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Setting up a PCR Based Method to Trace Animal Species in Processed Meat Products

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INTRODUCTION

The identification of food species is becoming a very important issue for the assessment of food composition, necessary for the provision of proper consumer information. The increasing demand for transparency in the food industry derives either from socio-religious or economic reasons and has provoked a strong demand for appropriate detection methods that allow identification of the different components in processed food. The conventional methodologies used for the determination of species origin in food products are mainly based on immunochemical and electrophoretic analysis of proteins and on liquid chromatography techniques. However, immunological and electrophoretic methods cannot distinguish between closely related species and are often not suitable for food products with complex matrixes. Chromatographic techniques allow detection of differences in the percentages of fatty acids but are rather laborious. Recently, DNA-based techniques such as the polymerase chain reaction (PCR) or DNA hybridisation have been applied to identify meat species. These methods are sensitive and can still be used if the meat has been cured or autoclaved. Therefore, the aim of this study was either to optimise the extraction procedure of genomic DNA or to evaluate the suitability of a PCR method employing mitochondrial primers in detecting the authenticity of processed meat products.

MATERIAL AND METHODS

The tests were conducted with species-specific primers; in particular a region encoding tRNA e ATPase8 genes in the mitochondrial DNA was selected for bovine (forward: 5′-CACAATCCAGAACTGACAC-3′; reverse: 5′-GTAGGCTTGGGAA TAGTACGA-3′), the gene coding for subunit 1 of NADH dehydrogenase was selected for Gallus-Gallus (forward: 5′-CAAACATTGTGGGCCCTTTT-3′; reverse:

5′-AGTGGGAGGGGGACTCAAAT-3′) and two primer pairs were selected for swine; the first nuclear pair capable of amplifying the porcine endogenous retroviruses (FORWARD: 5′-CCCAGTACCCCTTCTAGGT-3′; reverse 5′-TCATCTA ATTGGAGGGTCAA) and the second amplifying a segment of the mitochondrial cytochrome b gene (forward: 5′-AAATCTCCCCTCAATGGTAT-3′; reverse: 5′-GGTAGTCCTATTATCGTGGG-3′).

The specificity of the selected primers was assessed using genomic DNA obtained from cell lines derived from specific and closely related species (turkey, sheep, goat). Moreover, the DNA extraction efficiency was evaluated (QIAamp DNA stool Mini Kit Qiagen S.p.a. -MI) related to different samples of processed meat products, starting from 200 mg of each sample. In particular the following products were selected: 15 samples of mortadella (5 known as Bologna-IGP), 20 samples of gelatine (10 known as having certified bovine origin) and 25 samples of sausages (10 of certified swine origin, 5 of certified poultry origin and 5 of certified bovine origin). The extraction was measured using a spectrophotometer (260/280 nm). Specific PCR reactions were performed on the extracted DNA. The results were analysed using standard agarose gel electrophoresis with ethidium bromide stain and then photographed in the UV region (254 nm). The results were compared with those obtained using the conventional ELISA test.

RESULTS

The results of the tests carried out to evaluate the selected primers and performed on selected cell lines have shown their suitability and the complete absence of cross-correlations. Moreover, the efficiency of selected extraction kit was assessed since all samples showed a value of 3 $\mu g \pm 1$ DNA/μl.

The results related to the authentication of food samples by PCR technique, compared to those obtained by ELISA test are reported in Table I. The results suggest the higher overall sensitivity of the PCR method for processed meat products. The gelatines, for example, were not suitable for the ELISA test as they were highly degraded. Furthermore, the PCR method appeared to be able to detect very low levels of other species different from those expected. Among the primers used, those specific to porcine endogenous retroviruses (PERV) appeared to be unsuitable for food analysis since they are only able to detect swine origin for unprocessed meat.

DISCUSSION

The results obtained showed the high suitability of PCR-based methods in detecting the authenticity of origin of processed meat products. This technique, which is able to reveal very low levels of species-contamination, appeared to be more suitable than the conventional ELISA test, which is highly affected by thermal treatments. This

TABLE I
Comparison between ELISA and PCR results

Food samples	PCR results[1]				ELISA results[1]		
	Bovine	Poultry	Swine*	Swine**	Bovine	Poultry	Swine
MORTADELLA							
5IGP	5/5	0/5	0/5	5/5	5/5	0/5	5/5
10	10/10	4/10	0/10	10/10	10/10	1/10	10/10
GELATINE							
10	10/10	0/10	0/10	0/10	ND§	ND§	ND§
10	7/10	0/10	0/10	3/10	ND§	ND§	ND§
SAUSAGES							
10	0/10	0/10	10/10	10/10	0/10	0/10	10/10
5	0/5	5/5	0/5	2/5	0/5	0/5	0/5
5	5/5	0/5	0/5	1/5	5/5	0/5	0/5
5	1/5	0/5	5/5	5/5	0/5	0/5	5/5

*Nuclear sequences; **mitochondrial sequences §not detected.
[1]Expressed as ratio between number of confirmed/number of examined samples.

diagnostic approach could become a useful tool in detecting possible frauds, offering more safety and security to the final consumers.

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