POLYMERASE CHAIN REACTION IDENTIFICATION OF HORSE AND DONKEY MEAT IN HEAT-TREATED MEAT

H. Yetim[1], Z. Kesmen[1], F. Sahin[2] and H. W. Ockerman*[3]

[1.] Erciyes Univ., Coll. of Eng., Food Sci. Dept., 38039, Kayseri, Turkey, [2.] Yeditepe Univ., Coll. of Eng. Bioeng. Dept. 34755, Istanbul, Turkey, [3.] The Ohio State Univ., Dept of Anim. Sci. 43210, Columbus, OH, USA.
Email: ockerman.2@osu.edu

Keywords: species identification, PCR, horse, donkey, heat- processed meat products

Introduction

The differentiation of meat species in meat products has been considered important to preserve the consumers from the presence of unknown, less desirable meat types for economic, religious and health reasons (Koh *et al.*, 1998). However, species identification of cooked meat products to detect adulteration or fraudulent substitutions is a problem for the food control authorities (Meyer *et al.*, 1994). For this purpose, numerous analytical methods have been developed for meat species identification based on protein analysis; electrophoretic, chromatographic and immunological techniques (Patterson and Jones, 1990; Zerifi *et al.* 1992). Although most of these methods identified the species origin of raw meats, they were less sensitive when the meat had been thermally processed, because the temperatures during the heat treatment destroyed the species-specific proteins or the epitopes (Calvo *et al.*, 2001). Advances in DNA technology have led to rapid development of alternative approaches for species identification studies. Advantages of DNA analysis compared to protein analysis include stability at higher temperature, presence in all types of tissue and greater variation in genetic code (Wolf *et al.*, 1999). DNA methods have commonly been based on PCR analysis of species-specific mitochondrial DNA (mt-DNA) sequences. The superiority of mitochondrial based DNA analysis derives from the fact that each cell contains several copies of mt-DNA and have improved the possibility of amplifying template molecules of adequate size among the DNA fragments brought about by heat denaturation (Montiel-Sosa *et al.*, 2000). Also there is a relatively high mutation rate compared to nuclear genes to allow the differentiation between the closely-related species (Brodman *et al.*, 2001). PCR based methods exhibit high specifity, sensitivity, rapidity and suitability for complex identification. This study evaluated a method for species identification of horse and donkey meat in heat-processed meat by species-specific PCR of mt-DNA.

Materials and Methods

Preparation of Meat Samples: Bovine, ovine, swine, horse and donkey muscle tissues were used. A mixture of each specie was prepared from raw minced beef or sheep meats by adding donkey and horse meat at 5 different levels (0.0, 0.1, 0.5, 1.0 and 5.0%). Meat mixtures were subjected to heating for 30 min at 100°C and 120°C.

DNA extraction: DNA was extracted following the method of DeSalle *et al.* (1993) and Kesmen (2005).

Oligonucleotide Primers: Two sets of oligonucleotide primers were designed to amplify specific regions for the horse and donkey mt-DNA sequences. The first set amplifies a specific fragment of 153 bp which is part of the horse mitochondrial genome (ATPase8- ATPase6) using 5'-CTATCCGACACACCCAGAAGTAAAG-3' as forward primer and 5'-GATGCTGGGAAATATGATGATCAGA-3' as reverse primer. The second set detects a donkey species-specific amplicon of 145 bp from the donkey mitochondrial genome (NADH dehydrogenase subunit 2) using 5'-CATCCTACTAACTATAGCCGTGCTA-3' as forward pirmer and 5'-CAGTGTTGGGTT GTACACTAAGATG-3' as reverse primer.

PCR Amplification Specific Fragment: The PCR amplification reaction was performed in a total volume of 50 µl (Kesmen, 2005). The products of PCR amplification were analyzed by electrophoresis through a 2% agarose gel.

PCR Specificity and Sensitivity Test: 100ng/µl of bovine, ovine, horse and donkey genomic DNA and a negative control without DNA were used in PCR respectively, to evaluate the specificity of primers used in the study. Also serial 1:10 dilutions of raw horse and donkey DNA were made to 0.01 ng/µl. Each dilution was added separately to the reaction mixtures for the sensitivity test of the PCR assay.

Result and Discussion

The PCR results demonstrated that the specific primers for horse and donkey designed in this study hybridize on the species-specific DNA sequences of each specie and yield 153 and 145 bp fragments respectively in 0.01 ng DNA levels without any cross-reaction between the other species studied (Figure 1 and 2). Although detection of horse and donkey meat was successful in all mixtures heated at 100°C for 30min, regardless whether the product was prepared from beef or sheep (Figure 3). However, the same samples heated at 120°C for 30min could not be detected at the substitution level of 0.1% (Figure 4).

Conclusion

The use of a PCR method is shown to be a reliable, highly specific and sensitive method for the detection of horse and donkey meat in heat processed meat products. PCR assay was able to amplify DNA from the cooked and autoclaved meat since the heat treatment could not seriously destroy the DNA. Nevertheless, in autoclaved meat, DNA samples degrade to small fragments, averaging 300 bp (Meyer *et al.*, 1994). Therefore the PCR system developed in this research, targeting an

even shorter DNA fragments (153 and 145 bp), could be used succesfully in the analysis of highly degraded DNA. These results confirm the appropriateness of mt-DNA for meat species detection containing high number of copies per cell. Also the mt-DNA accepts the mutations fast enough to allow differentiation between closely related species. Particularly mt-DNA sequence encoding for subunit 8 and subunit 6 of ATP synthase complex was chosen as a target sequence to design horse specific primer. Such region was preferred to other mt-DNA coding sequences because both ATPase-8 and ATPase-6 exhibit a relatively high degree of variation among the vertebrates (Tartaglia *et al.,* 1998). Although phylogenetically close animal species are studied, identifications appear to be highly species-specific. It is a simple and reliable method for meat species identification that can be adapted to many differentiation problems.

