Food Control 88 (2018) 9–14

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Colourimetric detection of swine-specific DNA for halal authentication using gold nanoparticles

Zhenyun He [a, b, c], Hongshun Yang [a, b, *]

[a] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China
[c] Fujian Putian Sea-100 Food Co., Ltd, Putian, Fujian 355000, PR China

ARTICLE INFO

Article history:
Available online 2 January 2018

Keywords:
Colourimetric
DNA detection
Halal authentication
Food adulteration
Nanotechnology
Nanoparticle
Pork
Swine
Hazard detection
Food safety
Protein
Thin layer chromatography
Oligonucleotides
Rapid detection
Meat
Immunodetection

ABSTRACT

Detecting pork contamination is critical for halal food preparation. A fragment of species-specific DNA from swine mitochondrial genome was selected as a target, and two oligonucleotides complementary to the target were used as probes for pork identification. The probe-conjugated gold nanoparticles bound to the target DNA, leading to the aggregation of gold nanoparticles and thus causing the colour of the gold nanoparticles to change from red to blue. More than 0.1 μmol/L of DNA led to an obvious colour change, which was observed by naked eyes after dotting test solutions onto a thin layer chromatography (TLC) plate. Furthermore, 0.001−1 μmol/L of the target was semi-quantified by using a digital camera and image processing software. This colourimetric detection might be a promising technique for differentiation of pork from other animal meat products without using any sophisticated machines, and thus will be used for halal authentication.

1. Introduction

As of 2010, there were 1.6×10^9 Muslims around the world, which make up a quarter of the global population, and the number keeps increasing year by year (Hackett et al., 2015). As the rising population of Muslims, the halal food markets are growing unprecedentedly, and the global halal industry is estimated to be worth more than 1.6×10^{12} dollars (Lever & Miele, 2012; Lubis, Mohd-Naim, Alizul, & Ahmed, 2016; Sow et al., 2017). With halal foods becoming more available, the authenticity of the halal food has become a serious concern for the consumers (Feng, Fu, & Yang, 2017; Lubis et al., 2016; Pang, Deeth, Yang, Prakash, & Bansal, 2017). Especially, food adulteration by substituting beef with pork meat is one of the issues that irritate customers. This problem is particularly severe for Muslims live in non-Muslim countries where halal-certified foods are not easily available. To protect the Muslim consumers, the need for halal authentication is even greater today.

Currently, there are several food authentication methods for species identification, which can be categorised into lipid-based, protein-based and DNA-based methods (Alonso-Rebollo, Ramos-Gómez, Busto, & Ortega, 2017; Ballin, 2010; González, Pablos, Martın, León-Camacho, & Valdenebro, 2001; Lockley & Bardsley, 2000; Rao & Hsieh, 2014; Rao, Richt, & Hsieh, 2016; Rubert, Lacina, Zachariasova, & Hajslova, 2016). The methods include chromatographic analysis, immunological assays and DNA profiling, respectively. Gas chromatography and liquid chromatography are powerful techniques to separate and identify different components, and they have been used to detect pork and horse meat in halal food (von Bargen, Dojahn, Waidelich, Humpf, & Brockmeyer, 2013). Specific immunological assays such as enzyme linked immunological assay (ELISA) have been used to

* Corresponding author. Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Republic of Singapore.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.foodcont.2018.01.001
0956-7135/

detect species-specific and heat-stable proteins. However, specific antibody is needed for the detection (Asensio, González, García, & Martín, 2008; Rao et al., 2016). Moreover, proteins in raw meats can be modified after processing or cooking in some situations (Marikkar, Ng, & Man, 2011). Nucleic acids especially short fragments of DNA are normally can be detected in raw material as well as in processed food. It should be noted that no method has gained supremacy. The application will depend on the practical scenario to determine which method could be applied in food industry.

