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Application News

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MCE

Identification of Meat Species in Food Products by Molecular Biological Methods

Based on the Food Sanitation Act, Japan Agricultural Standard Law (JAS Law) and other related laws, producers and distributors of meat and meat products are required to display the place of origin, source species of meat, and part of the animal in order to protect the security and safety of foods, while the Islamic and Jewish religions strictly forbid consumption of pork for religious reasons. Since information concerning meat species contained in fresh meat and processed meat products is extremely important, a technology for identifying meat species is needed in order to assure product quality and the peace-of-mind of various consumers.

Methods for meat species indentification include protein-based methods (e.g., ELISA: Enzyme Linked Immunosorbent Assay) and molecular biological methods (PCR: Polymerase Chain Reaction). Protein-based methods are comparatively simple and analysis is inexpensive, but they are not suitable for identification of closely-related species or for analysis of processed food products. On the other hand, analysis of processed foods is considered possible by molecular biological methods because DNA has relatively high thermal stability. In meat species identification from their genetic characteristics, the cytochrome b gene region of mitochondrial DNA (mtDNA) is used as the target sequence. This article introduces an example of DNA detection in meat from beef, pork, chicken, lamb, horse meat, goat meat, and an analysis example in which the meat species were identified from processed meat products.

Y. Sogabe

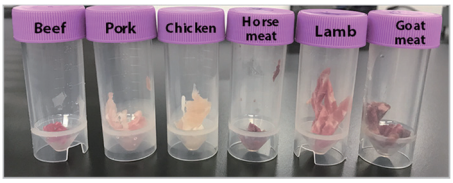


Fig. 1 Meat Samples: Beef, Pork, Chicken, Horse Meat, Lamb, Goat Meat

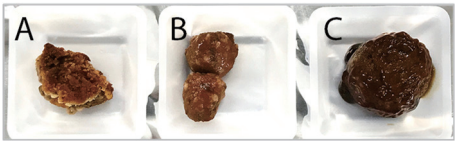


Fig. 2 Prosessed Meat Products (A, B, C)

Samples and Pretreatment

The samples used here were beef, pork, chicken, lamb, horse meat, goat meat and three kinds of processed meat products (A, B, C). The processes from sample pretreatment to identification of the meat species were carried out according to the protocol in Fig. 3.

First, 100 μL of the lysis buffer (Table 1) was added to a 5 mg single meat sample. For the processed meat samples, 500 μL of the solution was added to 50 to 100 mg of the sample. Zirconia beads with a size of φ2 mm were added to the above-mentioned sample solution, and the specimen was disintegrated under conditions of 5,000 rpm, 30 s, and 25 °C using a bead-type cell disruption system. The sample solution was then centrifuged at 5,000 rpm for 5 min at 25 °C and solids were removed as far as possible.

The supernatant was transferred to a different tube, and the proteinase K was inactivated by heating at 95 °C for 5 min. This sample solution was used as the PCR template sample.

Table 1 Lysis Buffer

Tris·HCl pH8.0	20 mM
EDTA	5 mM
NaCl	400 mM
SDS	0.30%
Proteinase K	200 μg/mL

PCR

A 0.5 μL of the sample solution obtained by pretreatment was used as the PCR template. The PCR method referred to the paper by Matsunaga et al. (Journal of the Japanese Society for Food Science and Technology, 46(3), 187, 1999). The composition of the PCR reaction mixture and the PCR program were as shown in Table 2.

Table 2 PCR Conditions

Reaction mixture		PCR program	
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	

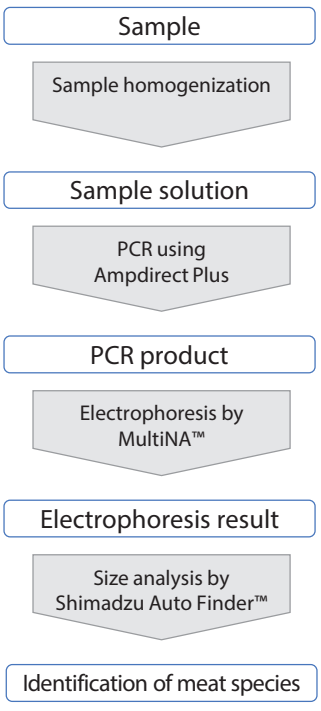


Fig. 3 Analysis Procedure

Electrophoresis and Identification of Meat Species

Electrophoresis of the PCR product was conducted with a MCE-202 MultiNA microchip electrophoresis system, and the size was confirmed. A MultiNA-dedicated DNA-500 Kit was used in the analysis with the MultiNA. For the analysis of the processed meat product samples, a Shimadzu Auto Finder was used to detect the sizes specific to the meat species, and the meat species were identified.

