

Review article

DNA-based detection of pork content in food



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A B S T R A C T

Determination of halal food is essential in ensuring the tranquillity of consumers, especially Muslims. Halal products mean they are free from prohibited ingredients according to Islamic law. One ingredient that is prohibited is food products containing pork and its derivatives. An accurate verification method with a fast result is necessary to meet this requirement for halal food. DNA quantification of pork is now believed to be able to make accurate and quick decisions, as DNA acts as a reservoir or biological characterization of all living things, including pigs, according to specific characteristics of molecular and connection settings. Various DNA-based methods developed include PCR, biosensor and CRISPR methods. This review discussed various DNA-based Keywords: biosensor, CRISPR, detection, DNA, pork, PCR methods, including PCR, biosensor and CRISPR, to detect pork content in food. Among these methods, CRISPR is considered the easiest, fastest and most accurate. Therefore, it is important to develop this method further in the future. In this article, we provide a short review on DNA-based methods for detection of pork content in food products.

1. Introduction

Pigs or pork are livestock widely diffused worldwide because of their various advantages. Almost all parts of the pig's body can be used for multiple human needs, ranging from fur, skin, meat, bones, and offal to blood. One pig can produce more than 185 derivatives for food and non-food products. Pork skin, for example, can be a source of gelatin which can be applied to various food, drug and cosmetic products [1].

Advancements in food science and technology and the era of globalization have made the fast spread of people and materials. Meeting the supply of raw materials for the food industry not only relies on a local scale but it can also be completed from other countries in a relatively short time. This fact is certainly a serious concern for people aware of animal food sources, for religious reasons, health, vegetarianism and food counterfeiting [2,3].

Diverse technologies in food processing and increasingly complex cooking methods lead to difficulties in monitoring ingredients of animal origin, especially pork, in food products. Like most meats, pork is mainly composed of protein, accounting for 26% of the total net weight. In dry conditions, the protein content of minimal fat flesh even reaches 89%. Therefore, the detection of protein content compared to other contents in pigs is very well known, based on nucleic acids.

Several protein-based analytical techniques, such as dielectric properties [2], spectroscopy [3,4], chromatography [5], and immunoassays [6,7] are used to identify animal products present in foodstuffs. However, the limitation about these methods is when testing heat-processed materials due to dissolved protein denaturation during the treatment. In addition, immunoassays analyses that rely on antibodies raised against specific proteins, are often hampered by cross-reactions occurring between closely related species [8].

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The use of analytical methods based on nucleic acid can overcome this limitation due to the very stable nature of DNA and its long-lived biological molecule found in all organisms tissues [9].

The rapid development of molecular biology technology in recent years has made DNA-based approaches increasingly superior in detection, specificity, and good stability [7,10]. This review discussed the various DNA-based methods capable of detecting pork present in food as well as the strengths and drawbacks of those methods. This overview can be used as a reference for developing new methods for detecting pork in foods by researchers and scientists.

2. DNA-based pork content detection method

2.1. Polymerase chain reaction (PCR) method

PCR is the method most commonly used to detect the presence of species-specific materials in food products due to its high sensitivity and specificity. This method becomes a useful technique for animal-derived product detection in foods. Several PCR-based methods have been proposed to identify species-specific materials in food, including conventional PCR (singleplex or multiplex), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP) and real-time PCR/qPCR.

2.1.1. Singleplex-multiplex PCR

Singleplex and multiplex PCR is a molecular biology technique, an adaptation of the PCR technique that allows the simultaneous amplification of various gene sequences. Singleplex PCR is used to detect single target sequences and does not require a special probe. Therefore, singleplex PCR is inherently simpler in its design, implementation, and optimization. This can be quick and easy to do with minimal requirements for optimization. Several studies used species-specific markers against Dloop mt DNA for the pork identification; thus, varying the length of the amplicon from 712 base pairs [11–13]. The PCR identification system based on genetic similarity also found a high rate of labeling error among meat products [14].

In multiplex assays, more than one target sequence can be amplified by using multiple primer pairs in the reaction mixture. The multiplex PCR method can amplify two or more target sequences (multi-target) simultaneously with one PCR reaction to save on tools, costs, processing time, and reagents. Multiplex PCR is widely used for various purposes, the most common [15] is for detecting pork content in food samples.

Since its initiation in 1988, multiplex PCR has been successfully exploited in numerous areas of DNA testing, with two or more DNA loci simultaneously enhanced in the same reaction [16]. The multiple and unique primer sets utilization in a mixture of single PCR enables the fragments production of different sizes that are specific for different sequences of DNA. Further information can be obtained by setting a target of multiple genes simultaneously via a single trial which requires the use of reagents which take a lot of time to complete [17].

For the simultaneous and fast identification of meat species, at least to detect contamination in complex meat products, it is required to develop multiplex PCR [17]. Akar and Abasiyanik [17] applied PCR assay to identify the most processed and raw meat species used in foodstuffs, such as ingredients derived from ruminants, poultry, fish, and pork. Some researchers proposed an accurate analytical method for adulteration detection of meat using conventional multiplex PCR analysis of the cytochrome *b* gene of animal mtDNA [11,18,19]. The method is applied to identify and track meat adulteration and distinguish the species present in meat mixtures. The method can also be used for quality control and monitoring in laboratories, industrial experimental verification of meat products, such as halal authentication and origin of certification raw materials. In addition, for accurate identification, there was research investigating the method of a multiplex PCR assay for determining five types of meat species, such as cat, dog, pork, monkey, and rat prohibited in the Islamic diet in a single test platform [20]. Research on capsule shells to determine the content of pork gelatin was also carried out by Shabani et al., [21]. Direct detection of food suspected of containing pork has also been successfully carried out with this PCR technique [22–25]. The presence of dietary supplements can also be found [26]. Detection of bread for the presence of porcine DNA can also be carried out by PCR techniques [27].

Multiplex PCR exhibits some advantages in detecting the presence of porcine DNA since the use of unique primer and multiple sets in mixture of a single PCR allows the production of fragments of different sizes that are specific for various DNA sequences, identify processed and raw meat species, accurate analytical techniques to detect adulteration of meat, and can determine pig DNA in dietary supplements and bread. However, there are still drawbacks to using Multiplex PCR in porcine DNA detection, including targeting multiple genes at once through a single trial that requires the use of reagents that is time-consuming. In addition, when testing cross-species specificity using the system of optimized multiplex PCR, only specific targets are amplified [10,14–19,21–23,28,29].

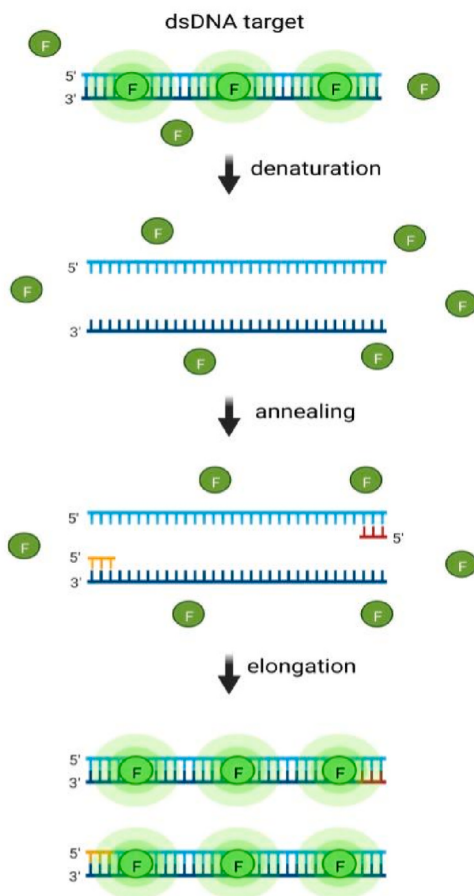
2.1.2. Random amplified polymorphic DNA (PCR-RAPD) dan restriction fragment length polymorphism (PCR-RFLP)

Polymerase Chain Reaction (PCR) is an in vitro technique that amplifies DNA, producing several million copies of a specific DNA segment from a small amount of a precursor material [28]. The specificity depends on the hybridization of the primer sequence, and the sensitivity depends on the amplification based on the polymerase enzyme. PCR usually comprises a series of temperature cycles repeated 20 to 40 times. Each cycle includes denaturation of the DNA duplex, hybridization of two DNA oligonucleotides (primary) flanking the target sequence and lengthening of the primers by DNA Random Amplified Polymorphic DNA (PCR-RAPD) and Restriction Fragment Length Polymorphism (PCR-RFLP).

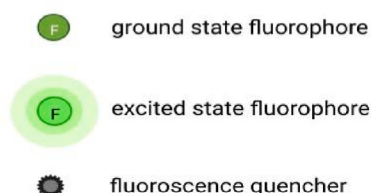
The development of molecular biology methods to obtain a genetic variation based on certain characteristics is growing so fast. Although many methods are new, in some cases, RAPD and RFLP are currently used in several molecular analyses and are still in great demand. RAPD is a method when the amplification is carried out randomly using a single primer; the results of the DNA pieces from the amplification process also run randomly according to the match of the nucleotide bases with the DNA template. The primers used are usually around 9–12 bp only. Existing DNA templates will be duplicated to produce millions of copies, and band analysis results are observed ranging from 100 bp - 3000 bp. The bands that appear also show different thicknesses [15].

RAPD has been used successfully for meat species identification [30–34]. The RAPD technique's underlying mechanism is based on DNA fragments amplification using short oligonucleotide primers, which are attached to multiple locations on genomic DNA. Sequentially, the amplified fragments size-based separation using gel electrophoresis occurs. The sample identification is performed via comparison of the DNA bands on the gel. In such a way, DNA fragments amplification uses arbitrary primers in various species; thus, obvious and distinct patterns with a high degree of polymorphism are identifiable according to different species [11]. Arslan et al. (2005) successfully identified the meat based on their origins, such as goat, beef, pig, wild boar, lamb, camel, pig, cat, rabbit, horse,

Fluorescence dye-based RT-PCR



Legend



DNA probe-based RT-PCR

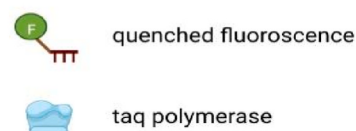
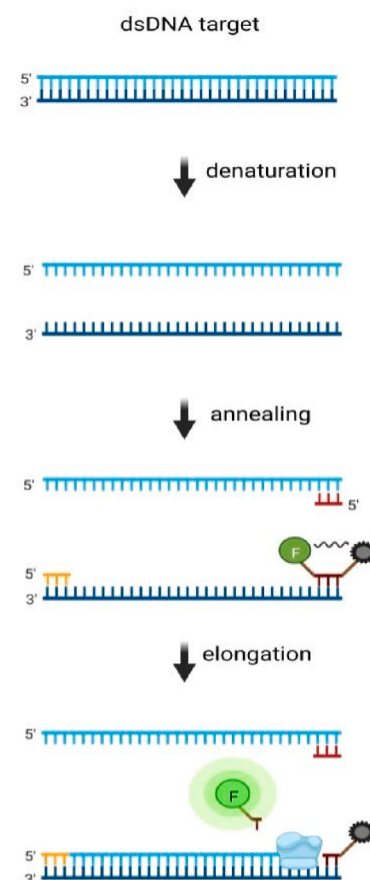


Fig. 1. PCR-RT technique for DNA detection between fluorescent and DNA probe.

donkey, dog, and bear by the deployment of the RAPD technique.

