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Application News

Microchip electrophoresis system MultiNA™ II MCE-301

Application of the Detection of Pork-Derived DNA in Food to Halal Food Management

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User Benefits

- Fingerprinting analysis allows for the objective identification of the animal species in meat products.
- It can detect even a 0.0001% admixture of pork.
- It is a useful tool for quality control and certification.

■ Introduction

From the perspective of ensuring food safety and traceability, the importance of verifying ingredients in food and identifying animal species is increasing. In processed meat products, in particular, verifying the species of the raw meat and detecting the contamination of meat from other species are critical quality control issues. Additionally, for foods subject to religious restrictions, such as halal foods, strict control over the contamination of pork-derived ingredients is required.

The PCR method, which utilizes DNA analysis, is widely used for animal species identification. Although agarose gel electrophoresis is commonly used to visualize PCR products, this method requires visual assessment of the presence or absence of bands, presenting challenges in terms of subjectivity, reproducibility, and data recording.

The MultiNA™ II microchip electrophoresis system automates the separation and detection of DNA fragments and allows results to be obtained as digital data. Also, the fingerprinting analysis function built into the analysis software enables automatic identification of DNA band patterns.

In this application, we investigated a method for identifying animal species in food using the MultiNA II. Specifically, we performed animal species identification on halal-certified foods and general processed foods, and evaluated detection sensitivity using model samples containing pork contamination.

■ Methods

The procedure, from sample preparation to fingerprinting analysis, was 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 and 100 µL of lysis buffer (20 mM Tris-HCl (pH 8.0), 5 mM EDTA, 400 mM NaCl, 0.3% SDS) were mixed with five 2-mm-diameter zirconia beads and disrupted using a bead-smash device at 5,000 rpm for 30 seconds. The sample suspension was heated at 95 °C for 5 min to inactivate Proteinase K, and then used as a template for PCR.

A 0.5 µL sample suspension obtained during sample preparation was used as the PCR template. The PCR target sequence was the cytochrome b gene on mitochondrial DNA, selected based on the literature by Matsunaga et al.1. The Ampdirect™ Plus PCR kit (Shimadzu Corporation) was used. The composition of the reaction mixture and the PCR program were carried out according to the protocol in Table 1.

The PCR products were analyzed using the MultiNA II microchip electrophoresis system.

Fingerprinting analysis of the resulting electrophoretic patterns was performed using the MultiNA II analysis software to evaluate the detection of species-specific DNA fragments for each animal species.

2. Adulteration Detection Sensitivity Test

To evaluate the sensitivity for detecting pork adulteration, we prepared model samples by gradually adding pork to beef. The adulteration rates were set at 1%, 0.1%, 0.01%, 0.001%, and 0.0001%. We conducted evaluations on four replicates (n=4) for each adulteration level. Additionally, we used a 100% beef sample as a negative control and a 100% pork sample as a positive control. After DNA extraction, we performed PCR using bovine-specific and porcine-specific primers, followed by electrophoresis analysis using MultiNA II.

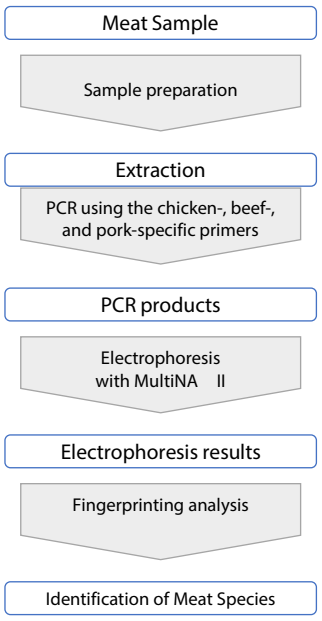

Fig. 1 Workflow for the identification of meat species

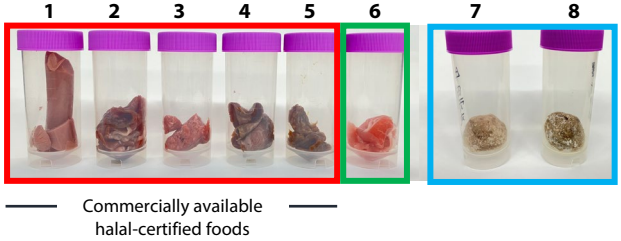

Fig. 2 Meat samples used in this application
1. Chicken sausage, 2. Diced Japanese beef, 3. Hamburger patties, 4. Roast beef, 5. Seasoned meat, 6. Japanese pork thigh 7. Processed beef product A, 8. Processed beef product B

Table 1 PCR conditions

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

■ Results

1. Animal Species Identification Test

Fig. 3 shows the results for halal-certified foods and general processed foods. The sizes of the DNA fragments amplified by PCR using the chicken-, beef-, and pork-specific primers employed in this method were 227 bp, 274 bp, and 398 bp, respectively.

