An innovative reverse line blot for simultaneous detection of animal species in food

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ORIGINAL PAPER

An innovative reverse line blot for simultaneous detection of animal species in food

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Abstract

Animal products play a significant role in human consumption. Therefore, identifying the food fraud is of great importance. We describe a simple but an innovative method for the simultaneous detection of meat sources from different animal species (cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck and turkey) and human as a control DNA was extracted from samples prepared from human and above-mentioned animals and amplified using a common primer pair derived from a region of the mitochondrial DNA. The PCR products were subsequently hybridized with the species-specific DNA probes covalently bonded to a Biodyne C blotting membrane. The results showed that the PCR products generated from mammalian reacted as expected, to catch-all probes, mammalian catch-all probes and the corresponding species specific DNA probes but not with bird catch-all probes. The avian PCR product reacted also as expected, to catch-all probes, bird catch-all probes and the corresponding species-specific DNA probes, and not with the mammalian catch-all probes. These results showed that the used detection system could discriminate simultaneously, each animal species from the others. To determine the sensitivity of the presented hybridization method, the meat samples from 2 to 6 animals were mixed and the extracted DNA was analyzed. Interestingly, the species-specific reaction could be detected in all mixtures consisting of different species. Furthermore, it could be shown that the mixed meat DNA ratio 1/100 also could be detected simultaneously.

Keywords Food fraud · DNA extraction · Hybridization · PCR · Simultaneous detection

Introduction

Control of food fraud is an important part of the management of the food hygiene and safety. In 2013, the horse meat scandal made headline news across Europe. In that case, horse meat was being passed off as beef for processing into hamburger [1]. It is considered that financial gain is behind this fraudulent activity given that the old horse meat is much cheaper than beef. Food fraud is important also for the consumers concerning consumption of meat prepared from pork, monkey, dog or some other more according to the religious and ethical behavior. Different methods were described for the detection of adulteration in meat products, based on the analysis of proteins or DNAs extracted from the probes. The most used methods based on the proteomics are peptide mass fingerprinting (PMF) [2] and peptide fragmentation fingerprinting (PFF) [3]. A study described by Ruiz Orduna et al. [4] presented a high-resolution mass spectrometry method to assess the authenticity of meat of beef, horse, pork and lamb. They reported that four proteolytic peptides myoglobin, myosin-1, myosin-2 and β-haemoglobin can be used for the meat authenticity. To mention that in the mentioned study, the amino acid sequence of some peptides was not species specific. Despite this problem, the above-mentioned method with some modification (use of species-specific peptides) can be considered as an optimal method for the detection of specific-species proteins in the meat. Another method used for the detection of the animal source in meat is ELISA which was described previously by Berger

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et al., Gonzales et al., Simontacchi et al., and Giovannacci et al. [5–8]. Although this method can be used successfully, but the range of available antibody against each species is limited. Another method based on DNA for determination of animal species in food is PCR with species-specific primers. The traditional PCR method was described by Calvo et al. [9]. Other PCR-based methods such as real-time PCR by Brodmann et al. [10], RFLP by Verkaar et al. [11] and RAPD by Calvo et al. [12] have also been reported. In contrast to the above-mentioned methods, the presented new and innovative PCR-based method can detect up to fifteen species simultaneously using common primers for mammalian and birds, followed by the hybridization of the PCR products with the species-specific probes of different animals (cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck, white-fronted goose and turkey).

Materials and methods

DNA extraction

DNA was extracted from the meat samples prepared from cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, mouse (as a negative control), chicken, wild duck, turkey and blood sample from human using DNA extraction kit (MBST, Iran) according to the manufacturer's instructions. All samples had been obtained with consent given accordingly by the institutional guidelines. Briefly, 50 mg of each sample was first lysed in a 180 µl lysis buffer, and the proteins were degraded with 20 µl proteinase K for 1–4 h at 55 °C. After addition of 360 µl binding buffer and incubation for 10 min at 70 °C, 270 µl of ethanol (100%) was added to the solution, and after vortexing, the complete volume was transferred into the MBST column. The MBST column was first centrifuged and then washed twice with 500 µl wash buffer. Finally, DNA was eluted from the carrier with 100 µl TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). The quantity of each extracted DNA sample was separately determined by spectrophotometer at OD 260. Additionally, 50 mg of meat from 1 to 6 different animal species was mixed as shown in Table 2 and the DNA was extracted and also analyzed.

