Kafkas Univ Vet Fak Derg
20 (6): 945-950, 2014
DOI: 10.9775/kvfd.2014.11428

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effect of Processing on PCR Detection of Animal Species in Meat Products

Özge ÖZGEN ARUN [1] Gürhan ÇİFTÇİOĞLU [1] Sema SANDIKÇI ALTUNATMAZ [2]
Sertaç ATALAY [3] Mustafa SAVAŞÇI [3] Hasan Semih EKEN [3]

[1] Istanbul University, Veterinary Faculty, Food Hygiene and Technology Department, TR-34320 Avcılar,Istanbul - TURKEY
[2] Istanbul University, Veterinary Faculty, Vocational High School, Food Technology Programme, TR-34320 Avcılar, Istanbul - TURKEY
[3] Military Food Control Laboratory of Keşan, TR-22800 Keşan, Edirne - TURKEY

Article Code: KVFD-2014-11428 Received: 11.04.2014 Accepted: 30.05.2014 Published Online: 10.06.2014

Abstract

Although most consumers are sensitive about the origin of the meat they consume, adulteration of meat products is not uncommon. For this reason, the development of reliable methods for animal species identification in meat products is an important research priority for food scientists. Species-specific protein- and DNA- identification methods generally used for this purpose. ELISA and protein electrophoresis are used for protein, PCR is used for species-specific DNA identification. Because DNA is known to be more resistant to processing than protein, PCR methods are generally considered as more sensitive for processed foods. However, processing conditions may also degrade DNA resulting in decreased DNA quality and yield. In this study, the individual and combined effects of heat treatment and low pH on the identification of animal species in meat products by PCR were evaluated. Beef sausage mixtures containing two different amounts of meat from a secondary species (either poultry, pork, or horse) were prepared and were subjected to heat treatment (65°C, 85°C, and 121°C) and pH adjustment (5.2 and 6.2). PCR screening for the four animal species was performed using DNA extracts of these meat samples. The results showed that, the combined effect of high temperature and low pH significantly affects the detection limit of the PCR method. Nevertheless, even low levels of adulteration can still be detected fallowing heat treatment.

Keywords: *Animal species identification, PCR, Heat processing, DNA degradation, Low pH*

Proses Koşullarının Et Ürünlerinde Hayvan Türünün PCR İle Teşhisine Etkisi

Özet

Birçok tüketicinin tükettiği etin orijini konusunda hassasiyetinin bulunmasına karşın, et ürünlerinde yapılan hileler oldukça sıktır. Bu sebeple, çeşitli hayvan türlerinin et ürünlerinde tespit edilmesinde kullanılacak güvenilir metotların geliştirilmesi, gıda bilimcilerinin öncelikli çalışma konularındandır. Bu amaçla, türe spesifik protein yada DNA'nın tanımlanması yöntemleri kullanılır. Protein tanımlanması için ELISA ve protein elektroforezi kullanılırken, türe spesifik DNA tanımlanmasında PCR yönteminden yararlanılır. DNA'nın proses koşullarına daha dayanıklı olduğu bilindiğinden, PCR işlem görmüş ürünlerde daha hassas olarak kabul edilmektedir. Buna karşın, proses koşulları DNA üzerine de yıkımlandırıcı etki göstererek, DNA kalite ve miktarını zayıflatabilir ve böylece PCR metotlarının işlenmiş ürünlerde kullanımını sınırlandırabilir. Bu çalışma ile ısıl işlem ve düşük pH'nın bağımsız ve kombine etkisinin et ürünlerinde hayvan türünün PCR ile teşhisine olan etkisini incelenmiştir. Bu amaçla, iki farklı seviyede ikincil bir türe ait (tavuk, domuz yada at) et katılmış olan deneysel sığır sosis karışımları yaygın olarak kullanılan proses şartlarını temsil etmek üzere ısıl işleme (65°C, 85°C, and 121°C) ve pH ayarlamasına (5.2 and 6.2) tabi tutuldu. Bu karışımların DNA ektraktlarının, PCR ile tür tayini testleri gerçekleştirildi. Elde edilen sonuçlar, düşük pH ve ısıl işlemin kombine etkisinin tespit limiti üzerine oldukça önemli etkisi olduğunu, ancak düşük miktarlardaki karışımın tespit edilmesinin mümkün olduğunu gösterdi.