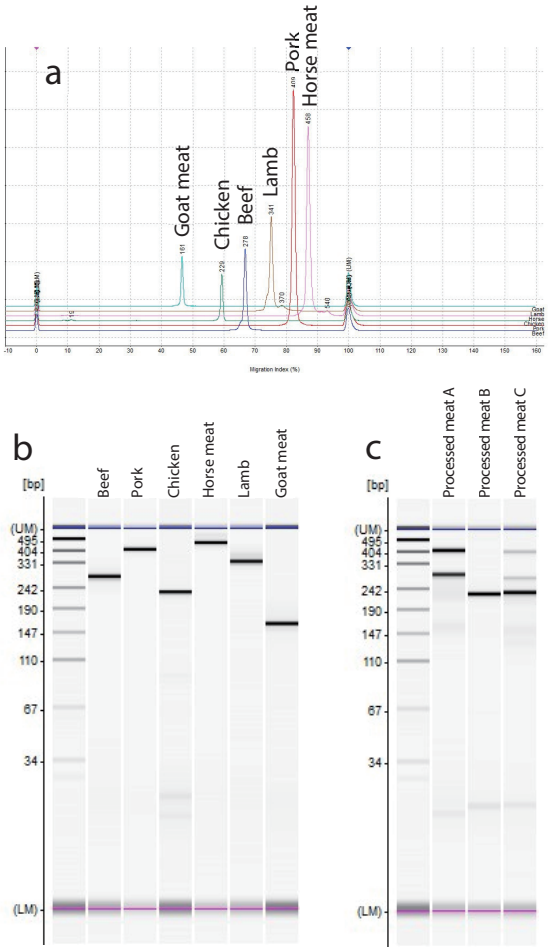
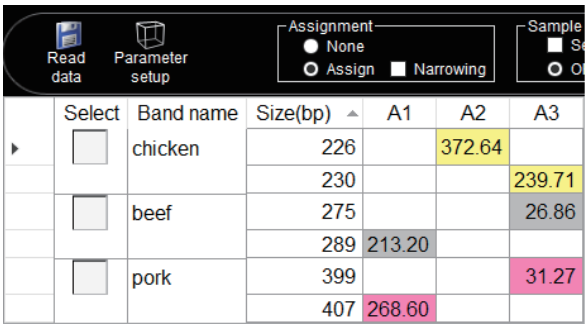


Fig. 4 Electrophoresis of PCR Products by MultiNA (Electropherogram and Gel Image)
a: Electropherogram of Meat Samples
b: Gel Image of Meat Samples
c: Gel Image of 3 Processed Meat Products



Select	Band name	Size(bp)	A1	A2	A3
	chicken	226		372.64	
		230			239.71
	beef	275			26.86
		289	213.20		
	pork	399			31.27
		407	268.60		

Fig. 5 Result of Identification of Meat Species of Prosessed Meats by Shimadzu Auto Finder
A1: Processed meat A, A2: Processed meat B, A3: Processed meat C
Numerical values in data indicate peak intensity (mV).

Results

Fig. 4 shows the results of the analysis of the six kinds of meats and the three kinds of processed meat products. With the method proposed by Matsunaga et al., the sizes of the DNA amplified by PCR from beef, pork, chicken, lamb, horse meat and goat meat are considered to be 274 bp, 398 bp, 227 bp, 439 bp, 331 bp, and 157 bp, respectively. These could also be detected clearly in this analysis (Fig. 4a, b).

In the processed meat products, two DNA fragments were detected from A, one was detected from B, and three were detected from C (Fig. 4c). When the data obtained by the MultiNA were analyzed with the Shimadzu Auto Finder, beef and pork were identified from A, chicken was identified from B, and chicken, beef, and pork were identified from C (Fig. 5). Samples B and C had commercial packages, and the contents identified in the analysis were the same as the source meat species indicated on the packages.

Conclusion

Extraction and purification of DNA is normally necessary in a molecular biological method. However, these processes are complex, and are time-consuming when a large number of samples is required. On the other hand, Ampdirect Plus has a neutralizing action for the PCR inhibitors protein and sugar in samples and enables direct PCR from the sample without DNA purification.

In electrophoresis by the MCE-202 MultiNA, a fully-automatic analysis was possible simply by setting the reagents and samples.

The Shimadzu Auto Finder, which is optional software for the MCE-202 MultiNA, can detect DNA of designated sizes from the digital data outputted by the MultiNA.

In conclusion, simple molecular biological identification of meat species is possible by using a combination of Ampdirect and the MultiNA system.

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