



Proofman Ladder-Shape Melting Temperature Isothermal Amplification for Rapid Detection of Duck-Derived Contaminants in Edible Oils

Fugang Xiao,^{1,*} Gang Hao,^{1,2} Xiaoqi Xi,^{1,3} Junxin Zhao,¹ Quanzeng Wei,¹ Caiwen Dong^{2,*} and Deguo Wang^{1,*}

Abstract

The behavior of mixing fake edible oil is not uncommon, and the addition of "gutter oil" to edible oil will have a serious impact on people's daily lives. A method for detecting duck components in edible oil using Proofman ladder-shape melting temperature isothermal amplification (Proofman-LMTIA) was established by combining Proofman enzyme-mediated probe cleaning with LMTIA technology. Proofman probes and LMTIA primers were designed for specific gene fragments in duck DNA, and the reaction system was optimized through experiments. The results showed that the established Proofman-LMTIA detection method had the best amplification effect at 66 °C and could detect duck DNA concentrations as low as 1 pg/μL. The method has strong specificity and can accurately distinguish duck source genes from DNA of other species, detecting adulteration of 0.1% of duck mass fraction. The amplification time is within 20 min. The established Proofman-LMTIA detection method can quickly detect the adulteration of duck components in edible oil, providing a reference for regulatory authorities.

Keywords: Edible oil; Duck derived components; Proofman probe; Ladder-shape melting temperature isothermal amplification (LMTIA); Gutter oil; Food fraud; Rapid diagnostics.

Received: 09 April 2025; Revised: 04 May 2025; Accepted: 20 May 2025.

Article type: Research article.

1. Introduction

"Gutter oil" (kitchen waste oil) is a type of waste edible oil from different sources. After refining and processing, it is difficult to detect trace amounts of "gutter oil" mixed with normal edible oil.^[1] Due to the severe supply situation of edible oil in China and the significant price differences of different types of edible vegetable oil, driven by interests, illegal businesses mix "gutter oil" that poses a huge threat to human health into normal edible oil to varying degrees for sale, in order to make huge profits.^[2] Due to the potential market

and huge profits, there are frequent safety issues such as adulteration and forgery in the edible oil industry.^[3-5] The blending of waste oil into edible oil is one of the most concerning issues in current food quality control in developing countries.^[6]

The quality and safety issues of edible oil are also world-class challenges faced by developed countries. The food ingredient fraud and economic profit driven adulteration database established by American scientists has sorted out the reports and papers on illegal addition and profit driven adulteration of prohibited ingredients in food worldwide. The analysis found that reports on illegal addition and adulteration of prohibited ingredients in edible oil ranked first among all food adulteration problems, accounting for 24% of the total,^[7] such as adulteration of olive oil, peanut oil,^[8] or sesame oil,^[9] etc. The illegal addition of prohibited ingredients in edible oil and profit driven adulteration are significant global food safety issues that have attracted widespread attention, as well as hot and difficult issues in the field of global food quality and safety. From this, the safety of edible oil is a difficult problem and challenge that threatens human health development, social

¹ Henan Key Laboratory of Biomarker Based Rapid-detection Technology for Food Safety, Food and Pharmacy College, Xuchang University, Xuchang, 461000, China

² College of Food and Biological Engineering, Zhengzhou University of Light Industry, Zhengzhou, 450002, China

³ Henan Provincial Engineering Laboratory of Insect Bio-reactor and College of Life Science and Agricultural Engineering, Nanyang Normal University, Nanyang, 473061, China

*Email: xfug@163.com (F. Xiao); biodcw@126.com (C. Dong); wangdg666@126.com (D. Wang)

harmony and stability,^[10-12] and identifying the adulteration of "gutter oil" in edible oil has become an urgent problem to be solved.^[2] Due to the complexity of the composition of "gutter oil" and the special requirements for rapid detection, there is an urgent need for a simple and fast detection method.^[2,13,14]

Due to duck being a frequently consumed food, there is a higher possibility of duck genes appearing in "gutter oil". This study aims to preliminarily identify "gutter oil" by detecting duck genes in edible oil. But as the saying goes, "If you mix oil with oil, you'll see your sorrows." It's extremely difficult to detect and identify different oils after mixing them. Due to the fact that "gutter oil" undergoes refining and high-temperature treatment, there is very little residual DNA and it is a small fragment, making it difficult to detect DNA using conventional methods.