Anahtar sözcükler: *Hayvan türü tayini, PCR, Isıl işlem, DNA yıkımlanması, Düşük pH*

INTRODUCTION

The composition of food is a major concern of consumers today. In the case of adulterated meat product consumption, several factors including economic, food safety (allergy) and moral reasons (religious belief), trigger

İletişim (Correspondence)
+90 212 4737070
oarun@istanbul.edu.tr

such apprehensions. Among these concerns, consumers are most sensitive because of religious factors and do not tolerate even trace amounts of adulteration of meat products with forbidden meats like pork. Nevertheless, the results of several studies and market cases have shown that meat products are frequently adulterated with forbidden/cheaper meats that undeclared on the product [1-4], and for this reason, there is an increased demand for effective methods for the identification of foreign animal species in meat products [5].

Several methods of species identification in meat products have been employed in a number of studies [6-9], and in most cases these methods are based on either protein- or DNA-analysis [5,8]. Species identification methods based on protein analysis include immunologic techniques such as ELISA, as well as electrophoretic methods [2,6,7,10].

However, species identification is often required for heat-treated meat products and heat processing can cause denaturation of proteins, thus limiting the sensitivity of protein-based tests [5,8,11,12]. On the other hand, due to its stability and resistance to degradation, most analytical methods today use DNA [5,8,13]. Moreover, conventional- and real time- PCR are frequently used because of the sensitivity and specificity of these methods [11,14-16]. The results of several studies have shown that these methods are also reliable for the analysis of heat-treated meat [12,17]; however, processing conditions have been shown to have an effect on DNA fragment size and thus on the sensitivity of PCR detection [11,18]. In this study, we aimed to evaluate the individual and combined effects of pH and heat treatment on the identification of certain animal species in meat samples using PCR.

MATERIAL and METHODS

Preparation of Experimental Meat Samples

Beef, poultry, domestic pork, and horse meat were used to prepare experimental sausage mixtures for use in this study. Beef sausage mixture was prepared from 73% beef, 15% beef fat, 0.03% $NaNO_3$, 0.5% paprika, 1% black pepper, 1% cumin, 3% salt, 0.5% mixed spices, 5% potato starch, and 1% sugar. To this mixture, selected amounts of poultry, pork, or horse meat were added to give final concentrations of 0.1 and 0.5% of a secondary animal species in the meat mixture. The mixtures were finely blended, after which each mixture was divided into two groups. The pH of one group was adjusted to both 5.2, and that of the second group to 6.2 by the addition of 0.5 N either lactic acid or NaOH. Following pH adjustment, each group was further divided into three subgroups. One was heated to 65°C, another to 85°C (internal temperature, 15 min heating) and the third subgroup was autoclaved at 121°C for 15 min.

DNA Extraction

DNA was extracted and purified in duplicate from all sausage mixtures as well as from raw meat samples of each species used for testing the primer specificities. DNA extraction was carried out using a NucleoSpin™ Tissue DNA isolation kit (MACHEREY-NAGEL GmbH and Co., Germany) according to the manufacturer's instructions. Extracted DNA was quantified by measuring UV absorption at 260 nm using a Spektonic Aquamate spectrometer (ThermoSpectronic, UK).

PCR Primers and PCR Conditions

Species-specific primers for horse, pork, poultry, and beef DNA were used. The sequences and origins of each primer set are summarized in *Table 1*. All PCR reactions were carried out using a Bioneer Thermocyler (Bioneer Corporation, South Korea). PCRs of poultry, horse, and pork primers were performed as multiplex-PCR reactions, while beef PCR reactions were performed separately as simplex reactions. Multiplex PCR amplification reactions were performed in total volumes of 50 µl containing 50 ng genomic DNA, 25 µl of the PCR mastermix 2^X (Fermentas, Turkey) and 3, 4, and 5 pmol of poultry, pork, and horse primer sets, respectively. Simplex PCR amplification reactions were performed in total volumes of 25 µl containing 50 ng genomic DNA, 12.5 µl of the PCR mastermix 2^X (Fermentas, Turkey) and 2.5 pmol of beef primer set.

