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ORIGINAL PAPER

Integrating LAMP-coupled Modification SPCE with SiNWs/PtNPs in an Electrochemical DNA Biosensor for Real-time Monitoring of Porcine DNA Amplification

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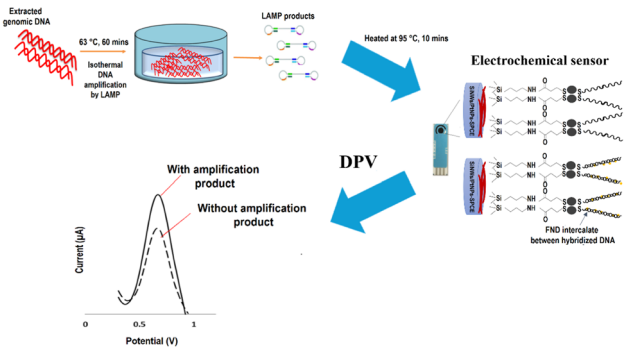
Abstract

This study presents a novel loop-mediated isothermal amplification (LAMP) method for the rapid and sensitive detection of porcine DNA, addressing critical needs in food safety and compliance with halal and kosher dietary laws. The key innovation lies in the design of highly specific primers targeting Sus scrofa mitochondrial DNA, ensuring accurate identification with minimal cross-reactivity. The LAMP technique was selected for its rapid amplification, isothermal operation, and simplified equipment requirements, making it suitable for on-site applications and reducing overall costs. Optimization of reaction conditions enabled reliable DNA amplification at 63°C within 60 minutes. The progress of the amplification was monitored in real time by measuring turbidity, while successful DNA synthesis was further confirmed by gel electrophoresis. For sensitive detection and quantification, an electrochemical DNA biosensor was integrated into the workflow. This biosensor utilizes a silicon nanowire–platinum nanoparticle–modified screen-printed carbon electrode and ferrocenylnaphthalene diimide as a dsDNA intercalator, enabling precise, real-time monitoring of the LAMP reaction. The optimized assay achieved a detection limit of 175.2 ng/µL, demonstrating high sensitivity for low-level porcine DNA detection. Specificity was validated across various meat sources and processed foods, with no false positives observed. The biosensor-based method also successfully detected porcine DNA in mixed meat samples, highlighting its strong potential for practical use in food authenticity testing and regulatory compliance.

Graphical Abstract



Keywords Loop-mediated isothermal amplification (LAMP) · *Sus scrofa* mtDNA · Electrochemical DNA biosensor · Screen-printed carbon electrode (SPCE) · Porcine DNA detection

Extended author information available on the last page of the article

Introduction

Accurate identification of species-specific DNA in food items is crucial for assuring food safety, authenticity, and compliance with labelling requirements [7]. The detection of porcine DNA, in particular, holds significant importance due to dietary restrictions observed by certain religious and cultural groups, such as those adhering to halal and kosher dietary laws [14]. These restrictions strictly prohibit the consumption of pork, making the reliable identification of porcine DNA essential to avoid unintentional violations of these dietary laws. Furthermore, the presence of porcine DNA in food products raises concerns related to food adulteration and fraud, as unscrupulous manufacturers might substitute cheaper pork for more expensive meats without proper labelling, misleading consumers and potentially causing serious ethical and health implications [5]. Although polymerase chain reaction (PCR) is a highly effective method for DNA detection, it frequently necessitates sophisticated thermal cycling equipment and lengthy processing periods. In order to denature DNA, anneal primers, and extend new DNA strands, PCR needs to be heated and cooled many times. This requires advanced lab tools and trained staff. This complexity makes PCR less suitable for rapid, field-based applications where quick and accurate results are crucial, such as in food safety inspections, border controls, and on-site testing in processing plants [18]. Additionally, the need for a controlled laboratory environment limits PCR's utility in remote or resource-limited settings. To address these limitations, the establishment of a quick and accurate technique for porcine DNA detection in food products is highly desirable.

Loop-Mediated Isothermal Amplification (LAMP) has gained recognition as a viable alternative, offering rapid DNA amplification under constant temperature conditions, thereby minimizing the equipment requirements and reducing the overall time needed for detection [13]. In contrast to PCR, LAMP enables amplification at a constant temperature, eliminating the need for a thermal cycler. This is achieved through the use of a DNA polymerase with strong strand displacement capability, which facilitates the efficient amplification of target DNA sequences without the need for temperature cycling. The isothermal nature of LAMP not only reduces the complexity of the required equipment but also speeds up the detection process, making it possible to obtain results within a shorter timeframe, often less than an hour. LAMP's effectiveness largely hinges on the design and optimization of its primers. The primer set generally comprises four to six oligonucleotides—including two inner primers (FIP and BIP) and two outer primers (F3 and B3)—each designed to bind to specific targets regions of the DNA sequence, thereby enhancing both specificity and detection sensitivity [16]. These primers are designed to bind six separate regions within the target DNA sequence, providing a high level of specificity. The inner primers (FIP and BIP) are particularly important as they initiate the strand displacement process, while the outer primers (F3 and B3) function to promote and accelerate the overall amplification reaction. Additional loop primers (LoopF and LoopB) can be incorporated to further enhance the speed of the reaction. The design of these primers is crucial as it directly influences the efficiency and target specificity of the LAMP reaction, determining the success of the DNA amplification and the reliability of the subsequent detection [1].

This work aimed to develop and optimize a LAMP-based technique for the sensitive detection of porcine DNA, addressing the critical need for reliable identification to ensure compliance with dietary laws and prevent food fraud. We designed and optimized a set of primers targeting specific regions of the *Sus scrofa* mitochondrial DNA to ensure high specificity and efficiency.

Additionally, we integrated the LAMP method with an advanced electrochemical DNA biosensor based on silicon nanowires and platinum nanoparticles to monitor amplification results. The electrochemical technique was selected due to its high sensitivity, rapid response, and compatibility with portable, miniaturized devices, making it ideal for field-based and point-of-need applications. Unlike traditional detection methods, electrochemical biosensing enables precise, real-time quantification of nucleic acids with minimal sample processing, thereby enhancing the practicality and efficiency of the LAMP assay for food authenticity testing.

