IDENTIFICATION OF MEATS USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE

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ABSTRACT

Use of a simpler, faster and reliable method for identification of species of origin in fresh and processed meat products is required to prevent unethical practices that may occur in the meat industry. The effectiveness of a random amplified polymorphic DNA (RAPD) method for identification of fresh meats from cattle, goat, sheep, camel, pork, wild swine, donkey, cat, dog, rabbit or bear origin was evaluated using a 10-base primer (ACGACCCACG). The method was also used to determine the species in a 1 : 1 mix of raw minced meat from sheep-pork, horse-beef or beef-sheep. Characteristic RAPD profiles for each species were obtained. However, efficacy of the technique in identifying species in meat mixtures varied depending on the species in the mix. These results indicate that RAPD may be useful for identification of meat samples from single species, such as intact meat samples, whereas caution should be exercised in identification of origin of species in minced meat that may consist of multiple species.

INTRODUCTION

Meat is a valuable source of nutrition rich in biologically valuable proteins, vitamins, phosphorus and iron. Amount of protein of animal source per capita is declining as the population of the world is increasing. As a result, demand for meat and meat products has become higher. It has been reported that some opportunist people may market the meat of animal species that the

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society normally does not consume to meet that demand and increase their profit. This unethical practice occurs primarily by mixing the meat of unacceptable species into that of livestock meat through grinding and/or processing, or less commonly, by direct marketing of the flesh. It is largely agreed that this practice is adulteration in regard to religious, ethical, economic and health aspects (Meyer *et al.* 1994; Saez *et al.* 2004). Methods used for identification of species of origin for raw meat include sensory analysis, anatomical differences, histological differentiation of the hair that may possibly exist on the meat, properties of tissue fat, level of glycogen in muscle tissue, as well as electrophoresis and hybridization (Chikuni *et al.* 1990; Ebbehoj and Thomsen 1991a; Buntjer and Lenstra 1998). Most of these methods have been reported to have limitations in use because of problems in specificity (i.e., sensory analysis, glycogen level, histological differentiation, properties of tissue fat, immunological methods), complexity (i.e., electrophoresis, DNA hybridization), high cost (i.e., DNA hybridization) and some requirements for baseline data about the differences in protein compositions (i.e., isoelectrofocusing) (Kang'ethe and Gathuma 1987; Chikuni *et al.* 1990; Ebbehoj and Thomsen 1991a,b; Rolf *et al.* 1994, 1995; Kamber 1996; Buntjer and Lenstra 1998; Koh *et al.* 1998; Matsunaga *et al.* 1999; Saez *et al.* 2004).

There is a need for development of a more accurate, faster and easy-to-use method (Matsunaga *et al.* 1999). Random amplified polymorphic DNA (RAPD) is a method successfully used for identification of meat species (Lee and Chang 1994; Koh *et al.* 1998; Martinez and Yman 1998; Partis *et al.* 2000; Ilhak and Arslan 2003; Saez *et al.* 2004).

The principle of RAPD technique is based on amplification of DNA fragments using a short oligonucleotide primer that ties multiple locations on the genomic DNA followed by separation of amplified fragments based on their sizes using gel electrophoresis. Samples are identified by comparing the DNA bands on the gel. This method has been successfully used for identification of plants, microorganisms and animals (Çetinkaya 1998; Welsh and McClelland 1990). Ilhak and Arslan (2003) identified raw meats from beef, lamb, goat and wild swine using a 10-base primer. Lee and Chang (1994) differentiated muscle samples of beef, goat, pork, dog, rat, rabbit, chicken, duck and man using RAPD technique with two different 10-base primers. Similarly, Martinez and Yman (1998) studied identification of raw and processed meats of horse, donkey, mule, swine, Canada deer, Ren deer, sheep, goat and kangaroo using three different 10-base primers. Partis *et al.* (2000) identified 22 different animal species.

The objective of the present study was to identify meats from beef, goat, sheep, camel, pig, wild swine, horse, donkey, cat, dog, rabbit and bear using the RAPD technique. In addition, efficacy of RAPD was tested for identifica-

tion of origin of species in 1 : 1 mixtures of sheep-pork, horse meat-beef and sheep-beef.