Table 1. *Summary of the primer pairs used in the study*
Tablo 1. *Çalışmada kullanılan primer çiftlerinin özeti*

Target Gene	Primer	Sequences (5' → 3')	Length of PCR product	Reference
Horse	Horse	CTCAgATTCACTCgACgAgggTAg	439 bp	[19]
	SIM	gACCTCCCAgCTCCATCAAACATCTCATCTTgATgAAA		
Pork	Pork1	CTA CAT AAg AAT ATC CAC CAC A	290 bp	[3]
	Pork2	ACA TTg Tgg gAT CTT CTA ggT		
Poultry	Poultry1	TgA gAA CTA CgA gCA CAA AC	183 bp	[3]
	Poultry2	ggg CTA TTg AgC TCA CTg TT		
Beef	Beef	CTAgAAAAgTgTAAgACCCgTAATATAAg	274 bp	[19]
	SIM	gACCTCCCAgCTCCATCAAACATCTCATCTTgATgAAA		

The amplification protocol used for multiplex PCR reactions was as fallows:10 min at 94°C; amplification for 30 s at 94°C, 60 s at 60°C and 60 s at 72°C for 35 cycles; and final extension for 5 min at 72°C. For simplex reactions the protocol used consisted of 10 min at 94°C; amplification for 30 s at 94°C, 30 s at 60°C and 30 s at 72°C for 35 cycles; and final extension for 5 min at 72°C [3,19].

Agarose Gel Electrophoresis

The PCR products were electrophoresed through a 2% agarose gel, containing 0.05% ethidium bromide. As a size reference, a 100 bp DNA ladder (Generuler, Fermentas, Turkey) was used. Visualization of the agarose gels was performed using a UV transilluminator, and the images were captured using DNR Minibis Pro analysis software (St. Paul, USA).

RESULTS

DNA Yield

The DNA concentration in extracts was calculated by using the 260 nm absorbance values for evaluation of the effects of processing conditions on DNA quantity. The overall average DNA concentrations were 30.8 ng/µl and 27.9 ng/µl for pH 5.2 and pH 6.2 sausages, respectively. The detailed concentrations of the samples are given in *Table 2*. The results did not showed a significant difference in relation with the processing conditions.

Species Detection

DNA extractions were carried out in duplicate for all experimental sausage batches, and PCR reactions were then repeated to obtain four amplification results for each sample. The results of the replicated amplifications are summarized in *Table 3*.

For quality control purposes, in each PCR reaction, a no template control (sterile MILLI Q water) was include (negative control), and a mixture of DNA extracted from raw meat samples of all four species was used as positive controls.

According to these results presence of all three secondary species could be detected in both concentrations (0.1 or 0.5%) after 65°C heating in both pH 5.2 and 6.2 sausage mixtures. The agar gel electrophoresis results of 65°C heated meat mixtures containing 0.1% foreign species meat are given in *Fig. 1*. The results were similar for 85°C heated sausage mixtures. The agar gel electrophoresis results of 85°C heated meat mixtures containing 0.1% foreign species meat are given in *Fig. 2*. However the results of 121°C heated mixtures different form the above results and detection could not be possible in pH 5.2 sausage mixtures while it was possible for pH 6.2 sausage mixtures *(Fig. 3)*.

The band intensities of bands corresponds to samples containing 0.1% secondary meat species were indistinct compare to samples containing 0.5% secondary meat

Table 2. *Absorbance values and concentration of the DNA extracts*
Tablo 2. *DNA ekstraktlarının absorbans değerleri ve konsantrasyonları*

pH	Heat	Absorbance	DNA Concentration
5.2	65°C	0.024	30.2
	85°C	0.029	35.2
	121°C	0.022	27.0
Mean		0.025	30.8
6.2	65°C	0.017	21.0
	85°C	0.022	27.5
	121°C	0.028	35.3
Mean		0.022	27.9

** absorbance values and concentrations are means of all 0.1 and 0.5% samples*

Table 3. *PCR screening results of experimental samples determined with primer pairs for poultry, horse and pork*
Tablo 3. *At, domuz ve kanatlı eti için primer çiftleri ile incelenen deneyesel örneklerin PCR tarama sonuçları*