The performance of the LAMP-biosensor system was rigorously evaluated using various parameters, including amplification temperature and time, to achieve optimal results. The system's specificity was further validated through cross-reactivity studies with different types of meat and processed food samples, ensuring no false positives and reliable detection of porcine DNA. Additionally, we investigated the biosensor's capability to detect porcine DNA in binary meat mixtures, simulating real-world scenarios, to demonstrate its practical applicability.

Materials and Methods

LAMP Primer Design

The LAMP primer sets were designed using the standard PrimerExplorer V5 software (http://primerexplorer.jp/e/), based on the complete mitochondrial DNA sequence of *Sus scrofa* (GenBank accession no. AJ002189.1) obtained from

the National Center for Biotechnology Information (NCBI) database. Five specific primer sequences, targeting an amplicon size of approximately 156 bp, were selected to meet the standard criteria for LAMP sensitivity and specificity.

The mitochondrial genome was specifically chosen as the target region because mitochondrial DNA is highly conserved within species yet exhibits sufficient sequence divergence between species, allowing for the development of primers with high specificity. In addition, the high copy number of mitochondrial DNA in animal cells increases the likelihood of successful DNA amplification, even in processed or degraded samples, thereby enhancing the assay's efficiency and sensitivity.

LAMP Reaction Condition

To optimize the LAMP reaction conditions, a method outlined by Ran et al. [17] was employed, with a few minor alterations. Each reaction had a volume of 25 µL, comprising 1.6 µM each of FIP and BIP, 0.2 µM each of F3 and B3, 0.8 µM LF primer, 1.8 mM dNTPs (Thermo Scientific, USA), 1x ThermoPol reaction buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100 (New England Biolabs, USA), 0.6 M betaine (TCI, USA), 8 U of B*st* DNA polymerase (SBS Genetech Co, Beijing), and 1 µL of template DNA. Nuclease-free water was added to get the final volume to 25 µL. After a quick centrifugation, the mixture was incubated for an hour at 63°C. Turbidity due to magnesium pyrophosphate precipitation indicated successful amplification, while a lack of turbidity indicated no amplification. Each LAMP assay was performed in triplicate.

Validation of LAMP Amplicon by Gel Electrophoresis

To validate the results, all LAMP products were subjected to 2% (v/v) TBE agarose gel electrophoresis, enhanced with 0.1% GelRed (Biotium). The sizes of the LAMP products were determined using a 100 bp DNA ladder (1st Base) as a reference marker. Electrophoresis was conducted at 80 V for 75 minutes in a 1x TBE buffer. Gels were visualized using a UV transilluminator.

Optimization of LAMP Condition

The reaction temperatures were varied to 60, 63, 65, and 68 °C for up to 60 minutes in order to find the perfect range for the porcine-specific LAMP assays. Three replicates (n=3) were prepared for each temperature. Additionally, LAMP amplification times of 20, 30, 40, 50, and 60 minutes were evaluated, with each time period assessed in triplicate to ensure reliability.

Food DNA Extraction

Processed food samples containing pork were sourced from a local store in Malaysia, along with fresh raw meat samples including beef (*Bos taurus*), lamb (*Ovis aries*), chicken (*Gallus gallus*), and pork (*Sus scrofa*). The DNeasy mericon Food Kit from Qiagen (Germany) was utilized for DNA extraction. A NanoDropTM 2000 spectrophotometer (ThermoFisher Scientific, USA) was used to test the concentration and purity of the DNA extracted. DNA samples with A_{260}/A_{280} ratios between 1.8 and 2.0 were used for LAMP amplification.

Electrochemical Sensor Preparation

First, cyclic voltammetry was used to activate the SPCE's carbon working electrode in 0.1 M NaOH. A 4 µL suspension of silicon nanowires (SiNWs) in 2% APTES was drop-cast onto the electrode and incubated at room temperature for 24 hours. The electrode was then rinsed with 95% ethanol and baked at 70 °C for 30 minutes. After cooling, 10 µL of 5 mM ethanolic 3,3'-dithiodipropionic acid (DTDPA) was applied and incubated for 2 hours at ambient temperature. Next, 10 µL of platinum nanoparticle (PtNPs) suspension was drop-cast onto the SiNWs-modified electrode. After incubating for 15 minutes at 50 °C, the electrode was rinsed with deionized water and then dried with nitrogen gas. The resulting modified SPCE served as the electrochemical sensing platform for detecting LAMP-amplified porcine DNA using the FND-based biosensor system.

Validation of the DNA Biosensor using LAMP Amplicon

To validate the developed DNA biosensor, LAMP products derived from various meats (including raw pork, beef, lamb, chicken, canned pork, and pork sausages) were subjected to denaturation at 95 °C for 10 minutes. This process converts double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA), which is crucial for the DNA biosensor, as it specifically detects ssDNA. Post-denaturation, the samples were rapidly cooled on ice to prevent re-annealing. A 10 µL aliquot of denatured LAMP amplicons was applied to the surface of the biosensor and incubated at 40 °C for two hours. Afterward, the biosensor was rinsed with TE buffer to remove any unbound DNA and was dried using nitrogen gas. The immobilized LAMP amplicons on the biosensor were incubated with ferrocenylnaphthalene diimide (FND) in 50 mM Tris-HCl (pH 7.6) for 20 minutes at room temperature without the application of any potential. In order to analyze the modified screen-printed carbon electrode (SPCE), differential pulse voltammetry (DPV) was conducted over a