MATERIALS AND METHODS

Raw Material

In the present study, postrigor muscle tissue samples from beef, goat, sheep, camel, pig, wild swine, horse, donkey, cat, dog, rabbit and bear were used. The samples of beef, sheep and goat were obtained from a local slaughterhouse whereas camel and pig was provided by slaughterhouses specializing in exotic animal in Aydin and Ankara, respectively. Samples for other species were obtained from the Department of Pathology, Faculty of Veterinary Medicine, Firat University, Elazig. Domestic animals sampled were of native breeds in Eastern Anatolia.

DNA Extraction

DNA was extracted from meat samples using a method reported by Koh *et al.* (1998) with slight modification. Briefly, approximately 1 g of sample was homogenized using 4 mL of TNES solution (20 mM Tris pH 8.0, 150 mM NaCl and 10 mM EDTA) in a 15 mL polypropylene tube. A 750 µL aliquot of the resulting homogenate was then transferred into a 1.5 mL-Eppendorf tube and 10 µL of proteinase K (200 mg/mL) and 50 µL of 10% SDS were added. The mixture was shaken vigorously and held overnight at 56C in a water bath. A 250 µL volume of 6 M NaCl was added and the resulting mixture was centrifuged at 11,000 r.p.m. for 15 min. A 500-µL portion of the aquatic phase of the sample was transferred into a separate Eppendorf tube and 300 µL of phenol-chloroform-isoamylalcohol (25 : 24 : 1) was added followed by vigorous shaking and centrifugation at 12,000 r.p.m. for 12 min. A 400 µL portion of the upper layer was transferred into another tube and 400 µL of chloroform was added followed by mixing and centrifugation. A 300-µL portion of the upper phase was taken and 300 µL of absolute ethanol at −20C and 30 µL of sodium acetate was added prior to vortexing and holding the sample at −80C for 2 h for precipitation of DNA. The resulting mixture was centrifuged at 13,000 r.p.m. for 10 min, then the liquid phase was removed. A 300-µL volume of 70% ethanol was added to the pellet followed by centrifugation at 13,000 r.p.m. for 5 min for washing of the DNA. Finally, ethanol was removed and the tube containing DNA was held at room temperature for 30 min for further removal of the residual ethanol via evaporation. The pellet, which is the extracted DNA, was diluted with sterile dH_2O and used as the target DNA for PCR reaction.

Polymerase Chain Reaction (PCR)

The PCR process was conducted using a touchdown thermocycler (Hybaid, Middlesex, England). A total volume of 50 µL of the reaction mixture was prepared in an Eppendorf tube containing 5 µL of 10xPCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 7.5 µL of 25 mM $MgCl_2$, 250 µM deoxynucleotidetriphosphate (dNTP), 2 U Tag DNA polymerase (Promega, Madison, WI, U.S.A.), 25 pmol 10-nt primer and 5 µL target DNA. The sequence of the 10-base primer used was ACGACCCACG (Integrated DNA technologies, Inc., U.S.A.). The thermocyler was programmed for a 45-cycle PCR. Each cycle was composed of denaturation at 95C for 5 min followed by holding at 94C for 1 min, at 34C for 1 min and at 72C for 2 min.

A 15-µL portion of the amplified DNA fragments was run on agarose gel (1.5%) at 100 volts for 2 h for electrophoresis. The resulting gel was stained using ethidium bromide (0.5 µg/mL) and visualized using a UV transilluminator and photographed using a Poloroid 322 camera and T667 film.

The study was composed of three replicates.

RESULTS

Results indicated that the RAPD profiles generated using the 10-base primer from beef, sheep, goat, horse, donkey, camel, dog, cat, rabbit and bear meats were distinctly different from each other and visually distinguishable. RAPD profiles of pig and wild swine, however, were not appreciably different (Fig. 1).

RAPD profiles of meat mixes and original species are presented in Fig. 2. In general, RAPD profiles of meat mixes of two original species were also a combination of their RAPD profiles. This combined profile was sufficiently characteristic in sheep-pork and horse-beef mixes for discrimination. However, the combined profile of sheep-beef mix was not discriminatory.

