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Araştırma Makalesi ***Research Article***

Authentication of Meat Species in Sucuk by Multiplex PCR

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Abstract

The identification of meat species used in meat products is important by reason of economic considerations, religious factors, verification of label, and prevention of unfair-market competition. In this paper, multiplex PCR method was experienced for routine detection of equine (horse and donkey), poultry (chicken and turkey), pig and cattle meat in sucuk (sausage). The primers used for these animals generated specific fragments, and they did not show cross reactions with the DNA from the other genus of animal. After multiplex PCR was successfully optimized, a field study was carried out to investigate the presence of horse, donkey, chicken, turkey and pig meat in 50 sucuks (30 beef and 20 beef + poultry) collected from markets. The result of the field study indicated that 23.3% of 30 beef sucuk samples were containing poultry meat. None of the 50 sucuk samples was containing pig meat, but one (2%) of the samples generated equine fragment. The present study showed that the multiplex PCR method can be used for routine analysis of meat species identification, verification and control of label information of meat products.

Özet

Multipleks PCR ile Sucuklarda Et Türlerinin Doğruluğunun Kanıtlanması

Et ürünlerinde kullanılan et türlerinin saptanması ekonomik sebepler, dini faktörler, etiketin doğrulanması ve haksız rekabetin önlenmesi açısından önemlidir. Bu çalışmada, sucuklarda tek tırnaklı (at ve eşek), kanatlı (tavuk ve hindi), domuz ve sığır etinin rutin tespiti için multipleks PCR metodu denendi. Bu hayvanların DNA'larının tespitinde diğer hayvan türleri ile çapraz eşleşme göstermeyen türe özgü primerler kullanıldı. Multipleks PCR başarılı bir şekilde optimize edildikten sonra, marketlerden toplanmış 50 adet sucukta (30 adeti sığır eti, 20 adeti sığır eti-kanatlı eti karışımı) at, eşek, tavuk, hindi ve domuz etinin varlığının araştırıldığı bir saha çalışması gerçekleştirildi. Saha çalışmasının sonuçları sığır etinden üretilmiş 30 adet sucuğun %23,3'ünün kanatlı eti içerdiğini gösterdi. Elli adet sucuk örneğinin hiçbirinde domuz eti bulunmadı fakat bir örnekten (%2) tek tırnaklı DNA parçası çoğaltıldı. Bu çalışma multipleks PCR metodunun et ürünlerinde et türlerinin belirlenmesi, doğrulanması ve etiket bilgilerinin kontrolü için rutin olarak kullanılabileceğini gösterdi.

Introduction

The identification of meat species in processed meat products has always been a concern by reason of fraudulence, religious factors, and control of unfair market competition in the meat industry. Advances in DNA technology and development of Polymerase Chain Reaction (PCR) technique have allowed to identification of animal species in meat products in a way faster, simpler and reliable (Bottero et al., 2003; Fajardo et al., 2006; Haunshi et al., 2009; Koh et al.,1998; Matsunaga et al., 1999; Pegels et al., 2011). PCR techniques used for the identification of meat species include RAPD-PCR (Koh et al., 1998), RFLP-PCR (Ali et al., 2012; Fajardo et al., 2006; Murugaiah et al., 2009), species-specific PCR (Haunshi et al., 2009; Kesmen et al., 2007; Lahiff et al., 2001; Mane et al., 2011), real-time PCR (Pegels et al., 2011; Sakalar and Abasiyanik, 2012; Ulca et al., 2013; Zhang et al., 2007) and multiplex PCR (Bai et al., 2009; Dalmasso et al., 2004; Ghovvati et al., 2009). The advantage of multiplex PCR is that, unlike species-specific PCR, there is no need for use a separate PCR reaction tube for each animal species. Templates DNA mixture can be simultaneously amplify in a single reaction tube by multiplex PCR technique, and thereby, the detection cost, time and labor force can be decreased.