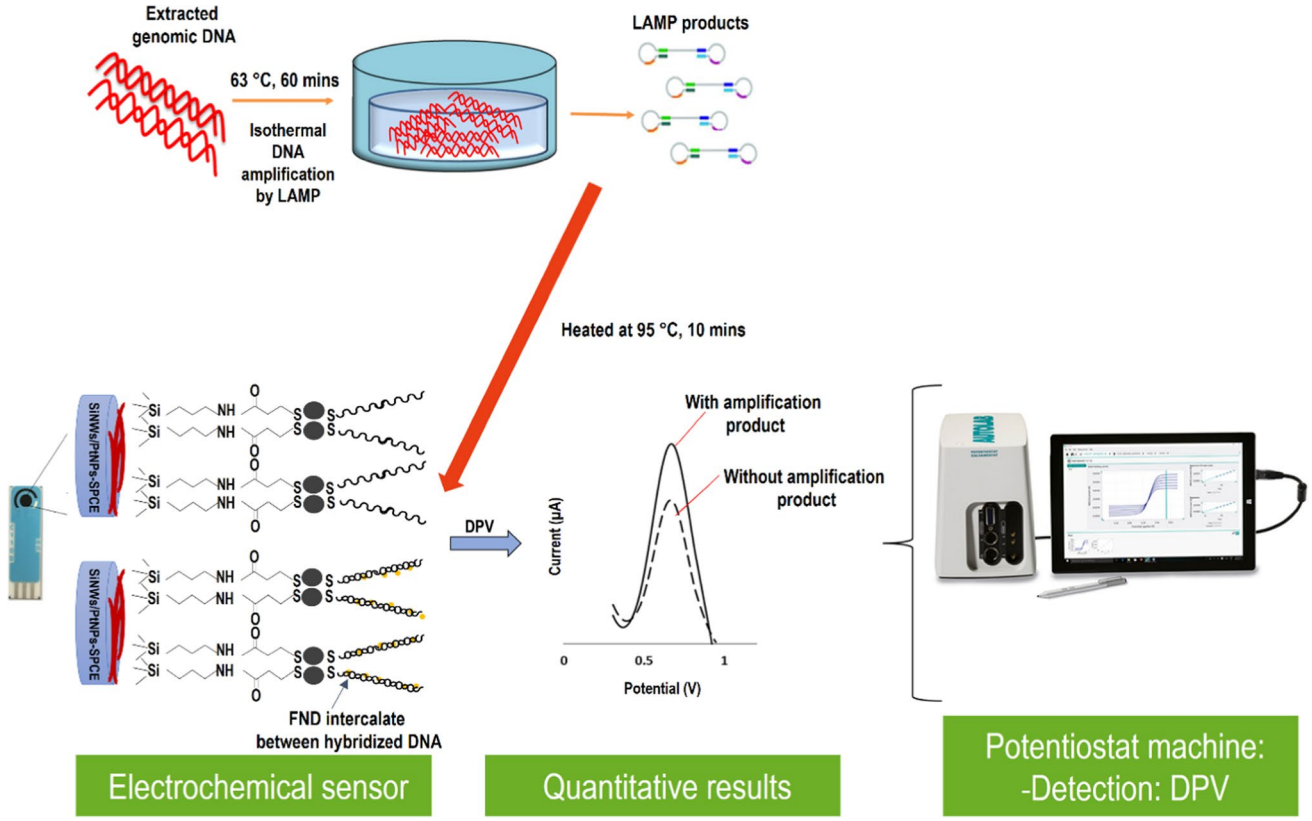


Fig. 1 Schematic diagram of LAMP amplicon detection using DPV method

potential range of −0.5 V to 0 V. The electrode was rinsed with Tris-HCl buffer to eliminate excess FND, and the modulation amplitude was adjusted to 0.5 V. The time interval was 0.64 seconds, and the measured step potential was 0.0005 volts. Figure 1 illustrates the schematic diagram of LAMP amplicon detection.

Results and Discussion

LAMP Primer Design

The LAMP assay was successfully implemented using the primer sets described in the Materials and Methods section. These primers facilitated highly specific and efficient amplification of *Sus scrofa* mitochondrial DNA, thereby ensuring the assay's sensitivity and reliability across all experiments. As detailed in Table 1, the primer set consisted of two outer primers (F3 and B3), two inner primers (FIP and BIP), and one loop primer (LF), all designed to target the mitochondrial genome (GenBank accession no. AJ002189.1). The strategic combination of these primers contributed to robust strand displacement and effective target DNA amplification, which are critical for the high performance observed in this study.

Table 1 A set of five primers was designed for LAMP, targeting the complete mitochondrial DNA of Sus scrofa (GenBank accession no. AJ002189.1). This set consists of two outer primers (F3 and B3), two inner primers (FIP and BIP), and one forward loop primer (LF)

LAMP Primer	DNA Sequences
F3	5'GATACCCCACTATGCCTAGC-3'
B3	5'ATTGTGCTTACTATTGTTCCTT-3'
FIP	5'AAGTCCTTTGAGTTTTAGGCAG T-CCAAATAGTTACATAACA-3'
BIP	5'TAATCGATAAACCCCGATAGACC T-GGGTTTGCTGAAGATGGC-3'
LF	5'TGCGAGTAGTACTCTGGCGAAT-3'

Optimization of LAMP Parameters (Amplification Temperature and Incubation Time)

Precipitate formation in the LAMP reaction is demonstrated by the subsequent reactions:

$$(\mathrm{DNA})_{n-1} + \mathrm{dNTP} \rightarrow (\mathrm{DNA})_n + {P_2O_7}^{4-} \quad (1)$$

$${P_2O_7}^{4-} + 2\mathrm{Mg}^{2+} \rightarrow \mathrm{Mg_2P_2O_7} \quad (2)$$

DNA polymerase generates pyrophosphate ions as a byproduct from dNTPs during the DNA polymerization process [10]. In the LAMP reaction buffer, these pyrophosphate ions

react with magnesium ions in substantial quantities, resulting in the formation of a precipitate. Detailed chemical and spectroscopic analyses identified this precipitate as magnesium pyrophosphate. The accumulation of magnesium pyrophosphate, which causes turbidity, is directly proportional to the amount of amplified DNA produced. This turbidity serves as a visible indicator of amplicon presence, providing an optical signal that is highly useful for real-time monitoring in LAMP assays. As amplification proceeds and more DNA strands are synthesized, the turbidity of the reaction mixture visibly increases, indicating the rising number of amplicons.