DNA profiling or fingerprinting is a prevailing technique to identify the species or individuals by their features of DNA, which is widely used for parentage, criminal investigation, animal pedigree confirmation, and disease diagnosis. An increasing number of DNA fingerprinting methods to analyse short tandem repeats (STR) and variable number of tandem repeats (VNTR) have been developed and more and more DNA index databases have been created (Jeffreys, Wilson, & Thein, 1985; Tautz, 1989). Polymerase Chain Reaction and other molecular technologies aid in the detection methods for food contamination (Li & Mustapha, 2004). However, STR or VNTR analysis usually needs long amplicons that are difficult to be recovered from extremely degraded samples (Ali, Hashim, Kashif, Mustafa, Man, & Hamid, 2012). Alternatively, mitochondrial genes are proven to be more appropriate in differentiating species in food matrix (Dalmasso, Civera, La Neve, & Bottero, 2011). Nonetheless, most of the methods are not only complicated and time-consuming, but also need technical specialists and expensive instruments (Ali, Hashim, Mustafa, Man, Yusop, Bari, et al., 2011). Even though those methods are worthwhile for criminal investigation or disease diagnosis, they are rarely used in food to identify the animal source due to the limitations.

Nanotechnology has been extensively used in medicine and food analyses (Dong et al., 2016; González-Sálamo, Socas-Rodríguez, Hernández-Borges, & Rodríguez-Delgado, 2017; Hou et al., 2016; Nguyen, Vardhanabhuti, Lin, & Mustapha, 2017). One of the applications is based on a unique optical property of metallic nanoparticles that the wavelength of light absorbed by nanoparticles relies on the shape, size and agglomeration state of the nanoparticles (Mody, Siwale, Singh, & Mody, 2010). By modifying these particles with functional oligonucleotides, DNA reactions can be indicated by the different colours of the nanoparticles without using a detector. The combination of DNA detection and gold nanoparticles was first introduced in 1997 (Elghanian, Storhoff, Mucic, Letsinger, & Mirkin, 1997). After then, a variety of colourimetric detection modes using gold nanoparticles were developed for DNA as well as proteins (Li & Rothberg, 2004; Nam, Jang, & Groves, 2007; Storhoff, Lucas, Garimella, Bao, & Müller, 2004). A few studies have addressed the application of gold nanoparticles in halal authentication (Ali, Hashim, Mustafa, Man, & Islam, 2012; Ali, Hashim, Mustafa, Man, Yusop, Bari, et al., 2011). These methods detected the presence of single stranded probes, and were based on salt-induced aggregation of nanoparticles. Therefore any single stranded DNA or RNA in the sample would interfere with the target detection, which would cause poor sensitivity and specificity.

The aim of this study was to develop a rapid and simple DNA detection method for halal verification. In this work, the nanotechnology combined with a species-specific DNA was used to achieve a colourimetric analysis for DNA identification. The result could be analysed by naked eyes without a need for specialised technicians or sophisticated instruments. The work focused on the pork identification has a potential to be used for other food adulteration issues such as undeclared horsemeat or mislabelled seafood.

2. Materials and methods

2.1. Chemicals and oligonucleotides

All chemicals and solvents used in this work were of analytical reagent grade or higher. The oligonucleotides listed in Table 1 were synthesised by the Integrated DNA Technologies, Singapore.

The swine-specific target DNA fragment was selected according to a previous research (Ali, Hashim, Kashif, Mustafa, Man, & Hamid, 2012). The target DNA was a 24-bp fragment from the mitochondrial genome of *Sus scrofa domesticus* (NCBI Reference Sequence: NC_012095.1). NCBI Nucleotide BLAST analysis against reference genomic sequence database confirmed the sequence was present in pig family and not in some common sources of meat including cattle, chicken, sheep and goat, deer and fish species. It should be noted that we did not examine some other meats like civets and pangolins, as the database is limited.

2.2. Synthesis and characterisation of gold nanoparticles

Approximately 11-nm diameter gold nanoparticles were synthesised by the citrate reduction of $HAuCl_4$ according to Liu and Lu (2006) with slight modifications. Briefly, 50 mL of 1 mM $HAuCl_4$ solution was added into a flask that was connected with a condenser and heated to boil while stirring. When the solution started boiling, 4 mL of sodium citrate solution (38.8 mM) was added. The reaction was heated for extra 20 min after the colour changed from colourlessness to burgundy red. The solution was cooled down to 25 °C under stirring, and then stored in a light-resistant container at 4 °C. Ultrasonic treatment was required prior to use if a small amount of metal plating was observed on the sides or bottom of bottles. The gold nanoparticles were imaged by transmission electron microscopy (TEM) (JEOL JEM-3011, Japan). The concentration of the gold nanoparticles was calculated by measuring the surface plasmon band using UV-Vis spectrometer (Shimadzu UV-2450, Japan) (Liu, Atwater, Wang, & Huo, 2007).