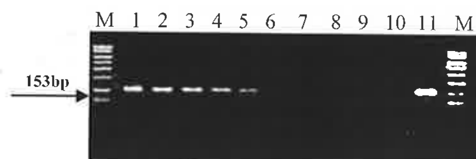

Figure 1: The result of PCR specificity and sensitivity test for the specific horse primers.M: Marker; 1: 100ng horse; 2: 10ng horse; 3: 1 ng horse; 4: 0.1 ng horse; 5: 0.01 ng horse; 6: donkey; 7: pig; 8:beef; 9: lamb; 10:Negative control; 11: Positive control .

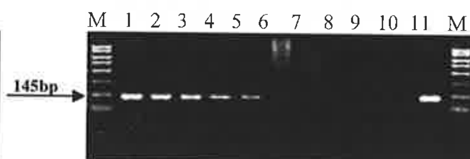

Figure 2: The result of PCR specificity and sensitivity test for the specific donkey primers. M: Marker; 1: 100ng donkey; 2: 10ng donkey; 3: 1 ng donkey; 4: 0.1 ng donkey; 5: 0.01 ng donkey; 6:horse; 7: pig; 8:beef; 9: lamb; 10:Negative control; 11: Positive control.

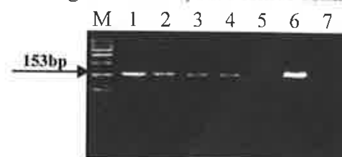

Figure 3: Electrophoretic gel of horse-beef mixtures heated at 100 °C for 30 min. M: Marker; 1: horse %5 + beef 95%; 2: horse %1 + beef 99%; 3: horse %0.5 + beef 99.5%; 4: horse %0.1 + beef 99.9%; 5:beef 100%; 6: Positive control; 7: Negative control.

Figure 4: Electrophoretic gel of donkey-beef mixtures heated at 120°C for 30 min. M: Marker; 1: donkey %5 + beef 95%; 2: donkey %1 + beef 99%; 3: donkey %0.5 + beef 99.5%; 4: donkey %0.1 + beef 99.9%; 5: beef 100%; 6: Positive control; 7: Negative control.

References

Brodmann, P.D., Nicholas, G., Schaltenbrand, P. and Ilg, E.C. (2001). Identifying unknown game species: with nucleotid sequencing of the mitochondrial sitokrom b gene and a subsequent basic local alignment search tool search. Z. Lebensm. Unters Forsch. 212, 491-496.

Calvo, J.H., Zaragoza, P. and Osta, R. (2001). Technical note: A quick and more sensitive method to identify pork in processed food by PCR amplification of a new specific DNA fragment. J. Anim. Sci., 79, 2108-2112.

DeSalle, R., Williams, A. and George, M. (1993). Isolation and characterization of animal mitochondrial DNA. Meth. Enzymol. 224, 174-204.

Guoli, Z., Mingguang, Z., Zhijiang, Z., Hongsheng, O. and Qiang, L. (1999). Establishment and application of a polymerase chain reaction fort he identification of beef. Meat Sci., 51, 233-236.

Kesmen, Z. (2005). A research on the meat species identification in fresh and processed meat products by using pcr techniques. PhD Dissertation, Ataturk Univ. Erzurum, 85 p.

Koh, M.C., Lim, C.H., Chua, S.B., Chew, S.T. and Phang, S.T.W. (1998). Random amplified polymorhic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci., 48, (3/4) 275-285.

Meyer, R., Candrian, U. and Lüthy, J. (1994). Detection of pork in heated meat products by Polymerase Chain Reaction (PCR). J. Assoc. Off. Anal. Chem. Int., 77, 617-622.

Montiel-Sosa, J.F., Ruiz-Pesini, E., Montoya, J, *et al.* (2000). Direct and highly species-specific determination of pork meat and fat in meat products by PCR amplification of mitochondrial DNA. J.Agric., Food Chem., 48 2829-2832.

Patterson,R. L. and Jones S.J. (1990). Review of current technique for verification of the species origin of meat. Analyst, 115, 501-506.

Tartaglia M., Ernestina, S., Pestalozza, S., Morelli, L., Antonucci, G. and Battaglia, P.A. (1998). Detection of bovine mitochondrial DNA in ruminant feeds: A molecular approach to test fort he presece of bovine derived materials. J. Food Prot., 61 (5), 513- 518.

Wolf, C., Rentsch, J. and Hübner, P. (1999). PCR-RFLP analysis of mitochondrial DNA: A reliable method for identification. J. Agric. Food Chem., 47, 1350-1355.

Zerifi, A., Labie, C. and Benard, G. (1992). SDS-PAGE Technique fort he species identification of cooked meat. Fleiswirtschaft 1. 54-59.