To enhance the efficiency and specificity of the LAMP assay, we investigated two critical parameters: amplification temperature and time. Optimizing these parameters was essential for refining the LAMP assay's performance. Reactions were carried out at different temperatures—specifically 60, 63, 65, and 68 °C—to identify the optimal temperature for achieving the fastest positive reaction. Experimental results identified 63 °C as the ideal temperature for the LAMP assay, yielding the maximum FND redox peak current (33.9 µA), which indicates effective hybridization at this precise temperature (Fig. 2a and b). This temperature setting not only facilitated rapid and efficient amplification but also significantly enhanced reaction kinetics when using 1 ng/µL of synthetic porcine oligonucleotide DNA as the target template. This discovery is consistent with the report by Zhang et al. [20], which determined that 63 °C is the optimal temperature for the LAMP amplification of *Toxoplasma gondii* DNA. Similarly, Ahmed et al. [4] employed 63 °C in their LAMP reaction for DNA-H33258 to detect and differentiate meat species based on DNA analysis. The substantial reduction in signal observed at 68 °C can be attributed to the reduced activity or denaturation of the Bst DNA polymerase at higher temperatures, which leads to inefficient or failed amplification. Additionally, elevated temperatures may destabilize primer-template binding, further impair the LAMP reaction and resulting in a significant reduction in signal output.

The effect of amplification time on the LAMP process is shown in Fig. 3. This study investigated optimization over five distinct time intervals: 20, 30, 40, 50, and 60 minutes. The visual turbidity of the LAMP amplicons was monitored, as shown in Fig. 3a, and the samples were subsequently

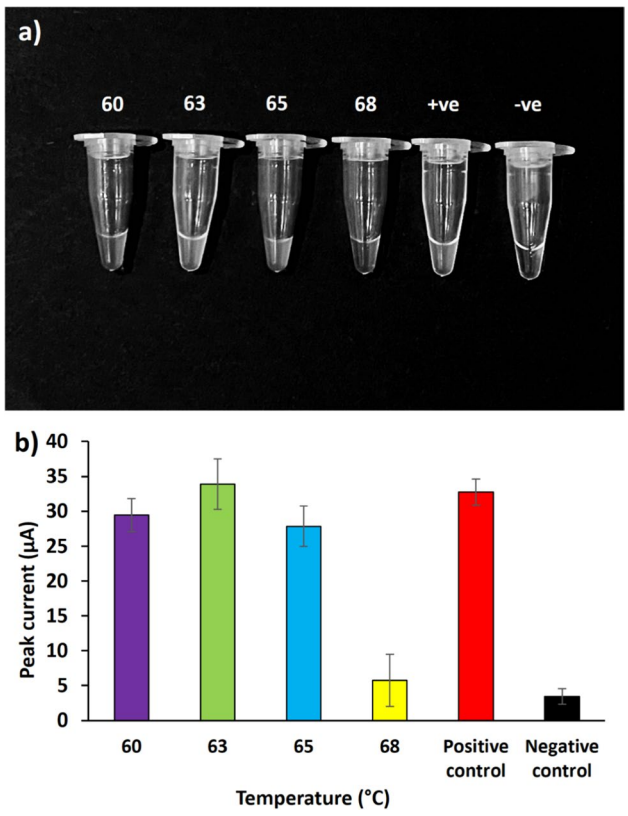


Fig. 2 Effect of different temperature for a 60-minute LAMP assay. **a**) Visual turbidity (the increased turbidity at 63 °C indicating successful amplification) and **b**) Bar chart represent FND peak current of LAMP temperature in 50 mM Tris-HCl containing 1.0 M NaCl (pH 7.6) using differential pulse voltammetry (DPV) measurement (n=3). A synthetic porcine oligonucleotide served as the positive control, while a no template reaction was used as the negative control

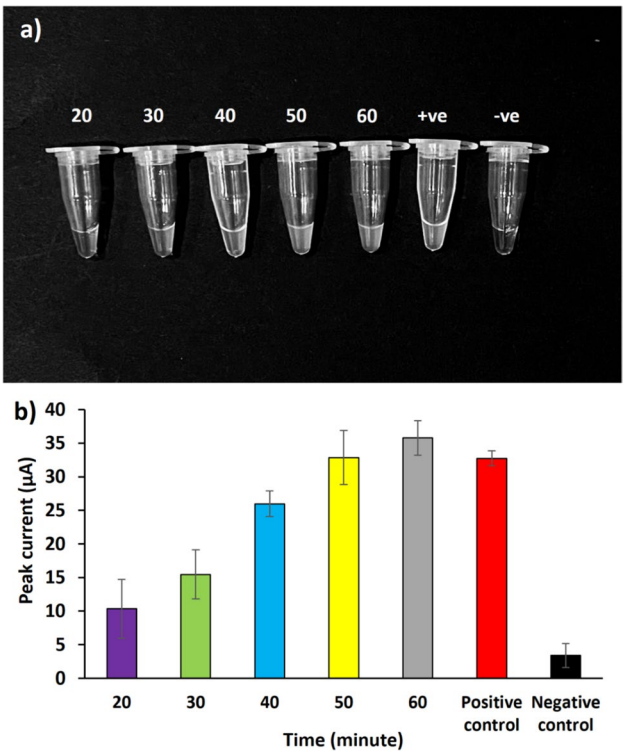


Fig. 3 Effect of different amplification time at 63 °C for LAMP assay. **a**) Visual turbidity and **b**) Bar chart represent FND peak current of LAMP amplification time in 50 mM Tris-HCl containing 1.0 M NaCl (pH 7.6) using differential pulse voltammetry (DPV) measurement (n=3). A synthetic porcine oligonucleotide served as the positive control, while a no template reaction was used as the negative control

quantified using a specially developed DNA biosensor. The results, presented in Fig. 3b, indicate that the optimal amplification time is 60 minutes. This is demonstrated by the highest peak current observed, which directly correlates with the maximum level of hybridization detected in the porcine DNA samples. Although the optimal time was identified as 60 minutes, this duration is still significantly shorter and more practical compared to conventional PCR methods, which typically require 90–120 minutes and sophisticated thermal cycling equipment. LAMP offers a simplified and time-effective alternative, making it suitable for rapid field testing and point-of-need diagnostics. Table 2 compares the various techniques.