At present, for the detection of adulteration in edible oil, the main methods adopted include rational methods, chromatography,^[15] spectroscopy,^[16,17] nuclear magnetic resonance spectroscopy,^[18] and molecular biotechnology. The commonly used molecular biotechnology method is digital polymerase chain reaction (dPCR) technology, and dPCR technology is the third generation PCR methods after traditional PCR methods and quantitative PCR (qPCR) methods.^[19] dPCR technology, with its unique advantages, has demonstrated outstanding performance in the field of food safety testing. This technology does not rely on calibration substances or standard curves, and is directly connected to the International Basic Units, ensuring absolute quantification of the results. Its high specificity, sensitivity, accuracy, and low sensitivity to matrix effects make dPCR unique in food safety testing. Compared with traditional PCR and fluorescence quantitative PCR, dPCR provides a faster, more efficient, and more accurate solution for food safety detection. These characteristics have made dPCR widely concerned in the field of food safety. However, digital PCR technology not only requires precision instruments in operation, but also requires complex chips with high prices and long detection times. These disadvantages restrict the widespread application of digital PCR. However, other detection methods, such as physical and chemical methods, have lower sensitivity, and spectroscopic instruments are more expensive. Nuclear magnetic resonance spectroscopy and chromatography not only use expensive instruments, but also require complex operations and specialized experimental personnel. Therefore, a simple, practical, low-cost, highly sensitive, and specific detection method is needed to detect adulteration of edible oil.

Our team reported the ladder-shape melting temperature isothermal amplification (LMTIA) technology in 2021.^[20] LMTIA technology is a novel nucleic acid isothermal amplification technique developed based on LAMP and PCR technology. LMTIA technology has higher sensitivity than LAMP technology and is simpler and faster than PCR technology, reducing PCR reaction time from 90 minutes to less than 20 minutes. In the preliminary research, we used LMTIA technology to establish LMTIA detection methods for

soybean components in edible oils,^[21] soybean components in dairy products,^[22] and corn components in edible oils.^[23]

This study established a method for identifying duck components in edible oil based on Proofman-LMTIA technology, providing a feasible detection method for identifying suspected "gutter oil".

2. Materials and methods

2.1 Materials and reagents

Edible oil, chicken, duck, pork, lamb, beef, and horse meat: purchase from major supermarkets in Xuchang; Blood/Cell/Tissue Genomic DNA Extraction Kit: Beijing Tiangen Biochemical Technology Co., Ltd; Nucleo Spin ® Food reagent kit: Macherey Nagel, Germany; Universal LMTIA reaction premix: Shandong Dege Biotechnology Co., Ltd; GPV8 ultra fidelity DNA polymerase (2U/ μ L): Universal Biosystems (Anhui) Co., Ltd.

2.2 Instruments and equipment

Gentier 96E Fully Automated Medical PCR Analysis System: Xi'an Tianlong Technology Co., Ltd; Nano Drop One Ultra Trace Nucleic Acid Protein Analyzer: Beijing Tiangen Biochemical Technology Co., Ltd; SCI-VS Adjustable Blender: Thermo Corporation, USA; 1-15K high-speed cryogenic centrifuge: Sigma, Germany; F6/10 homogenizer: Shanghai Jingxin Technology Co., Ltd; Dry thermostat (metal bath): Hangzhou Ruicheng Instrument Co., Ltd.

2.3 Methods

2.3.1 Research flowchart

The present study is summarized using the research flowchart shown in the Fig. 1.

2.3.2 DNA Extraction

Refer to the method established by Gu *et al* to extract DNA from edible oil.^[21] According to the instructions of the DNA extraction kit for meat, extract DNA from different types of meat separately. After extraction, the DNA concentration and purity were measured using an ultra-trace nucleic acid protein analyzer, and labeled separately for LMTIA amplification reaction. Store the extracted DNA samples at -20 °C.

2.3.3 Design of LMTIA primer and proofman probe

Search for duck source genes from the GenBank database, analyze them using Oligo 7 software, select fragments with a ladder like melting temperature curve as targets, and then use Primer3Plus (<http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi>) design LMTIA primers using online software and design Proofman probes based on the designed primers.^[20] The designed primers and probes were synthesized by Anhui General Biology Co., Ltd.