2.3. Preparation of DNA-modified gold nanoparticles

DNA modified nanoparticles were synthesised by conjugating 2 mL of the prepared nanoparticle solution (2.09×10^{-8} mol/L) with 30 μL of 0.1 mM oligonucleotides (probe A or probe B), respectively (Liu & Lu, 2006). The conjugation was reacted in two NaOH-treated glass vials placed on a shaker at room temperature. After shaking for 16 h, 20 μL of tris-acetate buffer (500 mM, pH 8.2) and 200 μL of NaCl solution (1 M) were respectively added dropwise and slowly to each vial. The two vials were kept shaking for another 24 h. The modified gold nanoparticles were obtained by centrifugation and washed with 10 mM pH 7.0 phosphate buffer solution (PBS) twice. The resulting nanoparticles were resuspended in 10 mM PBS and the concentrations were calculated as mentioned

Table 1
The sequences of oligonucleotides used in this work.

Oligonucleotides	Sequence
Probe A	5′-SH-$(CH_2)_6$-A_{10}-CTC GTC TGG TTT-3′
Probe B	5′-CGG GGT ATC TAG-A_{10}-$(CH2)_3$-SH-3′
Target	5′-CTA GAT ACC CCG AAA CCA GAC GAG-3′
Mismatch	5′-CTA GAT ATC CCG AAA CCA GAC GAG-3′
Deletion	5′-AT ACC CCG AAA CCA GAC GAG-3′
False	5′-TAT CGA AGG ACG GCT TTC TGC AGT-3′
Half	5′-GAA CTA CGAGTAAAA CCA GAC GAG-3′

above. The modified gold nanoparticles can be applied freshly or stored at 4 °C within two weeks, keeping in dark place.

2.4. Colourimetric detection and quantification

To capture the target DNA, 4.5 μL of two probes modified gold nanoparticles and 1 μL of DNA samples (target, mismatch, deletion, half match, false match, or digested product) were mixed (10 μL total volume) and incubated for 30 min at 50 °C. One microlitre of the solution was spotted onto a C18 reverse phase TLC plate (Analtech, Newark, DE, USA). The colour changes were observed by naked eyes.

The colours presented on the TLC plate were further semi-quantified by using the colour value (red channel) from the Histogram window of the Photoshop (Adobe, San Jose, CA, USA) (Nam et al., 2007). The dots result was recorded with a digital camera and the digital image could be adjusted using the software. The nanoparticle dots were selected and the areas were quantified with the histogram function on the red channel. The mean value of the colour intensity from the histogram panel was used to quantify the colour of every dot.

2.5. Statistical analysis

Each experiment was conducted at least in triplicates. The red channel value results were reported as mean ± standard deviation from three independent batches. Analysis of variance (ANOVA) and Duncan's test were conducted to determine the differences among groups by SAS software (SAS Institute Inc., Cary, NC, USA). Comparisons with $P < .05$ were considered significant.

3. Results and discussion

3.1. Characterisation of oligonucleotides-conjugated gold nanoparticles

Gold nanoparticles were generated by the citrate synthesis method, and the resulted sol was kept burgundy red during the storage at 4 °C. The morphology of the gold nanoparticles was observed by TEM (Fig. 2a). As shown in the TEM image, the nanospheres were uniformly dispersed in the colloidal suspension. Analysed by using the software ImageJ and Origin, the size distribution of the nanospheres was found to fit a Gaussian curve and the mean diameter of the nanospheres was estimated to be 11.0 ± 0.9 nm (Fig. 2b). Assuming that the density of the gold nanospheres was equal to that of bulk gold, the concentration of the modified gold nanoparticle suspension was calculated as 1.8×10^{-8} mol/L.

The nanoparticle was prepared with either probe A or probe B first. After chemical modification with 5′- or 3′-thiol-capped 22-base oligonucleotides (probe A or probe B), then nanoparticles modified with probe A were mixed with nanoparticles modified with probe B at 1:1 for the test. The poly A chain is the sequence of probe A (specific information is provided in Table 1). Poly B chain was also specifically defined in Table 1. The surface plasmon band (524 nm) of the gold nanoparticles did not shift (Fig. 2c). For probe A and probe B, there were two different 12-mer oligonucleotides both complementary to the target sequence and a 10-base chain of adenine nucleotides as a spacer. The 12-base sequences in the two probes were designed to capture the target DNA whereas the poly(A) chain was used to reduce the steric hindrance between the 12 bases and the gold surface. The poly(A) spacer was proven to significantly improve the hybridisation efficiency probably because the spacer decreased the steric hindrance and the interactions between the bases and the gold surface (Demers et al., 2000). The function of probe B is to increase the specifity of the reaction, as compared to the single-stranded approach. With oligonucleotides conjugated to the gold nanoparticles, the nanoparticles showed high stability (no significant shift in the surface plasmon band within three months) in PBS at pH 7.0 (data not shown), with optimimum storage temperature at 4 °C.