Similar optimization studies have been reported in literature. For example, Zhang et al. [20] optimized the amplification time for a LAMP assay targeting *Toxoplasma gondii* DNA, concluding that a 60-minute reaction time provided the best balance between sensitivity and specificity. Their findings are consistent with our results, reinforcing the importance of optimizing reaction conditions to achieve reliable DNA amplification.

Kono et al. [11] investigated the impact of different amplification times on the performance of a LAMP assay developed for detecting white spot syndrome virus in shrimp. They found that a 60-minute reaction time significantly enhanced sensitivity, which aligns with our observations that a 60-minute amplification period is optimal for porcine DNA detection. These studies collectively emphasize the importance of carefully calibrating amplification times to achieve the highest levels of accuracy and efficiency in LAMP assays.

Both the amplification time and temperature optimization results highlight the practicality of using LAMP for field testing applications. The ability of LAMP to perform DNA amplification under isothermal conditions at 63 °C within a relatively short 60-minute timeframe demonstrates its efficiency and simplicity. These characteristics are achieved using basic equipment, such as a water bath or a simple heating device, making LAMP a viable option for on-site testing where advanced laboratory infrastructure is unavailable [15].

Table 2 Comparison of various amplification techniques

Method	Amplification Time	Detection Type	Reference
Conventional PCR	90–120 min	Gel electrophoresis	Standard method
qPCR	60–90 min	Fluorescence-based	Real-time platforms
Colorimetric LAMP	30–60 min	Visual/turbidity	Ran et al., [17]
LAMP (This study)	60 min	Turbidity + Electrochemical (FND)	Current work

Table 3 Purity of extracted DNA determined by NanoDrop™ 2000 spectrophotometer

Food sample	DNA concentration (mg/mL)	DNA purity (A_{260}/A_{280})
Raw pork meat	0.187	1.80
Pork sausage	0.309	1.84
Canned pork	0.130	1.89
Raw chicken meat	0.089	1.89
Raw beef meat	0.430	1.84
Raw lamb meat	0.348	1.87

DNA Extraction from Food Samples

Genomic DNA was successfully extracted from all tested raw and processed food samples using the DNeasy mericon Food Kit, following the manufacturer's standard protocol without modification. The extracted DNA showed high purity, with A260/A280 ratios ranging from 1.80 to 1.89 (see Table 3), indicating minimal protein contamination and suitability for downstream LAMP amplification [8].

Mitochondrial sequences from these DNA samples were consistently amplified using the designated loop primer pairs. The mitochondrial 12S rRNA genes were targeted for species identification due to their appropriate length and high mutation rate, supporting accurate discrimination among meat types. These results confirm the reliability of the extraction method and its compatibility with subsequent molecular analyses. Previous studies have demonstrated the effectiveness of LAMP-based assays for the detection of porcine DNA, with methods targeting the D-loop region [10], and the RealAmp and LAMP approaches have shown high sensitivity, detecting porcine mitochondrial DNA at levels as low as 1 pg and 0.5 pg, respectively [17, 19].

Cross-Reactivity Study using Various Food Samples

Cross-reactivity studies are crucial for assessing the success of isothermal amplification. This study aimed to identify potential interference from non-target substances that might be present in the samples. Such interference can result from structural similarities, chemical interactions, or unintended binding of the assay components to substances other than the target. Figure 4a illustrates the visual turbidity due to the formation of magnesium pyrophosphate, which correlates with the quantity of amplified DNA products.

The developed biosensor was then used to quantify the LAMP amplicons. The FND peak current response of the biosensor (SPCE/SiNWs-PtNPs/ssDNA) after hybridization with various types of LAMP amplicons from raw pork meat, pork sausage, canned pork, raw beef meat, raw lamb meat, and raw poultry meat is illustrated in Fig. 4b. Compared

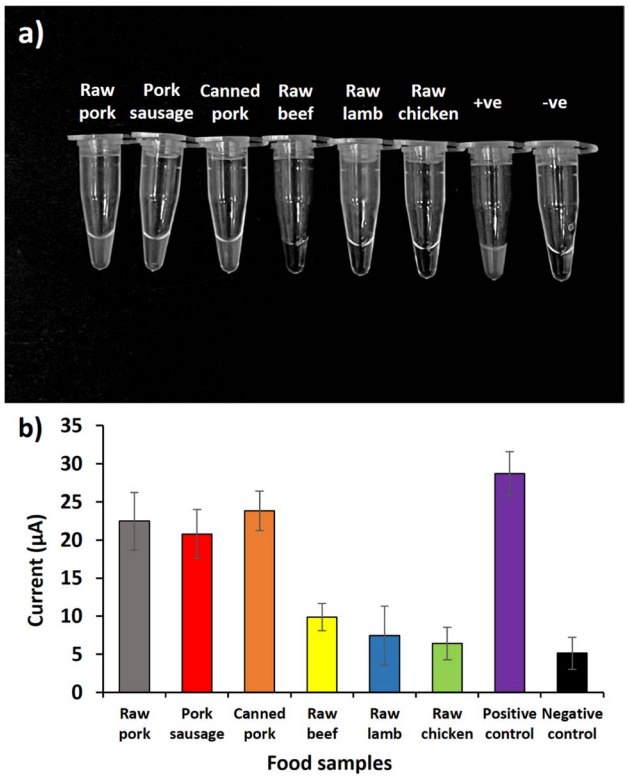


Fig. 4 Cross-reactivity study of the developed DNA biosensor towards different food samples. **a**) Visual turbidity and **b**) Bar chart represent the FND redox peak current of food samples in 50 mM Tris-HCl containing 1.0 M NaCl (pH 7.6) at the potential range −0.5 to 0 V, step potential 0.0005 V and modulation amplitude 0.5 V (n=3). A synthetic porcine oligonucleotide served as the positive control, while a no template reaction was used as the negative control

to the negative control (ssDNA probe), the DPV response for raw beef meat, raw lamb meat, and raw chicken meat did not exhibit any significant alterations, suggesting that no DNA hybridization occurred. This outcome is consistent with the absence of precipitation during the LAMP process. Nevertheless, the FND peak current increased when the capture DNA probe was exposed to fresh pork meat, pork sausage, and canned pork, with peak currents of 22.5 µA, 20.8 µA, and 23.9 µA, respectively. This result showed that FND has a high-affinity interaction with double-stranded DNA surfaces, resulting in a high FND redox current. The developed biosensor was only able to detect the Sus scrofa mtDNA targeted sequence.