2.3.4 LMTIA reaction temperature optimization

The LMTIA reaction temperature was optimized using a 10 μ L

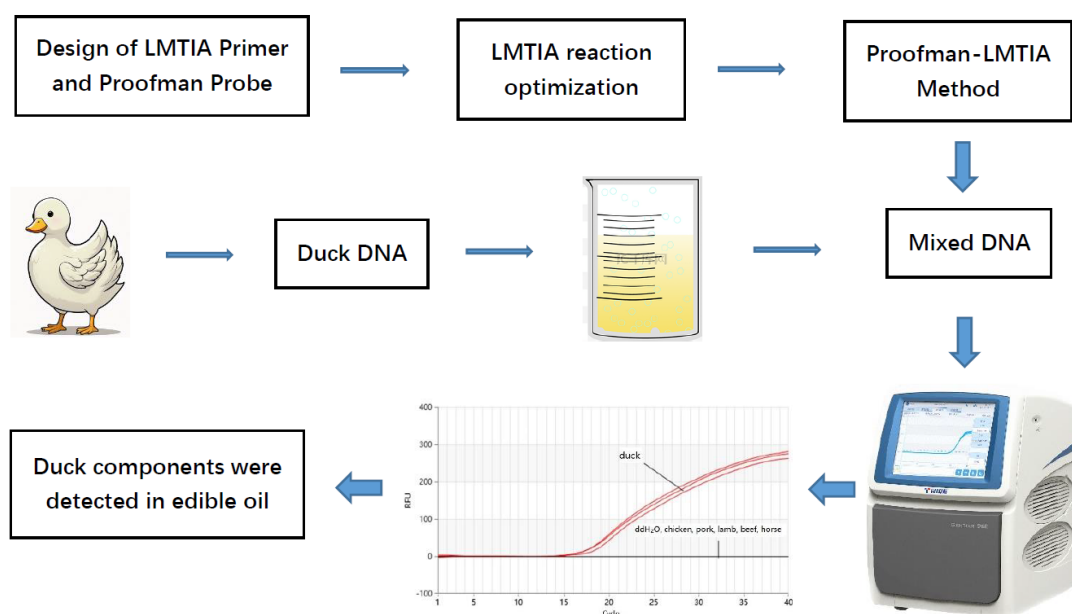


Fig. 1: Research flowchart.

system, each consisting of pre designed primers (1LF, LB, 1F2, 1B2), probes Pr-JOE, BstDNA polymerase, and 2 μ L of DNA or ddH₂O. During the experiment, a drop of paraffin oil was added to each single well to stabilize the reaction.^[21-23] The reaction temperatures were set to 65, 66, 67, and 68 $^{\circ}$ C, and the reaction conditions were to collect fluorescence signals every 30 seconds for one cycle, for a total of 40 cycles. Set up two parallel experiments for each temperature.

2.3.5 Relative sensitivity test

Add duck genomic DNA to soybean oil and dilute it to different concentrations such as 10 ng/ μ L, 1 ng/ μ L, 100 pg/ μ L, 10 pg/ μ L, 1 pg/ μ L, and 100 fg/ μ L. Extract DNA from edible oil according to the method established by Gu *et al.*^[21] and perform sensitivity detection at the optimal reaction temperature. Two parallel are set for each experiment.

2.3.6 Specific test

To verify whether the established method can distinguish DNA from different species, genomic DNA extracted from chicken, pork, lamb, beef, horse meat, and other sources was used as a reference, while ddH₂O was used as a negative control and duck genomic DNA was used as a positive control. According to the optimized experimental plan, each group of experiments will undergo three parallel experiments to

observe and analyze the amplification results.

2.3.7 Mixed sample detection limit test

To investigate the detection limit of the established experimental method, duck DNA was mixed with genomic DNA from other species to prepare a mixed DNA sample, and the LMTIA reaction system was used for detection. The proportions of duck DNA in the mixed DNA samples were 0.01%, 0.1%, 1.0%, 5.0%, 10.0%, and 20.0%, respectively. Add the mixed samples of duck DNA with different mass fractions to the Proofman-LMTIA reaction system after optimizing the temperature. Set two parallel samples for each experiment.

2.3.8 Data and graph processing

The experimental data and amplification curves were analyzed and processed using Archimed Analyzer real-time fluorescence quantitative PCR software (v1.4.4) and Gentier 96E fully automatic medical PCR analysis system (v1).

3. Results and discussion

3.1 Design results of LMTIA primer and Proofman probe

The primer and probe gene sequences designed based on the specificity of duck source gene fragments are shown in [Table 1](#).

Table 1: Sequence of LMTIA primer and Proofman probe for duck DNA.

Primer / probe	Sequences (5'-3')
Ya-ITS1-1LF	CTCCGCAGGTTACCT
Ya-ITS1-LB	GGCTTGGGCGTCGCA
Ya-ITS1-1F2	CAGCCCCGTAATGATTTAGTCGTAACAAGGTTTCCGT
Ya-ITS1-1B2	ACCGGGGCTGGGCTTTTGGGCTCCGTCGCTCAC
Ya-ITS1-LBPr	5'BHQ2-GGCTTGGGCGTCT-3'JOE

3.2 Results of LMTIA reaction temperature optimization

Different temperatures have an impact on DNA amplification, which in turn affects experimental results. The experimental results are shown in Fig. 2. At different temperatures of 65 °C, 66 °C, 67 °C, and 68 °C, only duck genomic DNA showed amplification reactions, while the negative control (ddH₂O) did not undergo amplification. When the reaction temperature is 66 °C, the duck genomic DNA amplification time is the earliest, the signal is the strongest, and the effect is the best. Therefore, 66 °C is selected as the optimal reaction temperature for this method.

3.3 Results of sensitivity test

According to Fig. 3, in the 40 cycles of reaction at a reaction temperature of 66 °C, amplification was observed in the groups with gradient diluted duck genomic DNA concentrations of 10 ng/μL, 1 ng/μL, 100 pg/μL, 10 pg/μL, and 1 pg/μL, while no amplification was observed in the 100 fg/μL duck genomic DNA and negative control (ddH₂O). This demonstration the Proofman-LMTIA method established in this experiment can accurately detect duck genomic DNA in edible oil samples at a concentration of 1 pg/μL or higher, indicating that the detection sensitivity of this method is 1 pg/μL. It shows that the sensitivity of detection is high in

samples mixed with a small amount of duck ingredients, providing a reliable method for detecting edible oil adulteration.