Sucuk is a traditional fermented sausage which is the most popular meat products consumed in Turkey. The English pronunciation of sucuk is soudjouk, and in some degree, it has typical properties of both Northern European and Southern European style fermented sausages (Ercoşkun and Özkal, 2011). The meat products such as sucuk can cause to be not distinguishing of meat species used in product by the consumers. Therefore, adulteration or fraudulent substitution can be easily made in such products. Consumers have a right to know that what meat species they eat. For this reason, meat products must be properly labeled by the producers, and routinely monitored by food authorities.

The aim of the present study was to experience a multiplex PCR for rapid detection and identification of species adulteration in sucuk. Instead of using separately primers for each animal species, an equine primer (for horse and donkey) and poultry primer (for chicken and turkey) were used to identification of meat species. In addition to the equine and poultry primers, the primers for the identification of pork and beef were added to multiplex-PCR reaction tube. This study also reported the results of a field study carried out on the presence of equine, poultry meat and pork in sucuk sold in the local markets by using this method.

Materials and Methods

Meat samples

Muscle tissue samples from beef, chicken, turkey, horse, donkey, and pig were used for positive control samples. A total of 50 sucuk samples were collected from local markets, butcher shops, and delicatessen stores, between September 2012 and January 2013. According to their label information, 30 out of 50 sucuk samples had been made from 100% beef, and 20 sucuk samples had been made from the mixture of beef and chicken or turkey meat. The samples were kept in a freezer (Uğur, UDD 600 BK, Nazilli, TR) until being analyzed.

DNA extraction from muscle tissue and sucuk samples

A method described by İlhak and Arslan (2007) was used for DNA extraction. Briefly, approximately 1-2 g tissue samples or sucuk samples were homogenized by 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 kept for 8 h at 58°C in a water bath. A 250 µl volume of 6 M NaCl was added to the resulting mixture and it was centrifuged at 11,600 × g for 5 min. Subsequently, 500 µl portion of the aquatic phase of the sample was used for the usual method of DNA extraction with phenol-chloroform-isoamyl alcohol (25:24:1) and finally precipitated with absolute ethanol at –20°C for 8 h, and washed with 70% ethanol, and the pellet was diluted with 100 µl of sterile dH_2O and used for PCR reaction.

Primers

PCR primers (Iontec Co., Istanbul, Turkey) and their base pair length for the amplification of cattle, poultry (chicken and turkey), equine (horse and donkey), and pig DNA were shown in Table 1.

Simplex PCR

The 50 µl reaction mixture was prepared in an Eppendorf tube containing 5 µl of 10 × PCR buffer (Promega, Madison, WI, USA), 5 µl $MgCl_2$ (25 mM), 250 µM deoxynucleotide triphosphate mix (dNTPs), 0.25 µl of Taq DNA polymerase (Promega, Madison, WI, USA), 2.5 µl from each primer of 25 pmol (total 2 primers (one forward and one reverse), 5 µl of target DNA, and about 25 µl of dH_2O. The thermocycler (PCR Sprint, ThermoHybaid, England) was programmed for 35 cycle PCR. Each cycle included holding at 94°C for 60 s, at 58°C for 60 s, and at 72°C for 60 s. At the end of the 35 cycle of PCR, final extension step at 72°C for 3 min was performed.

Multiplex PCR

The 50 µl reaction mixture was prepared in an Eppendorf tube containing 6 µl of 10 × PCR buffer (Promega, Madison, WI, USA), 7 µl $MgCl_2$ (25 mM), 7 µl of 250 µM deoxynucleotide triphosphate mix (dNTPs), 1 µl of Taq DNA polymerase (Promega, Madison, WI, USA), 2 µl from each primer of 25 pmol (total 8 primers (four forward and four reverse), 5 µl of target DNA, and about 8 µl of dH_2O. The thermocycler (PCR Sprint, ThermoHybaid, England) was programmed for 35 cycle. Each cycle was the same of the simplex PCR described above.

Agarose gel electrophoresis of amplified products

A 15 µl portion of the amplified DNA fragments was run on agarose gel (2%) at 100 V for 1 h for electrophoresis. The resulting gel was stained using ethidium bromide (0.5 µg/ml) and visualized by using a UV transilluminator (TC 312 E/F, Spectronics Corp., NY, USA) and photographed.

