasso, A. N., Goldberg, V., Navajas, E. A., Iriarte, W., Gimeno, D., Aguilar, I., … Ciappesoni, G. (2014). Genomic variation and population structure detected by single nucleotide polymorphism arrays in Corriedale, Merino and Creole sheep. *Genetics and Molecular Biology, 37*(2), 389-395. https://doi.org/10.1590/s1415-47572014000300011

Guan, F., Jin, Y.-T., Zhao, J., Xu, A.-C., & Luo, Y.-Y. (2018). A PCR method that can be further developed into PCR-RFLP assay for eight animal species identification. *Journal of Analytical Methods in Chemistry, 2018*, 1-6. https://doi.org/10.1155/2018/5890140

Hargin, K. D. (1996). Authenticity issues in meat and meat products. *Meat Science, 43*, 277-289. https://doi.org/10.1016/0309-1740(96)00072-1

Harlina, P. W., Maritha, V., Musfiroh, I., Huda, S., Sukri, N., & Muchtaridi, M. (2022). Possibilities of liquid chromatography mass spectrometry (LC-ms)-based metabolomics and lipidomics in the authentication of Meat Products: A mini review. *Food Science of Animal Resources, 42*(5), 744-761. https://doi.org/10.5851/kosfa.2022.e37

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Houhoula, D. P., Kouzilou, M., Tzogias, C., Kyrana, V., Sflomos, C., Tsaknis, J., & Lougovois, V. P. (2017). Effectual Gold Nanoprobe sensor for screening horse adulteration in Meat Products. *Journal of Food Research, 6*(4), 34. https://doi.org/10.5539/jfr.v6n4p34

Jia, W., Di, C., & Shi, L. (2023). Applications of lipidomics in goat meat products: Biomarkers, structure, nutrition interface and future perspectives. *Journal of Proteomics, 270*, 104753. https://doi.org/10.1016/j.jprot.2022.104753

Karniol, B., Shirak, A., Baruch, E., Singrün, C., Tal, A., Cahana, A., … Seroussi, E. (2009). Development of a 25-plex SNP assay for traceability in Cattle. *Animal Genetics, 40*(3), 353-356. https://doi.org/10.1111/j.1365-2052.2008.01846.x

Kawęcka, A., Gurgul, A., & Miksza-Cybulska, A. (2016). The use of SNP microarrays for Biodiversity Studies of sheep - a review. *Annals of Animal Science, 16*(4), 975-987. https://doi.org/10.1515/aoas-2016-0017

Kim, H. C., Ko, Y.-J., & Jo, C. (2021). Potential of 2D QNMR spectroscopy for distinguishing chicken breeds based on the metabolic differences. *Food Chemistry, 342*, 128316. https://doi.org/10.1016/j.foodchem.2020.128316

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: The coding rbcl gene complements the non-coding trnh-psba spacer region. *PLoS ONE, 2*(6). https://doi.org/10.1371/journal.pone.0000508

Laliotis, G., Koutsouli, P., & Bizelis, I. (2018). Implementation of contemporary DNA based techniques on traceability process of small ruminant species and products. *Journal of Advanced Veterinary and Animal Research, 5*(3), 255. https://doi.org/10.5455/javar.2018.e274

Lasagna, E., Ceccobelli, S., Di Lorenzo, P., Albera, A., Filippini, F., Sarti, F. M., … Di Stasio, L. (2015). Comparison of four Italian beef cattle breeds by means of functional genes. *Italian Journal of Animal Science, 14*(1), 3465.