3.4 Results of specific test

According to Fig. 4, only duck genomic DNA was amplified in 40 cycles, while the negative control (ddH₂O) and DNA from other species such as chicken, pork, lamb, beef, and horse meat did not undergo amplification. This demonstration the experimental method has strong specificity and can accurately distinguish the genes of ducks from those of other animals, thus enabling effective detection of food containing duck ingredients.

3.5 Results of mixed sample detection limit test

From Fig. 5, it was shown that within 40 cycles of reaction at 66 °C, the negative control (ddH₂O) and the sample with a duck DNA content of 0.01% did not undergo amplification, while the mixed simulated samples with duck DNA contents of 0.1%, 1.0%, 5.0%, 10.0%, and 20.0% showed amplification within 40 cycles. This demonstration the method established in this experiment can quickly detect adulteration of duck components with a mass fraction of 0.1% or higher in edible oil.

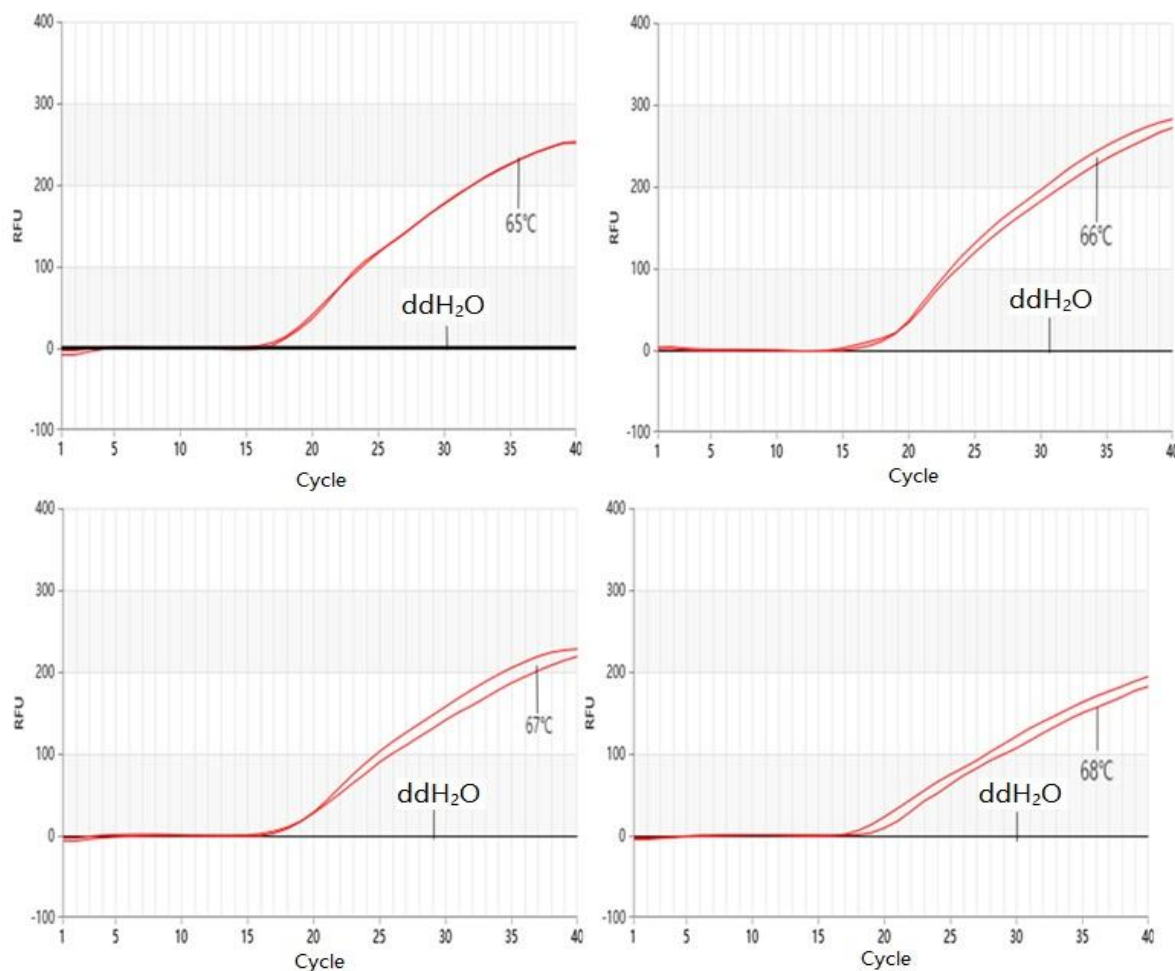


Fig. 2: LMTIA amplification curve of duck genomic DNA under different temperature conditions.