The RFLP method is a DNA marker whose detection is based on DNA-DNA hybridization. Several studies targeting PCR-RFLP have employed mt 12 S, 18 S, and 26 S rRNA genes for characterizing and tracing the meat origin for halal products authentication [35–38] used RFLP PCR for pork adulteration detection in raw meat via targeting the mitochondrial cytochrome *b* gene [38]. Murugaiah et al. (2009) employed this technique via mitochondrial genes (cytochrome *b*) analysis of beef (*Bostaurus*), buffalo (*Bubalus bubali*), chicken (*Gallus*), pork (*Sus scrofa*), quail (*Coturnixcoturnix*), goat (*Capra hircus*), and rabbit (*Orytolagus cuniculus*) for the identification and tracing of the adulteration origin in halal and non-halal mixed meat samples. A PCR-RFLP with a short nucleotide amplicon (109 bp) used to trace and identify porcine DNA in halal meat products has been developed [33]. Research conducted by Erwanto et al. (2012, 2014) developed an mtDNA-based PCR-RFLP method using the restriction enzyme *BseDI* to differentiate meat species. It determined the pork presence in meatballs, which could detect a 0.1% level of pork. Using the same technique, Haider et al. (2012) used the COI gene (mitochondrial cytochrome *c* oxidase subunit 1) to qualitatively identify and distinguish beef, buffalo, lamb, camel, turkey, and chicken “as halal meat”, and donkey and pork “as non-halal meat”. The advantage of using Random Amplified Polymorphic DNA (PCR-RAPD) and Restriction Fragment Length Polymorphism (PCR-RFLP) in the detection of pork DNA is that it can distinguish several types of meat circulating in the community, especially pork from a specific DNA identity, able to distinguish the presence of pork in meatballs. Meanwhile, the drawback of using PCR-RAPD and PCR-RFLP in detecting porcine DNA is that it takes a long time for two important analytical steps, such as PCR processing and cutting the PCR result DNA with restriction enzymes [26,27,30–35,37,41,42].

2.1.3. Real-time polymerase chain reaction (RT-PCR)

The DNA template is exponentially replicated throughout the PCR procedure. This results in a quantifiable link between the quantity of PCR product (amplicons) accumulated in a particular cycle and the initial concentration of the DNA template used. The number of amplicons counted during the exponential phase serves as the standard for estimating DNA template duplication. The PCR reaction operates at its best during the exponential phase, and this reaction continues until it is blocked by many conditions, including low template and reagent concentrations and an accumulation of pyrophosphate molecules. The PCR reaction enters the plateau phase as a result of this barrier. As a result, it is impossible to utilize the calculation of PCR products during the plateau phase as a reliable reference. An essential advancement in the application of PCR [41] is depicted in Fig. 1.

Numerous researchers have employed real-time PCR for meat authentication and specification because of its sensitivity, specificity, and speed. One of the most critical developments in molecular approaches is real-time PCR, particularly in identifying the source of adulteration in halal meat. Initially, this method was created to evaluate gene expression, recognise microbes, and measure genetically modified organisms [43]. It has also recently been widely applied to the identification of meat types [9,42,44]. The fundamental idea behind this method is the real-time measurement and quantification of the rise in fluorescence light intensity for each cycle of amplification throughout the process. Additionally, even in the exponential phase, the reaction components of this method are never limited [45].

Most often, pork substitutes other types of meat in food products. Ali et al. (2012) established a pork-specific real-time PCR assay for authentication of halal products with the detection limit of 0.001 ng of porcine DNA. In this context, this technique will be useful in detecting pork DNA in meat products. In addition, Edris et al. (2012) employed a similar approach for the halal and non-halal authenticity of meat products by aiming at the partial length cytochrome *b* gene from mtDNA. Targeting the same gene, some researchers combined species-specific primers and a TaqMan probe for specific amplification and detection of porcine mtDNA by real-time PCR to investigate pork adulteration in meatballs [46–48]. This method provides efficient detection to identify and quantitatively identify the pork presence in various processed meat products. Another study also compared the SYBR Green method and the Hydrolysis Probe method in the analysis of bovine gelatin DNA and pork gelatin DNA using Real-Time PCR [49–53]. Detection of contaminants from DNA fragments encoding pig *cyt b* in softgelly candy samples not labeled halal can also be carried out [54]. Another technique used to authenticate raw, processed and adulterated pork is done by Demirhan et al. (2012). The following research is to detect processed food samples that are positive for pig DNA [52,55]. In addition to food, detection can also be carried out on animal feed which is feared to affect the meat consumed [48].

The advantage of using RT-PCR in pork DNA detection is that it is an efficient method for pork detection, identification, and quantification in different types of products derived from meats. RT-PCR also combines species-specific primers and TaqMan probes for specific amplification. In addition, the detection of contaminants from DNA fragments encoding *cyt b* pigs in jelly candy samples not labeled halal can also be done. Detection can also be carried out on animal feed which is feared to affect the meat consumed. The disadvantage of using real-time polymerase chain reaction (RT-PCR) in detecting porcine DNA is that in the exponential phase the PCR reaction takes place optimally; this reaction continues until the PCR reaction is inhibited by several factors such as reduced levels of templates, reagents, and accumulation of pyrophosphate molecules. Therefore, the use of PCR is an introduction to the monitoring of DNA amplification in real time, requiring other supporting tools for maximum performance, for example, fluorescence techniques [6, 40,43–50,53,56–59].

2.2. DNA barcoding method

The molecular approach through DNA Barcoding technology is a fast and appropriate alternative choice to identify plant and animal species that have or have not been described. DNA barcode generally consists of a central variable moiety that distinguishes species of interest and specific regions and allows for loading of long primer pairs amplified by barcode sequences in different species which for most animal species is the ~650 bp region of the mitochondrial COI gene [60].

The application of DNA barcoding in pork detection has been applied, including the determination of uncooked pork sausage and

chorizo products and then identified by mini-barcoding [61]; minor DNA components are also reported to be strong enough to detect minor components up to 1% of the total DNA [62]. Another study carried out simulations of DNA sequence and sequencing data on sausages (35% cattle, 1% horses, 9% pigs, and 55% sheep), resulting in species quantification that could be achieved at a discrimination level of 1% [63]. The method of DNA metabarcoding was developed on the MiSeq® platform that targets the 16 S rDNA region, and it was deployed to analyze DNA extracts from muscle meat and DNA mixtures from sausages [64,65]. Sequencing of the targeted mt-DNA region using the DNA barcode method yielded a total of 1,363,351 sequence reads. They identified pork adulteration in the kebab that is normally not present [66].

The advantage of using the DNA barcoding method is the capability of detecting multiple DNA sources in one analysis; the DNA barcodes combination with NGS, allowing multiplexing; so that it is stronger than traditional DNA barcodes. By employing short oligonucleotide sequences for DNA indexation, different sample amplicons can be in the simultaneous collection and sequence in a single loop [60]. Disadvantages of using the DNA Barcoding method are not being able to identify many species in the same product, and the requirement for high-quality DNA extracts from the meat matrix to enable genomic fragments amplification and sequencing of at least 500 base pairs. This method also requires a long analytical time span from DNA extraction to analyzing final bioinformatics, requires skilled personnel and specialized laboratories (Demirhan et al., 2012; Al-Kahtani et al., 2017; Zia et al., 2020; Kane & Hellberg, 2016; Tillmar et al., 2013; Ripp et al., 2014).

2.3. Loop-mediated isothermal amplification (LAMP)

LAMP is a specific, sensitive, and fast amplification method that can be utilized to identify species. This method uses four to six primers and specific DNA polymerases capable of inducing DNA through automatic cyclical strand switching in a relatively short time. The reaction takes place under isothermal conditions at a temperature of 63 °C. The LAMP method can amplify up to 109 copies in an hour because multiple primers are used to recognise different DNA target sequences.

A recent research review focused on the LAMP application in species identification of a variety of meat products [68]. The primer sets of *Bos taurus*, *Sus scrofa* domesticus and *Equus caballus* (BSE)-LAP to target species-specific mt-DNA to differentiate raw and cooked meat species have also been investigated [69]. The four pig-specific primers in the LAMP assay were designed according to the mt-ND1 gene sequence to clearly show pig DNA in meat products [70]. Another study using a special D-loop-based pig primer designed for the LAMP assay detected 1 pg of raw pork DNA and 0.1% pork in a beef mixture within 30 min [71]. A new method based on LAMP in combination with LFD was developed for mammalian DNA, offering a detection limit of 10 pg isolated porcine DNA and 0.01% DNA in additives [72]. Similarly, a LAMP and DNA strips combination was developed to detect a mixture of 0.01% pork in beef meatballs in about 1 h [73]. In a recent study using LAMP, researchers used the Alkaline Lysis method to extract porcine DNA before amplifying the mt-D loop, and heated meat samples at 121 °C for 30 min. The results showed that pork in beef could be detected up to a mixed level of 0.1%, and the DNA detection limit was at 0.5 ng/μL [74].

The advantage of using the LAMP method in porcine DNA detection is its high amplification efficiency and without requiring skilled personnel and sophisticated equipment. Meanwhile, the disadvantage of using the LAMP method to detect porcine DNA is due to the lack of sensitivity compared with PCR to inhibition of complex sample cases such as blood, possibly due to the use of Bst DNA polymerase from Taq polymerase as in PCR. In addition, the multiplexing approach for LAMP is less developed than for PCR. The greater number of primers per target in LAMP leads to an increase in the primer-primer interaction [60,63,65,68,70,71].

2.4. Biosensor method

The basic components of a common biosensor are shown in Fig. 2 wherein, biomolecules can be enzymes, DNA, proteins, whole cells, antibodies etc [75]. The sensor platform, where chemical reactions between the analyte and biomolecules occur, is the transducer surface [76]. The transducer converts one type of energy into another such as chemical energy into electrical signals. Furthermore, the electronic circuit processes the signal, to obtain the signal in a useable form [77,78].

2.4.1. Optical biosensor method

In optical sensors, direct measurements are made through a process of color recognition or cause a change in optical properties (for instance, an antibody-antigen complex formation), an optical signal (fluorescence, chemiluminescence, color), and environment. The resulting optical signal can be observed directly or measured with a photodetector, which can convert optical signals into measured electrical signals. The signals are classified based on thermal (i.e., thermopiles) or photon detectors (i.e., photodiodes or photo-multipliers) [79,80].