Polymerase chain reaction

The PCR was performed with 100 µl total volume, including approximately 100 ng DNA, 1X PCR buffer, 2.5 U Taq Polymerase (Cina gene, Iran), 2 µl (20 mM) of each common forward primer (sense all) and common reverse biotin-linked primer (antisense biotin) both from MWG, Germany (Table 1), 200 µM each of dNTPs (Fermenta), and 1.5 mM $MgCl_2$ in an automated thermocycler (MWG, Germany) with the following program: 5 min incubation at 95 °C to denature double-stranded DNA, 38 cycles of 45 s at 57 °C (annealing step), 45 s at 72 °C (extension step). Finally, PCR was completed with an additional extension step for 10 min. The primers were derived from the mitochondrial genome as listed in Table 1. Designing of the primers was performed by alignment of the corresponding

Table 1 The primer used were derived from mitochondrial genome of each animal species

No.	Name	Nucleotide sequences	Accession No.
1	Sense all	5′ TAGAGGAGCCTGTTCTATAATCGAT 3′	HQ18404
2	Antisense Biotin	5′ CACTTTCCAGTATGCTTACCTTGTTACGAC 3′	HQ18404
3	Mammalian catch-all	5′ CTATATACCGCCATCTTCAGCA 3′	HQ18404
4	Bird catch-all	5′ CCGCCGTCGCCAGCCCACC 3′	AY235570
5	All catch-all	5′ CACGCACACACCGCCCGTCACCCTC 3′	HQ18404
6	Horse	5′ AGAACTTTAACCCGGACGA 3′	NC_001640
7	Sheep	5′ AATATGATATACTTAAAC 3′	NC_001941
8	Goat	5′ AATACAATGCACTCAAGC 3′	NC_005044
9	Cattle	5′ AAATAGATTCAGTGCATCTA 3′	HQ18404
10	Camel	5′ AGTTCAACGAGCCTGCAAA 3′	EU159113
11	Dog	5′ AGTAATAAGACACAACC A 3′	EU789763
12	Cat	5′ AGTGGTAACTCCCAAAA 3′	U20753
13	Rabbit	5′ AGTGACAAATATTTACTT 3′	AJ001588.1
14	Human	5′ AGTATACTTCAAAGGACATT 3′	FJ800808
15	Pig	5′ ATGTAGT AATAAAAATAACCT 3′	AP003428.1
16	Chicken	5′ GCCATCAACATCAATAAATATATA 3′	AY235570
17	Turkey	5′ ATACCCAACCCTAGCTAAAG 3′	NC_010195
18	Wild duck	5′ TACCACGTAAATGCCAAA 3′	NC_009684
19	Buffalo	5′ AAGTAAATATAATGCATCCA 3′	NC_006295

region of the species-specific DNA fragments to choose the suitable species-specific oligonucleotides. The PCR products were loaded on 1.5% agarose gel in 0.5X Tris–Borate–EDTA (TBE) buffer (5.4 g Tris base, 2.75 g boric acid, and 2 ml of 0.5 M EDTA, pH 8.0, in 1000 ml aqua bidest) and visualized using ethidium bromide and a UV Illuminator.

Hybridization assay

The procedure of the RLB assay was described in the study published in detail by Bahadori-Ranjbar et al. [13] and Soltani et al. [14]. Briefly, a Biodyne C blotting membrane was activated in 16% EDAC solution at room temperature for 10 min. subsequently, the membrane was shortly washed in aqua bidest and placed in a miniblotter (MBST, Iran). The oligonucleotide probes with N-terminal (TFA)-C6 amino linker (MWG, Germany) at the concentration of 1000 pmol in 250 µl 500 mM sodium hydrogen carbonate (pH 8.4) was transferred into the slots of miniblotter and incubated for 1 min at RT. After aspiration of solutions, the membrane was incubated for 10 min in 100 mM NaOH at RT. Subsequently, the membrane was washed for 5 min once in 2 × saline–sodium phosphate–EDTA (SSPE; 1 l 20 × SSPE = 175.3 g NaCl, 27.6 g NaH_2PO_4, 9.4 g EDTA, pH 7.4), 0.1% sodium dodecyl sulfate (SDS) at 60 °C, and once in the above-mentioned buffer at 42 °C for 5 min. The membrane was then placed into the miniblotter with the slots perpendicular to the line pattern of the applied probes. Forty-five microliters of PCR products were diluted with 200 µl 2 × SSPE, 0.1% SDS, heated to 95 °C for 10 min, and then cooled on ice. The slots were aspirated from remaining probe solutions and then filled with the diluted PCR product, and hybridization was performed at 37 °C for 60 min. After aspiration of the solution from the slots, the membrane was washed twice in preheated 2 × SSPE, 0.5% SDS at 42 °C under gentle shaking. The membrane was then incubated with 25 ml 2 × SSPE, 0.1% SDS, and 8 µl streptavidin-POD (Roche, Germany) for 30 min at 42 °C. The membrane was subsequently washed twice in preheated 2 × SSPE, 0.5% SDS at 37 °C for 10 min under gentle shaking and twice at room temperature. Finally, chemo luminescence detection was performed on X-ray film, according to the standard procedure and according to the manufacturer's instructions (Amersham, UK).

