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Identification of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*) using polymerase chain reaction targeting specific sequences from the mitochondrial 12S rRNA gene

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Abstract

Polymerase chain reaction (PCR) based on oligonucleotide primers targeting the mitochondrial 12S rRNA gene was applied to the specific identification of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*). The use of a common reverse primer, together with forward specific primers for red deer, fallow deer, and roe deer, allowed the selective amplification of the desired cervid sequences. The specificity of each primer pair was verified by PCR analysis of DNA from various game and domestic meats. The assay can be useful for the accurate identification of meats from cervid species, avoiding mislabeling or fraudulent species substitution in meat products.

Keywords: Game meat; 12S rRNA gene; Polymerase chain reaction (PCR); Species-specific primers

1. Introduction

Determining the species origin of meat is an integral part of food regulatory control with respect to economic fraudulence. For example, game meat products are often a target for fraudulent labeling, because of the different prices between game and other meat species (Brodmann, Nicholas, Schaltenbrand, & Ilg, 2001; Wolf, Rentsch, & Hübner, 1999). Apart from the possible economic loss, correct species identification is important to the consumer for other reasons such as medical requirements of individuals who may have specific food allergies, or religious dietary restrictions. To detect such frauds and protect consumers, the development of reliable and simple tools which facilitates routine control throughout the food chain is required (Verkaar, Nijman, Boutaga, & Lenstra, 2002).

Most analytical methods for meat species identification are based on the analysis of proteins by either electrophoretic (Vallejo, González, Mazorra, & Rodríguez, 2005), chromatographic (Ashoor, Monte, & Stiles, 1988; Toorop, Murch, & Ball, 1997), or immunochemical assays (Chen & Hsieh, 2000). However, most proteins suffer denaturation in heated products, resulting in changed antigenicity and electrophoretic mobility of molecules (Giovannacci et al., 2004).

Methods for meat identification are also based on DNA analysis. In comparison with protein-based techniques, DNA-based ones have proved to be more reliable because DNA is more stable under conditions associated with the high temperatures, pressures, and chemical treatments used in the preparation of some food products (Arslan, Ilhak, & Calicioglu, 2006; Frezza et al., 2003). In particular, the

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introduction of Polymerase Chain Reaction (PCR) methodology in food analysis has provided a vast range of techniques for rapid detection and identification of organisms at species and intraspecies level. The main advantage of PCR is its high power of sensitivity and specificity, permitting a million-fold increase of the starting template target fragment (Chikuni, Tabata, Kosugiyama, Monma, & Saito, 1994). Among PCR-based techniques employed in the food industry to monitor adulterations of products from animal origin, the most frequently used are: (i) PCR amplification of marker gene fragment(s) with universal primers, coupled with techniques like nucleotide-sequencing or restriction fragment length polymorphism (RFLP) (Colombo, Cardia, Renon, & Cantoni, 2004; Girish et al., 2004, 2005); and (ii) PCR amplification with species-specific primers (Hird, Goodier, & Hill, 2003; Matsunaga et al., 1999). PCR using specific primers can selectively detect DNA sequences from a food matrix and offers the advantages of being less expensive, and more useful for routine analysis of large numbers of samples (Herman, 2001).

The increasing trend towards reducing the fat content in the diet has led to increased interest in consuming meats from game species. Particularly, venison is becoming more and more popular in European markets, with red deer (Cervus *elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*) the three main species demanded by consumers. In this paper, we describe a method for the specific identification of meats from red deer, fallow deer, and roe deer, based on selective PCR amplification of DNA fragments on the mitochondrial 12S rRNA gene.

2. Materials and methods

2.1. Selection of meat samples

Authentic muscle samples of red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), mouflon (*Ovis ammon*), and pyrenean ibex (*Capra pyrenaica*) were obtained from the Department of Animal Pathology (Facultad de Veterinaria, Universidad Autónoma de Barcelona, Spain) and from several meat-cutting plants of the Comunidad de Madrid (Parque Natural "El Pardo"), Andalucía (Parque Natural Sierra de Cazorla, Segura y Las Villas), Castilla-La Mancha (Ciudad Real and Toledo), and Extremadura (Badajoz).

Cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*), swine (*Sus scrofa domestica*), horse (*Equus caballus*), rabbit (*Oryctolagus cuniculus*), duck (*Cairina muschata*), turkey (*Meleagris gallipavo*), chicken (*Gallus gallus*), and goose (*Anser anser*) muscle samples were obtained from several local abattoirs and retail markets (Madrid, Spain). Each species were morphologically identified before the samples were obtained.

Fresh muscle portions from all selected specimens were processed immediately or stored frozen at −20 °C until use. Red deer, fallow deer and roe deer meats were also analyzed after being subjected to experimental pasteurization (72 °C for 30 min) and sterilization (121 °C for 20 min) treatments.

Several dry-cured (ham and sausage) and cooked meat products from red deer, fallow deer, and roe deer species purchased from different retail markets were also included in the analysis.

2.2. DNA extraction

Genomic DNA was obtained from meat using a Wizard® DNA Clean-up System kit (Promega Corp., Madison, WI) as described in previous work (Fajardo et al., 2006).

The DNA extracted from raw and processed tissues was electrophoresed in a 1% D1 (Hispanlab S.A., Torrejón, Spain) agarose gel containing 1 μg/ml ethidium bromide in Tris–acetate buffer (0.04 M Tris–acetate and 0.001 M EDTA, pH 8.0) for 30 min at 110 V. Genomic DNA was visualized by UV transillumination using a Geldoc 1000 UV Fluorescent Gel Documentation System-PC (Bio-Rad Laboratories, Hercules, CA).

2.3. Design of deer-specific primers and PCR amplification of the selected DNA fragments from red deer, fallow deer, and roe deer meats

Amplification and sequencing of a conserved 12S rRNA gene fragment (~720 bp) from red deer, fallow deer, roe deer, chamois, mouflon, pyrenean ibex, cattle, sheep, goat, and swine meats was accomplished following a previously described procedure (Fajardo et al., 2006). The set of primers used for this purpose were: 12S-FW and 12S-REV oligonucleotides (Table 1).

Alignment of 12S rRNA gene sequences obtained from red deer (AJ 885204, AJ 885205, AJ 885206), fallow deer (AJ 885203), roe deer (AJ 885202, AJ 972679, AJ 972680,

Table 1
DNA sequences of the primers used in this study

Primers	Length (bp)	Sequence (5′ to 3′)	Description
12S-FW	20	GGTAAATCTCGTGCCAGCCA	Conserved forward primer (for sequencing)
12S-REV	25	TCCAGTATGCTTACCTTGTTACGAC	Conserved reverse primer (for sequencing)
12SCE-FW	32	CAAAAACATATAACGAAAGTAACTTTCCGACC	Red deer specific forward primer
12SDD-FW	24	TAAACAACGAAGGTAACCTTATCG	Fallow deer specific forward primer
12SCC-FW	32	TGAAAATAGATAACGAAAGTAGCTTTGAACTA	Roe deer specific forward primer
12SCERV-REV	19	AAAGCACCGCCAAGTCCTT	Common reverse primer