Leaché, A. D., & Oaks, J. R. (2017). The utility of single nucleotide polymorphism (SNP) data in Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics, 48*(1), 69-84. https://doi.org/10.1146/annurev-ecolsys-110316-022645

Li, Z., Bai, X., Peng, T., Yi, X., Luo, L., Yang, J., … Hu, D. (2020). New insights into the skin microbial communities and skin aging. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.565549

Lidiya, W. (2020). Nucleic acids probes in seafood authentication. *International Journal of Food and Fermentation Technology, 10*(2). https://doi.org/10.30954/2277-9396.02.2020.3

Magiati, M., Myridaki, V. M., Christopoulos, T. K., & Kalogianni, D. P. (2019). Lateral flow test for meat authentication with visual detection. *Food Chemistry, 274*, 803-807. https://doi.org/10.1016/j.foodchem.2018.09.063

Marmiroli, N., Peano, C., & Maestri, E. (2003). Advanced PCR techniques in identifying food components. *Food Authenticity and Traceability*, 3-33. https://doi.org/10.1533/9781855737181.1.3

Notomi, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research, 28*(12). https://doi.org/10.1093/nar/28.12.e63

OECD (2023). Meat consumption (indicator). *Agricultural Output*. https://doi.org/10.1787/fa290fd0-en

Orrù, L., Catillo, G., Napolitano, F., De Matteis, G., Scatà, M. C., Signorelli, F., & Moioli, B. (2009). Characterization of a snps panel for meat traceability in six cattle breeds. *Food Control, 20*(9), 856-860. https://doi.org/10.1016/j.foodcont.2008.10.015

Özbay Doğu, S. (2016). Determination tools of origin in the food traceability. *Journal of Food and Health Science*, 140-146. https://doi.org/10.3153/jfhs16015

Roy, S., Rahman, I. A., & Ahmed, M. U. (2016). Paper-based rapid detection of pork and chicken using lamp-magnetic bead aggregates. *Analytical Methods, 8*(11), 2391-2399. https://doi.org/10.1039/c6ay00274a

Santiago-Felipe, S., Tortajada-Genaro, L. A., Carrascosa, J., Puchades, R., & Maquieira, Á. (2016). Real-time loop-mediated isothermal DNA amplification in compact disc micro-reactors. *Biosensors and Bioelectronics, 79*, 300-306. https://doi.org/10.1016/j.bios.2015.12.045

Shi, Z., Yin, B., Li, Y., Zhou, G., Li, C., Xu, X., … Liu, L. (2019). N-glycan profile as a tool in qualitative and quantitative analysis of meat adulteration. *Journal of Agricultural and Food Chemistry, 67*(37), 10543-10551. https://doi.org/10.1021/acs.jafc.9b03756

Stachniuk, A., Sumara, A., Montowska, M., & Fornal, E. (2019). Liquid chromatography-mass spectrometry bottom-up proteomic methods in animal species analysis of processed meat for food authentication and the detection of adulterations. *Mass Spectrometry Reviews, 40*(1), 3-30. https://doi.org/10.1002/mas.21605

Subara, D., & Jaswir, I. (2018). Gold nanoparticles: Synthesis and application for halal authentication in meat and meat products. *International Journal on Advanced Science, Engineering and Information Technology, 8*(4-2), 1633. https://doi.org/10.18517/ijaseit.8.4-2.7055

Sudmoon, R., Tanee, T., Mokkamul, P., & Chaveerach, A. (2007). Species identification and sex determination of the genus nepenthes (nepenthaceae). *Pakistan Journal of Biological Sciences, 10*(4), 561-567. https://doi.org/10.3923/pjbs.2007.561.567

Suratno, Windarsih, A., Warmiko, H. D., Khasanah, Y., Indrianingsih, A. W., & Rohman, A. (2023).

Metabolomics and proteomics approach using LC-orbitrap HRMS for the detection of pork in tuna meat for halal authentication. *Food Analytical Methods, 16*(5), 867-877. https://doi.org/10.1007/s12161-023-02472-x

Tanner, N. A., & Evans, T. C. (2014). Loop-mediated isothermal amplification for detection of nucleic acids. *Current Protocols in Molecular Biology, 105*(1). https://doi.org/10.1002/0471142727.mb1514s105

Teletchea, F., Bernillon, J., Duffraisse, M., Laudet, V., & Hänni, C. (2008). Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples. *Journal of Applied Ecology, 45*(3), 967-975. https://doi.org/10.1111/j.1365-2664.2007.01415.x