There is an increased use of optical methods for single-use sensors, particularly in incorporation with smartphones. In addition, optical detection principles such as surface plasmon resonance (SPR) [81] or localized SPR [82,82], and surface-enhanced Raman spectroscopy (SERS) [83] also provide the label of free from chemical and biological methods sensing [84].

SPR is a sensing technique in which the incident light stimulates the resonant oscillation of electrons at the inter-face between positive and negative material [85]. Wavelength-dependent SERS sensing was highlighted in the current research with the morphology of the developed sensor [86]. The detection involved visual (fluorescence) observation of the colloidal solution color shift due to the

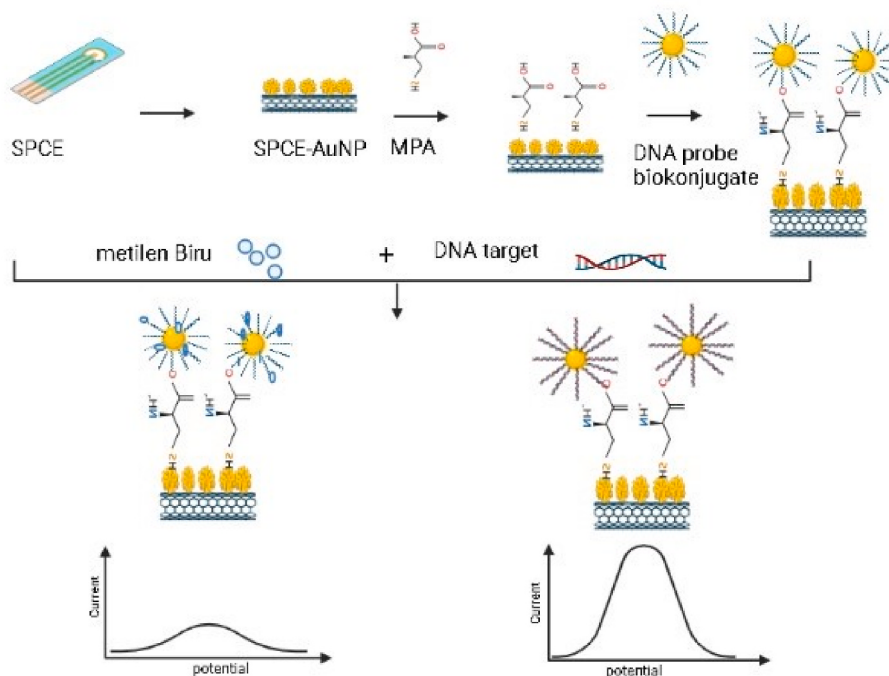


Fig. 2. Schematic of DNA biosensor based on AuNP-DNA probe bioconjugate on SPCE-Gold surface

salt-induced aggregation of nanoparticles, which could be prevented by the presence of single-stranded DNA (the probe only) [87]. The most essential functional materials for optical sensing are dyes, silver, gold, graphene nanoparticles, quantum dots, and photonic crystals.

Several optical method studies have been carried out to detect the quality of meat containing pork. The optical thin film microarray method on silicon-based surfaces to identify venison, rabbit, duck, chicken, beef, horse, lamb and pork, resulting in optical biosensor chips, has also been developed [88]. The study targeting the porcine mitochondrial genome was designed by Emanuel et al. with a chemiluminescent fiber optic biosensor [89]. Covalent integration of pig probes into gold nanoparticles successfully detected and quantified the presence of porcine DNA [36,90,91]. Sensitive and specific paper-based methods have also been developed to detect DNA from chickens and pigs using magnetic beads (MB) [92]. They could incorporate the loop-mediated isothermal amplification (LAMP) with ECL technique to detect and quantify porcine DNA. Developed surface enhanced Raman spectroscopy (SERS) using silver nanopartikel (AgNPs) on Si matrix to identify the pork DNA in a rapid way and sensitive to ultra-low molarity detection [86].

The optical biosensor method allows for the detection and quantification of potential targets in samples that have undergone considerable degradation or high levels of processing, making them suitable for direct detection. The optical biosensor approach can be used as a portable biosensor for on-site monitoring because it is an isothermal method that does not require a thermal cycle process. The optical biosensor approach for detecting pig DNA has some drawbacks, including the need for a lengthy autoclave process and inaccurate results with 100% pork when used in large amounts, reflecting saturation. With non-target flesh, specific primers only generate short DNA strands; in the stain test, these short strands do not aggregate to form dark blotches on the paper. There will be less ECL signal strength than the original due to the presence of DNA [84,89,90,93–95].

2.4.2. Electrochemical biosensor method

In the electrochemical sensing-based method, the indirect of direct generation of electric signal, with electroactive species, or via biorecognition events or enzyme-mediated electrodes [96] that are proportional to its concentration. One of the most critical factors when applying electrochemical sensors is material selection for the electrodes since they have to be suitable for the specific application (e.g., chemical resistance to the sample) and conform to specifications, such as selectivity, sensitivity, or long-term stability. Common electrode materials used for the manufacture of disposable sensors include inert metals 1,806,739 [97]. The most vital electrochemical techniques include amperometry, potentiometry, voltammetry, impedance spectroscopy and conductometry [97,98]. While electrochemical chemo-chemistry and biosensors primarily require high conductivity liquid electrolytes containing ions, solid electrolytes, such as yttria stabilized zirconia, can be used in gas (potentiometric) sensors.

Potentiometry determines the change in potential of an open circuit between two electrodes, i.e., working and reference electrodes at equilibrium without current flow, caused by the analyte that depends on the concentration. However, amperometry and voltammetry typically use a third or additional electrode in a potentiostatic system to set the desired voltage at the sensing (working electrode) that does not depend on the drop of voltage within the solution. In amperometry, current that arises from the reduction or oxidation process of electroactive molecules, is determined at a constant or single potential amperometry; or time gradual potential (chronoamperometry). Voltammetric metrics involve the measurement of the current when a possible sweep occurs. It can be linear, cyclic, or combined with pulses, for instance, differential pulse or square wave voltammetry. When the impedance spectroscopy technique is used, a sinusoidal potential over a frequency range is applied to an electrochemical cell. By applying the current response measurement, the system capacitance and resistance can be measured, enabling the investigation of the properties of surface and material. In conductometry, the electrolyte resistance is measured using an alternating potential [99]. A novel electrochemical biosensor strategy has been used to detect isothermal amplicons of pork species as a model system, in which picogram levels of starting materials were used as template DNA. ELISA/immunosensor was developed for sensitive detection of adulteration of pork in meat [6]. In Fig. 2, an electrochemical DNA biosensor based on the AuNP–DNA probe bioconjugate was able to differentiate DNA samples from pork, chicken, and beef [100,101]. Another study suggested that the formed short-length DNA-linked dual platform SERS biosensor were able to substitute the traditional method of porcine DNA detection that was the less sensitive. It could be adopted as an approach for the universal quantitative and qualitative DNA detection from various sources [101].

With samples containing 10% pork, the electrochemical biosensor approach for identifying pork DNA can be used as an alternative to differentiate between raw meat and processed meat. Based on isothermal target gene amplification and electrochemical detection using square wave voltammetry, the electrochemical biosensor approach could distinguish between different meat species. There is no process necessary for ferrocene labeling, which is the chemical bonding of ferrocene molecules to DNA. This technique uses a low-cost dielectric analyzer to discriminate between complementary and non-complementary target DNA detection. The performance of DNA biosensors depends on the DNA probe's length, the platform's makeup, the presence and placement of Raman tags, and the sensing strategy chosen, which is a drawback of electrochemical biosensor methods used to detect porcine DNA. Additionally, mixing or interference with both specific and generic DNA should be impossible. The probe DNA, AuNP, and SiNS from the electrode all degrade in this approach. The biosensor response and the amount of immobilized probe DNA are both decreased by the degrading effect. Additionally, the device's resistance was reduced as a result of complementary target hybridization [3,57,58,101].

2.4.3. CRISPR method (clustered regularly interspaced short palindromic repeats)

CRISPR is a method of genome editing that edits internal DNA/RNA in a reprogrammable and sequence-specific way; In a process known as gene editing, the CRISPR-associated Cas endonuclease protein has been employed in a variety of ways. It has been successfully utilized in bioenergy, medicines for infectious diseases, and agriculture [102].

CRISPR-Cas12 is the newest method for detection. This detection platform has been applied to detect pig DNA combined with the fluorescence technique. This technique enables the detection of target genes that are amplification-free and mix-to-read, thereby enabling fast detection of porcine DNA in complex samples and presenting excellent selectivity in authenticating halal food to other processed meat products. All detection processes can be achieved in one test tube at 37 °C.

The regularly clustered short palindromic repeat system (CRISPR) is recognized for its strong function in gene editing. In recent years, the highly specific recognition capability of sequence of the CRISPR system has been proven to be a quick and highly sensitive detection tool for target nucleic acid analysis [103,104]. In particular, the proteins associated with CRISPR type II (such as Cas12a, Cas13a) are revealed with the activity of indiscriminate cleavage in single-stranded DNA (ssDNA) that can rapidly magnify the detection signal and significantly improve the detection [105,106]. Incorporating the technology of isothermal amplification has proven that nucleic acid detection has some advantages in a multitude of specificities, ensuring the detection results accuracy, and making more convenient and faster detection [107,108]. A previous study has demonstrated the method possible application in meat authentication [109]. However, it did not provide a complete detection process in detail, nor its verification and application in the altered foods identification.

The rapid and particular visual RPA-CRISPR/Cas12a assay created in this study to identify ingredients derived from pork in meat products is particularly appropriate for first-line promotion and application of analysis of low-resource meat adulteration, particularly for detection on-site [110]. In food detection, it has been reported that a nucleic acid analysis strategy based on amplification-free CRISPR-Cas12 and readable mixtures has been reported, which allows rapid identification and analysis of pork components [111], as shown in Fig. 3.



Fig. 3. The working principle illustration of halal food authentication via CRISPR-Cas12-based nucleic acid test.

The advantage of the CRISPR method in detecting swine DNA is that it can test processed meat products such as sausages and dried pork slices. A CRISPR-Cas12-based nucleic acid assay strategy holds promise for fast food authentication. Combined with a simple biosensor method to obtain DNA samples for fast and accurate identification of pork-derived components in meat products within 1 h. Incorporated with a simple biosensor method to obtain DNA samples, pork-derived components in meat products can be identified quickly and accurately within an hour. The method can be incorporated with the CTAB technique to derive high purity DNA samples to satisfy the meat products detection requirements with lower LOD. The CRISPR/Cas12a can be performed in a single tube under 37 °C, and the results can be easily assessed directly under a portable mini blue light transilluminator. The CRISPR method is associated with disadvantages in the detection of porcine DNA with limitations by off-target effects, and chromosomal translocations due to off-target cleavage. These problems persist and are complicated when editing complex genomes [109–112].