Results

DNA was extracted from meat prepared from cattle, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck, turkey and blood sample from human. As a negative control, DNA extracted from mouse and buffalo was used. The extracted DNA from different samples was analyzed on agarose gel to be sure that the extraction was performed successfully (Fig. 1a).The extracted DNA was amplified using common primers which were designed from the region of the mitochondrial genome with flanking common nucleotide sequences to produce simultaneously a PCR product with species-specific animal information. The PCR product had a length of approximately 420 bp (Fig. 1b).The PCR products were loaded on membrane on which the species-specific animal probes still were covalently bound. Figure 2 showed that as expected, all PCR products generated from the DNA extracted from mammalian, namely cattle, sheep, goat, horse, camel, pig, dog, cat, rabbit, mouse, and human can recognize the catch-all probes, corresponding species-specific animal probes and catch-all mammalian probes except sheep, horse and mouse. In our further experiments, we could find out that putting two common oligonucleotide probes (catch-all and catch-all mammals) side by side can lead to some hybridization problems consequently to different visualization intensity. In many cases, such problems can be solved if the developing time by chemo luminescence procedure is more. A good result could also be achieved if these two oligonucleotide probes applied not side by side but in distance to each other. The lane between wild duck and turkey in Fig. 2, due to penetration of the samples, was not evaluated.

Since the DNA probes specific for mouse and buffalo were missing on the membrane, the PCR products generated from the DNA extracted from the meat of these animals showed no cross reactivity with other species-specific DNA probes. As expected, the PCR product generated from chicken, wild duck and turkey could react as inspecting not only with the DNA probes specific for animals and bird (catch-all and catch-all birds) but also with the corresponding species-specific probes (Fig. 2).In the next experiment, the meat of different animals was mixed (Table 2) and DNA

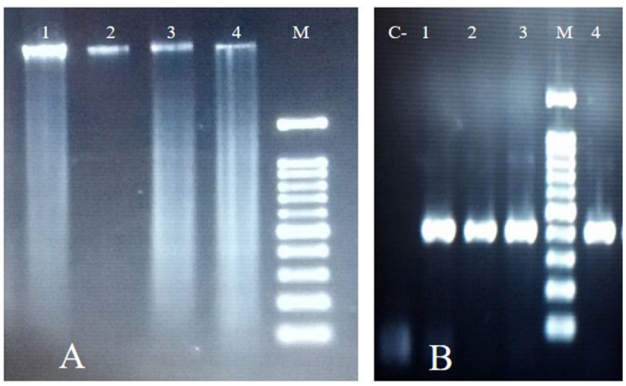

Fig. 1 DNA was extracted from meat prepared from different species (lane 1–4 were cattle, sheep, goat and chicken respectively) and analyzed on 1% agarose gel (**a**). The extracted DNA was amplified using common primer pair resulting in a PCR product of approximately 420 bp in length (**b**). *M* 100 bp DNA marker, *C* control negative

