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Original Research Article

Applicability of species-specific polymerase chain reaction for fraud identification in raw ground meat commercially sold in Iran

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ABSTRACT

Identifying of the species origin in meat and meat products is important for preventing adulteration and protecting consumers in terms of health and religious convictions. Species-specific polymerase chain reaction (PCR) has been known as a suitable method for identifying meat species; however, there is little information on applicability of this method for the detection of fraudulent actions in different food products. This study aimed to evaluate a species-specific PCR assay for the detecting of chicken and donkey meats as adulterants in raw ground meats. Specificity of the primer sets was tested against the target species. The method was applied to the binary meat mixtures of the target species with the detection limits ranged from 0.1% to 10% (w/w). Also, 91 ground beef samples and 53 mixtures of ground beef and lamb samples were collected from local butcheries and tested in order to evaluate the applicability of this method. The oligonucleotide primers amplified mitochondrial DNA sequences and revealed PCR products with expected sizes of 300, 225, 183 and 145 base pair from cattle, sheep, chicken and donkey respectively. PCR assay performed on the experimental meat mixtures showed the detection limit of 0.1% for all primer sets. Results demonstrated that 47.2% and 0.7% of all the samples contained chicken and donkey meats respectively. This method of detection can be applied by quality control laboratories and inspection services to determine adulteration in raw ground meat under certain circumstances.

1. Introduction

In recent years, awareness of food safety and quality has been increased. In this regard, meat adulterations have become a very important issue from health, economic, religious and regulatory aspects (Mane et al., 2009). Raw ground meat is one of the most popular meat products which is frequently adulterated with cheaper meat species. It is used in the manufacturing process of hamburger in food industry and preparation of many recipes. The ground meat which is commercially available in local butcheries in Iran is generally made of either beef or combination of beef and lamb. Use of other meat species accounts for adulterant, unless it is declared by suppliers.

Meat adulterations have been a widespread problem in meat and meat products. In turkey, Ayaz et al. (2006) reported that out of 100 different meat products they analyzed, 22% contained

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undeclared meat species. Doosti et al. (2011) also reported the presence of undeclared meat species in 7.58% of 244 meat products analyzed in their study in Iran. This wide range of adulteration could cause a great concern for consumers and also result in more stringent quality control measures held by inspection services (Hernández-Chávez et al., 2011). Therefore, it is necessary to develop a suitable method for detecting undeclared meat species in meat products.

So far, a considerable number of protein and DNA-based methods have been developed to identify the species origin in food products. Protein-based methods include sodium lauryl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) (Huang et al., 2008; Azira et al., 2012), isoelectric focusing (IEF) (Ortea et al., 2010), ELISA (Liu et al., 2006) and HPLC (von Bargen et al., 2014) which are less specific. DNA-based methods, particularly Polymerase chain reaction (PCR) have been proved to be very useful for species identification in foods (Jia-qin et al., 2008) and mislabeling detection (Machado-Schiaffino et al., 2008). These methods include the use of specific primers for the amplification of conserved mitochondrial or nuclear DNA sequences by the PCR reaction, followed by restriction fragment length polymorphism studies (PCR-RFLP) (Abdel-Rahman et al., 2009; Wang et al., 2010), randomly amplified polymorphic DNA (Martinez and Yman, 1998), real-time PCR (Safdar and Abasıyanık, 2013; Soares et al., 2013), multiplex PCR (Kitpipit et al., 2014; Safdar and Junejo, 2015) and single-stranded conformational polymorphisms PCR (Rehbein et al., 1999).

Species-specific PCR has been shown to be suitable for fraud identification in meat and meat products. Using this technique, a specific targeted sequence can be detect in sequences of different origins without further sequencing or digestion of the PCR products (Biesalski, 2005; Che Man et al., 2007).

The objective of this study was to evaluate the practical use of qualitative PCR assay for detecting undeclared meat species in raw ground meat.

2. Materials and methods

2.1. Sample collection

Ground meat samples, 91 ground beef and 53 mixtures of ground beef and lamb were randomly collected from 144 different butcheries. Authentic meat samples of cattle, sheep, and chicken were prepared from certified slaughter houses and donkey meat samples were obtained from Veterinary Hospital (Faculty of Veterinary Medicine, University of Tehran, Karaj, Iran) to be used as positive controls. All the samples were transported to the laboratory in the refrigeration and immediately processed or stored frozen at −20 °C.