3. Conclusions and future prospects

Large laboratories can test the majority of food products. However, at least four drawbacks exist about utilization of centralized quality control, i.e., i) because there are so many different analytes, even a centralized laboratory might only focus on one or two pollutants. Each sample must therefore be sent simultaneously to various laboratories. ii) In order to prevent potential change, samples must be sent under extremely controlled circumstances, which may call for low temperatures. Costs for shipment are consequently increased. iii) Transportation delays can lower food quality by delaying distribution to customers or retailers. iv) Consumers pay higher prices for food due to centralized testing and the associated costs. For this reason, it would be more effective if food testing is conducted where they are required (homes, packaging centers, manufacturing premises, etc.) using single-use sensors [99]. Likewise, food containing pork should be detected easily, quickly and precisely. DNA-based detection has answered this challenge where the introduction of heat-stable DNA and its presence in all cells of the target organism has made DNA-based methods widely used as a reference for researchers. The types of DNA studied were quite diverse, but the mitochondrial gene was most widely used for detecting pork content (cytochrome *b*). This is because the cytochrome *b* gene has been regarded as one of the most meaningful genes for works related to phylogenetic and is the most well-known mitochondrial gene regarding the function and structure of its protein products [113]. In addition, the mitochondrial Cyt *b* gene was selected as the target gene that can be replicated multiple times within the cell. A great amount of Cyt *b* gene DNA can be derived regardless of severely damaged DNA under harsh processing conditions [114].

Each method (PCR, DNA Barcode, LAMP, Biosensor and CRISPR) has advantages and disadvantages (Table 1) that can be chosen according to the conditions of the researcher, both in terms of the ability to use tools and the availability of existing materials. The most important thing is how the detection can provide the right results and can be done directly on the spot to make it easier to make consumption safety decisions regarding religion and dietary habits (see Table 2).

Table 1
Advantages and disadvantages of DNA-based pig detection method.

DNA-based detection	Advantages	Disadvantages	Reference
PCR Method Singleplex- Multiplex	PCR mixture allows the production of fragments of different sizes that are specific for various DNA sequences, identify processed and raw meat species, accurate analytical techniques for detecting adulteration of meat, and can determine pork DNA in dietary supplements and breads. .	Targeting multiple genes at once through a single trial requires the use of reagents that take a lot of time to do and when cross-species specificity is tested using the multiplex PCR system developed only the specific targets are amplified.	[10,14–23,28, 29]
RAPD and PC- RFLP	To distinguish several types of meat circulating in the community, especially pork from a specific DNA identity, able to distinguish the presence of pork in meatballs.	It takes a long time for two important analytical steps, such as PCR processing and cutting the PCR result DNA with restriction enzymes	[26,27,30–35, 37,41,42]
<i>Real Time</i>	RT-PCR also combines species-specific primers and TaqMan probes for specific amplification. In addition, the detection of contaminants from DNA fragments encoding cyt <i>b</i> pigs in jelly candy samples not labeled halal can also be done. Detection can also be carried out on animal feed which is feared to affect the meat consumed.	In the exponential phase the PCR reaction takes place optimally, this reaction continues until the PCR reaction is inhibited by several factors such as reduced levels of templates, reagents, and accumulation of pyrophosphate molecules. DNA amplification in real time, requiring other supporting tools for maximum performance, for example by using fluorescence techniques.	[6,40,43–50, 53,56–59]
DNA Barcoding	Flexible use, high sensitivity and selectivity with one-step DNA sequencing and quantification	It is necessary to use high quality DNA with a long period of bioinformatics analysis, complex library preparation protocols, the number of extraneous DNA traces and in computer programs are often biased. Requires specialized laboratories and skilled personnel.	[55,60–63, 67]

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Table 1 (continued)

DNA-based detection	Advantages	Disadvantages	Reference
LAMP Method	Fast detection, high selectivity and efficiency. No thermal cycle required.	Sometimes the method is considered to be less versatile and less sensitive to inhibitors with little or no multiplexing plus less availability of reagents. There is a large number of primers per target will increase the interaction.	[63,65,68,70,71]
Biosensor Method Optic	This method offers detection and quantitation of potential targets in highly processed meat products or extensively degraded samples where PCR-based identification techniques may not work making them suitable for direct detection. The optical biosensor method is an isothermal method that does not require a thermal cycle process, this method can be implemented in a portable biosensor format for on-site monitoring.	Requires a relatively long autoclave process, inaccuracies were also observed in 100% pork when used in large quantities, reflecting saturation. Specific primers that produce only short DNA strands with non-target flesh, and these short strands will not produce aggregates (dark spots) on paper in the stain test. The presence of DNA will indeed result in a lower ECL signal intensity than the original	[84,89,90,93–95]
Electrochemistry	Can be distinguish between raw meat and processed meat, namely in samples with 10% pork content. The electrochemical biosensor method was able to identify different meat species based on isothermal amplification of the target gene followed by electrochemical detection with square wave voltammetry. It does not require any procedure for ferrocene labeling, namely the chemical attachment of ferrocene compounds to DNA. This method is capable of distinguishing complementary and non-complementary target DNA detection using a low-cost dielectric analyzer	The performance of the DNA biosensor depends on the length of the DNA probe, the composition of the platform, the presence and position of the Raman tag, and the sensing strategy chosen. There should also be no possibility of mixing or interfering with specific and nonspecific DNA. In this method, there is also degradation of the probe DNA, AuNP and SiNS from the electrode. The degradation effect reduces the amount of immobilized probe DNA and reduces the biosensor response. In addition, complementary target hybridization resulted in a decrease in the resistivity of the device.	[3,57,58,101]
CRISPR Method	Can test processed meat products such as sausages and dried pork slices. The CRISPR-Cas12-based nucleic acid assay strategy holds promise for fast food authentication. Combined with a simple biosensor method to obtain DNA samples for identification of pork-derived components in meat products quickly and accurately within an hour. The technique can be run with the CTAB method with lower LOD. CRISPR/Cas12a can be performed in a single tube under 37 °C, and the results can be easily assessed directly under a portable mini blue light transilluminator.	Limited by off-target effects and chromosomal translocations due to off-target cleavage and these problems persist and are complex when editing complex genomes	[109–112]

Table 2

Variety of DNA-based pork content detection.

Method	Type	Specialization	Analytical data	Reference
PCR	RAPD and RFLP	using mt 12 S, 18 S, and 26 S rRNA genes to detect and trace the meat origin of hala products detect pork adulteration in raw meat short ifragment (109 bp) pork cytochrome <i>b</i> gene	PCR products of pork and lard were digested with RE <i>Bsa</i> JI, which resulted in the expected fragments of 131 and 228 bp This assay is sensitive enough to detect 0.0001 ng of pork DNA in pure format and 0.01% (w/w) pork	[31,32] [33]
		Gene of mitochondrial (cytochrome <i>b</i>) beef (<i>Bostaurus</i>), buffalo (<i>Bubalusbubali</i>), goat (<i>Carprahicus</i>), chicken (<i>Gallus</i>), rabbit, quail (<i>Cotumixcoturnix</i>), pork (<i>Sus scrofa</i>) trace and determine 25-nucleotide (nt) single-stranded (ss) DNA annealing probes with denatured DNA from meatballs	Pork produced better banding intensity when present in 1%, 3% or 5% in the mix mixture, compared to the other meat species tested. Twenty nm GNP changed color from pink-red to purple-gray, and its absorption peak at 525 nm was redshifted by 30–50 nm in 3 mM phosphate buffer saline (PBS). can detect 0.1% pork content.	[30] [34] [35]
		cytochrome <i>b</i> gene to identify the contamination of pork in meatballs into 2 fragments (i. e., 131 and 228 bp) identify and differentiate beef, buffalo, lamb, camel, turkey, and chicken. Amplicons were digested with seven restriction endonucleases (<i>Hind</i> II, <i>Avall</i> , <i>Rsa</i> I, <i>Taq</i> I, <i>Hpa</i> II, <i>Tru</i> II and <i>Xba</i> I) selected based on preliminary in silico analysis. meat species identification design primer A:ACGACCCACG and primer B:CACCACGCCT	The resulting PCR product was electrophoresed on 1.8% ethidium bromide (Fluka)-stained agarose (EuroClone, Italy) gel in 0.5X Tris Borate EDTA (TBE). PCR was performed at 100 microL containing 50 ng genomic DNA, 2.5 mM MgCl, 200 ng primer, 2 units SUPER TAQ (HT Biotechnology), 200 p.m.. Each dATP, dGTP, dCTP and dTTP (Promega) in 10 mM Tris-HCL, pH 8 0.3.50 mM	[38] [37]
		primer 60-01 dan primer 60–03. Lanes: M, 100-bp DNA markers amplify the 359 bp region in the cytochrome <i>b</i> gene and digest the amplified product using <i>Hae</i> III and <i>Hinf</i> I Mitochondrial DNA (mt DNA) 439, 322, 274, 271, 225, 212, and 157 bp for horses, dogs, cats, cattle, sheep, pigs, and goats, respectively, were amplified. 10-base primers: OPL-01 (50 GGC ATG ACC T30), OPL-02 (50 TGG GCG TCA A30), OPL-04 (50 GAC TGC ACA C30) and OPL-05 (50 ACG CAG GCA C30) region (D-loop) resulting in a unique 712 base pair (bp) amplicon.	GC content varies from 50 to 80% Pigs dominate all tested species, even at the 1% level	[27] [42]
			PCR was performed at 30 cycles for the mixture at the level of 5%, 2.5%, 1%, and 0.5%, while at 35 cycles for the mixture at the level of 0.1%. The profiles obtained with OPL-04 primers contain from 5 to 8 DNA bands with molecular sizes between 2.0 and 0.5 kbp.	[36] [39,40]
			The pork detection sensitivity in other species meat employing a unique pork-specific PCR was made at 0.1%; detection limit (LOD) of pig DNA is 10 pg (pico gram).	[56]
			The detection sensitivity of pork in meat of other species using unique pig-specific PCR was set at 0.1%; limit of detection (LOD) pig DNA is 10 pg (pico gram). 1% pork detected in heat treatment Detected 0.01% (w/w) contamination of pork revealed a high labeling error rate of about 57% 40% of commercially labeled products carry a different meat species that is not indicated on the label	[11] [12] [13] [14] [17]
			The assay was analyzed to trace 0.01–0.02 ng of DNA in the raw condition and 1% suspected meat in the formulation of meatball. Specific detection of pigs at 0.1%; detection limit (LOD) 10 pg (pico grams)	[18] [11]
			In pure state, DNA detection limit at 0.01–0.001 ng and in meatball product is 0.5% meat in 32 Detection limit 0.01–0.02 ng in pure state and 1% suspected pork content in meatballs	[19] [20]
			The method sensitivity was investigated on binary gelatin mixtures that contain 0.1%, 1%, 10%, and 100% (w/w) pork gelatin in gelatin derived from beef and vice versa.	[21]
	targeted at mitochondrial DNA (mtDNA) cytochrome <i>b</i> (cyt <i>b</i>) gene sequences	[22]		
	Singelplex-Multiplex	pig mitochondrial displacement region (DI-loop) generating a unique 712 base pair (bp) amplicon. mitochondrial (mt) DNA h 83-bp and 531-bp gen sitokrom b (cyt-b) gen sitokrom b (cyt-b) conservative region of 16 S rRNA. The primers produced specific DNA fragments with lengths of 183, 224, 290 and 374 bp for poultry, fish, pigs, and ruminants, respectively. n mitochondrial genes ND5, ATPase 6, and cytochrome <i>b</i> to amplify 172, 163, 141, 129 and 108 bp DNA fragments from cat, dog, pork, monkey and mouse meat VPH-PF 50 -AAT TTT TGG GGA TGC TTA GAC T-30 (22mer Tm 52.5 C) and reverse VPH-PR 50 -TAT TTT GGG AGG TTA TTG TGT A-30 (25mer Tm 52.6 C). mitochondrial cytb, ND5, and 16s rRNA genes mitochondrial ND5, ATPase 6, dan gen cytochrome <i>b</i> mitochondrial DNA region (cytochrome <i>b</i> gene) targeted at mitochondrial DNA (mtDNA) cytochrome <i>b</i> (cyt <i>b</i>) gene sequences		