DISCUSSION

RAPD profiles exhibit variations within the species as well as among the species, because different bands are obtained depending on the primer used (Koh *et al.* 1998). As the sequence of the primer changes, different locations on the DNA are amplified, resulting in different bands on the gel (Williams *et al.* 1990). Different results are obtained when a different primer is used for each species. It is advantageous that a primer of choice should be able to

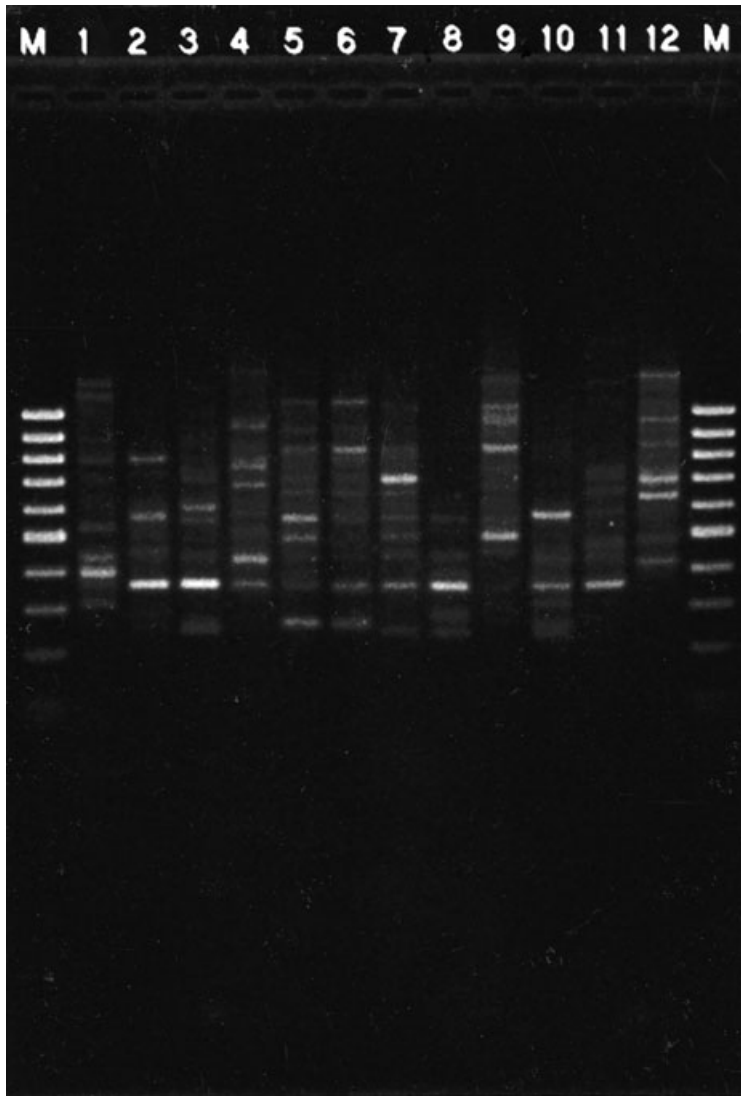

FIG. 1. RAPD PROFILES OF MEATS FROM VARIOUS SPECIES
M, marker; 1, bear; 2, rabbit; 3, dog; 4, cat; 5, donkey; 6, horse; 7, wild swine; 8, pig; 9, camel; 10, sheep; 11, goat; 12, beef.