Previous studies have corroborated the specificity and reliability of LAMP assays in detecting species-specific DNA. For instance, Cho et al. [6] successfully developed a LAMP assay to identify and discriminate eight meat species, demonstrating excellent target discrimination and detection efficiency, making it suitable for rapid diagnostic applications. Similarly, Yang et al. [19] reported a real time loop-mediated isothermal amplification (RealAmp) method for identifying of pork in meat products based on mitochondrial DNA sequences, which could differentiate between various meats (cattle, sheep, chicken and duck) with high accuracy.

To confirm these findings, the LAMP amplicons from all samples were further validated using agarose gel electrophoresis. As shown in Fig. 5, the LAMP amplification products displayed a characteristic ladder pattern, which is distinct from the single discrete band typically seen in conventional PCR. This pattern results from the formation of branched DNA structures, caused by the annealing of inverted repeat regions within the same strand and the generation of multiple loop formations. The genomic DNA extracted from the porcine samples (S1, S2, S3) was effectively amplified by the LAMP primers, whereas no visible bands were observed for beef, lamb, or chicken samples (S4, S5, S6), indicating high specificity of the assay.

A notably intense lowest band was observed in the positive samples but was absent in the negative control (C2). This bright band likely represents the accumulation of smaller LAMP amplification products, which are a common

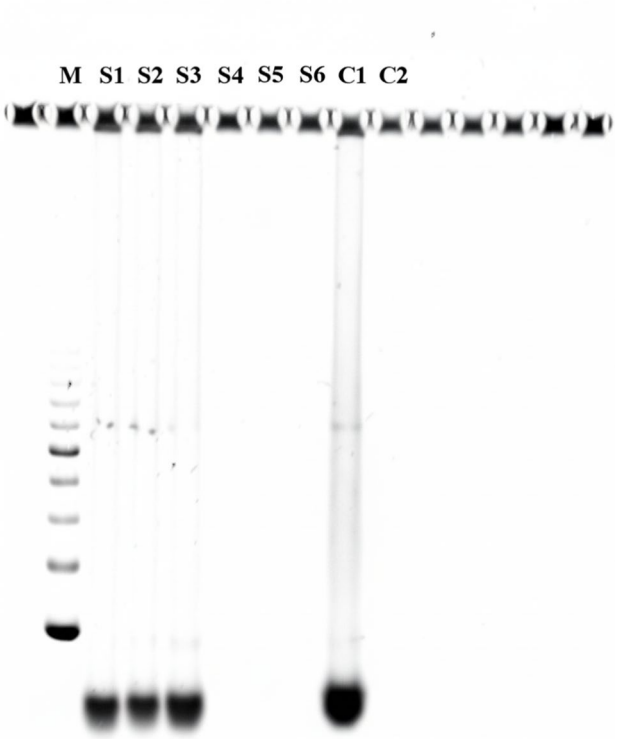


Fig. 5 Enhanced agarose gel image with a light background, highlighting LAMP-amplified products from various samples: raw pork, pork sausage, canned pork, raw beef, raw lamb, and raw chicken (labelled S1 to S6). A synthetic porcine oligonucleotide served as the positive control (C1), while a no template reaction was used as the negative control (C2). Each reaction used 1 µg of extracted DNA. M indicated the 100 bp molecular weight marker

feature of the LAMP reaction due to its mechanism of generating various sizes of stem-loop DNA structures. The absence of this band in the negative control suggests it is not the result of non-specific amplification, primer-dimer formation, or experimental artifacts, thereby supporting the specificity and reliability of the assay.

The use of a synthetic porcine oligonucleotide as a positive control (C1) and the absence of amplification in the negative control further validate the experimental procedure. These results are consistent with the electrochemical detection findings, providing strong evidence for the specific detection of *Sus scrofa* DNA. The parallel validation by both agarose gel electrophoresis and electrochemical methods demonstrates the robustness and accuracy of the developed DNA sensing approach.

Validation of Developed DNA Biosensor Specificity and Capability

In order to verify the specificity and efficacy of the DNA biosensor detection system that was developed, a study was conducted using binary raw meat mixtures that contained variable proportions of chicken and pork. The aim of this study was to assess the system's ability to accurately identify and amplify target DNA from mixed samples, thereby imitating real-world situations where precise species discrimination is crucial.

Figure 6 illustrates the use of the designated LAMP primer combination, specifically designed for pork DNA amplification, in this study. Remarkably, the results demonstrated an exceptional level of specificity. These LAMP primers exhibited distinct behaviour when applied to binary meat mixtures with different pork-to-chicken ratios. Figure 6a visually showcases the notable turbidity patterns observed in the binary meat mixtures, providing a clear contrast between samples containing pork and those composed entirely of chicken meat. When pork was present in the mixture, the turbidity levels showed noticeable changes, creating a visual effect that distinguished between the two scenarios. Conversely, no changes in turbidity or formation of precipitates were observed in samples consisting solely of chicken meat. To further validate these observations, statistical analysis was performed on the peak current values obtained from the electrochemical measurements (Fig. 6b). One-way ANOVA followed by post hoc multiple comparisons revealed statistically significant differences ($p < 0.05$) between samples containing pork (samples 2–7) and those without pork (samples 1 and 8). These differences are indicated in Fig. 6b by asterisks above the data points. The results confirm that the LAMP assay exhibits a significant increase in electrochemical signal in the presence of pork DNA compared to chicken-only or negative control

