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Identification of meat species in different types of meat products by PCR

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Summary: Species identification in food of animal origin is an important subject for food control. Adulteration in foods is relevant for economical, religous, legislation or public health concerning reasons. The aim of this study was to determine the adulteration in different meat products such as sucuk (n=37), sausage (n=33) and salami (n=32). A total of 102 different meat products obtained from various markets in Ankara were analyzed for species identification by PCR. According to the analyzes, five (13.5%) poultry, and one (2.7%) poultry and equine meats together were found in the sucuk samples that are not declared in their labels. Also, seven (21.8%) and two (6.1%) poultry meat were detected in 32 salami and 33 sausage samples, respectively. These results indicate that 15 (14.7%) of the total samples were found to contain undeclared species. As a result, there were meat products which were not in compliance with their labels in various markets, presenting a potential public health risk and economical losses of consumers.

Keywords: Adulteration, meat species identification, mislabeling, PCR.

Farklı tip et ürünlerinde PZR ile tür tayini

Özet: Et ürünlerinde tür tayini gıda güvenliği açısından önemli bir konudur. Etikette bildirilmeyen çeşitli hayvan türlerine ait etlerin kullanımı, tüketici sağlığı, ülke ekonomisi, dini ve yasal düzenlemeler ile ilişkilendirilmektedir. Bu çalışmada, sucuk, salam, sosis gibi ürünlerde farklı türlere ait etlerin varlığının tespiti amaçlanmıştır. Araştırmada Ankara'da çeşitli marketlerde satışa sunulan toplam 102 adet işlenmiş et ürünü tür tayini amacıyla PZR yöntemi kullanılarak incelenmiştir. Yapılan analizler sonucunda, incelenen 37 adet sucuk örneğinden etikette belirtilmeyen 5 (%13.5) kanatlı, 1 (%2.7) kanatlı ve tek tırnaklı eti tespit edilmiştir. Benzer şeklide, 32 salam örneğinin yedisinde (%21.8), 33 adet sosis örneğinin de ikisinde (%6.1) kanatlı eti bulunmuştur. Ürünlerin etiket bilgileri karşılaştırıldığında 15 adet (% 14.7) örneğin etiketinde belirtilmeyen farklı hayvan türlerine ait etlerden üretildiği tespit edilmiştir. Sonuç olarak, Ankara'da farklı marketlerden alınan işlenmiş et ürünleri örneklerinde, etiketlerinde belirtilmeyen hayvan türlerine ait etlerin kullanıldığı ve bu durumun potansiyel halk sağlığı sorunları yanında tüketicinin ekonomik olarak da kayba uğramasına sebep olabileceği ortaya konmuştur.

Anahtar sözcükler: Hile, et tür tayini, yanlış etiketleme, PZR.

Introduction

The consumption of meat and meat products continues to escalate in most regions of the world, especially in developing countries (6). In Turkey, total meat consumption is estimated at 12 kg/person per year. Correspondingly, the prices of beef and other meat products have increased. Red meat is still considered a luxury item in Turkey with a price double than that of chicken (1). Due to escalating prices, the globalization of the food trade and increased processing, meat adulteration and fraud has become common (3).

Species identification in food of animal origin is an important aspect of food control (7). Adulteration in food is relevant for economic, religious and public health reasons (26). Generally, adulteration in meat products encompasses the fraudulent substitution or addition of animal proteins such as cheaper varieties or plant proteins like soybean. The other common feature is mislabeling and the use of lower amounts of meat than is declared on the product (9). Determination of fraud in meat production is not only important for economic, health and ethical reasons but also to ensure fair trade and compliance with legislation (17, 23). Due to several reasons including food scandals and socio-economic changes, consumers are demanding increase in the detection of meat species and fraudulent labeling in different foods (5). Also, authentication has become more important for Muslim countries in recent years, because of halal food status (21).

Various methods based on analysis of species-specific components like protein and DNA have been developed to detect meat and meat products coming from different animal species (14). However, detection of

proteins might be impossible because of their degradation or severe alteration during the processing of meat. Immunological, chromatographic and electrophotometric methods based on protein detection may be inadequate to discriminate between species which are close relatives. Furthermore, these techniques are not suitable for routine use because the isolation procedure is difficult and time-consuming (4, 16).