Species mixture	pH	Heat	Horse*	Pork*	Poultry*
0.1%	5.2	65°C	4/4	4/4	4/4
		85°C	4/4	4/4	4/4
		121°C	0/4	0/4	0/4
	6.2	65°C	4/4	4/4	4/4
		85°C	4/4	4/4	4/4
		121°C	4/4	4/4	4/4
0.5%	5.2	65°C	4/4	4/4	4/4
		85°C	4/4	4/4	4/4
		121°C	0/4	0/4	0/4
	6.2	65°C	4/4	4/4	4/4
		85°C	4/4	4/4	4/4
		121°C	4/4	4/4	4/4

** number of the positive results in all 4 replicated amplifications*

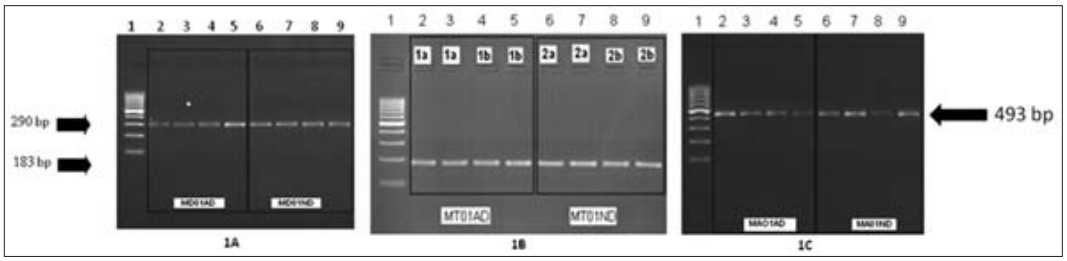

Fig 1. Multiplex PCR results of 65°C heated 0.1% samples
A- Pork (Lane-1:100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9:pH 6.2), **B-** Poultry (Lane-1:100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9:pH 6.2), *C-* Horse (Lane-1:100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9:pH 6.2)

Şekil 1. 65°C'de ısıtılmış %0.1 örneklerin multipleks PCR sonuçları
A- Domuz (Sıra-1:100 bp DNA marker, Sıra 2-5:pH 5.2, Lane 6-9:pH 6.2), **B-** anatlı (Sıra-1:100 bp DNA marker, Sıra 2-5:pH 5.2, Sıra 6-9:pH 6.2), *C-* At (Sıra-1:100 bp DNA marker, Sıra 2-5:pH 5.2, Sıra 6-9:pH 6.2)

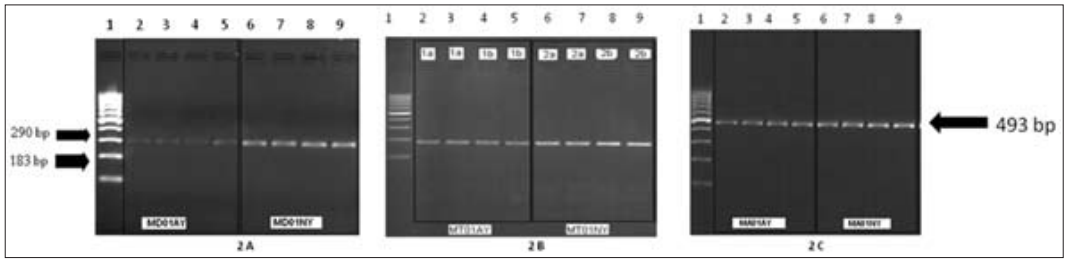

Fig 2. Multiplex PCR results of 85°C heated 0.1% samples
A- Pork (Lane-1:100 bp DNA ladder, Lane 2-5: pH 5.2, Lane 6-9: pH 6.2), **B-** Poultry (Lane-1: 100 bp DNA ladder, Lane 2-5: pH 5.2, Lane 6-9: pH 6.2), **C-** Horse (Lane-1: 100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9: pH 6.2)

Şekil 2. 85°C'de ısıtılmış %0.1 örneklerin multipleks PCR sonuçları
A- Domuz (Sıra-1:100 bp DNA marker, Sıra 2-5: pH 5.2, Sıra 6-9: pH 6.2), **B-** Kanatlı (Sıra-1: 100 bp DNA marker, Sıra 2-5: pH 5.2, Sıra 6-9: pH 6.2), **C-** At (Lane-1: 100 bp DNA marker, Sıra 2-5:pH 5.2, Sıra 6-9: pH 6.2)