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Table 2 (continued)

Method	Type	Specialization	Analytical data	Reference
		e cytochrome <i>b</i> (cyt <i>b</i>)	The PCR-southern hybridization primer sensitivity to trace each meat from species is 0.1 ng.	
		Cytochrome B (CytB) gene in chicken and -actin (ActB) gene in pork.	produced two fragments, namely 274 bp for cattle and 389 bp for pigs	[23]
			Sensitive to 16 pg DNA per reaction and detection limit of 0.01% (w/w) for each type	[24]
		The 294-bp mitochondrial DNA D-loop. Region	Detected 1% pig content	[25]
		mitochondrial cytb genes, and the 16 S rRNA gene	Detection limit 0.005 ng/ μ L.	[26]
		Gen mitochondrial cytochrome <i>b</i> (mt-Cytb)	Detection was found (1%, w/w) in bread samples	[27]
	Real time	The PCR product of 359-bp was successfully obtained from the cyt <i>b</i> . Gene	pork produced better banding intensity when present in 1%, 3% or 5% in the mix mixture, compared to the other meat species tested.	[42]
		test for <i>S. scrofa</i> -specific probe stains,	To standardize the method with maximum sensitivity in mixed samples, the probe concentration was set at 20 M and Total DNA for PCR amplification at 10 ng.	[9]
		the COI gene	at least 5 pg of tested DNA	[44]
		from nuclear DNA (nDNA) or mitochondrial DNA (mtDNA)	The incorporation of highly sensitive and specific probes can greatly improve the specificity and sensitivity of the test	[45]
		<i>Sus scrofa</i> cytb 567	LOD (4 μ g/mL–1).	[36]
		Gen cytochrome <i>b</i> or cyt <i>b</i> dari mitochondrial DNA (mtDNA)	Successfully detects at least 0.05 pg (5%) adulterated meat	[18]
		Primers and probes used for endogenous control were previously designed targeting the conserved 141-bp fragment in the 18 S rRNA gene.	Detection limit of 0.1 pg heat -treated pork and 0.1% (w/w) pork in a mixture of beef and chicken.	[46]
		mtDNA babi	detection of 1% pig contamination	[47]
		Sus-ACTB-97bp-F CGTAGGTGCACAGTAGGTCTGAC Beta-Actin gene Sus-ACTB-97bp-R GGCCAGACTGGGGACATG DQ452569 Sus-ACTB-97bp-P VIC-CCAGGTCGGGGAGTC-MGB Gallus-TGFB3-129bp-F GGCTGCAAGTCACCGTGGTA TGFB3 gene Gallus-TGFB3-129bp-R CCGCTAGCCAGAAGCTCAGC AY685072 Gallus-TGFB3-129bp-P FAM-CAGGAGCCACGTGAGCAGCACAG-BHQ [18]	Obtained weight of chicken = 0.04 μ L - 4, and pig = 0.2 μ L + 2.5	[48]
		The isolates of beef gelatin DNA and pork gelatin DNA were obtained as much as 19.38 ng/ μ L and 13.63 ng/ μ L with a purity of 1.566 and 1.573 respectively.	bovine gelatin DNA and pork gelatin DNA	[49]
		Gene selection and DNA extraction	Limit of detection (LOD) from 0.1 to 1 pg/ μ L and LODs of gelatin from 0.1% to 5% (w/w)	[50]
		18 S rRNA gene	Successful identification of 100%–0.01% in meat products	[51]
		12 S rRNA-tRNA	Found 1% contamination of gelatin samples	[52]
		(Prk-F: CTG CCC TGA GGA CAA ATA TCA TTC and Prk-R: AAG CCC CCT CAG ATT CAT TCT ACG	Rate of tracing and quantification less than 0.0001% and 0.01% (w/w) of pork	[53]
		Sus Fw (50 –CTACATAAGAATATC CACCAC–30) and Sus Rv (50 –CATTTGTGGGATCTTCTAGGT–30) and Pg Fv (50 –CTA CATAAGAATATCCACCAC–30) and Pg Rv (50 –AGCCTACACCACAGCCA CAG–30)	Detection limits 0.22, 0.047, 0.048, 0.0000037, 0.015 ng/l	[55]
		DNA at 22nd-197th basal orders with an amplification of 176 bp.	Coefficient of variation (CV) for repetition is 16.28%, and when mixed with meat produces 7.46%	[54]
		cytochrome <i>b</i> (cyt <i>b</i>)	Detection limit 1.0% w/w for marsmelo and candy	[67]
		n ~650 bp from COI gene	Meat samples purchased from online specialty meat distributors had a higher label error rate (35%) than samples purchased from local butchers (18%) and samples purchased at supermarkets (5.8%).	[61]
		mitochondrial 16 S rRNA gene	The results distinguish over 99.9% of mammalian species and also detect small components up to 1% of mixed samples.	[62]
		short mtDNA	able to measure the material correctly at 1% discrimination level	[63]
		Mitochondrial 16 S rDNA. Gene region	The results showed that there were 61 species that could be identified, differentiated and detected up to 0.1% proportion.	[64]
		mitochondrial DNA fragment 120 bp gene	given 1% (w/w) pork, the NGS technique sensitivity is near to 1%	[65]
		mitochondrial DNA (mtDNA) gene		[66]
DNA	Barcoding			

(continued on next page)

Table 2 (continued)

Method	Type	Specialization	Analytical data	Reference
Metode LAMP		mt-DNA spesifik	the presence of one species of two of the three targeted mtDNA fragments 350 accounted for at least 0.2% of the aligned reads on the corresponding species-specific reference 351 sequences. This 0.2% threshold determined in the case studies evaluated 354 should be further validated in the process. Other NGS.	
		mt-ND1 gene	LOD 10 pg/μL-100 fg/μL on raw and cooked meat, and LOD of 0.01%–0.0001% on raw meat and cooked meat mixture	[69]
		18 S rRNA gene	The sensitivity of analysis of the LAMP assay equals to 0.5 pg	[70]
		Glucagon gene chromosome (Gcg)	0.1% pork in mixed beef	[71]
Biosensor	Optic	A special primer for swine detection is designed by attaching a unique oligonucleotide as a modified tag on the 5-terminal primary in the front. In addition, the 5-terminal inner primary terminal which is retarded has been modified mitochondrial D loop gene	Limit of detection of 10 pg (that is equivalent to 4 copies) porcine DNA isolated and 0.01% DNA in additives	[72]
			can detect a mixture of 0.01% pork in beef meatballs in it about 1 h	[73]
		Controlling positive biotin-dA20 (5'-ALD AAAAAAAAAAAAAAAAAA-3'-biotin)	The test was able to detect pork in 0.1% mixed beef and the DNA detection limit was at 0.5 ng/μL	[74]
		genom mitokondria Sus Scrofa.	The absolute detection limit of this method is 0.5 pg deer/bovine DNA, and the practical detection limit is 0.001%.	[88]
		<i>Sus scrofa</i> (pig) mt-genome 16, 613 bp,	The sensitivity obtained from the optical fiber and the short time required for results (about 2.5 h) demonstrate the usefulness of the system	[89]
	Electrochemical	pig cytochrome <i>b</i> (cytb) gene	This hybrid probe detects up to 1% of pork in the pork-beef binary	[115]
		5'-CTG ATA GTA GAT TTG TGA CCG TAG AAA-C6 spacerSH/-3'	to detect 1% pork in raw and cooked meatball	[90]
		An example of an oligonucleotide	biosensor sensitivity up to ~59%. L	[91]
		Cytochrome <i>b</i> gene and rpoB gene	Detection limits for chicken and pork are low at 1 pg/mL and 100 pg/mL	[95]
		dsDNA	the detection limit was determined to be 1 pg/μL (3.43 × 10 ⁻¹ copies/μL), target DNA detection limits were 1 pg/mL and 100 pg/mL for chicken and pig species, respectively.	[95]
CRISPR		The mtDNA samples from various raw and processed meats were isolated and cut with the restriction enzyme SalI.	selective against 10% of pig DNA content in the mixture.	[100]
		The directly extracted IgG was immobilized in microplates	Standard: detection of 0.1% adulterated pork in 45 min.	[6]
		The porcine 24-mer DNA probe sequence was selected from the cytochrome <i>b</i> (cytb) gene.	Competitive: 0.01% adulteration of pork in 20 min.	
		Preparation of CrRNA and ssDNA	pork with a limit of detection (LOD) of 100 aM validated with DNA extracted from pork samples (LOD 1 fM)	[101]
		mtDNA sequence homology analysis in both intraspecies and interspecies, finally, primer pairs P16-F and P21-R are screened from 25 primer pairs in mitochondrial gene of porcine (AY337045.1, nt 12,911–13,306) as the best pig species-specific RPA oligo.	The entire system completes the full process within 40 min, providing fast and high-sensitivity detection.	[109]
		Guide RNA (gRNA) targeting the pig cytochrome <i>b</i> (Cyt <i>b</i>) gene	The detection limit can reach 10 ⁻³ ng for porcine DNA and is completed within 30 min.	[110]
			n detection limit 2.7 ng/μL total DNA from pork.	[111]

Author contribution statement

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Data availability statement

No data was used for the research described in the article.

Declaration of interest's statement

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.