DNA is a more stable molecule compared to proteins under most conditions, so methods based on amplification of target DNA regions have been applied in the recent years (22). PCR using species-specific primers would allow direct species identification without the need for further analysis of the PCR products. In addition, the use of species-specific primers can be affected by the existing intra specific polymorphisms. PCR can be combined with a nucleotide sequencing or restriction fragment length polymorphism (RFLP) analysis. However, their increased complexity make their application as routine quality control tests less realistic (18). Multiplex PCR is highly repeatable, time saving and more affordable than the other methods. Therefore, genomic and mitochondrial genes, such as cytochrome *b* gene, D-loop, *12S rRNA* and *16S rRNA* gene, have been used frequently for species determination by multiplex PCR (6, 15).

The aim of this study was to determine adulteration in different meat products such as sucuk, sausage and salami obtained from various markets in Ankara by PCR.

Materials and Methods

Preparation of the samples and DNA extraction: Different meat products including sucuk, sausage and salami were collected from local shops (n=11), hypermarkets (n=5) and butchers (n=3) in Ankara region, Turkey. In total, 37 sucuk, 33 sausage and 32 salami samples were stored at -20°C until DNA isolation. Some of the local shops, hypermarkets or butchers were sampled twice and collected samples were marked alphabetically. The products' meat contents were declared in their labels. DNA isolation was performed with a commercial DNA isolation kit DNeasy® Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sucuk samples included high levels of oil, which prevented DNA isolation or decreased the DNA yield. To improve the amount of DNA yield, an additional step was added to the recommended isolation protocol, which was incubation of sucuk samples at 56°C for 1 h.

PCR amplification: The *12S rRNA* region within mitochondrial DNA was amplified with species-specific primers for differentiation of seven animal species. Sequences and references of the primers and amplicon lengths are listed in Table 1. Species-specific PCR optimizations were performed to define chemical density of $MgCl_2$ and annealing temperatures of primers. Optimum PCR results are summarized in Table 2. PCR reactions were performed in total 25 µl volumes by using BioRad C1000 Thermal Cycler (Hercules, CA). PCR cycles were as follows: initial denaturation step at 94°C for 4 min; 30 cycles of 94°C for 30 s, annealing at 57-64°C for 30 s (Table 2), extension at 72°C for 30 s; and a final elongation at 72°C for 30 s; and a final elongation at 72°C for 10 min. To enhance PCR's reliability, known DNA was used as positive control for each species (Figure 1).

Table 1. The sequences and references of the primers and amplicon lengths.
Tablo 1. Primer sekansları, kaynakları ve amplikon uzunlukları.

Species	Forward and Reverse primers, respectively	References	Amplicon (bp)
Cattle	5'-TTAGTTGAATTAGGCCATGAAGCA-3' 5'-GTTTAAATAGGGTTAAGATGCACTCAATC-3'	Martín et al. (2007)	84
Sheep	5'-CTAAGAATAGAGTGCTTAGTTGAACCAGG-3' 5'- GTCTCCTCTCGTGTGGTTCAGATA-3'	Martín et al. (2007)	121
Goat	5'-AAACGTGTTAAAGCACTACATC-3' 5'-GTCTTAGCTATAGTGTATCAGCTGCA-3'	Martín et al. (2007)	122
Horse	5'-GACACACCCAGAAGTAAAGACA-3' 5'-TGCTGGGAAATATGATGATCAGA-3'	Kesmen et al. (2009)	145
Donkey	5'-TGCTAGCCTCATTATCAGTAT-3' 5'-GTGATGAGGATACGTGCT-3'	Kesmen et al. (2009)	83
Pork	5'-CTACATAAGAATATCCACCACA-3' 5'-ACATTGTGGGATCTTCTAGGT-3'	Dalmasso et al. (2004)	290
Chicken&Turkey	5'-TGAGAACTACGAGCACAAAC-3' 5'-GGGCTATTGAGCTCACTGTT-3'	Dalmasso et al. (2004)	183

Table 2. Optimum PCR conditions.
Tablo 2. Optimum PZR koşulları.