Tomita, N., Mori, Y., Kanda, H., & Notomi, T. (2008). Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nature Protocols, 3*(5), 877-882. https://doi.org/10.1038/nprot.2008.57

Tortereau, F., Moreno, C. R., Tosser-Klopp, G., Servin, B., & Raoul, J. (2017). Development of a SNP panel dedicated to parentage assignment in French sheep populations. *BMC Genetics, 18*(1). https://doi.org/10.1186/s12863-017-0518-2

Verma, S., Karihaloo, J. L., Tiwari, S. K., Magotra, R., & Koul, A. K. (2006). Genetic diversity in Eremostachys Superba Royle ex Benth. (Lamiaceae), an endangered Himalayan species, as assessed by RAPD. *Genetic Resources and Crop Evolution, 54*(2), 221-229. https://doi.org/10.1007/s10722-006-9118-0

Vishnuraj, M. R., Devatkal, S., Vaithiyanathan, S., Uday Kumar, R., & Mendiratta, S. K. (2021). Development and validation of MIRNA based method for rapid identification of offal meats in processed chicken meat products. *Food Control, 121*, 107593. https://doi.org/10.1016/j.foodcont.2020.107593

Wang, J., Jun, S., Bittenbender, H. C., Gautz, L., & Li, Q. X. (2009). Fourier transform infrared spectroscopy for Kona Coffee Authentication. *Journal of Food Science, 74*(5). https://doi.org/10.1111/j.1750-3841.2009.01173.x

Wong, Y.-P., Othman, S., Lau, Y.-L., Radu, S., & Chee, H.-Y. (2018). Loop-mediated isothermal amplification (LAMP): A versatile technique for detection of micro-organisms. *Journal of Applied Microbiology, 124*(3), 626-643. https://doi.org/10.1111/jam.13647

Woolfe, M., & Primrose, S. (2004). Food forensics: Using DNA technology to combat misdescription and fraud. *Trends in Biotechnology, 22*(5), 222-226. https://doi.org/10.1016/j.tibtech.2004.03.010

Wu, S.-H., Hwang, C.-Y., Lin, T.-P., Chung, J.-D., Cheng, Y.-P., & Hwang, S.-Y. (2006). Contrasting phylogeographical patterns of two closely related species, Machilus thunbergii and Machilus Kusanoi (Lauraceae), in Taiwan. *Journal of Biogeography, 33*(5), 936-947. https://doi.org/10.1111/j.1365-2699.2006.01431.x

Xu, R., Wei, S., Zhou, G., Ren, J., Liu, Z., Tang, S., … Wu, X. (2018). Multiplex Taqman locked nucleic acid real-time PCR for the differential identification of various meat and meat products. *Meat Science, 137*, 41-46. https://doi.org/10.1016/j.meatsci.2017.11.003

Xu, Y., Xiang, W., Wang, Q., Cheng, N., Zhang, L., Huang, K., & Xu, W. (2017). A smart sealed nucleic acid biosensor based on endogenous reference gene detection to screen and identify mammals on site. *Scientific Reports, 7*(1). https://doi.org/10.1038/srep43453

Zeng, S., Yong, K.-T., Roy, I., Dinh, X.-Q., Yu, X., & Luan, F. (2011). A review on functionalized gold nanoparticles for Biosensing Applications. *Plasmonics, 6*(3), 491-506. https://doi.org/10.1007/s11468-011-9228-1

Zhang, J. Y., Yuan, Q. H., Meng, Y. Q., Li, X. L., Nan, Z. B., Wang, Y. R., & Zhang, W. S. (2007). A genetic diversity analysis of Wild Lespedeza populations based on morphological characters, allozyme and RAPD methods. *Plant Breeding, 126*(1), 89-94. https://doi.org/10.1111/j.1439-0523.2007.01311.x

Zhang, T., Chen, C., Xie, K., Wang, J., & Pan, Z. (2021). Current State of Metabolomics Research in meat quality analysis and authentication. *Foods, 10*(10), 2388. https://doi.org/10.3390/foods10102388