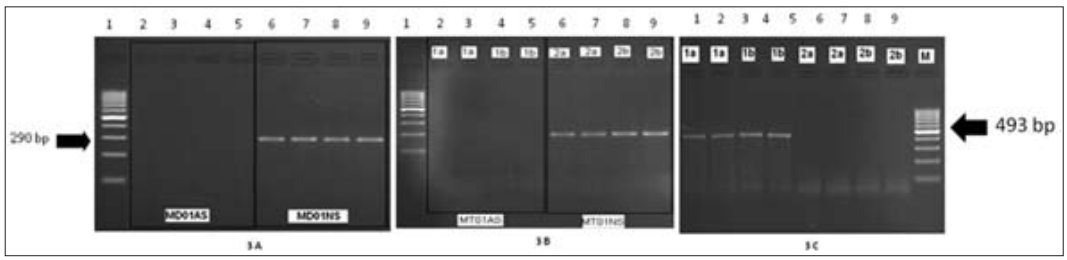

Fig 3. Multiplex PCR results of 121°C heated 0.1% samples
A- Pork (Lane-1: 100 bp DNA ladder, Lane 2-5: pH 5.2, Lane 6-9: pH 6.2), **B-** Poultry (Lane-1:100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9:pH 6.2), **C-** Horse (Lane-1:100 bp DNA ladder, Lane 2-5:pH 6.2, Lane 6-9:pH 5.2)

Şekil 3. 121°C ısıtılmış %0.1 örneklerin multipleks PCR sonuçları
A- Domuz (Sıra 1: 100 bp DNA marker, Sıra 2-5: pH 5.2, Sıra 6-9: pH 6.2), **B-** Kanatlı (Sıra 1:100 bp DNA marker, Sıra 2-5:pH 5.2, Sıra 6-9: pH 6.2), **C-** At (Sıra 1:100 bp DNA marker, Sıra 2-5:pH 6.2, Sıra 6-9:pH 5.2)

species. The results of sausage mixtures containing pork meat are given in *Fig. 4*.

The presence of beef could be possible in all samples regardless of the PH and heat treatment *(Fig. 5)*. However band intensities of pH 5.2 sausages were weaker compare to pH 6.2 samples.

DISCUSSION

The results of several studies have shown that heat processing strongly effects the detectability of species-specific DNA in meat products and thus significantly decreases the sensitivity of PCR [11,12,17,19]. Furthermore, the results of a study by Bauer et al.[20] showed that the combined effect of low pH and heat is stronger than the individual effects of conditions on PCR detection of Genetically Modified DNA in food products. The effects of a similar set of conditions on the detectability of species-specific DNA in meat products was assessed in the present study.

The first and important step of PCR screening is to extract sufficient amount of detectable DNA [21]. Therefore, the amount of DNA extracted from experimental sausages was screened by measuring the absorbance at 260 nm in

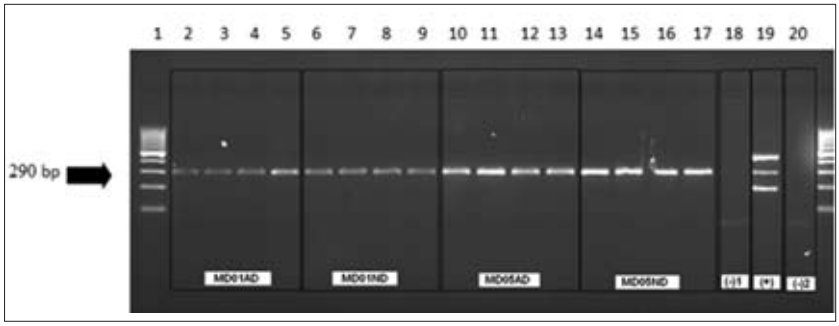

Fig 4. Multiplex PCR results of 65°C heated pork samples (Lane-1:100 bp DNA ladder, Lane 2-5: 0.1% pH 5.2, Lane 6-9:0.1%, pH 6.2, Lane 10-13:0.5%, pH 5.2, Lane 14-17: 0.5% pH 6.2, Lane 18: Ext negative control, Lane 19: meat mixture positive control, Lane-20: PCR negative control)