Conditions	Pork	Horse	Chicken Turkey	Donkey	Cattle	Goat	Sheep
10x Buffer	1x	1x	1x	1x	1x	1x	1x
$MgCl_2$ (mM)	2	3	1,5	4	1,5	1,5	1,5
dNTP (mM)	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Forward primer (pmol)	3	3	3	5	5	5	3
Reverse primer (pmol)	3	3	3	5	5	5	3
Taq DNA polymerase (IU)	1	1	1	1	1	1	1
DNA (ng)				50-100			
Annealing temperature ºC	57	64	57	57	57	60	57

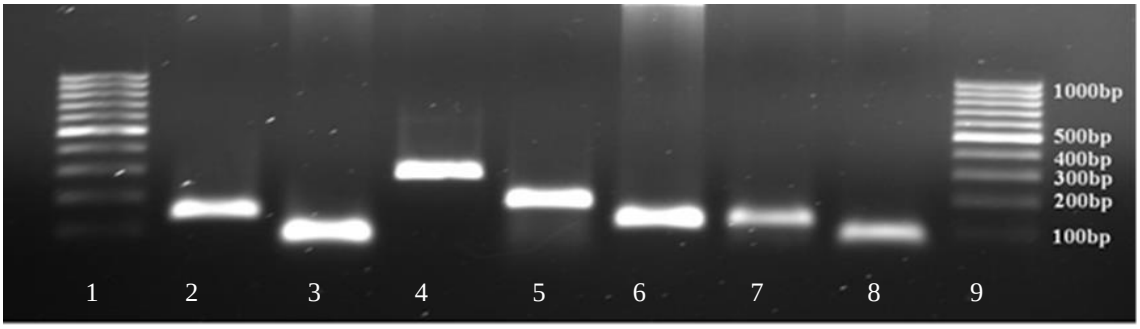


Figure 1. PCR results from positive control samples. Lane 1 (L1) and L9 is 100bp ladder. L2 is positive control for horse, L3 is positive control for donkey, L4 is positive control for pork, L5 is positive control for chicken & turkey, L6 is positive control for goat, L7 is positive control for sheep, L8 is positive control for cattle.
Şekil 1. Pozitif kontrollerin PZR sonuçları. 1.sıra (L1) ve L9 100 bp marker. L2 at, L3 eşek, L4 domuz, L5 tavuk&hindi, L6 keçi, L7 koyun, L8 sığır için pozitif kontroller.

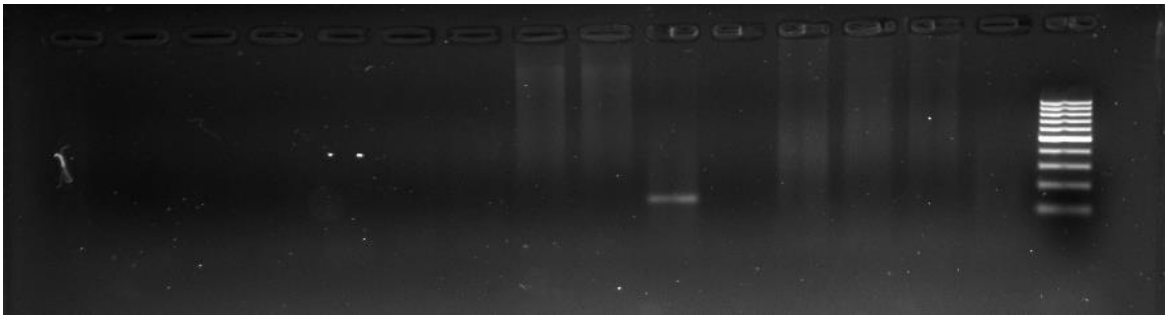
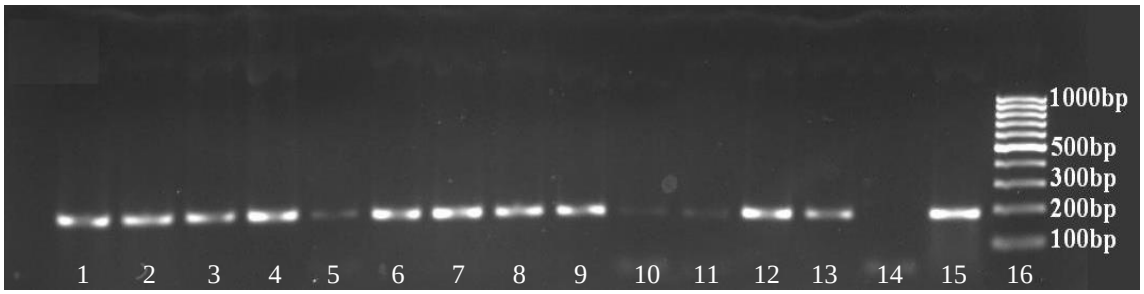


Figure 2. PCR result of the sucuk sample with positivity regarding to horse meat (L6), 100 bp ladder (L16).
Şekil 2. At eti tespit edilen sucuk örneğine ait PZR sonucu (L6).