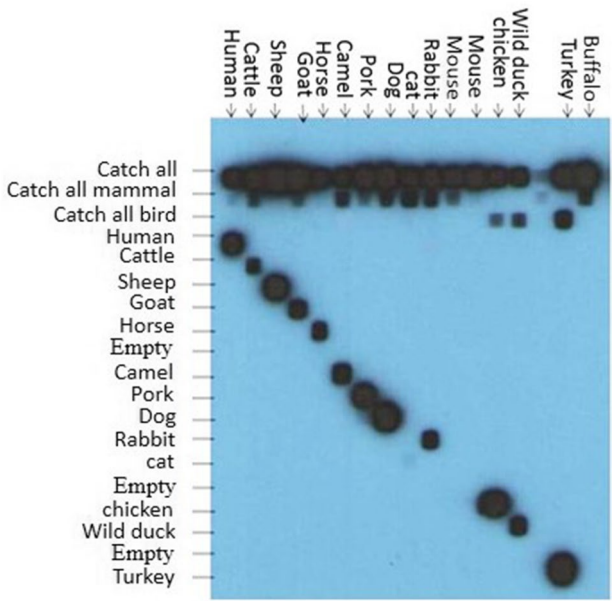

Fig. 2 The species-specific DNA probes for cattle, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck, turkey, human, Catch-all, Catch-all mammalian and Catch-all birds were bond on the membrane and the membrane was hybridized with the PCR products generated from cattle, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck, turkey and human. The reaction was visualized by chemoluminescence assay

was extracted. The extracted DNA was amplified using common primers and after confirmation on the agarose gel, analyzed by hybridization. The results showed that the DNA extracted from one species (buffalo, Fig. 3 lane 2), two species (buffalo and dog, Fig. 3 lane 5), four species (buffalo, pig, dog and chicken, Fig. 3 lane 3) and six species (cattle, buffalo, pig, dog, rabbit and chicken, Fig. 3 lane 4) could be detected by hybridization. It is to mention that sometimes the expected reaction with catch-all mammals and catch-all birds were missing, but the positive reaction with the catch-all was in all examined experiments always reproducible. In the third experiment, the PCR product generated from DNA extracted from the meat of cattle and sheep in the different ratio (1/10 and 1/100) was analyzed by hybridization. Our results showed that the corresponding species-specific probes could be detected by the ratio 1/10 rather than 1/100.

Discussion

In the meat industries, the adulteration process represents the presence of unknown species of meat, use of meat varieties of commercially lower value, use of low-quality raw materials, replacement of animal or plant proteins and incorrect labeling of ingredients. Nowadays, the mislabeling fraud is a common event in the food products industry like meat products trading [15, 16]. According to the United States Pharmacopeial Convection, the definition of food fraud is deliberate and purposed mislabeling of food products for financial gain with the intent of deceiving and cheating the consumers regarding what is actually in the packages of food products. Food fraud sometimes causes damage to health and the economy. Here, it must show the role of the private authorities responsible for detecting fraud and controlling this condition, which sometimes causes dangerous health effects, especially when non-certified substances are used in the food industry [17]. Also, any substitution, addition, misrepresentation of food, food ingredients, packaging of food or misleading and mislabeling statements made about a food product for greed financial benefits intentionally is known as a food fraud [18]. Due to the high cost of beef in most countries, and the difficulty of detecting adulterations in different meat products like hamburger, the replacement of beef with cheaper animal and plant proteins in these products is possible [19]. The important question is why the detection of food fraud is important? The detection of food fraud is a necessary process to protect the consumer from various risks of health, as well as due to many considerations, nutritional and religious reasons, in addition, to prevent losing consumer confidence in the food products in the markets and as a result of this, maintain health and economy at the same time. Consequently, there is a special need for using new methods enabling rapid, sensitive and with appropriate cost and time to detect species-specific differentiation of food adulterations in different food products. The development of new and advanced techniques for the authentication of food products continues rapidly with increasing consumer awareness of food safety, authenticity affairs and constant care to maintain the health. Food authentication

Table 2 50 mg of meat prepared from each of the different animals (buffalo, pork, dog, cattle, chicken and rabbit) was mixed as given in the table for DNA extraction and analyzing by hybridization test

Number	Buffalo	Pork	Dog	Cattle	Chicken	Rabbit
1	X	–	–	–	–	–
2	X	–	X	–	–	–
3	X	X	X	–	X	–
4	X	X	X	X	X	X