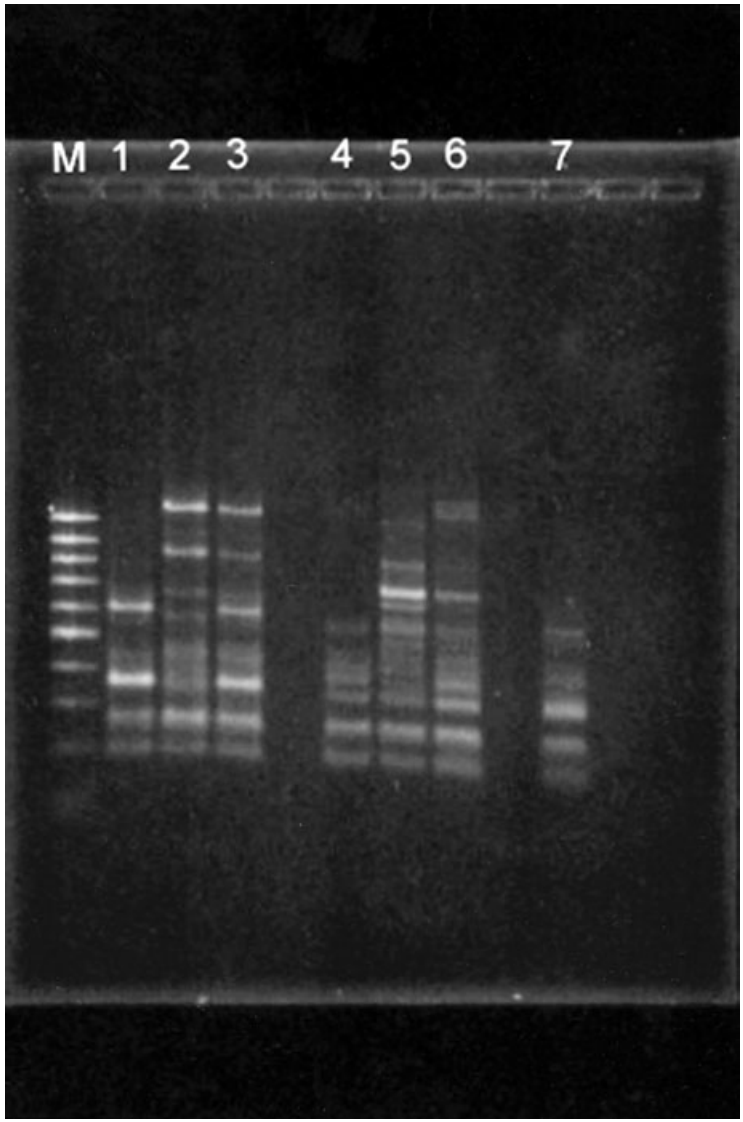

FIG. 2. EFFICACY OF RAPD IN DIFFERENTIATING SPECIES IN MIXED MEATS FROM DIFFERENT SPECIES

M, marker; 1, sheep; 2, wild swine; 3, 1 : 1 sheep and wild swine; 4, beef; 5, horse; 6, 1 : 1 beef and horse; 7, 1 : 1 beef and sheep.

generate specific RAPD profiles for each species so that a single primer can be used for species identification (Koh *et al.* 1998).

Numbers of the bands obtained from RAPD method vary depending on the primer used. It has been recommended that primers yielding fewer bands, preferably one band, should be used for more accurate and rapid interpretation of the results (Martinez and Yman 1998). Koh *et al.* (1998) studied the efficacy of 29 different 10-base primers to find the most ideal primer. Their results showed that some primers were not suitable for RAPD method.

Lee and Chang (1994) identified cattle, goat, swine, rat, rabbit, chicken, duck and human using RAPD method with a primer with sequence of ACGACCCACG on DNA extracted from blood. The researchers reported that the RAPD technique could be used for identifying the differences between species, within species, even among individuals.

The most crucial advantage of the restriction fragment length polymorphism (RFLP) method is that, unlike PCR, alone or RFLP methods, there is no need for use of specific primers for each animal species or for separate PCR reactions. In addition, the DNA sequence of the species does not have to be known. Therefore, species of the meat can be identified inexpensively in a short period of time (Koh *et al.* 1998; Martinez and Yman 1998).

In the present study, 12 different animal species were identified using RAPD with a primer of ACGACCCACG sequence. Obviously, there is a large variation among the bands of different species. However, the primer failed to differentiate within a species such as domesticated and wild swine. More specific primers need to be developed for separation of individuals within the same species.

In some communities, attempts to sell mixed meats from various species through grinding can occur. In the present study, application of RAPD to mixtures of meats of different species produced a profile that was a combination of RAPD profiles of original species. In general, such a combined profile was difficult to interpret. Profiles of sheep-pork and horse-beef were still distinguishable from other species. However, bands in that of sheep-beef were not clearly separated from each other. This might be attributed to the fact that the target DNA concentration was not standardized in the present study, which has been reported as an important factor to obtain clear bands (Koh *et al.* 1998). To our knowledge, there was no previous study in the literature about use of RAPD for identification of species in mixtures of meats from different species.

It can be concluded that use of RAPD for identification of single species may be useful for samples from intact meat. However, this method was not found to be helpful for identifying meat species in a minced mixed meat sample. The present study confirms the results of previous studies about the benefits and efficacy of RAPD method and also extends the identifiable species.

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