3.2. Detection of synthesised target DNA using modified gold nanoparticles

The colourimetric detection method discussed herein is based on the property of gold nanoparticles which agglomeration triggers a shift in the surface plasmon resonance and leads to a colour change. In this study, both 12-base sequences at the end of probe A and probe B were able to bind to the synthesised target DNA simultaneously (Fig. 1). Accordingly, when enough amount of the target DNA was added into the mixture containing each (probe A and probe B) modified nanoparticles, the colour of the reaction would change from red to purple after 10 min of incubation at 50 °C. The colour change resulted from the DNA-induced agglomeration of gold nanoparticles was evidenced by a red shift in the surface plasmon band from 524 to 556 nm at the presence of 1 μmol/L target DNA (Fig. 2c). Alternatively, the colour change was obviously observed by transferring 1 μL of the reaction solution to a TLC plate, which achieved a visual detection. Different concentrations of the target DNA led to various colours as shown in Fig. 3a. When the concentration of the added target increased to 0.1 μmol/L (equals to 10^{-13} mol), the significant colour shift from red to blue was differentiated by naked eyes. However, the sensitivity was lower than that of another gold nanoparticle-based colourimetric assay where 10 fmol (10^{-14} mol) of DNA could be detected (Elghanian et al., 1997). Elghanian et al. (1997) detected salmon sperm DNA. The sensitivity was very high. However, the working temperature was very narrow, only workable between 58 °C and 58.5 °C, which would be hard for food industry to apply. The current approach we reported had less but good enough sensitivity;

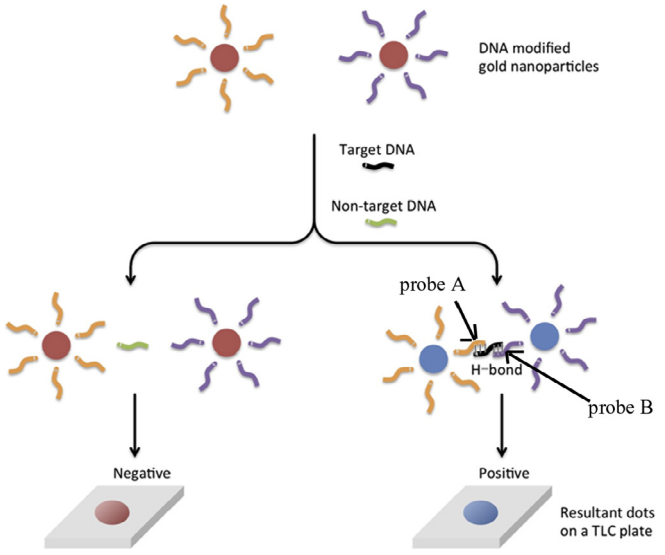

Fig. 1. The schematic of the gold nanoparticle-based colourimetric detection of DNA. The target was a short fragment of DNA from the swine mitochondrial genome, which could be extracted from pork products. The modified gold nanoparticles could bind to the target DNA, leading to the agglomeration and the colour change from red to blue. By transferring the reaction to a TLC plate, the result could be observed and preserved. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