Results

In the first stage of the study, simplex PCR was performed for amplifying the DNA extracted from muscle tissue samples of beef, chicken, turkey, horse, donkey and pig. The primers which used in the present study were generated specific fragments of 439, 256, 212, and 183 bp for equine (horse and donkey), cattle, pig, and poultry (chicken and turkey), respectively (Figure 1). Primers did not show any cross reactions with the DNA of the other genus of animals which used in the study. Then, the DNAs extracted from muscle tissue samples of beef, chicken, turkey, horse, donkey and pig were mixed in a tube separately and prepared DNA mixture of the animals. The multiplex PCR was performed to the DNA mixtures for amplifying of the each animals' DNA. The multiplex PCR was successfully carried out, and the amplified products were shown in Figure 1.

In the second stage, a field study was carried out to investigate the presence of equine, poultry and pig meat in sucuk sold in the local markets by using this method. The results of the multiplex PCR analysis of the sucuk samples were showed in Figure 2. The results showed that 7 out of 30 (23.3%) sucuk samples that have been labeled as 100% beef by the producers were found as containing poultry meat, and 20 (100%) sucuk samples that have been labeled as beef + poultry meat by the producers were found to be labeled properly. None of the sucuk samples were found as containing pork, but one sample (2%) of 50 sucuk samples was found as containing equine (horse or donkey) meat.

Table 1. Primer pairs used for the identification of animal species and their amplicon size.

Tablo 1. Hayvan türlerinin belirlenmesinde kullanılan Primer çiftleri ve amplikon boyutları.

Animal Species	Primers Design	Amplicon Size (bp) and References
Cattle	5'- GTACTACTAGCAACAGCTTA-3' 5'- GCTTGATTCTCTTGGTGTAGAG-3'	256 (Bottero et al., 2003)
Poultry (Chicken and Turkey)	5'-TGAGAACTACGAGCACAAAC-3' 5'-GGGCTATTGAGCTCACTGTT-3'	183 (Dalmasso et al., 2004)
Equine (Horse and Donkey)	5'- GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3' 5'-CTCAGATTCACTCGACGAGGGTAGTA-3'	439 (Matsunaga et al., 1999)
Pig	5'-GCCTAAATCTCCCCTCAATGGTA-3' 5'-ATGAAAGAGGCAAATAGATTTTCG-3'	212 (Lahiff et al., 2001)

Discussion

Species identification of meat which used in meat products has an importance because of economic reasons, religious factors, fraud and control of unfair-market competition. This issue is being more important as the halal market has expanded in global trade. According to the food labeling regulations, animal species used in meat products should be indicated on the label of food. In Turkey, according to the Turkish Food Codex: Notification of meat and meat products (Notification no: 2012/74), minced meat or prepared meat mixtures may not be produced by mixing the red meat with poultry meat, after March 1, 2013 (Anonym, 2012). The present study did not investigate whether the related article of the Turkish Food Codex were met by the producers, because sucuk samples were collected between September 2012 and January 2013. However, the present study investigated whether the information given by the manufacturers on the label was correct.

Results of the present study showed that 23.3% of total 30 beef sucuk samples had been mislabeled. In a study carried out by Şakalar and Abasiyanik (2011), it has been noted that 35.1% of the collected red meat samples includes poultry meat, although it was not indicated on their label. Özpınar et al. (2013) reported that 53.4% of the meat and meat products collected in İstanbul province were labeled incorrectly. In another study carried out by Ulca et al. (2013), it was found that 4.76% of the processed meat products collected from retail markets had incorrect labeling. In the present study, no sucuk sample was containing pig meat, but one sample was containing equine (horse or donkey) meat. There are several studies indicating that horse meat (between 0 and 9.8%) and pork (between 0 and 7.1%) were detected in meat and meat products sold in Turkey (Ayaz et al., 2006; Çetin et al., 2008; Çetin et al., 2010; Günşen et al., 2006; Türk et al., 2005; Türkyılmaz et al., 2009; Yalçın and Alkan, 2012).