References

- [1] C. Meiderstma, PIG 05049, 2007. <https://christienmeindersma.com/PIG-05049>. (Accessed 23 June 2022). accessed.
- [2] Z.Z. Abidin, F.N. Omar, D.R.A. Biak, Y.C. Man, Alternative for rapid detection and screening of pork, chicken, and beef using dielectric properties in the frequency of 0.5 to 50 GHz, *Int. J. Food Prop.* 19 (5) (2016) 1127–1138, <https://doi.org/10.1080/10942912.2015.1058274>.
- [3] F. Gao, J. Wang, H. Zhang, H. Jia, Z. Cui, G. Yang, Role of ionic strength on protein fouling during ultrafiltration by synchronized UV–vis spectroscopy and electrochemical impedance spectroscopy, *J. Membr. Sci.* 563 (2018) 592–601, <https://doi.org/10.1016/j.memsci.2018.06.030>.
- [4] F. Mabood, et al., Fast detection and quantification of pork meat in other meats by reflectance FT-NIR spectroscopy and multivariate analysis, *Meat Sci.* 163 (2020), 108084, <https://doi.org/10.1016/j.meatsci.2020.108084>.
- [5] M. Kumar, Evaluation of the isotopic abundance ratio in biofield energy treated resorcinol using gas chromatography-mass spectrometry technique, *A. B. Pharm. Anal. Acta* 7 (5) (2016), <https://doi.org/10.4172/2153-2435.1000481>, 0–7.
- [6] J. Mandli, I. El Fatimi, N. Seddaoui, A. Amine, Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat, *Food Chem.* 255 (2018) 380–389, <https://doi.org/10.1016/j.foodchem.2018.01.184>.
- [7] R.M.H. Raja Nhari, I. Hanish, N.F.K. Mokhtar, M. Hamid, A.F. El Sheikh, Authentication approach using enzyme-linked immunosorbent assay for detection of porcine substances, *Qual. Assur. Saf. Crop Foods* 11 (5) (2019) 449–457, <https://doi.org/10.3920/QAS2018.1415>.
- [8] Y. Ayaz, N.D. Ayaz, I. Erol, Detection of species in meat and meat products using enzyme-linked immunosorbent assay, *J. Muscle Foods* 17 (2) (2006) 214–220, <https://doi.org/10.1111/j.1745-4573.2006.00046.x>.
- [9] V. Fajardo, I. González Isabel, M. Rojas, T. García, R. Martín, A review of current PCR-based methodologies for the authentication of meats from game animal species, *Trends Food Sci. Technol.* 21 (8) (2010) 408–421, <https://doi.org/10.1016/j.tifs.2010.06.002>.
- [10] S. Rahmati, N.M. Julkapli, W.A. Yehye, W.J. Basirun, Identification of meat origin in food products-A review, *Food Control* 68 (2016) 379–390, <https://doi.org/10.1016/j.foodcont.2016.04.013>.
- [11] N.S. Karabasanavar, S.P. Singh, D. Kumar, S.N. Shebannavar, Detection of pork adulteration by highly-specific PCR assay of mitochondrial D-loop, *Food Chem.* 145 (2014) 530–534, <https://doi.org/10.1016/j.foodchem.2013.08.084>.
- [12] A. Hamzah, Porcine DNA detection in finished meat products using different mitochondrial DNA (mt-DNA) on polymerase chain reaction, *J. Nutr. Food Sci.* 4 (6) (2014) 2–5, <https://doi.org/10.4172/2155-9600.1000323>.
- [13] H. Barakat, H.A.S. El-Garhy, M.M.A. Moustafa, Detection of pork adulteration in processed meat by species-specific PCR-QIAxcel procedure based on D-loop and cytb genes, *Appl. Microbiol. Biotechnol.* 98 (23) (2014) 9805–9816, <https://doi.org/10.1007/s00253-014-6084-x>.
- [14] A. Di Pinto, et al., Occurrence of mislabeling in meat products using DNA-based assay, *J. Food Sci. Technol.* 52 (4) (2015) 2479–2484, <https://doi.org/10.1007/s13197-014-1552-y>.
- [15] M. Jannah, et al., *Metode Biologi Molekuler, Widina Bhakti Persada, Bandung*, 2021.
- [16] T. Balacs, Research reports, *Int. J. Aromather.* 8 (2) (1997) 43–45, [https://doi.org/10.1016/s0962-4562\(97\)80025-2](https://doi.org/10.1016/s0962-4562(97)80025-2).
- [17] E. Şakalar, M.F. Abasiyanik, Qualitative analysis of meat and meat products by multiplex polymerase chain reaction (PCR) technique, *Afr. J. Biotechnol.* 10 (46) (2011) 9379–9386, <https://doi.org/10.5897/ajb10.1683>.
- [18] S. Edris, et al., Conventional multiplex polymerase chain reaction (PCR) versus real-time PCR for species-specific meat authentication, *Life Sci.* J. 9 (4) (2012) 5831–5837 [Online]. Available: <http://www.lifesciencesite.com/870>.
- [19] M.A.M. Hossain, et al., Heptaplex polymerase chain reaction assay for the simultaneous detection of beef, buffalo, chicken, cat, dog, pork, and fish in raw and heat-treated food products, *J. Agric. Food Chem.* 67 (29) (2019), <https://doi.org/10.1021/acs.jafc.9b02518>.
- [20] M.E. Ali, et al., Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods, *Food Chem.* 177 (January 2022) 214–224, <https://doi.org/10.1016/j.foodchem.2014.12.098>.
- [21] H. Shabani, et al., Halal authenticity of gelatin using species-specific PCR, *Food Chem.* 184 (2015) 203–206, <https://doi.org/10.1016/j.foodchem.2015.02.140>.
- [22] S. Aravindran, A.M. Sahilah, A. Aminah, Market surveillance on non-halal additives incorporated in surimi based products using polymerase chain reaction (PCR)-southern hybridization analysis, *AIP Conf. Proc.* 1614 (2014) 202–207, <https://doi.org/10.1063/1.4895196>.
- [23] M. Indriati, E. Yuniarsih, Multiplex PCR method of detecting pork to guarantee halal status in meat processed products, *J. Ilmu Produksi dan Teknol. Has. Peternak.* 7 (3) (2019) 96–101, <https://doi.org/10.29244/jipthp.7.3.96-101>.
- [24] K. Balakrishna, S. Sreerohini, M. Parida, Ready-to-use single tube quadruplex PCR for differential identification of mutton, chicken, pork and beef in processed meat samples, *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* 36 (10) (2019) 1435–1444, <https://doi.org/10.1080/19440049.2019.1633477>.
- [25] J. Ha, et al., Identification of pork adulteration in processed meat products using the developed mitochondrial DNA-based primers, *Korean J. Food Sci. Anim. Resour.* 37 (3) (2017) 464–468, <https://doi.org/10.5851/kosfa.2017.37.3.464>.
- [26] S. Sultana, M.A.M. Hossain, I.S.M. Zaidul, M.E. Ali, Multiplex PCR to Discriminate Bovine, Porcine, and Fish DNA in Gelatin and Confectionery Products, vol. 92, Elsevier Ltd, 2018, <https://doi.org/10.1016/j.lwt.2018.02.019>.
- [27] M. De Cicco, et al., Multianalytical detection of pig-derived ingredients in bread, *Food Anal. Methods* 12 (3) (2019) 780–790, <https://doi.org/10.1007/s12161-018-01410-6>.
- [28] M. Arya, I.S. Shergill, M. Williamson, L. Gommersall, N. Arya, Taqman-PCR-Exp-Rev-Mol-Diag, 2005, pp. 1–11, <https://doi.org/10.1586/14737159.5.2.xxx>.