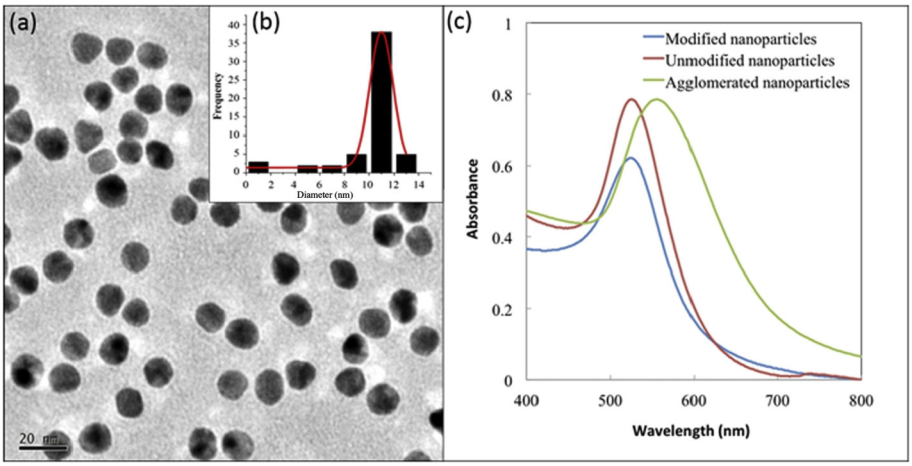

Fig. 2. TEM image (a) and size distribution (b) of the gold nanoparticles with the diameter of 11 ± 0.9 nm; Comparison of UV-Vis spectra (c) for 11 nm diameter of unmodified, modified and agglomerated gold nanoparticles.

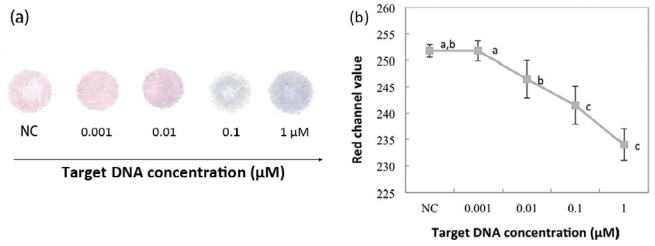

Fig. 3. Colourimetric detection of different concentrations of the target DNA based on gold nanoparticles. (a) Resultant dots on a TLC plate; (b) semi-quantification data. Dots were captured by a camera and the average colour intensity values were obtained with the histogram of the red channel using Adobe Photoshop. Different superscript letters indicate significant difference ($P < .05$) compared with the others as assessed by ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

one great advantage of it was that it worked at a wider temperature range (45 °C-60 °C), though the optimimum temperature was at 50 °C.

There are several approaches to increase the sensitivity. Storhoff el al. (2004) reported that scattered light was able to detect lower concentrations of gold nanoparticles in comparison with absorbed or reflected light, which improved the sensitivity by four orders of magnitude. Besides, a larger nanoparticle is proven to cause a larger colourimetric shift. Larger gold nanoparticles with the diametres of around 24 nm and 50 nm were synthesised. However, the size distributions of larger nanoparticles were less uniform as compared to that of small nanoparticles (data not shown). Generally, larger nanoparticles that have less uniform size distribution lead to lower reproducibility (Nam et al., 2007). To solve this problem, commercial gold nanoparticles with larger diametres and acceptable size distribution might be used in our future work. Considering the limit of detection, 10^{-13} mol of the target DNA is equal to 1 μg of mitochondrial DNA (the length of the pig mitochondrial DNA is 16,679 nucleotides (Ursing & Arnason, 1998)), which can be easily extracted from 10^7 cells that are estimated to be 0.1 g of meat. Therefore, 0.1 g of pork could be detected theoretically by naked eyes.

To quantify the colours displayed on the TLC plate, a digital camera was used to obtain the image and the RGB model was used for semi-quantification. As shown in Fig. 3b, a linear range (0.001–1 μM, 10^{-15} - 10^{-12} mol) for the detection of target DNA was achieved. Even though the nuance of the colours for the negative control, 0.001 and 0.01 μM DNA were not distinguished by naked eyes, it could be differentiated by the distinct intensities of the red colour. As a consequence, the limit of detection has been improved 100 fold from 0.1 to 0.001 μM by using a digital camera and Adobe Photoshop. The quantification method was proposed in a colourimetric bio-barcode assay in which as low as attomolar (10^{-18} M) of barcode DNA could be detected (Nam et al., 2007). The ultrahigh sensitivity was due to the use of silica beads and magnetic particles for enrichment. To further improve the sensitivity, silica and magnetic beads will be introduced in this scheme to capture the target DNA prior to colourimetric detection using gold nanoparticles. Alternatively, a three-layer composite nanoparticle that is composed of SiO_2, Fe_3O_4, and Au particles can be applied for highly sensitive detection systems (Stoeva, Huo, Lee, & Mirkin, 2005). It should be highlighted that our method is based on double stranded DNA thus it has enhanced sensitivity, compared to single-stranded DNA approach. In addition, it is workable in a wide temperature range, thus would be practical for applications in the industry.