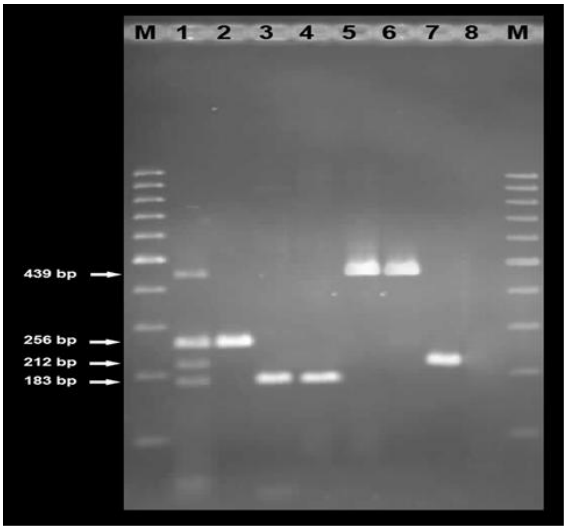

Figure 1. Agarose gel analysis of the multiplex PCR and simplex PCR products.

M: Molecular marker (100 bp), 1: Multiplex PCR products of the mixture of cattle: turkey: chicken: pig: horse: donkey DNA 2: cattle (256 bp), 3: turkey (183 bp), 4: chicken (183 bp), 5: horse (439 bp), 6: donkey (439 bp), 7: pig (212 bp), 8: negative control (Multiplex PCR result of sterilized distilled water with the primers)

Şekil 1. Multipleks PCR ve basit PCR ürünlerinin agaroz jel analizleri.

M: moleküler marker (100 bp), 1: sığır: hindi: tavuk: domuz: at: eşeğin multipleks PCR ürünlerinin DNA karışımı 2: sığır (256 bp), 3: hindi (183 bp), 4: tavuk (183 bp), 5: at (439 bp), 6: eşek (439 bp), 7: domuz (212 bp), 8: negatif kontrol (primerin sterilize distile su ile karıştırılmış multipleks PCR sonuçları)

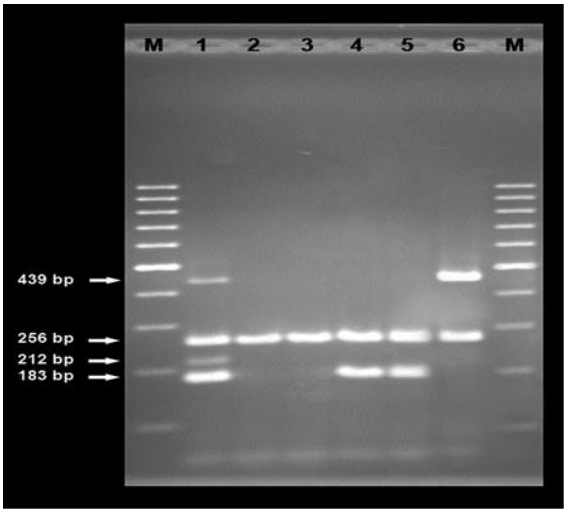

Figure 2. Multiplex-PCR results of the some sucuk samples collected from local markets.

M: moleculer marker (100 bp), 1: positive control for the multiplex PCR products of the mixture of cattle: turkey: chicken: pig: horse: donkey DNA 2-6: Multiplex-PCR results of the some sucuk samples.

Şekil 2. Yerel marketlerden toplanmış bazı sucuk örneklerinin multipleks PCR sonuçları.

M: moleküler marker (100 bp), 1: Pozitif kontrol için sığır: hindi: tavuk: domuz: at: eşek DNA karışımlarının multipleks PCR ürünleri 2-6: Bazı sucuk örneklerinin multipleks PCR sonuçları.