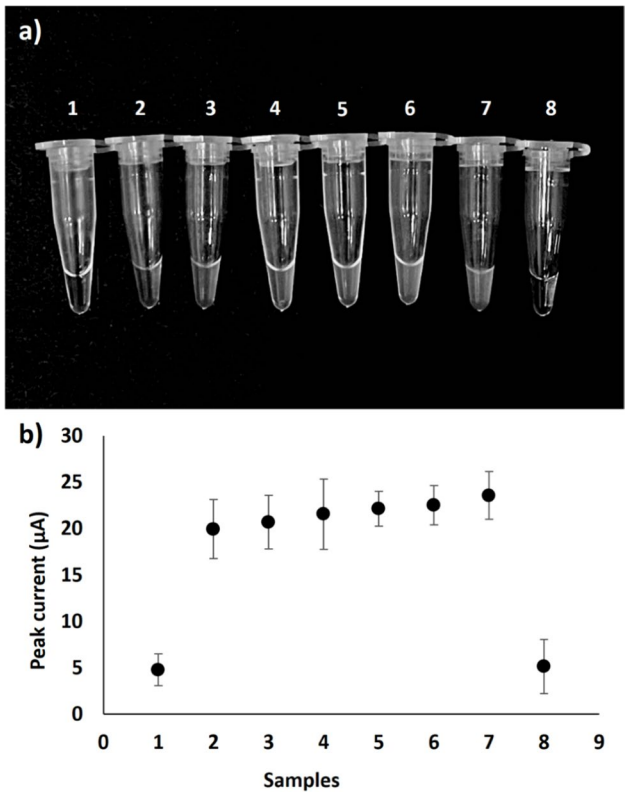


Fig. 6 **a**) Visual turbidity and **b**) Electrochemical redox peak current responses of LAMP amplicons obtained from isolated DNA of pork-chicken binary mixtures, amplified using a loop primer set specific to pork DNA. Samples 1–8 represent the following compositions: 1) 0% pork/100% chicken, 2) 20% pork/80% chicken, 3) 40% pork/60% chicken, 4) 60% pork/40% chicken, 5) 80% pork/20% chicken, 6) 100% pork/0% chicken, 7) 100% porcine synthetic oligonucleotide DNA (reference control), and 8) negative control (LAMP without any template DNA). Statistically significant differences ($p < 0.05$) between sample groups are indicated by asterisks above the corresponding data points in Figure 6b

samples, further supporting the specificity and sensitivity of the developed method.

The LAMP amplicons were then quantified using the DNA biosensor, applying the calibration curve and validation procedures that were previously developed and published in our earlier work [12]. This established and validated calibration curve served as a reference for quantitative evaluation in the present study. Notably, the LAMP primers did not produce any amplification signal in the 0% pork and 100% chicken binary mixtures, which effectively supported the absence of false positives and negligible cross-reactivity with chicken DNA. The binary mixtures that contained a minimum quantity of pork DNA produced the most compelling validation, specifically at the 20% pork composition (19.9 µA) (Fig. 6b).

In this scenario, the designated LAMP primers successfully amplified the DNA within the mixture, clearly demonstrating the biosensor's sensitivity in detecting even

small traces of pork-specific target DNA. The detection of pork DNA in the mixed samples was further substantiated by the observed electrochemical responses, displayed as a significant increase in the FND redox current. This distinctive electrochemical signal serves as a direct indicator of substantial amplicon generation when pork-specific target DNA was present within the binary mixtures. While visual differences in turbidity were apparent (Fig. 6a), the electrochemical peak current measurements in Fig. 6b showed no significant statistical difference between the various pork-chicken mixtures. This suggests that the electrochemical method may be effective for qualitative detection (presence/absence) of pork DNA but may not precisely quantify the relative concentration of pork in mixed samples.

These findings are consistent with previous studies that have utilized LAMP for species-specific DNA detection in mixed samples. For instance, Abdulmawjood et al. [3] demonstrated the effectiveness of a LAMP assay in detecting ostrich DNA in meat products, even at low contamination levels. Their research highlighted the high sensitivity and specificity of LAMP in identifying meat adulteration, similar to our results with porcine DNA detection in binary meat mixtures. Similarly, Abdullahi et al. [2] developed a LAMP assay to detect pork DNA in mixed meat samples (pork and cattle beef), achieving reliable differentiation between target and non-target species, thereby confirming the robustness of LAMP for food authenticity testing. Furthermore, the integration of the LAMP method with an electrochemical biosensor in our study parallels the work of Jaroenram et al. [9], who employed a LAMP-electrochemical biosensor system to detect *Mycobacterium tuberculosis* in sputum samples. Their study demonstrated the system's rapid and reliable detection capabilities, essential for timely clinical safety interventions. Our results corroborate these findings, emphasizing the practicality of using an integrated LAMP-biosensor system for on-site applications, where rapid and accurate species identification is critical.

Conclusions

This research reports the successful development and optimization of a Loop-Mediated Isothermal Amplification (LAMP) method for rapid and sensitive porcine DNA detection. The carefully designed primer sets, targeting specific regions of the *Sus scrofa* mitochondrial DNA, exhibited strong target selectivity and reliable amplification performance, positioning LAMP technique as an ideal choice for this application. The amplification parameters, including temperature and time, were meticulously optimized to ensure maximum sensitivity and reliability, with optimal results observed at 63 °C over a 60-minute incubation period. Our findings indicate that the formation of a white insoluble byproduct during loop-mediated isothermal amplification serves as a dependable visual and optical signal for detecting the target DNA. The optimized LAMP method, coupled with an advanced DNA biosensor based on silicon nanowires and platinum nanoparticles, enabled precise quantification of the amplified products. The biosensor exhibited high sensitivity, detecting porcine DNA at very low concentrations, and demonstrated exceptional specificity, with no cross-reactivity observed with DNA from other meat species. The validation of the DNA biosensor using binary raw meat mixtures further underscored its capability to accurately identify and quantify pork DNA in complex samples. The distinct turbidity patterns and electrochemical responses provided clear differentiation between samples containing pork and those composed solely of chicken meat. This specificity was maintained even at low concentrations of pork DNA, highlighting the biosensor's potential for real-world applications in food authenticity testing and species identification.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10876-026-03041-y.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and Consent to Participate Not applicable

Competing Interests The authors declare no competing interests.