3.3. Specificity of the colourimetric detection

In order to evaluate the specificity, non-target DNA fragments including a mismatch (1-base mismatch), a deletion (4-base deletion), a half (half-complementary) and a false (non-complementary) DNA were tested in this detection system along with the target DNA (Table 1). These fragments include a part of sequences that share with the target sequence. For instance, the mismatch and the target DNA have 23 bp in common, but they differ in only one position.

The colour results of the reactions are presented in Fig. 4. The colours for the target and non-target DNA fragments were distinguishable: the target was displayed as a blue dot and the non-targets were red dots. It is important to note that several bases of

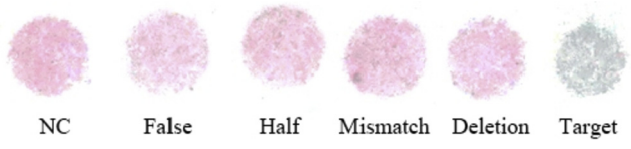

Fig. 4. Specificities of the reactions between the modified nanoparticles and the given sequences: NC (negative control), false (a non-complementary target), half (a half-complementary target), mismatch (1-base mismatch), deletion (4-base deletion) and target DNA.

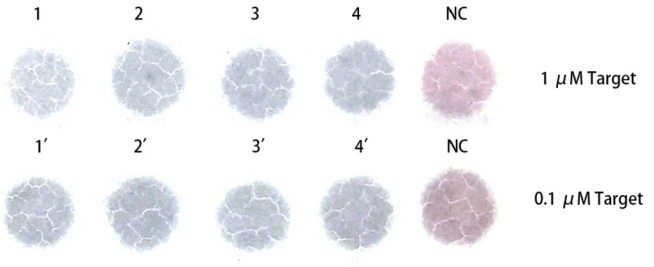

Fig. 5. Interference assessment of non-target DNA by TLC plate-based colourimetric assay. 1–4: 0, 1, 10, 100 μM of the false match DNA mixed with 1 μM of the target, respectively. 1′-4′: 0, 1, 10, 100 μM of the false match DNA mixed with 0.1 μM of the target, respectively.

mismatch and deletion did not lead to the aggregation of the nanoparticles in the tail-to-tail alignment. Even the only one different base failed the aggregation, indicating the high specificity of the reaction. This result can be explained by the alignment of the gold nanoparticle probes. In this study, the probe A-gold nanoparticle, probe B-gold nanoparticle together with the target DNA form a "tail-to-tail" complex (Fig. 1). It was reported that a tail-to-tail alignment of gold nanoparticle probes could lead to a highly specific differentiation of targets that contain one base mismatches, insertions or deletions (Storhoff, Elghanian, Mucic, Mirkin, & Letsinger, 1998).

3.4. Interference assessment of non-target DNA

The interference of non-target DNA in the assay was evaluated by using different concentrations of false match oligonucleotides (0, 1, 10, 100 μM) mixed with the target DNA. As shown in Fig. 5, the spot colour stayed blue when 1 or 0.1 μM target DNA was mixed with the increasing concentrations of false match oligonucleotides. This indicates that the false match DNA used in this work does not have an influence on the detection of the target DNA. Moreover, the target DNA could still be detected when its concentration decreased to 0.1 μM, which is the limit of detection for the method. Hence, the TLC plate-based colourimetric assay was developed with good resistance to non-target DNA interference and showed the potential in sensitive detection of real food samples. However, the current results were based on the synthesised DNA fragments though they were relatively specific to pork samples. Real food sample or practical scenario is complex. Therefore, various real food samples would be tested in the next stage to study the efficiency and effectiveness of this proposed method.

4. Conclusions

In this study, a synthesised swine-specific DNA target was detected by a gold nanoparticle-based colourimetric detection method. As low as 0.1 μmol/L of the target could be visualised by naked eyes, more sensitive semi-quantification of 0.001–1 μmol/L of DNA was achieved by using the RGB colour model to quantify the intensity of the spot colour. To improve the sensitivity and specificity, more factors involving the nanoparticle material and size, monodispersity, choice of media for visualisation, design of the probes, probe concentration, etc. will be optimised in the future work. The current results are based on the synthesised DNA fragments, though it is relatively specific to pork samples. Real food sample or practical scenario is complex. Therefore, various real food samples would be tested in the next stage to study the efficiency and effectiveness of this proposed method.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

Projects 31371851 and 31471605 supported by National Natural Science Foundation of China contributed to this study. We also thank the financial support from the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112) and an industry project from Fujian Putian Sea-100 Food Co., Ltd (R-143-000-633-597).