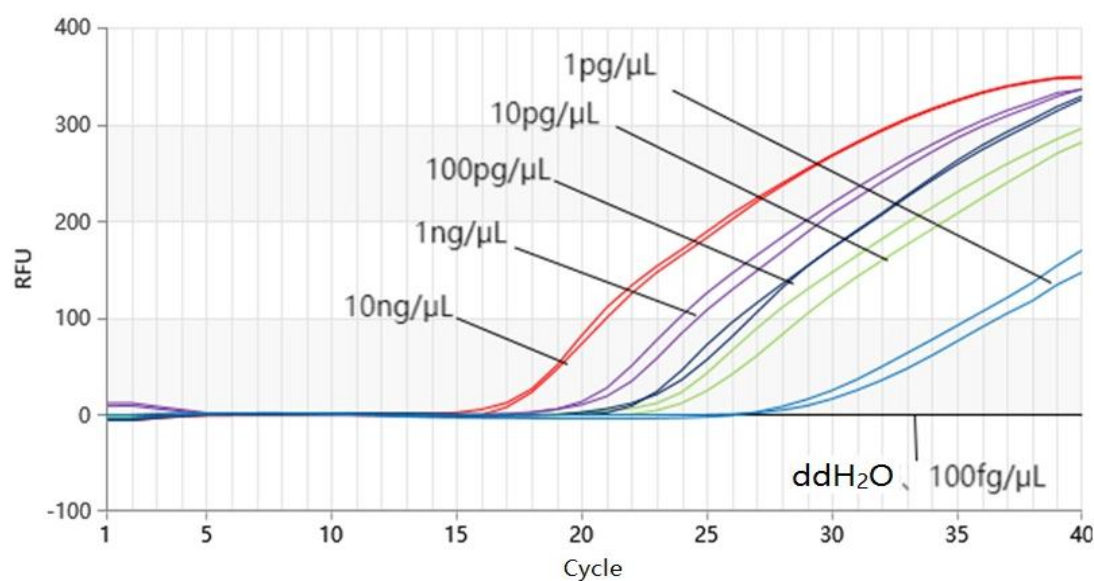


Fig. 3: LMTIA amplification curve during sensitivity test of duck genomic DNA.

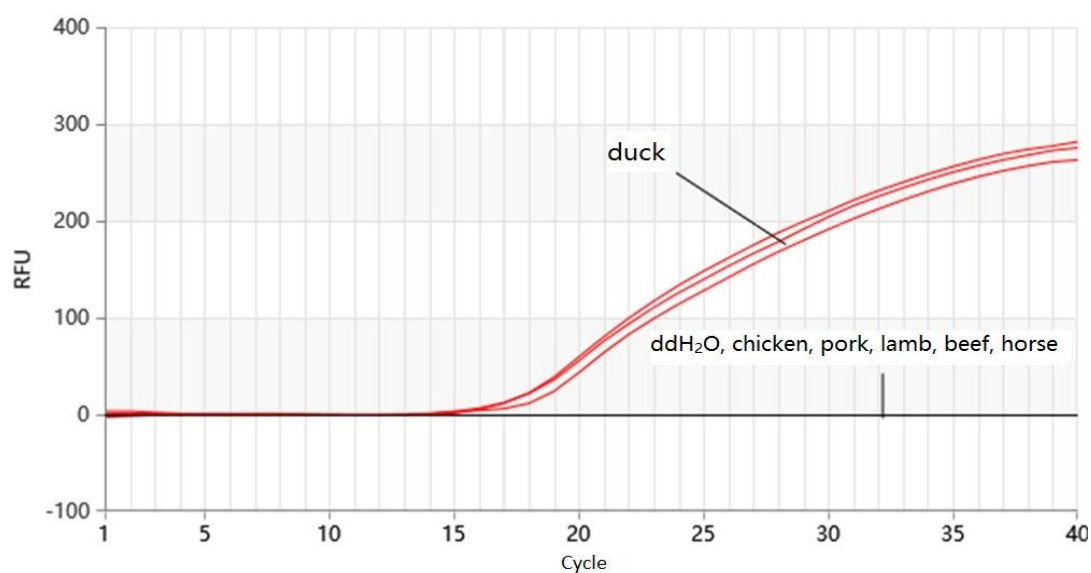


Fig. 4: LMTIA amplification curve during specific test of duck genomic DNA.