Şekil 4. 65°C'de ısıtılmış domuz sosis örneklerinin multipleks PCR sonuçları (Sıra-1:100 bp DNA marker, Sıra 2-5: %0.1 pH 5.2, Sıra 6-9:%0.1, pH 6.2, Sıra 10-13: %0.5, pH 5.2, Sıra 14-17: %0.5 pH 6.2, Sıra 18: Ektraksiyon negatif kontrol, Sıra 19: et karışımı pozitif kontrol, Sıra 20: PCR negatif kontrol)

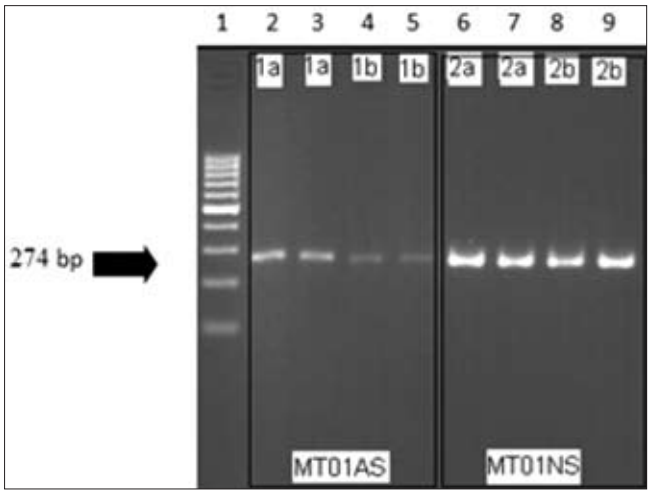

Fig 5. Simplex (beef) PCR results of 121°C heated samples (Lane-1:100 bp DNA ladder, Lane 2-5:pH 5.2, Lane 6-9:pH 6.2)

Şekil 5. 121°C sısıtlmış örneklerin simpleks (sığır) PCR sonuçları (Sıra-1:100 bp DNA marker, Sıra 2-5:pH 5.2, Sıra 6-9:pH 6.2)

order to determine the effect of temperature and pH. The results were not found to differ significantly between low- and high- pH meat samples or between batches subjected to different temperatures.

The results of the PCR testing performed on DNA extracts are given *Table 3* and the PCR amplification products of heat-processed experimental sausage samples visualized on agarose gels are shown in *Fig. 1, 2, 3, 4* and *5*. The results of the multiplex PCR reactions carried out on sausage meat prepared from binary meat mixtures showed that the meat species of all three secondary species can be detected after heating at 65 and 85°C in both 0.1 and 0.5% mixtures regardless of the pH (*Fig. 1* and *2*). These findings are in keeping with those reported by Kesmen et al.[12], which showed that 0.1% of foreign meat in sausage mixture was detectable after heating at 72°C. Despite a combination of low pH and high temperature being applied in our study, the detection limit of PCR method, like that in the study by Kesmen et al.[12], was low (0.1%).

As shown in *Fig. 4*, agarose gel bands corresponding to samples containing smaller amounts of foreign meat (0.1%) were indistinct compared to those corresponding to samples containing larger amounts of foreign meat (0.5%). Band intensities of PCR products on agarose gels are known to be proportional to DNA concentration [22], and thus although these observed differences cannot be used for quantitative purposes, they demonstrates the negative effect of processing on the amplification of DNA by PCR.