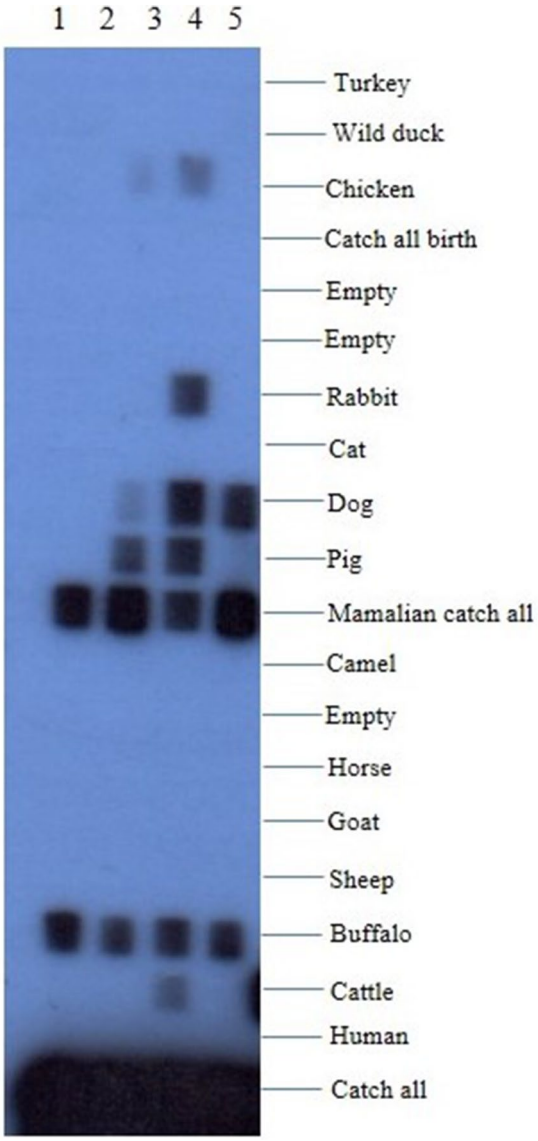

Fig. 3 The species-specific DNA probes for cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck, turkey, human, Catch-all, Catch-all mammalian and Catch-all birds were bond on the membrane and the membrane was hybridized with the PCR products generated from one species (buffalo, Fig. 3 lane 2), two species (buffalo and dog, Fig. 3 lane 5), four species (buffalo, pig and dog, chicken, Fig. 3 lane 3) and six species (cattle, buffalo, pig, dog, rabbit and chicken, Fig. 3 lane 4). The reaction was visualized by chemoluminescence assay. Lane 1 was negative control

is also of concern to food processors that do not wish to be subjected to inequitable competition from fraudulent food processors. The specialists designed different diagnostic methods to detect food cheating. These methods were different in diagnostic accuracy. Scientists were working continuously on developing these methods to reach a food product free from fraud, especially in industrial meat products. The methods of meat identification include physical techniques such as color, texture and odor, anatomical techniques which are very classical technology, histological techniques, chemical techniques and immunological methods as complement fixation test described by Singh et al. [20], enzyme-linked immune sorbent assay by Ong et al. [21] and radio immuno assay described by Lowenstein et al. [22]. Molecular techniques such as DNA-based detection methods have been used for meat species identification, including PCR sequencing, PCR restriction fragment length polymorphism (RFLP), species-specific PCR, random amplified polymorphic DNA (RAPD), and single-stranded conformational polymorphism (SSCP). Naturally, all these methods have special advantages and disadvantages. The most important disadvantage of these methods is time consuming, high cost and in some cases, low sensitivity [23–25]. The comparison of different methods of species identification based on protein analysis with those related to DNA analysis appears to indicate that the first group deals with the identification of species-specific proteins in the food, which shows directly the used species-specific meat in the processed food and is associated with high cost.