- [29] Anonymous, Compare and Contrast: Multiplex vs. Singleplex PCR, 2015. https://lifescience.roche.com/global_en/blog/lab-life/real-time-pcr/compare-and-contrast-multiplex-vs-singleplex-pcr.html#:~:text=In conventional singleplex PCR%2C a, labeled with spectrally distinct fluorophores. (Accessed 23 June 2022). accessed.
- [30] O.I. Ilhak, A. Arslan, Identification of meat species by polymerase chain reaction (PCR) technique, *Turk. J. Vet. Anim. Sci.* 31 (3) (2007) 159–163.
- [31] M.C. Koh, C.H. Lim, S.B. Chua, S.T. Chew, S.T.W. Phang, Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species, *Meat Sci.* 48 (3–4) (1998) 275–285, [https://doi.org/10.1016/S0309-1740\(97\)00104-6](https://doi.org/10.1016/S0309-1740(97)00104-6).
- [32] J.C.I. Lee, J.G. Chang, Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species identification, *Forensic Sci. Int.* 67 (2) (1994) 103–107, [https://doi.org/10.1016/0379-0738\(94\)90325-5](https://doi.org/10.1016/0379-0738(94)90325-5).
- [33] L. Partis, D. Croan, Z. Guo, R. Clark, T. Coldham, J. Murby, Evaluation of a DNA fingerprinting method for determining the species origin of meats, *Meat Sci.* 54 (4) (2000) 369–376, [https://doi.org/10.1016/S0309-1740\(99\)00112-6](https://doi.org/10.1016/S0309-1740(99)00112-6).
- [34] R. Saez, Y. Sanz, F. Toldrá, PCR-based fingerprinting techniques for rapid detection of animal species in meat products, *Meat Sci.* 66 (3) (2004) 659–665, [https://doi.org/10.1016/S0309-1740\(03\)00186-4](https://doi.org/10.1016/S0309-1740(03)00186-4).
- [35] A.A. Aida, Y.B.C. Man, C.M.V.L. Wong, A.R. Raha, R. Son, Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication, *Meat Sci.* 69 (1) (2005) 47–52, <https://doi.org/10.1016/j.meatsci.2004.06.020>.
- [36] M.E. Ali, U. Hashim, S. Mustafa, Y.B. Che Man, K.N. Islam, Gold nanoparticle sensor for the visual detection of pork adulteration in meatball formulation, *J. Nanomater.* 2012 (2012), <https://doi.org/10.1155/2012/103607>.
- [37] M. Kargar, F. Moein Jahromi, A. Doosti, S. Handali, Molecular investigation of quinolone resistance of quinolone resistance-determining region in *Streptococcus pneumoniae* strains isolated from Iran using polymerase chain reaction-restriction fragment length polymorphism method, *Osong Public Heal. Res. Perspect.* 5 (5) (2014) 245–250, <https://doi.org/10.1016/j.phrp.2014.08.010>.
- [38] M.E. Ali, U. Hashim, S. Mustafa, Y. Che Man, Swine-specific PCR-RFLP assay targeting mitochondrial cytochrome B gene for semiquantitative detection of pork in commercial meat products, *Food Anal. Methods* 5 (3) (2012) 613–623, <https://doi.org/10.1007/s12161-011-9290-5>.
- [39] Y. Erwanto, M.Z. Abidin, E.Y.P. Muslim, S. Sugiyono, A. Rohman, Identification of pork contamination in meatballs of Indonesia local market using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, *AJAS (Asian-Australas. J. Anim. Sci.)* 27 (10) (2014) 1487–1492, <https://doi.org/10.5713/ajas.2014.14014>.
- [40] Y. Erwanto, M.Z. Abidin, X. Sisindari, A. Rohman, Pig species identification in meatballs using polymerase chain reaction- restriction fragment length polymorphism for Halal authentication, *Int. Food Res. J.* 19 (3) (2012) 901–906.
- [41] P.M. Holland, R.D. Abramson, R. Watson, D.H. Gelfand, Detection of specific polymerase chain reaction product by utilizing the 5' → 3' exonuclease activity of *Thermus aquaticus* DNA polymerase, *Proc. Natl. Acad. Sci. U. S. A.* 88 (16) (1991) 7276–7280, <https://doi.org/10.1073/pnas.88.16.7276>.
- [42] C. Murugaiah, Z.M. Noor, M. Mastakim, L.M. Bilung, J. Selamat, S. Radu, Meat species identification and Halal authentication analysis using mitochondrial DNA, *Meat Sci.* 83 (1) (2009) 57–61, <https://doi.org/10.1016/j.meatsci.2009.03.015>.
- [43] A.K. Shalek, et al., Single-cell RNA-seq reveals dynamic paracrine control of cellular variation, *Nature* 510 (7505) (2014) 363–369, <https://doi.org/10.1038/nature13437>.
- [44] C.C. Lin, L.L. Fung, P.K. Chan, C.M. Lee, K.F. Chow, S.H. Cheng, A rapid low-cost high-density DNA-based multi-detection test for routine inspection of meat species, *Meat Sci.* 96 (2) (2014) 922–929, <https://doi.org/10.1016/j.meatsci.2013.09.001>.
- [45] N.A. Mohamad, A.F. El Sheikh, S. Mustafa, N.F.K. Mokhtar, Comparison of gene nature used in real-time PCR for porcine identification and quantification: a review, *Food Res. Int.* 50 (1) (2013) 330–338, <https://doi.org/10.1016/j.foodres.2012.10.047>.
- [46] M. Kim, I. Yoo, S.Y. Lee, Y. Hong, H.Y. Kim, Quantitative detection of pork in commercial meat products by TaqMan® real-time PCR assay targeting the mitochondrial D-loop region, *Food Chem.* 210 (2016) 102–106, <https://doi.org/10.1016/j.foodchem.2016.04.084>.
- [47] T.J. Raharjo, Y.N.C. Chudori, F.W. Agustina, TaqMan probe real-time polymerase chain reaction targeting the ATPase 6 gene for the detection of pork adulteration in meat and meatballs, *J. Food Saf.* 39 (6) (2019) 1–6, <https://doi.org/10.1111/jfs.12715>.
- [48] Y. Cai, et al., Quantitative analysis of pork and chicken products by droplet digital PCR, *BioMed Res. Int.* 2014 (2014), <https://doi.org/10.1155/2014/810209>.
- [49] Z. Zilhadia, A.N. Izzah, O.S. Betha, Perbandingan metode SYBR green dan Hydrolysis probe dalam analisis DNA gelatin sapi dan gelatin babi menggunakan real time polymerase chain reaction, *J. Sains Farm. Klin.* 4 (1) (2017) 16, <https://doi.org/10.29208/jsfk.2017.4.1.194>.
- [50] Y. Yang, L. Li, H. Wang, M. Liu, Y. Wu, Development and verification of a quantitative real-time PCR method to identify and quantify gelatin derived from animal hide, *J. Food Sci.* 85 (9) (2020) 2762–2772, <https://doi.org/10.1111/1750-3841.15362>.
- [51] T.S. Kang, T. Tanaka, Comparison of quantitative methods based on SYBR Green real-time qPCR to estimate pork meat adulteration in processed beef products, *Food Chem.* 269 (2018) 549–558, <https://doi.org/10.1016/j.foodchem.2018.06.141>.
- [52] H.I.A. Amqizaa, H.A. Al-Kahtani, E.A. Ismail, K. Hayat, I. Jaswir, Identification and verification of porcine DNA in commercial gelatin and gelatin containing processed foods, *Food Control* 78 (2017) 297–303, <https://doi.org/10.1016/j.foodcont.2017.02.024>.
- [53] J.S. Amaral, G. Santos, M.B.P.P. Oliveira, I. Mafra, Quantitative detection of pork meat by EvaGreen real-time PCR to assess the authenticity of processed meat products, *Food Control* 72 (2017) 53–61, <https://doi.org/10.1016/j.foodcont.2016.07.029>.
- [54] A. Rahmawati, B. Kuswandi, Y. Retnaningtyas, Deteksi gelatin babi pada sampel permen lunak jelly menggunakan metode fourier transform infra red (FTIR) dan kemometrik (detection of porcine gelatin in jelly soft candy sample using fourier transform infra red and chemometrics), *J. Pustaka Kesehat.* 3 (2) (2015) 278–283 [Online]. Available: <http://jurnal.unej.ac.id/index.php/JPK/index>.
- [55] H.A. Al-Kahtani, E.A. Ismail, M. Asif Ahmed, Pork detection in binary meat mixtures and some commercial food products using conventional and real-time PCR techniques, *Food Chem.* 219 (2017) 54–60, <https://doi.org/10.1016/j.foodchem.2016.09.108>.
- [56] N. Haider, I. Nabulsi, B. Al-Safadi, Identification of meat species by PCR-RFLP of the mitochondrial COI gene, *Meat Sci.* 90 (2) (2012) 490–493, <https://doi.org/10.1016/j.meatsci.2011.09.013>. *Meat Sci.*, vol. 90, no. January 2012, pp. 490–493.
- [57] A. Suea-Ngam, P.D. Howes, A.J. Demello, An amplification-free ultra-sensitive electrochemical CRISPR/Cas biosensor for drug-resistant bacteria detection, *Chem. Sci.* 12 (38) (2021) 12733–12743, <https://doi.org/10.1039/d1sc02197d>.
- [58] S. Roy, I.A. Rahman, J.H. Santos, M.U. Ahmed, Meat species identification using DNA-redox electrostatic interactions and non-specific adsorption on graphene biochips, *Food Control* 61 (2016) 70–78, <https://doi.org/10.1016/j.foodcont.2015.09.029>.
- [59] S.A. Motalib, et al., Sensitivity of polymerase chain reaction (PCR) -southern hybridization and 2 conventional PCR analysis for Halal, *LWT-Food Sci. Technol.* 63 (1) (2015) 714–719, <https://doi.org/10.1016/j.lwt.2015.03.006>.
- [60] Q. Zia, M. Alawami, N.F.K. Mokhtar, R.M.H.R. Nhari, I. Hanish, Current analytical methods for porcine identification in meat and meat products, *Food Chem.* 324 (2020), 126664, <https://doi.org/10.1016/j.foodchem.2020.126664>.
- [61] D.E. Kane, R.S. Hellberg, Identification of species in ground meat products sold on the U.S. commercial market using DNA-based methods, *Food Control* 59 (2016) 158–163, <https://doi.org/10.1016/j.foodcont.2015.05.020>.
- [62] A.O. Tillmar, B. Dell'Amico, J. Welander, G. Holmlund, A universal method for species identification of mammals utilizing next generation sequencing for the analysis of DNA mixtures, *PLoS One* 8 (12) (2013) 1–9, <https://doi.org/10.1371/journal.pone.0083761>.
- [63] F. Ripp, et al., All-Food-Seq (AFS): a quantifiable screen for species in biological samples by deep DNA sequencing, *BMC Genom.* 15 (1) (2014), <https://doi.org/10.1186/1471-2164-15-639>.
- [64] S. Dobrovolny, et al., Development of a DNA metabarcoding method for the identification of fifteen mammalian and six poultry species in food, *Food Chem.* 272 (2019) 354–361, <https://doi.org/10.1016/j.foodchem.2018.08.032>.
- [65] G. Cottenet, C. Blancpain, P.F. Chuah, C. Cavin, Evaluation and application of a next generation sequencing approach for meat species identification, *Food Control* 110 (November 2019) (2020), <https://doi.org/10.1016/j.foodcont.2019.107003>.
- [66] A. Ribani, G. Schiavo, V.J. Utzeri, F. Bertolini, C. Geraci, This is the final peer-reviewed accepted manuscript of : when citing , please refer to the published version, *Accepted Manuscript* 246 (2018) 90–98.