The multiplex PCR experiments carried out in this study showed that even 0.1% of foreign species meat is detectable in samples subjected to heating at 121°C (15 min, pH 6.2). These results differ from those reported by Kesmen et al.[12], which showed that the limit of detection of foreign meat mixture was increased to 0.5% when the mixture was heated at 120°C for 30 min; however, this difference may accounted for by the difference in heat exposure time (15 min *vs* 30 min). In another study by Arslan et al.[17] it was shown, like our study, that PCR detection of beef was possible for autoclaved beef meat samples. In another study which horse meat was cooked at 120ºC [19], findings differed from those reported here and by Arslan et al.[17]: horse DNA was not detectable fallowing autoclaving process despite the samples consisting of 100% horse meat compared with the 0.1% horse meat used in our study. Arslan et al.[17], attribute the discrepancy between findings of the two studies to difference in target DNA fragment size. The primers used in our study, however target fragment equal in size to those targeted in the study by Matsuagana et al.[19] and thus the difference is more likely to result from the different extraction methods used in the two studies. Hird et al.[13] have shown that autoclaving and canning processes strongly affects the DNA fragmentation. Fragmented DNA is not extracted with the same efficiency as intact DNA by some DNA extraction methods; and because it is more difficult to precipitate short DNA fragments compared with larger ones, this is particularly true for methods including a DNA precipitation step. The extraction method used in our study is a spin column-based method, while that used in the study by Matsuagana et al.[19] is precipitation-based method.

PCR experiments in this study showed differences for meat samples adjusted to different pHs, specifically between low pH (pH 5.2), 121°C heat-treated samples and pH 6.2, 121°C heat-treated samples. Foreign species present in meat samples at either 0.1 or 0.5% were not detectable in low pH (5.2) samples subjected to heating

at 121ºC, while they were detectable in pH 6.2 samples *(Fig. 3)*. Kesmen et al.[22] previously showed that 0.1% of foreign species in a meat mixture is detectable in fermented sausages. The final pH of the fermented meat was not reported; however, the pH of the meat is likely to have been low due to 15 days ripening process applied. Considering the results of Kesmen et al.[22] (low pH-no heat treatment) and our results for pH 6.2 autoclaved meat samples showed that the effect of combined processing conditions (low pH and high temperature) is stronger than the conditions applied individually. Similarly, Pascoal et al.[1] reported that the combined effect of heat and high pressure might result in stronger DNA degradation.

In the case of simplex PCR experiments for the detection of beef in meat samples, detection was possible in all samples irrespective of the processing conditions applied *(Fig. 5)*. This finding can most likely be attributed to the high concentration of beef present in the final sample product. As shown in *Fig. 5*, the agarose gel bands corresponding to pH 5.2 samples that were autoclaved were significantly weaker than those corresponding to pH 6.2 samples subjected to autoclaving, demonstrating that the degrading effect of 121°C heat treatment on DNA was more pronounced in samples subjected to low pH.

Certain cases, the low limit of detection of PCR methods can be considered a disadvantage in the identification of foreign animal species in meat products: accidental contamination across processes can result false- positive identification of meat adulteration. However, the low limit of detection associated with PCR is required in instances in which even trace amounts of foreign animal species are unacceptable, such as in cases where certain species are not tolerated for religious reasons.

In conclusion, our results demonstrated that PCR identification of foreign animal species represented at low levels in meat products is possible even after severe heat processing. Food additives commonly used in meat production did not interfere with the results of this study. It was also shown that the combined effect of pH and high temperature on DNA integrity was stronger than the effect of either condition individually, and the limit of detection of the PCR method used was significantly increased by these conditions. During industrial meat processing, various conditions are applied in combination. Considering the likelihood that the combination of such processes negatively affects the detectability of foreign animal species in meat products as demonstrated in this study, we recommend that method validation studies for PCR detection of foreign animal species should be performed for different types of meat products subjected to different processing conditions. We also recommend that further studies on animal species quantification methods be conducted, especially in the case of meat from animal species, which are of economical concern such as poultry.

REFERENCES

1. Pascoal A, Prado M, Castro J, Cepeda A, Barros-Velázquez J: Survey of authenticity of meat species in food products subjected to different technological processes, by means of PCR-RFLP analysis. *Eur Food Res Technol,* 218 (3): 306-312, 2004.

2. Ayaz Y, Ayaz ND, Erol I: Detection of species in meat and meat products using Enzyme Linked Immunosorbent Assay. *J Muscle Foods,* 17 (2): 214-220 2006.

3. Ghovvati S, Nassiri MR, Mirhoseini SZ, Moussavi AH, Javadmanesh A: Fraud identification in industrial meat products by multiplex PCR assay. *Food Control,* 20 (8): 696-699, 2009.

4. Lawrence F: Horsemeat scandal: Timeline. The Guardian. http://www.theguardian.com/uk/2013/may/10/horsemeat-scandal-timeline-investigation, 2013, *Accessed:* 27.01.2014.