References

Ali, M., Hashim, U., Kashif, M., Mustafa, S., Man, Y. C., & Hamid, S. A. (2012). Development of swine-specific DNA markers for biosensor-based halal authentication. *Genetics and Molecular Research, 11*(2), 1762–1772.
Ali, M., Hashim, U., Mustafa, S., Man, Y., & Islam, K. N. (2012). Gold nanoparticle sensor for the visual detection of pork adulteration in meatball formulation. *Journal of Nanomaterials*, 1–7.
Ali, M., Hashim, U., Mustafa, S., Man, Y. C., Yusop, M., Bari, M., et al. (2011). Nanoparticle sensor for label free detection of swine DNA in mixed biological samples. *Nanotechnology, 22*(19), 195503–195510.
Alonso-Rebollo, A., Ramos-Gómez, S., Busto, M. D., & Ortega, N. (2017). Development and optimization of an efficient qPCR system for olive authentication in edible oils. *Food Chemistry, 232*, 827–835.
Asensio, L., González, I., García, T., & Martín, R. (2008). Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). *Food Control, 19*(1), 1–8.
Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Science, 86*(3), 577–587.
von Bargen, C., Dojahn, J. r., Waidelich, D., Humpf, H.-U., & Brockmeyer, J. (2013). New sensitive high-performance liquid chromatography–tandem mass spectrometry method for the detection of horse and pork in halal beef. *Journal of Agricultural and Food Chemistry, 61*(49), 11986–11994.
Dalmasso, A., Civera, T., La Neve, F., & Bottero, M. T. (2011). Simultaneous detection of cow and buffalo milk in mozzarella cheese by real-time PCR assay. *Food Chemistry, 124*(1), 362–366.
Demers, L. M., Mirkin, C. A., Mucic, R. C., Reynolds, R. A., Letsinger, R. L., Elghanian, R., et al. (2000). A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles. *Analytical Chemistry, 72*(22), 5535–5541.
Dong, F., Dong, X., Zhou, L., Xiao, H., Ho, P. Y., Wong, M. S., et al. (2016). Doxorubicin-loaded biodegradable self-assembly zein nanoparticle and its anti-cancer effect: Preparation, in vitro evaluation, and cellular uptake. *Colloids and Surfaces B: Biointerfaces, 140*, 324–331.
Elghanian, R., Storhoff, J. J., Mucic, R. C., Letsinger, R. L., & Mirkin, C. A. (1997). Selective colourimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science, 277*(5329), 1078–1081.
Feng, X., Fu, C., & Yang, H. (2017). Gelatin addition improves nutrient retention, texture and mass transfer of fish balls without altering their nanostructure during boiling. *LWT-Food Science and Technology, 77*, 142–151.
González-Sálamo, J., Socas-Rodríguez, B., Hernández-Borges, J., & Rodríguez-Delgado, M.Á. (2017). Core-shell poly (dopamine) magnetic nanoparticles for the extraction of estrogenic mycotoxins from milk and yogurt prior to LC–MS analysis. *Food Chemistry, 215*, 362–368.
González, A., Pablos, F., Martın, M., León-Camacho, M., & Valdenebro, M. (2001). HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters. *Food Chemistry, 73*(1), 93–101.
Hackett, C., Connor, P., Stonawski, M., Skirbekk, V., Potancoková, M., & Abel, G. (2015). The future of world religions: Population growth projections, 2010-2050. In S. Stencel, M. Lipka, & A. Sandstrom (Eds.), *Overview* (pp. 5–23). Washington, DC: Pew Research Center.
Hou, R., Zhang, Z., Pang, S., Yang, T., Clark, J. M., & He, L. (2016). Alteration of the nonsystemic behavior of the pesticide ferbam on tea leaves by engineered gold nanoparticles. *Environmental Science & Technology, 50*, 6216–6223.
Jeffreys, A. J., Wilson, V., & Thein, S. L. (1985). Hypervariable'minisatellite' regions in human DNA. *Nature, 314*(6006), 67–73.
Lever, J., & Miele, M. (2012). The growth of halal meat markets in Europe: An exploration of the supply side theory of religion. *Journal of Rural Studies, 28*(4), 528–537.
Li, Y., & Mustapha, A. (2004). Simultaneous detection of *Escherichia coli* O157: H7, *Salmonella*, and *Shigella* in apple cider and produce by a multiplex PCR. *Journal of Food Protection, 67*(1), 27–33.
Li, H., & Rothberg, L. J. (2004). Label-free colourimetric detection of specific sequences in genomic DNA amplified by the polymerase chain reaction. *Journal of the American Chemical Society, 126*(35), 10958–10961.