Sensitivity and specificity of the multiplex PCR has been proved and reported by the researchers (Dalmasso et al., 2004; Ghovvati et al., 2009; Şakalar and Abasiyanik, 2011). In Turkey, horse, donkey, poultry meat and pig meat are the most used species for adulteration in meat products. However, in meat plants processing poultry and ruminant species together, contamination of meat products with another meat species may be inevitable during meat operation such as cutting and grinding via knives, bowl cutters, cutting boards etc.,. The result of the multiplex PCR analyses of such samples may show the product as if it is adulterated. On the other hand, the presence of equine meat or pork in meat products is unacceptable by the Muslim consumers, even though contamination is unintentional and incidental level. Because of that, meat processing plants should process a single species, or should process their products in a separated production line.

The Regulation related to Notification of meat and meat products (Notification no: 2012/74) entered into force after March 1, 2013. Absence of horse meat in a meat product does not mean that this product does not contain donkey or mule meat, similarly absence of chicken meat in a meat product does not mean that this product does not contain turkey or other poultry meat. Therefore, equine primer and poultry primer were used to identification of horse, donkey, and chicken, turkey meat, respectively, instead of using separately primers for each animal species. In this way, the present study describes the application of the multiplex PCR to detect equine (horse and donkey), poultry (chicken and turkey), pork and beef in sucuk samples with a single reaction tube and four specific primers. This technique, unlike simplex PCR, does not require more than one PCR reaction tube for one meat product. Hence, it is cheap and time saving method. This method can be routinely used by the food inspection laboratories for the verification and control of animal species indicated in the label of sucuk.

REFERENCES

Ali, M.E., Hashim, U., Mustafa, S., Man, Y.B.C., 2012. Swine-Specific PCR-RFLP assay targeting Mitochondrial Cytochrome B gene for semiquantitative detection of pork in commercial meat products. Food Analytical Methods 5, 613-623.

Anonymous, 2012. Turkish Food Codex Notification No. 2012/74. (Türk Gıda Kodeksi Et ve Et Ürünleri Tebliği (Tebliğ No: 2012/74).

Ayaz, Y., Ayaz, N.D., Erol, İ., 2006. Detection of species in meat and meat products using Enzyme-linked immunosorbent assay. Journal of Muscle Foods 17, 214-220.

Bai, W., Xu, W., Huang, K., Yuan, Y., Cao, S., Luo, Y., 2009. A novel common primer multiplex PCR (CP-M-PCR) method for the simultaneous detection of meat species. Food Control 20, 366-370.

Bottero, M.T., Civera, T., Nucera, D., Rosati, S., Sacchi, P., Turi, R.M., 2003. A multiplex polymerase chain reaction for the identification of cows', goats' and sheep's milk in dairy products. International Dairy Journal 13, 277-282.

Çetin, O., Bingöl, E.B., Akkaya, H., 2008. The microbiological, serological and parasitological quality of çig kofte (raw meatball) and its lettuce marketed in Istanbul. Polish Journal of Environmental Studies 17, 701-706.

Çetin, O., Bingöl, E.B., Çolak, H., Ergün, O., Demir, C., 2010. The microbiological, serological and chemical quality of mincemeat marketed in İstanbul. Turkish Journal of Veterinary and Animal Sciences 34, 407-412.

Dalmasso, A., Fontanella, E., Piatti, P., Civera, T., Rosati, S., Bottero, M., 2004. A multiplex PCR assay for the identification of animal species in feedstuffs. Molecular and Cellular Probes 18, 81-87.

Ercoşkun, H., Özkal, S.G., 2011. Kinetics of traditional Turkish sausage quality aspects during fermentation. Food Control 22, 165-172.

Fajardo, V., Gonzalez, I., Lopez-Calleja, I., Martin, I., Hernandez, P.E., Garcia, T., Martin, R., 2006. PCR–RFLP authentication of meats from red deer (Cervus elaphus), fallow deer (Dama dama), roe deer (Capreolus capreolus), cattle (Bos taurus), sheep (Ovis aries), and goat (Capra hircus). Journal of Agricultural and Food Chemistry 54, 1144-1150.

Ghovvati, S., Nassiri, M.R., Mirhoseini, S.Z., Heravi Moussavi A., Javadmanesh, A., 2009. Fraud identification in industrial meat products by multiplex PCR assay. Food Control 20, 696-699.

