

Identification of Meat Species by DNA Detection

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User Benefits

- ◆ The electrophoresis operation can be performed almost fully automatically.
- ◆ Meat species can be identified from trace amounts of samples.
- ◆ The target size can be automatically detected by fingerprinting analysis.

Introduction

To ensure food safety and security, the Food Sanitation Law, JAS Law, and other laws and regulations require labeling of the origin, meat species, and parts of meat. In addition, the consumption of pork is strictly forbidden in the Muslim and Jewish religions, and the labeling of information on the species of meat contained in meat and meat products is very important. Thus, there is a need for meat species identification technology to guarantee peace of mind and quality for various consumers.

There are two methods to identify meat species: protein-based (ELISA: enzyme-linked immunosorbent assay) and gene-based (PCR: polymerase chain reaction). Protein-based methods are relatively simple and inexpensive to analyze, but are not suitable for discriminating between close species or from processed foods. On the other hand, gene-based methods can be used to analyze even processed foods because DNA is relatively stable against heat. Here, in addition to examples of DNA detection from meat using PCR and electrophoresis, examples of analysis for meat species identification from processed meat products will be presented.

Sample Preparation

The workflow from sample preparation to meat identification was conducted as shown in Fig. 1. Beef, pork, chicken, horse meat, mutton, and goat meat were used as meat samples.

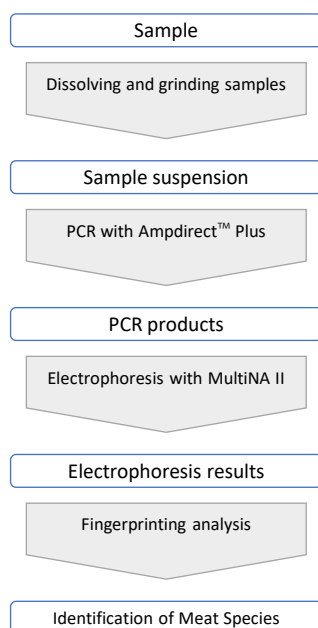


Fig. 1 Workflow for the identification of meat species

Three commercial processed meat samples (A, B, and C) were used (Fig. 2). To each 5 mg of meat sample, 100 μ L of sample dissolving solution in Table 1 was added. For processed meat products, 500 μ L of sample solution was added for each 50 to 100 mg sample. Zirconia beads with a particle diameter of 2 mm were added to the above sample solution, and the samples were crushed in a bead-type cell disruption device at 5000 rpm for 30 sec at 25 $^{\circ}$ C. Proteinase K was inactivated by heat treatment of the sample suspension at 95 $^{\circ}$ C for 5 min and used as a template sample for PCR.

Table 1 Sample dissolving solution

Tris \cdot HCl pH 8.0	20 mM
EDTA	5 mM
NaCl	400 mM
SDS	0.3 %
Proteinase K	200 μ g/mL

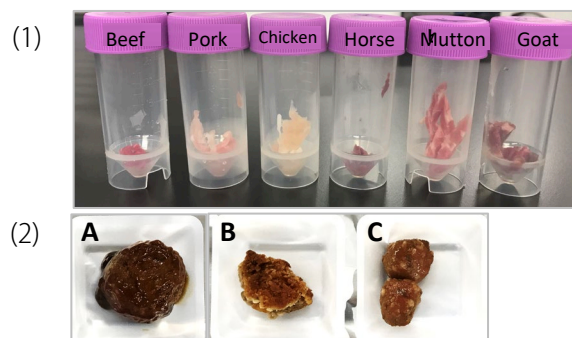


Fig. 2 (1) Beef, pork, chicken, horse meat, mutton, and goat meat
(2) Three commercial processed meat samples (A, B, and C)

PCR

A 0.5 μ L of the sample suspension obtained in preparation was used as the template for PCR. The target sequence for PCR was the cytochrome b gene on mitochondrial DNA, based on the method of Matsunaga et al. (1999)¹. The kit for PCR was Ampdirect Plus (Shimadzu Corporation). The composition of the reaction solution and PCR program were performed according to the attached protocol, as shown in Table 2.

Table 2 PCR conditions

Reaction buffer		PCR programs	
2 x Ampdirect plus	10 μ L	95 $^{\circ}$ C, 10 min	} 35 cycles
BIOTAQ	0.5 U	94 $^{\circ}$ C, 30 sec	
primer-F	2 μ M	60 $^{\circ}$ C, 60 sec	
primer-R	2 μ M	72 $^{\circ}$ C, 90 sec	
Distilled Water	up to 20 μ L	72 $^{\circ}$ C, 7 min	