Liu, X., Atwater, M., Wang, J., & Huo, Q. (2007). Extinction coefficient of gold nanoparticles with different sizes and different capping ligands. *Colloids and Surfaces B: Biointerfaces, 58*(1), 3–7.
Liu, J., & Lu, Y. (2006). Preparation of aptamer-linked gold nanoparticle purple aggregates for colourimetric sensing of analytes. *Nature Protocols, 1*(1), 246–252.
Lockley, A., & Bardsley, R. (2000). DNA-based methods for food authentication. *Trends in Food Science & Technology, 11*(2), 67–77.
Lubis, H. N., Mohd-Naim, N. F., Alizul, N. N., & Ahmed, M. U. (2016). From market to food plate: Current trusted technology and innovations in halal food analysis. *Trends in Food Science & Technology, 58*, 55–68.
Marikkar, J., Ng, S., & Man, Y. C. (2011). Composition and thermal analysis of lipids from pre-fried chicken nuggets. *Journal of the American Oil Chemists Society, 88*(6), 749–754.
Mody, V. V., Siwale, R., Singh, A., & Mody, H. R. (2010). Introduction to metallic nanoparticles. *Journal of Pharmacy and Bioallied Sciences, 2*(4), 282–289.
Nam, J.-M., Jang, K.-J., & Groves, J. T. (2007). Detection of proteins using a colourimetric bio-barcode assay. *Nature Protocols, 2*(6), 1438–1444.
Nguyen, T. H. D., Vardhanabhuti, B., Lin, M., & Mustapha, A. (2017). Antibacterial properties of selenium nanoparticles and their toxicity to caco-2 cells. *Food Control, 77*, 17–24.
Pang, Z., Deeth, H., Yang, H., Prakash, S., & Bansal, N. (2017). Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt. *Journal of Dairy Science, 100*, 3436–3447.
Rao, Q., & Hsieh, Y. H. P. (2014). Enhanced immunodection of bovine central nervous tissue using an improved extraction method. *Food Control, 46*, 282–290.
Rao, Q., Richt, J. A., & Hsieh, Y. H. P. (2016). Immunoassay for the detection of animal central nervous tissue in processed meat and feed products. *Journal of Agricultural and Food Chemistry, 64*, 3661–3668.
Rubert, J., Lacina, O., Zachariasova, M., & Hajslova, J. (2016). Saffron authentication based on liquid chromatography high resolution tandem mass spectrometry and multivariate data analysis. *Food Chemistry, 204*, 201–209.
Sow, L. C., Peh, Y. R., Pekerti, B. N., Fu, C., Bansal, N., & Yang, H. (2017). Nanostructural analysis and textural modification of tilapia fish gelatin affected by gellan and calcium chloride addition. *LWT-Food Science and Technology, 85*, 137–145.
Stoeva, S. I., Huo, F., Lee, J.-S., & Mirkin, C. A. (2005). Three-layer composite magnetic nanoparticle probes for DNA. *Journal of the American Chemical Society, 127*(44), 15362–15363.
Storhoff, J. J., Elghanian, R., Mucic, R. C., Mirkin, C. A., & Letsinger, R. L. (1998). One-pot colourimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. *Journal of the American Chemical Society, 120*(9), 1959–1964.
Storhoff, J. J., Lucas, A. D., Garimella, V., Bao, Y. P., & Müller, U. R. (2004). Homogeneous detection of unamplified genomic DNA sequences based on colourimetric scatter of gold nanoparticle probes. *Nature Biotechnology, 22*(7), 883–887.
Tautz, D. (1989). Hypervariabflity of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research, 17*(16), 6463–6471.
Ursing, B. M., & Arnason, U. (1998). The complete mitochondrial DNA sequence of the pig (Sus scrofa). *Journal of Molecular Evolution, 47*(3), 302–306.