Günşen, U., Aydın, A., Ovalı, B.B., Coşkun, Y., 2006. Çiğ et ve ısıl işlem görmüş et ürünlerinde ELISA tekniği ile farklı et türlerinin tespiti. İstanbul Üniversitesi Veteriner Fakültesi Dergisi 32, 45-52.

Haunshi, S., Basumatary, R., Girish, P.S., Doley, S., Bardoloj, R.K., Kumar, A., 2009. Identification of chicken, duck, pigeon and pig meat by species-specific markers of mitochondrial origin. Meat Science 83, 454-459.

İlhak, O.İ., Arslan, A., 2007. Identification of meat species by polymerase chain reaction (PCR) technique. Turkish Journal of Veterinary and Animal Sciences 31, 159-163.

Kesmen, Z., Sahin, F., Yetim, H., 2007. PCR assay for the identification of animal species in cooked sausages. Meat Science 77, 649-653.

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

Lahiff, S., Glennon, M., O'Brien, L., Lyng, J., Smith, T., Maher, M., Shilton, N., 2001. Species-specific PCR for the identification of ovine, porcine and chicken species in meat and bone meal (MBM). Molecular and Cellular Probes 15, 25-35.

Mane, B.G., Mendiratta, S.K., Tiwari, A.K., Sharma, B.D., Bhilegaokar, K.N., Anjaneyulu, A.S.R., 2011. Detection of pork in admixed meat and meat products by species-specific PCR technique. The Indian Journal of Animal Sciences 81, 1178-1181.

Matsunaga, T., Chikuni, K., Tanabe, R., Muroya, S., Shibata, K.,Yamada, J., Shinmura, Y., 1999. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Science 51, 143-148.

Murugaiah, C., Noor, Z.M., Mastakim, M., Bilung, L.M., Selamat, J., Radu, S., 2009. Meat species identification and halal authentication analysis using mitochondrial DNA. Meat Science 83, 57-61.

Özpınar, H., Tezmen, G., Gökçe, İ., Tekiner, İ.H., 2013. Detection of animal species in some meat and meat products by comparatively using DNA microarray and real time PCR methods. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 19, 245-252.

Pegels, N., Gonzalez, I., Martin, I., Rojas, M., Garcia, T., Martin, R., 2011. Applicability assessment of a real time PCR assay for the specific detection of bovine, ovine and caprine material in feedstuffs. Food Control 22, 1189-1196.

Sakalar, E., Abasiyanik, M.F., 2012. The development of dublex real-time PCR based on SYBR green florescence for rapid identification of ruminant and poultry origins in foodstuff. Food Chemistry 130, 1050-1054.

Şakalar, E., Abasiyanik, M.F., 2011. Qualitative analysis of meat and meat products by multiplex polymerase chain reaction (PCR) technique. African Journal of Biotechnology 10, 9379-9386.

Ulca, P., Balta, H., Cagin, I., Senyuva, H.Z., 2013. Meat species identification and Halal authentication using PCR analysis of raw and cooked traditional Turkish food. Meat Science 94, 280-284.

Türk, N., Kafa, B., İzan, Y., 2005. Et ve et ürünlerinde tür tayini. 5. Gıda Kongresi, 19-21 Nisan İzmir, Türkiye.

Türkyılmaz, Ö., Kafa, B., İzan, Y., Sava, Ş., 2009. Çiğ et ve et ürünlerinde AGID yöntemi ile türlerin tespiti. Bornova Veteriner Kontrol Araştırma Enstitüsü Dergisi 31, 15-20.

Yalçın, H., Alkan, G., 2012. Investigation of the presence of horse and pig meat in meat and meat products using uhlenhuth precipitation ring, agar gel immuno-diffusion and enzyme-linked immuno sorbent assay techniques. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 18, 923-927.

Zhang, C.L., Fowler, M.R., Scott, N.W., Lawson, G., Slater, A., 2007. A TaqMan real-time PCR system for the identification and quantification of bovine DNA in meats, milks and cheeses. Food Control 18, 1149-1158.