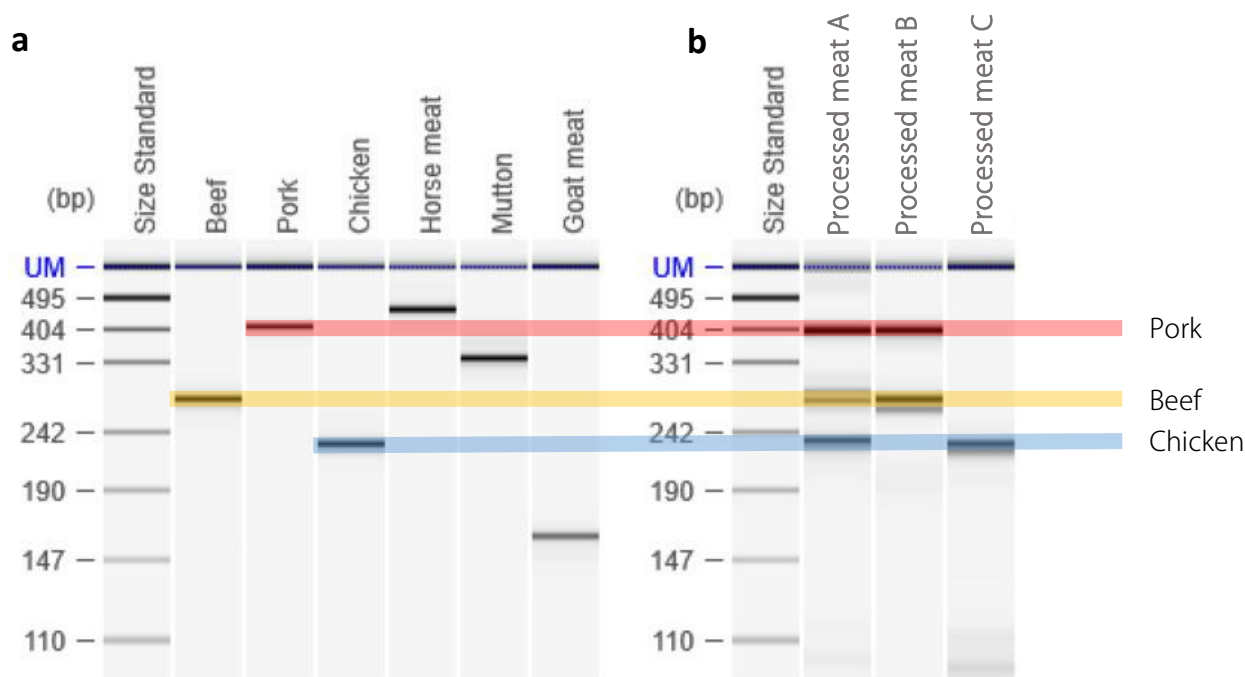


Fig. 3 Electrophoresis results by MultiNA II
 a: Results of PCR products from six different meats
 b: Results of PCR products from three processed meat products

Peak Table		DNA Report		Fingerprinting (Reference size information)		
Reference name:		Meat				
Comment:						
No.	Fragment name	(9) A7 Processed A	(10) A8 Processed B	(11) A9 Processed C		
1	Goat					
2	Chicken	✓		✓		
3	Beef	✓	✓			
4	Mutton					
5	Pork	✓	✓			
6	Horse					

Fig. 4 Results of the fingerprinting analysis

■ Electrophoresis and its analysis

PCR products were electrophoresed with MultiNA II to confirm the size of DNA. Analysis with MultiNA II was performed using the dedicated reagent DNA-500 kit. For the analyzed data of each processed meat product, the fingerprinting analysis was used to detect the size specific to the meat species and to identify the meat species.

■ Results

The electrophoresis results of six meats and three processed meat products by MultiNA II are shown in Fig. 3a. The sizes of DNA amplified by PCR from beef, pork, chicken, horse meat, mutton, and goat meat are 274 bp, 398 bp, 227 bp, 439 bp, 331 bp, and 157 bp, respectively, according to Matsunaga et al. Each of these was clearly detected in this analysis.

For processed meat products, three DNAs were detected from A, two from B, and one from C (Fig. 3b). When the data obtained by MultiNA II was analyzed using the fingerprinting analysis of the data analysis software, chicken, beef and pork were identified from A, beef and pork from B, and chicken from C (Fig. 4). The meat species detected matched the meat species indicated in the ingredient names on the product packaging.

■ Conclusions

Generally, gene-based methods require extraction and purification of DNA. However, these operations are cumbersome and take a lot of time when the number of specimens is large. Ampdirect plus neutralizes PCR inhibitors such as proteins and sugars in the sample, allowing PCR directly from the sample without DNA purification.

Electrophoresis by MultiNA II can be analyzed fully automatically by simply setting reagents and samples. In addition, the fingerprinting analysis of MultiNA II's data analysis software can detect DNA of a specific size in each data. As shown here, the combination of MultiNA II and Ampdirect plus makes it possible to easily determine the meat species based on genes.

<References>

- 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).

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