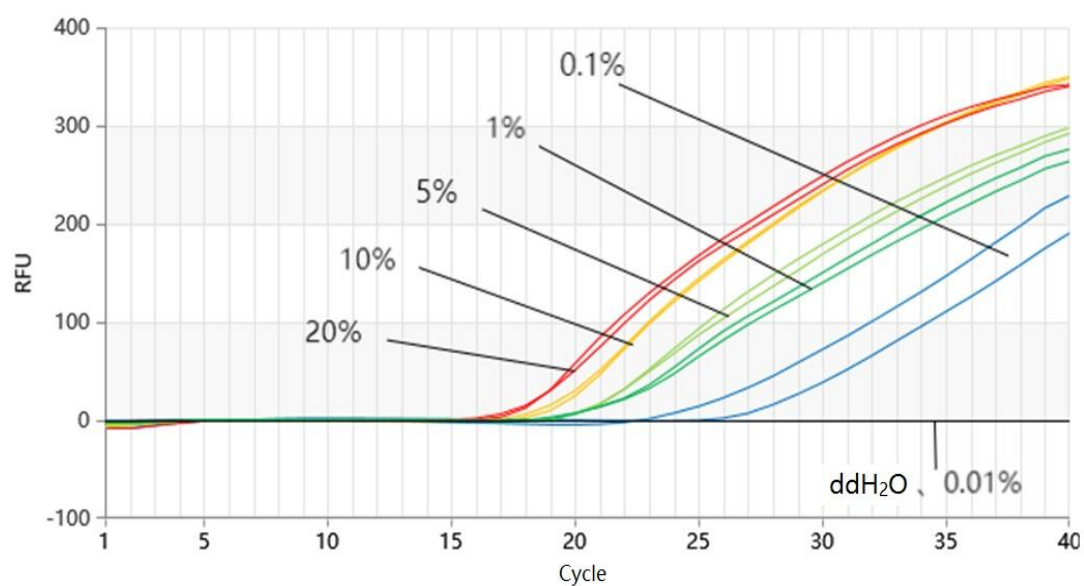


Fig. 5: LMTIA amplification curve during detection limit test of duck genomic DNA mixed sample.

3.6 Discussion

DNA plays an important role in biogenic identification.^[24,25] Although traditional PCR methods can perform qualitative and quantitative analysis, they are time-consuming, have complex temperature control, and slightly lower sensitivity.^[26,27] In contrast, Notomi *et al* developed the Loop mediated Isothermal Amplification (LAMP) technique in 2000,^[28] which can rapidly and accurately amplify target gene fragments at a constant temperature, with high sensitivity and specificity.^[29] It is gradually replacing traditional PCR in food adulteration detection. The unique property of constant temperature eliminates the need for complex temperature control equipment, making operation simpler and faster, reducing costs, and adapting to grassroots detection needs. However, LAMP technology still has drawbacks such as high experimental design requirements, difficulty in primer design, and high requirements for target sequence length.^[30]

In 2021, Wang *et al* solved the problem of LAMP false positives caused by aerosol pollution and non-specific amplification, and invented LMTIA based on the mechanism of single chain template generation in LAMP reaction.^[20] LMTIA is a novel nucleic acid detection technology that combines the unique advantages of PCR and LAMP. The LMTIA method not only requires complementary pairing between primers and target sequences, but also requires a ladder like melting curve of the target sequence to provide a single stranded template for amplification reactions.^[20] Therefore, LMTIA technology should have higher specificity in theory. In this study, specificity tests were conducted on the established LMTIA method. DNA tests for chicken, pork, lamb, beef, and horse meat showed negative results and did not interfere with duck DNA detection, demonstrating the good specificity of the LMTIA method. The reaction mechanism of LMTIA technology is easy to understand, the primer design is simple, the reaction speed is fast and has strong specificity, high sensitivity, low requirements for the length of the target sequence, and wide applicability.^[21-23] LMTIA technology, as a new technology, will have significant advantages in grassroots testing scenarios in the market.

The edible oil detection method we currently establish can only qualitatively determine whether it is adulterated, but cannot detect the adulteration content. Therefore, using LMTIA technology to establish a quantitative detection method for adulteration is our next direction of effort. DNA has high genetic stability, and DNA based detection methods can effectively avoid interference from other factors. However, extracting edible oil DNA is still an urgent challenge that needs to be overcome. Both the reagent kit method and the improved method have problems such as time-consuming operation and low extraction success rate. It is necessary to continue optimizing the edible oil DNA extraction method, shorten the extraction time, and extract all long-chain DNA in the oil as much as possible. It is worth noting that during the processing of edible oil, DNA is severely damaged and gradually degraded over time until it completely disappears.

On the one hand, the success rate of detection can be improved through nucleic acid enrichment methods, and on the other hand, primer fragments can be shortened or primers can be added to improve sensitivity and amplification efficiency to achieve detection goals.

4. Conclusion

This study established a novel Proofman-LMTIA detection method for duck components in edible oil, aiming to quickly and conveniently detect duck components in edible oil (suspected to be "gutter oil" detection). This method designs primers and probes for specific target sequences of duck genes, and achieves the best amplification effect at 66 °C by optimizing the reaction conditions. At 66 °C, this method demonstrated extremely high detection sensitivity for duck genomic DNA, reaching a level of 1 pg/μL. At the same time, this method has no cross reactivity with common animal derived components and has good specificity. The Proofman-LMTIA detection method established in this experiment can quickly and accurately detect duck DNA in mixed samples, with a detectable limit mass fraction of 0.1%. The method is easy to operate and can complete the detection within 20 min. The Proofman-LMTIA method established in this experiment provides an efficient and feasible solution to the problem of adulteration of edible oil, and provides a reference for food safety supervision.

Acknowledgments

This research was funded by the Technology Project of Henan Province (Grant No. 242102321134, 252102111048).

Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

References

- [1] T. Wang, H. L. Wu, W. J. Long, Y. Hu, L. Cheng, A. Q. Chen, R. Q. Yu, Rapid identification and quantification of cheaper vegetable oil adulteration in camellia oil by using excitation-emission matrix fluorescence spectroscopy combined with chemometrics, *Food Chemistry*, 2019, **293**, 348-357, doi: 10.1016/j.foodchem.2019.04.109.
- [2] J. Tan, R. Li, Z. Jiang, S. Tang, Y. Wang, M. Shi, Y. Xiao, B. Jia, T. Lu, H. Wang, Synchronous front-face fluorescence spectroscopy for authentication of the adulteration of edible vegetable oil with refined used frying oil, *Food Chemistry*, 2017, **217**, 274-280, doi: 10.1016/j.foodchem.2016.08.053.
- [3] E. J. Rifna, R. Pandiselvam, A. Kothakota, K. S. Rao, M. Dwivedi, M. Kumar, R. Thirumdas, S. V. Ramesh, Advanced process analytical tools for identification of adulterants in edible oils - A review, *Food Chemistry*, 2022, **369**, 130898, doi: 10.1016/j.foodchem.2021.130898.
- [4] G. Peng, M. Chang, M. Fang, C. Liao, C. Tsai, S. Tseng, Y.