After optimization of PCR for each species, amplifications were performed. Each amplicon 2 µl sample was loaded with 2 µl of 2x DNA loading dye (Fermentas, Cat. R0611) and loaded into a 2.0% agarose gel containing ethidium bromide (Gene Choice) for electrophoresis. Electrophoresis (BioRad, Power Pac-Basis, Singapore, BioRAD, electrophoresis tank, Wide Mini Singapore) was carried out at 120V for approximately 20 min. PCR products were visualized under UV light in gel-doc system (Kodak, Gel-logic 200).

Results

According to the analysis, 5 (13.5%) poultry, and 1 (2.7%) poultry and horse meat together were found in the sucuk samples that are not declared in their labels (Figure 2). Also, 7 (21.8%) and 2 (6.06%) poultry meat were detected in 32 salami and 33 sausage samples, respectively (Figure 3). These results indicate that 15 (14.7%) of the total samples were found to contain undeclared species (Table 3). None of the 102 samples contained donkey, goat or pork meat and in total 5 samples with poultry positive PCR band were considered as poultry meat contamination due to the weak bands.



L1-L4, L6 and L7 are positive PCR results from salami samples; L8, L9, L12 and L13 are positive PCR results from sausage samples; L5, L10 and L11 are suspicious positive PCR result from salami and sausage samples; L14 is PCR negative control; L15 is PCR positive control; L16 is 100bp DNA ladder.
L1-L4, L6 ve L7 salam örneklerinde; L8, L9, L12 ve L13 sosis örneklerinde pozitif PZR sonuçları; L5, L10 ve L11 salam ve sosis örneklerinde şüpheli pozitif sonuçlar; L14 negatif kontrol, L15 pozitif kontrol, L16 100bp marker.

Figure 3. PCR results of salami and sausages samples with positive and suspicious positivity regarding to chicken & turkey meat.
Şekil 3. Kanatlı eti tespit edilen salam ve sosis örneklerine ait PZR sonuçları.

Table 3. Samples contain undeclared meat species.
Tablo 3. Etikette belirtilmeyen et türü içeren örnekler.

Samples	Pork	Horse	Chicken/Turkey	Donkey	Cattle	Goat	Sheep	Unidentified
Sucuk (n=37)	-	1	6	-	-	-	-	-
Salami (n= 32)	-	-	7	-	-	-	-	1
Sausage (n=33)	-	-	2	-	-	-	-	-
Total (n=102)	-	1	15	-	-	-	-	1

Discussion and Conclusion

Recently, the need for information on the composition of meat products has increased so detecting the species used in a product is of considerable importance for food safety, consumer demands and laws. This study showed that there were 14.7% mislabeling in analyzed samples.

In a similar research in Ankara, Ayaz et al. (3) reported that 11 of 28 (39.2%) sausage samples and five of 14 (35.7) salami samples that were declared as beef, were found to contain a mix of beef and poultry meat. Günşen et al. (11) analyzed 50 sucuk, 75 salami and 60 sausage samples. According to their results, no pork meat was identified in the samples. Among the sucuk samples, 58 (46.4%) and 10 (8%) were found to contain chicken and horse meat, respectively. Only chicken meat was detected in sausage (4%) and salami (13.3%) samples. It was determined that, from the 260 meat product samples, 49 (18.8%) of them were mislabeled. Türkyılmaz and Irmak (24) detected the authenticity of 116 different meat and meat products. Within the meat products, there were no frauds in sausage and salami samples. However, in 26 sucuk samples, five (19.2%) were mislabeled and substituted with chicken. In the survey of Ulca et al. (25), 42 traditional Turkish meat products (sucuk, doner kebap, salami, sausage etc.) were monitored for Halal authentication. Each of the meat products was initially demonstrated to be free from pork. In this study, 12 randomly selected samples were used to test whether there was any evidence of beef, chicken or turkey DNA in the products. Nine of the samples were correctly labeled, and one of the three mislabeled products was a sausage that was found to contain only chicken, not beef as declared.