5. Lockley AK, Bardsley RG: DNA-based methods for food authentication. *Trends Food Sci Tech,* 11 (2): 67-77, 2000.

6. Özgen-Arun Ö, Uğur M: Using the pseudoperoxidase staining method in the polyacrylamid gel isoelectric focusing (PAGIF) technique for determining the origin of meat in sausages. *Turk J Vet Anim Sci*, 23, 599-603,1999.

7. Macedo-Silva A, Barbosa SFC, Alkmin MGA, Vaz AJ, Shimokomaki M, Tenuta-Filho A: Hamburger meat identification by dot-ELISA. *Meat Sci,* 56 (2): 189-192, 2000.

8. Ballin NZ, Finn KV, Anders HK: Species determination - Can we detect and quantify meat adulteration? *Meat Sci*, 83 (2): 165-174, 2009.

9. Yalçin H, Alkan G: Et ve et ürünlerinde at ve domuz eti varlığının Uhlenhuth Presipitasyon Halka, Agar Gel Immuno Diffuzyon ve Enzyme-Linked Immuno Sorbent Assay metotları ile araştırılması. *Kafkas Univ Vet Fak Derg*, 18 (6): 923-927, 2012.

10. Özgen-Arun Ö, Uğur M: Animal species determination in sausages using a SDS-PAGE technique. *Arch Lebensmittelhyg,* 51, 49-53, 2000.

11. Guoli Z, Mingguang Z, Zhijiang Z, Hongsheng O, Qiang L: Establishment and application of a polymerase chain reaction for the identification of beef. *Meat Sci,* 51 (3): 233-236, 1999.

12. Kesmen Z, Sahin F, Yetim H: PCR assay for the identification of animal species in cooked sausages. *Meat Sci*, 77 (4): 649-653, 2007.

13. Hird H, Chisholm J, Sánchez A, Hernandez M, Goodier R, Schneede K, Popping B: Effect of heat and pressure processing on DNA fragmentation and implications for the detection of meat using a real-time polymerase chain reaction. *Food Addit Contam*, 23 (7): 645-650, 2007.

14. Köppel R, Ruf J, Zimmerli F, Breitenmoser A: Multiplex real-time PCR for the detection and quantification of DNA from beef, pork, chicken and turkey. *Europ Food Res Technol*, 227 (4): 1199-1203, 2008.

15. Kesmen Z, Yetiman AE, Şahin F, Yetim H: Detection of chicken and turkey meat in meat mixtures by using Real-Time PCR Assays. *J Food Sci*, 77 (2): 167-173, 2012.

16. Köppel R, Zimmerli F, Breitenmoser A: Heptaplex real-time PCR for the identification and quantification of DNA from beef, pork, chicken, turkey, horse meat, sheep (mutton) and goat. *Europ Food Res Technol*, 230 (1): 125-133, 2009.

17. Ulca P, Balta H, Çağın İ, Senyuva H Z: Meat species identification and Halal authentication using PCR analysis of raw and cooked traditional Turkish foods. *Meat Sci*, 94 (3): 280-284, 2013.

18. Arslan A, Ilhak OI, Çalıcıoğlu M: Effect of method of cooking on identification of heat-processed beef using polymerase chain reaction (PCR) technique. *Meat Sci*, 72 (2): 326-330, 2006.

19. Ebbehoj KF, Thomsen PD: Species differentiation of heated meat products by DNA hybridization. *Meat Sci,* 30 (3): 221-234, 1999.

20. Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata K, Yamada J, Shinmura: A quick and simple method for the identification of meat species and meat products by PCR assay. *Meat Sci,* 51 (2): 143-148, 1999.

21. Bauer T, Weller P, Hammes WP, Hertel C: The effect of processing parameters on DNA degradation in food. *Europ Food Res Technol* 217, 338-343, 2003.

22. Gryson N: Effect of food processing on plant DNA degradation and PCR based GMO analysis: A review. *Anal Bioanal Chem*, 396, 2003-2022, 2010.

23. Kesmen Z, Yetim H, Sahin F: Identification of different meat species used in sucuk production by PCR assay. *GIDA,* 35 (2): 81-87, 2010.