2.2. Sample preparation and examination

Authentic meat samples of the target species were mixed to form binary meat mixtures containing different percentages of 0.1, 1, 5, and 10 (w/w) of lamb in beef, donkey in beef, chicken in beef, donkey in lamb, chicken in lamb, and beef in lamb to the final weight of 100 g.

2.3. DNA extraction

DNA was extracted from all samples according to the salt extraction (SALT) method, utilized previously for the extraction of DNA from shrimp muscle (Aljanabi and Martinez, 1997). First ground meat samples (70 mg) were immersed in 400 μl of lysis buffer (10 mM Tris–HCl, pH 8.0; 2 mM EDTA pH 8.0; 0.4 M NaCl), 40 μl of 20% (m/v) SDS and 20 μl of 10 mg ml^{-1} proteinase K and were mixed, using a vortex. Following incubation at 65 °C for 1 h, 300 μl of 6 M NaCl was added. Samples were mixed, using a vortex at maximum speed for 30 s and then centrifuged at 10,000 × *g* for 30 min. The upper aqueous phase from each sample was collected in new sterile microcentrifuge tubes and an equal volume of isopropanol was added to each sample and mixed. Samples were incubated at −20 °C for 1 h and were then centrifuged at 10,000 × *g* for 20 min. The DNA pellet was washed with 70% (v/v) ethanol, air dried and eluted in 100 μl of sterile deionized water. All regents purchased from Merck Company (Darmstadt, Germany) in analytical grade.

2.4. Checking quality and purity of DNA

Concentration and purity of extracted DNA were measured by UV absorption at 260 nm and calculating absorbance ratio at 260 nm to absorbance at 280 nm using biophotometer plus apparatus (Eppendorf, Hamburg, Germany). The quality of DNA extracts was checked by electrophoresis running of the extracted DNA on a 1% agarose gel (Merck).

2.5. Species-specific primers and PCR amplification

Species-specific DNA segments in cattle, sheep, chicken and donkey were amplified by the use of primer sequences as described in Table 1. All primers were checked in NCBI (National Center for Biotechnology Information) and investigated for highly suitable annealing temperatures using Eppendorf PCR System. The reaction mixture was prepared in a 500 μl PCR tube in a total volume of 20 μl containing 2 μl of 10× PCR buffer (CinnaGen, Karaj, Iran), 0.4 μl of 10 mM of dNTPs (CinnaGen), 0.6 μl of 50 mM $MgCl_2$ (CinnaGen), 0.2 μl of 5 U/μl *Taq* DNA polymerase (CinnaGen), 0.5 μM each of forward and reverse primers (Eurofins MWG Operon, Ebersberg, Germany), 100 ng of DNA template and nuclease free water to adjust the volume (CinnaGen). The PCR conditions programmed on gradient thermo cycler (Eppendorf) were as follows.

Initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at different temperatures (Table 1) for 40 s and extension at 72 °C for 30 s. Then final extension was done at 72 °C for 5 min. The PCR products were kept at −20 °C for further use.

PCR amplified in parallel with all specimens and along with positive and negative controls.

2.6. Electrophoresis of PCR products

PCR amplified products were analyzed by electrophoresis on 2% agarose gel (Merck) stained with 10 μg/ml of DNA safe stain (CinnaGen), run in 0.5× TBE buffer (Sigma–Aldrich, St. Louis, USA) for 1 h at 100 V, and finally visualized by UV transilluminator (Vilberlourmat, Marne La Vallée, France).

3. Results and discussion

3.1. PCR assay

The present study evaluated a qualitative PCR assay for detecting chicken and donkey meats as adulterants in raw ground meat. Spectrophotometric results of the isolated DNA using salt extraction method indicated enough DNA templates of high quality and purity for PCR amplification. DNA concentrations were between 150 and 1350 μg/ml with the A260:A280 ratio ranging from 1.6 to 1.9. After obtaining sufficient DNA template, analysis on 1% agarose gel revealed DNA integrity.