- [67] Y. Demirhan, P. Ulca, H.Z. Senyuva, Detection of porcine DNA in gelatine and gelatine-containing processed food products-Halal/Kosher authentication, *Meat Sci.* 90 (3) (2012) 686–689, <https://doi.org/10.1016/j.meatsci.2011.10.014>.
- [68] N.A. Tasrip, et al., Loop mediated isothermal amplification; a review on its application and strategy in animal species authentication of meat based food products, *Int. Food Res. Journal* 26 (1) (2019) 1–10.
- [69] A.R. Cho, H.J. Dong, S. Cho, Meat species identification using loop-mediated isothermal amplification assay targeting species-specific mitochondrial DNA, *Korean J. Food Sci. Anim. Resour.* 34 (6) (2014) 799–807, <https://doi.org/10.5851/kosfa.2014.34.6.799>.
- [70] G. Ran, et al., Development of a rapid method for the visible detection of pork DNA in halal products by loop-mediated isothermal amplification, *Food Anal. Methods* 9 (3) (2016) 565–570, <https://doi.org/10.1007/s12161-015-0246-z>.
- [71] S.Y. Lee, M.J. Kim, Y. Hong, H.Y. Kim, Development of a rapid on-site detection method for pork in processed meat products using real-time loop-mediated isothermal amplification, *Food Control* 66 (2016) 53–61, <https://doi.org/10.1016/j.foodcont.2016.01.041>.
- [72] Y. Xu, et al., A smart sealed nucleic acid biosensor based on endogenous reference gene detection to screen and identify mammals on site, *Sci. Rep.* 7 (December 2016) (2017) 1–10, <https://doi.org/10.1038/srep43453>.
- [73] K. Takasaki Riztzyan, Y. Yamakoshi, S. Futo, Single-laboratory validation of rapid and easy DNA strip for porcine DNA detection in beef meatballs, *J. AOAC Int.* 101 (5) (2018) 1653–1656, <https://doi.org/10.5740/jaoacint.17-0394>.
- [74] P.S. Girish, et al., Rapid detection of pork using alkaline lysis- Loop Mediated Isothermal Amplification (AL-LAMP) technique, *Food Control* 110 (2020), 107015, <https://doi.org/10.1016/j.foodcont.2019.107015>.
- [75] R.P. Corcuera, Cavalieri, "Encyclopedia of Agricultural, Food, and Biological Engineering, J.I.R.D., 2003, pp. 119–123.
- [76] L. Gugoasa, R.-I. Staden, A. Ciucu, J. Staden, Influence of physical immobilization of dsDNA on carbon based matrices of electrochemical sensors, *Curr. Pharmaceut. Anal.* 10 (1) (2014) 20–29, <https://doi.org/10.2174/157341291001140102104740>.
- [77] G.A. Evtugyn, H.C. Budnikov, E. Nikolskaya, Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination, *Talanta* 46 (4) (1998) 465–484.
- [78] I. Karube, Y. Nomura, Enzyme sensors for environmental analysis, *J. Mol. Catal. B Enzym.* 10 (1–3) (2000) 177–181, [https://doi.org/10.1016/S1381-1177\(00\)00125-9](https://doi.org/10.1016/S1381-1177(00)00125-9).
- [79] G. Zanchetta, R. Lanfranco, F. Giavazzi, T. Bellini, M. Buscaglia, Emerging applications of label-free optical biosensors, *Nanophotonics* 6 (4) (2017) 627–645, <https://doi.org/10.1515/nanoph-2016-0158>.
- [80] O.S. Wolfbeis, *Springer Series on Chemical Sensors and Biosensors: Method and Applications*, vol. 4, 2006 [Online]. Available: <http://link.springer.com/10.1007/b100321>.
- [81] H. Guner, et al., A smartphone based surface plasmon resonance imaging (SPRi) platform for on-site biodetection, *Sensor. Actuator. B Chem.* 239 (2017) 571–577, <https://doi.org/10.1016/j.snb.2016.08.061>.
- [82] P. Chen, et al., Multiplex serum cytokine immunoassay using nanoplasmonic biosensor microarrays, *ACS Nano* 9 (4) (2015) 4173–4181, <https://doi.org/10.1021/acsnano.5b00396>.
- [83] X. Fu, Z. Cheng, J. Yu, P. Choo, L. Chen, J. Choo, A SERS-based lateral flow assay biosensor for highly sensitive detection of HIV-1 DNA, *Biosens. Bioelectron.* 78 (2016) 530–537, <https://doi.org/10.1016/j.bios.2015.11.099>.
- [84] G.A. Lopez, M.C. Estevez, M. Soler, L.M. Lechuga, Recent advances in nanoplasmonic biosensors: applications and lab-on-a-chip integration, *Nanophotonics* 6 (1) (2017) 123–136, <https://doi.org/10.1515/nanoph-2016-0101>.
- [85] A. Ishaq, et al., Potentiality of analytical approaches to determine gelatin authenticity in food systems: a review, *LWT (Lebensm.-Wiss. & Technol.)* 121 (December 2019) (2020), 108968, <https://doi.org/10.1016/j.lwt.2019.108968>.
- [86] K. Ramachandran, K. Daoudi, H.H. Kacem, S. Columbus, H. Benaoum, M. Gaidi, Rapid and ultra-sensitive detection of pork DNA with surface enhanced Raman spectroscopy for onsite food quality inspection, *Sens. Bio-Sensing Res.* 38 (August) (2022), 100524, <https://doi.org/10.1016/j.sbsr.2022.100524>.
- [87] J.M.R. Flauzino, L.M. Alves, V.R. Rodovalho, J.M. Madurro, A.G. Brito Madurro, Application of biosensors for detection of meat species: a short review, *Food Control* 142 (April) (2022), 109214, <https://doi.org/10.1016/j.foodcont.2022.109214>.
- [88] W. Wang, Y. Zhu, Y. Chen, X. Xu, G. Zhou, Rapid visual detection of eight meat species using optical thin-film biosensor chips, *J. AOAC Int.* 98 (2) (2015) 410–414, <https://doi.org/10.5740/jaoacint.14-230>.
- [89] E. Torelli, M. Manzano, R.S. Marks, Chemiluminescent optical fibre genosensor for porcine meat detection, *Sensor. Actuator. B Chem.* 247 (2017) 868–874, <https://doi.org/10.1016/j.snb.2017.03.064>.
- [90] M.E. Ali, U. Hashim, S. Mustafa, Y.B. Che Man, T. Adam, Q. Humayun, Nanobiosensor for the detection and quantification of pork adulteration in meatball formulation, *J. Exp. Nanosci.* 9 (2) (2014) 152–160, <https://doi.org/10.1080/17458080.2011.640946>.
- [91] K.L. Foo, U. Hashim, C.H. Voon, M. Kashif, M.E. Ali, Au decorated ZnO thin film: application to DNA sensing, *Microsyst. Technol.* 22 (4) (2016) 903–910, <https://doi.org/10.1007/s00542-015-2572-x>.
- [92] S. Roy, I.A. Rahman, M.U. Ahmed, Paper-based rapid detection of pork and chicken using LAMP-magnetic bead aggregates, *Anal. Methods* 8 (11) (2016) 2391–2399, <https://doi.org/10.1039/c6ay00274a>.
- [93] S.S. Ac, et al., Markers in serum, *Nano Lett.* (2014) 6–11, <https://doi.org/10.1021/nl500574n>.
- [94] X.L. Cheng, et al., Identification of five gelatins by ultra performance liquid chromatography/time-of-flight mass spectrometry (UPLC/Q-TOF-MS) using principal component analysis, *J. Pharm. Biomed. Anal.* 62 (2012) 191–195, <https://doi.org/10.1016/j.jpba.2011.12.024>.
- [95] S.X. Roy, Sharmili Wei, J.L.Z. Ying, M. Safavieh, M.U. Ahmed, A novel, sensitive and label-free loop-mediated isothermal amplification detection method for nucleic acids using luminophore dyes, *Biosens. Bioelectron.* 86 (2016) 346–352, <https://doi.org/10.1016/j.bios.2016.06.065>.
- [96] A.E.G. Cass, et al., Ferrocene-mediated enzyme electrode for amperometric determination of glucose, *Anal. Chem.* 56 (4) (1984) 667–671, <https://doi.org/10.1021/ac00268a018>.
- [97] J.A. Rogers, A.A. Maznev, M.J. Banet, K.A. Nelson, Optical generation and characterization of acoustic waves in thin films: fundamentals and applications, *Annu. Rev. Mater. Sci.* 30 (2000) 117–157, <https://doi.org/10.1146/annurev.matsci.30.1.117>.
- [98] A.P.F. Turner, I. Karube, G.S. Wilson, *Biosensors: Fundamentals and Applications*, 1989.
- [99] C. Dincer, et al., Disposable sensors in diagnostics, food, and environmental monitoring, *Adv. Mater.* 31 (30) (2019), <https://doi.org/10.1002/adma.201806739>.
- [100] Y.W. Hartati, A.A. Suryani, M. Agustina, S. Gaffar, A. Anggraeni, A gold nanoparticle–DNA bioconjugate–based electrochemical biosensor for detection of Sus scrofa mtDNA in raw and processed meat, *Food Anal. Methods* 12 (11) (2019) 2591–2600, <https://doi.org/10.1007/s12161-019-01593-6>.
- [101] I. Khalil, et al., Dual platform based sandwich assay surface-enhanced Raman scattering DNA biosensor for the sensitive detection of food adulteration, *Analyst* 145 (4) (2020) 1414–1426, <https://doi.org/10.1039/c9an02106j>.
- [102] S. Nidhi, et al., Novel crisper–cas systems: an updated review of the current achievements, applications, and future research perspectives, *Int. J. Mol. Sci.* 22 (7) (2021) 1–42, <https://doi.org/10.3390/ijms22073327>.
- [103] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a and Csm6, *Science* 360 (6387) (2018) 439–444, <https://doi.org/10.1126/science.aag0179>.
- [104] K. Pardee, et al., Portable, on-demand biomolecular manufacturing, *Cell* 167 (1) (2016) 248–259.e12, <https://doi.org/10.1016/j.cell.2016.09.013>.
- [105] C.H. Chen, et al., Dual inhibition of PIK3C3 and FGFR as a new therapeutic approach to treat bladder cancer, *Clin. Cancer Res.* 24 (5) (2018) 1176–1189, <https://doi.org/10.1158/1078-0432.CCR-17-2066>.
- [106] S.Y. Li, Q.X. Cheng, J.K. Liu, X.Q. Nie, G.P. Zhao, J. Wang, CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA, *Cell Res.* 28 (4) (2018) 491–493, <https://doi.org/10.1038/s41422-018-0022-x>.
- [107] J.S. Chen, et al., J.S. Chen, E. Ma, L.B. Harrington, M. Da Costa, X. Tian, J.M. Palefsky, et al., CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity, *Science* 6245 (April) (2018) 1–5, eaar6245, "Science (80-).".
- [108] S.Y. Li, et al., CRISPR-Cas12a-assisted nucleic acid detection, *Cell Discov* 4 (1) (2018) 18–21, <https://doi.org/10.1038/s41421-018-0028-z>.

- [109] H. Liu, et al., A frontline nucleic acid rapid detection system for food safety based on CRISPR-Cas12a combined with recombinase polymerase amplification, RPA-Cas12a-FS, *Food Chem.* 334 (February 2020) (2021), 127608, <https://doi.org/10.1016/j.foodchem.2020.127608>.
- [110] G. Zhao, et al., Alkaline lysis-recombinase polymerase amplification combined with CRISPR/Cas12a assay for the ultrafast visual identification of pork in meat products, *Food Chem.* 383 (October 2021) (2022), 132318, <https://doi.org/10.1016/j.foodchem.2022.132318>.
- [111] Y. Wu, et al., CRISPR-Cas12-Based rapid authentication of halal food, *J. Agric. Food Chem.* 69 (35) (2021) 10321–10328, <https://doi.org/10.1021/acs.jafc.1c03078>.
- [112] J.P. Broughton, et al., CRISPR–Cas12-based detection of SARS-CoV-2, *Nat. Biotechnol.* 38 (7) (2020) 870–874, <https://doi.org/10.1038/s41587-020-0513-4>.
- [113] M.D. Esposti, S. De Vries, M. Crimi, A. Ghelli, T. Patarnello, A. Meyer, Mitochondrial cytochrome b: evolution and structure of the protein, *BBA - Bioenerg.* 1143 (3) (1993) 243–271, [https://doi.org/10.1016/0005-2728\(93\)90197-N](https://doi.org/10.1016/0005-2728(93)90197-N).
- [114] F. Bellagamba, V.M. Moretti, S. Comincini, F. Valfrè, Identification of species in animal feedstuffs by polymerase chain reaction - restriction fragment length polymorphism analysis of mitochondrial DNA, *J. Agric. Food Chem.* 49 (8) (2001) 3775–3781, <https://doi.org/10.1021/jf0010329>.
- [115] M.E. Ali, U. Hashim, M. Kashif, S. Mustafa, Y.B. Che Man, S.B. Abd Hamid, Development of swine-specific DNA markers for biosensor-based halal authentication, *Genet. Mol. Res.* 11 (2) (2012) 1762–1772, <https://doi.org/10.4238/2012.June.29.9>.