- Kao, H. Chou, H. Cheng, Incidents of major food adulteration in Taiwan between 2011 and 2015, *Food Control*, 2017, **72**, 145-152, doi: 10.1016/j.foodcont.2016.07.043.
- [5] P. Visciano, M. Schirone, Food frauds: Global incidents and misleading situations, *Trends in Food Science & Technology*, 2021, **114**, 424-442, doi: 10.1016/j.tifs.2021.06.010.
- [6] Z. Yang, Q. Zhou, W. Wu, D. Zhang, L. Mo, J. Liu, X. Yang, Food fraud vulnerability assessment in the edible vegetable oil supply chain: A perspective of Chinese enterprises, *Food Control*, 2022, **138**, 109005, doi: 10.1016/j.foodcont.2022.109005.
- [7] J. C. Moore, J. Spink, M. Lipp, Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010, *Journal Of Food Science*, 2012, **77**, R118-R126, doi: 10.1111/j.1750-3841.2012.02657.x.
- [8] W. Zhu, X. Wang, L. Chen, Rapid detection of peanut oil adulteration using low-field nuclear magnetic resonance and chemometrics, *Food chemistry*, 2017, **216**, 268-274, doi: 10.1016/j.foodchem.2016.08.051.
- [9] L. Zhang, X. Huang, P. Li, W. Na, J. Jiang, J. Mao, X. Ding, Q. Zhang, Multivariate adulteration detection for sesame oil, *Chemometrics and Intelligent Laboratory Systems*, 2017, **161**, 147-150, doi: 10.1016/j.chemolab.2016.11.009.
- [10] M. Wang, B. Gao, Y. Xing, X. Xiong, Preparation of an electrochemical sensor based on multi-walled carbon nanotubes/molecularly imprinted polymers for the detection of capsaicin in gutter oil by differential pulse voltammetry, *International Journal of Electrochemical Science*, 2020, **15**, 8437-8449, doi: 10.20964/2020.09.09.
- [11] Q. Ye, X. Meng, Highly efficient authentication of edible oils by FTIR spectroscopy coupled with chemometrics, *Food Chemistry*, 2022, **385**, 132661, doi: 10.1016/j.foodchem.2022.132661.
- [12] Z. Huang, J. Xin, S. Sun, Y. Li, D. Wei, J. Zhu, X. Wang, J. Wang, Y. Yao, Rapid identification of adulteration in edible vegetable oils based on low-field nuclear magnetic resonance relaxation fingerprints, *Foods*, 2021, **10**, 3068, doi: 10.3390/foods10123068.
- [13] Q. Wu, L. Yao, P. Qin, J. Xu, X. Sun, B. Yao, F. Ren, W. Chen, Time-resolved fluorescent lateral flow strip for easy and rapid quality control of edible oil, *Food Chemistry*, 2021, **357**, 129739, doi: 10.1016/j.foodchem.2021.129739.
- [14] A. Amirav, B. Neumark, K. J. Margolin Eren, A. B. Fialkov, N. Tal. Cannabis and its cannabinoids analysis by gas chromatography-mass spectrometry with Cold EI, *Journal of Mass Spectrometry*, 2021, **56**, e4726, doi: 10.1002/jms.4726.
- [15] D. Yuan, S. Li, L. Zhang, F. Ma, H. Wang, Q. Zhang, P. Li, Rapid and sensitive quantification of capsaicinoids for edible oil adulteration by immunomagnetic solid-phase extraction coupled with time-resolved fluorescent immunochromatographic assay, *Food Chemistry*, 2023, **404**, 134552, doi: 10.1016/j.foodchem.2022.134552.
- [16] M. Mburu, C. Komu, O. Paquet-Durand, B. Hitzmann, V. Zettel, Chia oil adulteration detection based on spectroscopic measurements, *Foods*, 2021, **10**, 1798, doi: 10.3390/foods10081798.
- [17] N. Su, S. Weng, L. Wang, T. Xu, Reflectance spectroscopy with multivariate methods for non-destructive discrimination of edible oil adulteration, *Biosensors*, 2021, **11**, 492, doi: 10.3390/bios11120492.
- [18] K. Lim, K. Pan, Z. Yu, R. Xiao, Pattern recognition based on machine learning identifies oil adulteration and edible oil mixtures, *Nature Communications*, 2020, **11**, 5353, doi: 10.1038/s41467-020-19137-6.
- [19] D. Verginelli, S. Ciuffa, K. Spinella, D. La Rocca, M. Misto, C. Quarchioni, P. Bonini, C. Fusco, L. Peroni, S. Peddis, U. Marchesi, In-House validation of four duplex droplet digital PCR assays to quantify GM soybean events, *Foods*, 2024, **13**, 4011, doi: 10.3390/foods13244011.
- [20] D. Wang, Y. Wang, M. Zhang, Y. Zhang, J. Sun, C. Song, F. Xiao, Y. Ping, C. Pan, Y. Hu, C. Wang, Y. Liu, Ladder-shape melting temperature isothermal amplification of nucleic acids, *BioTechniques*, 2021, **71**, 359-370, doi: 10.2144/btn-2020-0173.
- [21] M. Gu, F. Xiao, B. Wang, Y. Zhang, C. Ding, G. Zhang, D. Wang, Study on detection of soybean components in edible oil with ladder-shape melting temperature isothermal amplification (LMTIA) assay, *Analytical Methods*, 2023, **15**, 581-586, doi: 10.1039/d2ay01719a.
- [22] F. Xiao, M. Gu, Y. Zhang, Y. Xian, Y. Zheng, Y. Zhang, J. Sun, C. Ding, G. Zhang, D. Wang, Detection of soybean-derived components in dairy products using proofreading enzyme-mediated probe cleavage coupled with ladder-shape melting temperature isothermal amplification (Proofman-LMTIA), *Molecules*, 2023, **28**, 1685, doi: 10.3390/molecules28041685.
- [23] F. Xiao, Y. Zhang, M. Gu, X. Xi, Y. Zhang, J. Sun, Q. Wei, B. Hu, G. Zhang, D. Wang, Comparative study on the target sequences of thaumatin-like protein (TLP) and internal transcribed spacer (ITS) in the detection of corn component in edible oil by ladder-shape melting temperature isothermal amplification (LMTIA), *LWT - Food Science and Technology*, 2024, **197**, 115903, doi: 10.1016/j.lwt.2024.115903.
- [24] S. Barrias, J. Ibáñez, J.R. Fernandes, P. Martins-Lopes, The role of DNA-based biosensors in species identification for food authenticity assessment, *Trends in Food Science & Technology*, 2024, **145**, 104350, doi: 10.1016/j.tifs.2024.104350.
- [25] A. Lanubile, L. Stagnati, A. Marocco, M. Busconi, DNA-based techniques to check quality and authenticity of food, feed and medicinal products of plant origin: A review, *Trends in Food Science & Technology*, 2024, **149**, 104568, doi: 10.1016/j.tifs.2024.104568.
- [26] S. S. Kim, Application of comparative genomics for the development of pcr primers for the detection of harmful or beneficial microorganisms in food: mini-review, *Foods*, 2025, **14**, 1060, doi: 10.3390/foods14061060.
- [27] A. S. M. Abuelnaga, K. A. E. H. ABD El-Razik, N. S. Ata, R. H. Hedia, E. A. E. Elgabry, M. M. H. Soliman, H. S. A. W. Marie, Bacteriological assessment and multiplex-PCR test for the detection of meat adulteration of different animal species, *Food Science and Technology*, 2021, **41**, 98-104, doi: 10.1590/fst.11520.

- [28] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Loop-mediated isothermal amplification of DNA, *Nucleic Acid Research*, 2000, **28**, E63, doi: 10.1093/nar/28.12.e63.
- [29] S. Fuertes-Perez, M. Hilgarth, R.F. Vogel, Development of a rapid detection method for *Photobacterium* spp. using Loop-mediated isothermal amplification (LAMP), *International Journal of Food Microbiology*, 2020, **334**, 108805, doi: 10.1016/j.ijfoodmicro.2020.108805.
- [30] S. Wang, H. Song, T. Wang, H. Xue, Y. Fei, X. Xiong, Recent advancements with loop-mediated isothermal amplification (LAMP) in assessment of the species authenticity with meat and seafood products, *Critical Reviews in Food Science and Nutrition*, 2025, **65**, 2214-2235, doi: 10.1080/10408398.2024.2329979.

Publisher's Note: Engineered Science Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits the use, sharing, adaptation, distribution and reproduction in any medium or format, as long as appropriate credit to the original author(s) and the source is given by providing a link to the Creative Commons license and changes need to be indicated if there are any. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

©The Author(s) 2025