Chicken-specific DNA fragments were detected in chicken products, and beef-specific DNA fragments were detected in processed beef products. In addition, no pork-derived DNA was detected in the halal-certified foods (Fig. 3a).

On the other hand, in general processed beef products, DNA from beef, chicken, and pork was detected in Product A, while DNA from beef and chicken was detected in Product B. The packaging for these products indicated that they were manufactured on production lines that also handle pork and chicken, and the results were consistent with the product labeling (Fig. 3b).

Moreover, when the electrophoresis results were evaluated using fingerprinting analysis, species-specific DNA peaks were automatically recognized, confirming that objective species identification is possible.

2. Adulteration Detection Sensitivity Test

Fig. 4 shows the results for model samples containing pork contamination.

Pork-specific DNA fragments were detected in all contaminated samples. In particular, it was confirmed that detection is possible even at an extremely low concentration of 0.0001% pork contamination.

These results demonstrate that this method is capable of highly sensitive detection of animal species-specific DNA*.

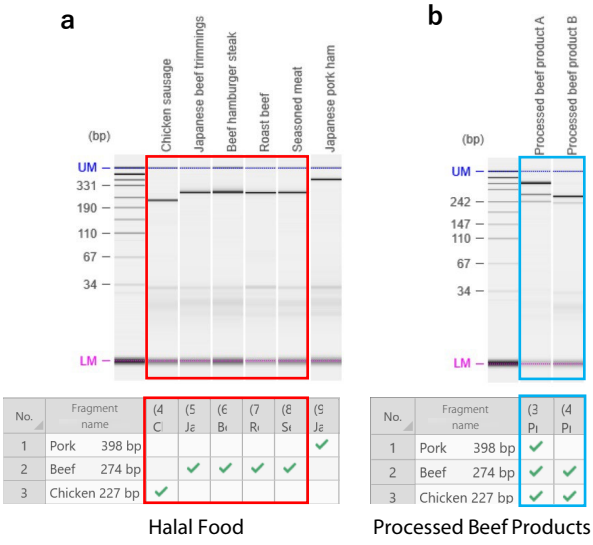

Fig. 3 Results for halal foods and processed beef products using MultiNA II

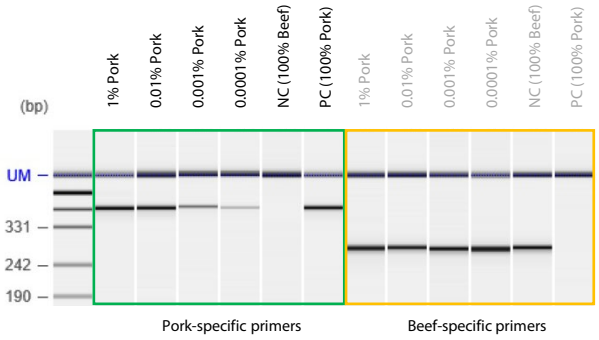

Fig. 4 Detection Sensitivity of Pork Contamination in Model Samples

Table 2 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

■ Conclusions

We investigated a method for identifying animal species in food using MultiNA II.

In this method, PCR products are analyzed using microchip electrophoresis, and by further applying fingerprinting analysis, it has become possible to perform objective identification that does not rely on visual assessment using conventional agarose gel electrophoresis. Furthermore, since the results can be saved as digital data, this contributes to improved reproducibility and record-keeping.

In species identification tests targeting halal foods and processed foods, we were able to clearly detect the DNA of each animal species. Furthermore, in contamination tests, we confirmed that this highly sensitive analysis method is capable of detecting pork contamination as low as 0.0001%.

This method is expected to be useful in the field of food analysis for applications such as raw material verification, quality control, and halal food inspection*.

* The MultiNA II test for detecting pork-derived DNA is an analytical method used to assess the risk of pork contamination in food. Even if no pork-derived DNA is detected, this does not conclusively prove the 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)

<Related Application>

1. Meat Type Identification Using DNA Detection
   Application News No. 01-00849-EN

01-01190-EN First Edition: Aug. 2026

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