Ghovvati et al. (10) collected three types of industrial meat products, sausages (10), cold cuts (10) and ground meat (10) from different companies. No porcine meat was found in the samples, but 40% of sausage and 30% of cold cut samples contained poultry meat, which was not in accordance with the ingredients mentioned by the companies. In another study, the prevalence of undeclared plant and animal-derived species in a total of 139 processed meat products collected from retail markets and butcheries were evaluated. The results revealed that sausages had the highest incidence of adulteration and mislabeling, while pork (52%) and chicken (39%) were the most commonly detected animal species (6). In an American study, 42 ground pork and 87 fresh pork sausage samples collected throughout Alabama were examined for four species: pork, beef, poultry and sheep. 54% of the sausage samples were found to contain undeclared species (12). A total of 806 raw and 96 cooked meat samples collected from Florida retail markets were examined for regulatory control of these products. Results indicated that the overall rate of substituted species in both cooked and

raw meat samples was 16.6%. Percentage of violation in cooked products was higher than that in raw meats (22.9% versus 15.9%). The undeclared species found in ground beef and veal products included sheep, pork and poultry, respectively (13).

Different species; beef, horse, mule, donkey, buffalo, elk, reindeer, pork, lamb, goat, kangaroo, and ostrich were tested in a study in Norway of the products sliced, frozen red meat, pork salami, boiled canned pork and "Lammerull". All products were correctly labeled except for one of the sliced frozen red meat sold as beef. This product did not contain any of the above-mentioned species (20).

The results obtained from this study are very similar to the others mentioned above. In all of the studies, adulteration or fraudulent labeling were reported. Chicken was the most frequently detected undeclared animal species in our study. The main reason for the substitution of cheaper chicken flesh or fat for more expensive beef and mutton constituents is economic. Another potential source could be the use of mechanically deboned meat (MDM). The MDM is mostly produced from chicken carcasses and can be included in sausages and burgers as a cheap protein source. A further reason that should not be forgotten is accidental cross contamination. Because of improper handling and the use of shared equipment, sometimes spice contamination can occur during processing.

Our results and most of the other studies indicated that the meat species substitution occurs commonly in processed meats like sausages, salami and sucuk. The most significant reason for this kind of adulteration in meat products is processing techniques. These techniques cause indiscriminate changes in the texture, appearance, color and flavor of the product. Also, most of the ingredients like species added to the meat mixture can be disguised by the original constituents of the product. So, these make detection by visual observation more difficult in such products than in fresh meat.

In terms of where the products were collected, the highest rate of adulteration was discovered in the samples obtained from local butcheries. It is known that the highest percentage of low income groups generally shop at these butchers, so it can be expected that cheaper components can be found in such locations since the individuals may often be more concerned about the cost of the products rather than the composition. The same situation was also discussed in the study of Cawthorn et al. (6).

Our study and the others showed that every country has specific concerns and requirements about authenticity, labeling and compositional regulations. Because of Halal food status, generally chicken meat substitution is common in Muslim countries. Islamic dietary law is universal and according to that in Muslim countries pork production is very low and pork meat is expensive. The studies in other countries have shown that the tendency to mix pork meat and especially pork fat into the product is more frequent. Due to over production of pork meat and its cheapness, pork flesh and derivatives could have been illicitly incorporated into the meat products without indication in the label to provide greater profit for the food manufacturers. Another potential reason for choosing pork could be its use in MDM production like chicken.

This study was conducted to detect poultry, ovine, equine, donkey, goat and pork materials in industrial meat products by PCR. PCR is a highly repeatable, time saving and affordable method, but it gives only qualitative results. In order to prevent false detections because of cross contamination during processing, quantitative analysis can be performed. According to the Turkish Food Codex (24), food contents and amounts of components must be labeled. Also, using mixtures or MDM in meat products has been banned. So, based on our results, there are some meat products that do not comply with the laws. Adulteration of meat products with their cheaper counterparts is a problem in Turkish meat industry. To protect public health and avoid unfair profit, meat products must be regularly analyzed by governmental institutions using effective methods.

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