Development of Automated Animal Species Identification for Halal Foods Using Multiplex PCR and Microchip Electrophoresis

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1. Introduction

Ensuring food safety and traceability requires accurate identification of food ingredients and animal species. In processed meat products and halal foods, verification of meat species and detection of pork contamination are essential for quality control. PCR-based DNA analysis is widely used for this purpose; however, conventional agarose gel electrophoresis relies on subjective visual interpretation of band patterns. The MultiNA™ II microchip electrophoresis system automates DNA fragment analysis and enables digital data acquisition with fingerprinting-based pattern identification. In this study, animal species identification was performed for halal-certified and processed foods, and the detection sensitivity for pork contamination was evaluated.

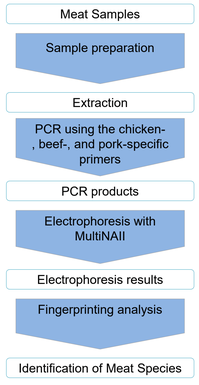
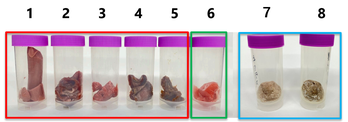


Fig. 1 Workflow for the identification of meat species



Commercially available halal-certified foods

1. Chicken sausage, 2. Diced Japanese beef, 3. Hamburger patties, 4. Roast beef, 5. Seasoned meat, 6. Japanese pork thigh 7. Processed beef product A, 8. Processed beef product B

Fig. 2 Meat samples used in this study

Fig. 3 MultiNA™ II microchip electrophoresis system

Table1 Workflow of pretreatment

Rection buffer		PCR programs	
2x Ampdirect plus	10 µL	95 ℃, 10 min	
BIOTAQ™	0.5 U	94 ℃, 30 sec	35 cycles
primer-F	2 µM	60 ℃, 60 sec	
primer-R	2 µM	72 ℃, 90 sec	
Distilled Water	up to 20 µL	72 ℃, 7 min	

2. Methods

The procedures from sample preparation to fingerprinting analysis were carried out as shown in Fig. 1.

1. Animal Species Identification Test

Commercially available halal-certified foods, domestically produced pork, and processed products made primarily from beef were used as samples (Fig. 2). Approximately 20 mg of sample was homogenized with 100 µL of lysis buffer and five 2-mm zirconia beads using a bead-smashing device (5,000 rpm, 30 s). The homogenate was heated at 95 °C for 5 min to inactivate Proteinase K, and the lysate was used as the PCR template. PCR amplification targeted the mitochondrial cytochrome b gene using the primer set reported by Matsunaga et al.[1]. The Ampdirect™plus PCR kit (Shimadzu Corporation) was used, and the reaction mixture and PCR conditions followed the protocol shown in Table 1. PCR products were analyzed with the MultiNA™ II microchip electrophoresis system (Fig. 3), and fingerprinting analysis was performed using the analysis software to identify species-specific DNA fragment patterns.

2. Detection Sensitivity Test for Contamination

To evaluate the detection sensitivity simulating pork contamination, model samples containing 1%, 0.1%, 0.01%, 0.001%, and 0.0001% pork in beef were prepared. Each contamination level was analyzed in four replicates (n = 4), with 100% beef and 100% pork used as negative and positive controls, respectively. After DNA extraction, PCR was performed with bovine- and porcine-specific primers, followed by analysis using the MultiNA II.

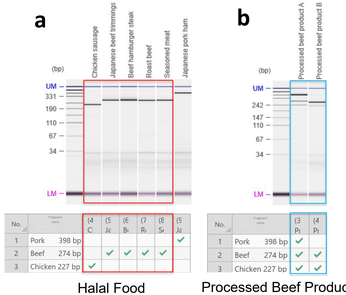


Fig. 4 Results for halal foods and processed beef products using MultiNA II

3. Results

1. Animal Species Identification Test

Fig. 3 shows the results for halal-certified foods and general processed foods. PCR amplification generated species-specific DNA fragments of 227 bp (chicken), 274 bp (beef), and 398 bp (pork).

Chicken-specific DNA fragment was detected in chicken products and beef-specific DNA fragment in processed beef products. The pork-derived DNA fragment was not detected in the halal-certified foods (Fig. 4a). In contrast, beef, chicken, and pork DNA fragment were detected in Product A, while beef and chicken DNA were detected in Product B (Fig. 4b). These results were consistent with the product labeling. Fingerprinting analysis automatically identified the species-specific DNA peaks, demonstrating objective animal species identification.

2. Detection Sensitivity Test for Contamination

Fig. 5 shows the results for model samples containing pork contamination. Pork-specific DNA fragments were detected at all contamination levels, including 0.0001% pork. These results demonstrate the high sensitivity of this method for detecting animal species-specific DNA.

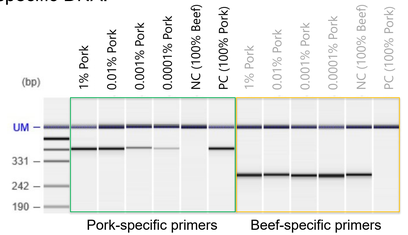


Fig. 5 Results of model samples simulating pork contamination

Table2 Detection Results for Model Samples Containing Pork Contamination

Pork mg/100mg Beef	Pork admixture by weight	Number of detections out of n attempts
1 mg	1.0000%	4/4
0.01 mg	0.0100%	4/4
0.001 mg	0.0010%	4/4
0.0001 mg	0.0001%	4/4
NC	(Beef 100%)	0/4
PC	(Pork100%)	4/4

4. Conclusion

This study demonstrated that the MultiNA II microchip electrophoresis system is a useful tool for animal species identification in food. By combining microchip electrophoresis with fingerprinting analysis, the system enabled objective identification of PCR products without visual interpretation of band patterns using conventional agarose gel electrophoresis. Furthermore, since the results can be saved as digital data, this contributes to improved reproducibility and record-keeping

In analyses of halal foods and processed foods, species-specific DNA fragment was clearly detected. In addition, this system showed high sensitivity for pork contamination detection, with a detection limit of 0.0001%.

These results suggest that this method is useful for applications in raw material verification, quality control, and halal food inspection.

*This assay is an analytical method used to assess the risk of pork-derived ingredient contamination in food. However, even if pork-derived DNA is not detected, this does not conclusively prove the complete absence of pork contamination.

Reference
1) T Matsunaga et al.: Effects of processing conditions on species identification of meat products, Nippon Shokuhin Kagaku Kogaku Kaishi, 46, 3, 187-194 (1999)