References

1. U. Abdullahi, E. Igwenagu, S. Aliyu, A. Mu'azu, R. Naim, and W. R. Wan-Taib (2017). A rapid and sensitive Loop-mediated isothermal amplification assay for detection of pork DNA based on porcine tRNA lys and ATPase 8 genes. *Int Food Res J* **24** (4), 1357–1361.
2. U. Abdullahi, R. Naim, W. R. Wan-Taib, A. Saleh, A. Muazu, S. Aliyu, and A. A. Baig (2015). Loop-mediated isothermal amplification (LAMP), an innovation in gene amplification: bridging the gap in molecular diagnostics; a review. *Ind J Sci Technol* **8** (17), 1–12. https://doi.org/10.17485/ijst/2015/v8i17/55767.
3. A. Abdulmawjood, N. Grabowski, S. Fohler, S. Kittler, H. Nagengast, and G. Klein (2014). Development of loop-mediated isothermal amplification (LAMP) assay for rapid and sensitive identification of ostrich meat. *PLoS one* **9** (6), e100717. https://doi.org/10.1371/journal.pone.0100717.
4. M. U. Ahmed, Q. Hasan, M. Mosharraf Hossain, M. Saito, and E. Tamiya (2010). Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor. *Food Control* **21** (5), 599–605. https://doi.org/10.1016/j.foodcont.2009.09.001.
5. S. Bourguiba-Hachemi and M. D. Fathallah (2016). DNA testing of meat foods raises issues beyond adulteration. *Sky J Food Sci* **5** (1), 1–7.
6. A. R. Cho, H. J. Dong, and S. Cho (2014). Meat species identification using loop-mediated isothermal amplification assay targeting species-specific mitochondrial DNA. *Korean J Food Sci Anim Resour* **34** (6), 799–807. https://doi.org/10.5851/kosfa.2014.34.6.799.
7. Farag MDEDH (2020) Detecting adulteration in halal foods. In: Al-Teinaz YR, Spear S, Abd El-Rahim IHA (eds.) The Halal Food Handbook. pp. 283-319. https://doi.org/10.1002/9781118823026.ch18
8. Gautam A (2022) DNA and RNA Isolation techniques for non-experts. Cham, Switzerland: Springer, Print.
9. W. Jaroenram, J. Kampeera, N. Arunrut, C. Karuwan, A. Sappat, P. Khumwan, S. Jaitrong, K. Boonnak, T. Prammananan, A. Chaiprasert, A. Tuantranont, and W. Kiatpathomchai (2020). Graphene-based electrochemical genosensor incorporated loop-mediated isothermal amplification for rapid on-site detection of Mycobacterium tuberculosis. *J Pharm Biomed Anal* **186**, 113333. https://doi.org/10.1016/j.jpba.2020.113333.
10. P. Kanchanaphum, S. Maneenin, and W. Chaiyana (2014). Analysis of pork meat using loop mediated isothermal amplification (LAMP) to confirm halal status. *Int J Biosci* **4** (9), 62–68. https://doi.org/10.1016/j.jpba.2020.113333.
11. T. Kono, R. Savan, M. Sakai, and T. Itami (2004). Detection of white spot syndrome virus in shrimp by loop-mediated isothermal amplification. *J Virol Methods* **115** (1), 59–65. https://doi.org/10.1016/j.jviromet.2003.09.015.
12. N. Kusnin, N. A. Yusof, J. Abdullah, S. Sabri, F. Mohammad, S. Mustafa, N. A. Ab Mutalib, S. Sato, S. Takenaka, N. A. Parmin, and H. A. Al-Lohedan (2020). Electrochemical sensory detection of Sus scrofa mtDNA for food adulteration using hybrid ferrocenylnaphthalene diimide intercalator as a hybridization indicator. *RSC Adv* **10** (46), 27336–27345. https://doi.org/10.1039/d0ra03585h.
13. Hardianto A. Muflihah, P. Kusumaningtyas, S. Prabowo, and Y. W. Hartati (2023). DNA-based detection of pork content in food. *Heliyon* **9** (3), e14418. https://doi.org/10.1016/j.heliyon.2023.e14418.
14. M. Muhammad, A. Elistina, and S. O. Ahmad (2020). The challenges faced by halal certification authorities in managing the halal certification process in Malaysia. *Food Res.* **4** (1), 170–178. https://doi.org/10.26656/fr.2017.4(S1).S17.
15. T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* **28** (12), e63–e63. https://doi.org/10.1093/nar/28.12.e63.
16. K. Ptitsyn, S. Khmeleva, L. Kurbatov, O. Timoshenko, E. Suprun, S. Radko, and A. Lisitsa (2024). Lamp primer designing software: The overview. *Biomed Chem RM* **7** (4), e00226. https://doi.org/10.18097/BMCRM00226.
17. G. Ran, L. Ren, X. Han, X. Liu, Z. Li, and D. Pang (2016). Development of a rapid method for the visible detection of pork DNA in halal products by loop-mediated isothermal amplification. *Food Anal Methods* **9**, 565–570. https://doi.org/10.1007/s12161-015-0246-z.
18. J. Wang, S. Kaur, A. Kayabasi, M. Ranjbaran, I. Rath, I. Benschikovski, B. Raut, K. Ra, N. Rafiq, and M. S. Verma (2024). A portable, easy-to-use paper-based biosensor for rapid in-field detection of fecal contamination on fresh produce farms. *Biosens Bioelectron* **259**, 116374. https://doi.org/10.1016/j.bios.2024.116374.
19. L. Yang, S. Fu, X. Peng, L. Li, T. Song, and L. Li (2014). Identification of pork in meat products using real-time loop-mediated isothermal amplification. *Biotechnol Biotechnol Equip* **28** (5), 882–888. https://doi.org/10.1080/13102818.2014.963789.
20. H. Zhang, O. M. Thekisoe, G. O. Aboge, H. Kyan, J. Yamagishi, N. Inoue, Y. Nishikawa, S. Zakimi, and X. Xuan (2009). Toxoplasma gondii: sensitive and rapid detection of infection by loop-mediated isothermal amplification (LAMP) method. *Exp Parasitol* **122** (1), 47–50. https://doi.org/10.1016/j.exppara.2009.01.012.

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