The most used methods based on the proteomics are peptide mass fingerprinting (PMF) [2] and peptide fragmentation fingerprinting (PFF) [3]. High-resolution mass spectrometry method was previously reported to assess the authenticity of meat of beef, horse, pork and lamb. Ruiz Orduna et al. [4] reported that four proteolytic peptides myoglobin, myosin-1, myosin-2 and β-haemoglobin can be used for meat authenticity. It is to mention that the used amino acid sequence of myosin 1 isoform peptide was identical in donkey (Accession No. XP_014719640.1) and horse (Accession No. NP_001075228.1), also in cattle (Accession No. NP_776542.1) and buffalo (Accession No. XP_010841309.1), or amino acid sequence of myosin 2 isoform peptide was identical in cattle (Accession No. NP_001159699.1) and buffalo (Accession No. XP_010841306.1), also in sheep (Accession No. 011977812.1), in goat (Accession No. XP_017920148.1) and in Tibetan antelope (Accession No. XP_005959865.1). The used amino acid sequence of peptide β-haemoglobin was also identical in Wild Bactrian camel (Accession No. XP_006195511.1), in alpaca (Accession No. XP_006203487.1), in mouflon (Accession No. XP_014946723.1), in dromedary (Accession No. 3GDJ_B),in cattle (Accession No. NP_776342.1) and in buffalo (Accession No. XP_006061645.1). We believe that the mentioned methods with some modification can be considered as an optimal method for the detection of species-specific proteins in the meat. The second group detects the species-specific DNA, which can be found in the processed food and can have different solid or liquid sources from the used animals. DNA is independent of the type of tissue it resides in. Therefore, it is not a problem, whether the sample

taken for analysis is collected from muscles, blood, liver or other sites. Especially in food fraud, the DNA provides higher information compared to the protein analysis, because the DNA, due to the character of a genetic code, can represent the use of nearly all materials prepared for species-specific animals in food [26]. DNA hybridization technology represents the hybridization of complementary DNA oligonucleotides which is a basic principle of molecular biology used in a variety of methods with possible applications in species-specific identification of different food products. This qualitative basic technology has the advantage of enabling the simultaneous detection of multiple species in a sample. Such methods were also used to obtain the expression levels of many different genes simultaneously because it can search for genes that are expressed specifically under certain condition [22, 27–29] or for detection of infectious agent in certain materials as studies of Bahadori-Ranjbar et al. [13] and Soltani et al. [14]. The importance of preparing a highly sensitive, inexpensive and rapid method led to design the animal species-specific detection system, which is able to detect simultaneously more animal species which is normally used in multiplex PCR. Cottenet et al. [30] described a macro-LCD array system for detection of different meat species in food and its evaluation needed LCD array slide scanner and corresponding software. The disadvantage of this method compared to the presented method is that it needs special equipment which cannot normally be found in all laboratories worldwide. In the present study, DNA was extracted from 13 animals (cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, chicken, wild duck and turkey) and subsequently, each corresponding DNA was amplified with the common primer derived from the mitochondrial genome. The PCR products were then hybridized with the membrane coated with species-specific DNA probes and the hybridization results were then visualized using streptavidin-conjugated peroxidase in the chemo luminescence system. The results showed that each PCR product could recognize the corresponding species DNA probes on the membrane only. Interestingly, the experiment of mixed meats from different animals showed that all different species could be detected in the mixture prepared from the meat of six species, which denoted for high accuracy of the presented method. Since the species specific reaction could be achieved by the analysis of the DNA prepared from two different animals in 1:100 ration in the mentioned hybridization method, the method can be considered as a detection method with high sensitivity. Furthermore, in this way, many animals can be traced simultaneously; therefore, the presented method has the considerable advantage of the methods based on the DNA like real-time PCR or multiplex PCR.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements The present manuscript did not contain any studies with human or animal subjects.

References

1. O'mahony P (2013) Finding horse meat in beef products—a global problem. QJM 106(6):595–597
2. Pappin DJ, Hojrup P, Bleasby AJ (1993) Rapid identification of proteins by peptide-mass fingerprinting. Curr Biol 3(6):327–332
3. Eng JK, McCormack AL, Yates JR (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J Am Soc Mass Spectrom 5(11):976–989
4. Ruiz Orduna A, Husby E, Yang CT, Ghosh D, Beaudry F (2015) Assessment of meat authenticity using bioinformatics, targeted peptide biomarkers and high-resolution mass spectrometry. Food Addit Contam Part A 32(10):1709–1717
5. Berger R, Mageau R, Schwab B, Johnston R (1988) Detection of poultry and pork in cooked and canned meat foods by enzyme-linked immunosorbent assays. J Assoc Off Anal Chem 71(2):406–409
6. González-Córdova A, Calderón de la Barca A, Cota M, Vallejo-Cordoba B (1998) Deteccion inmunoquimica de la adulteracion de chorizo de cerdo con proteinas de soja: Immunochemical detection of fraudulent adulteration of pork chorizo (sausage) with soy protein. Food Sci Technol Int 4(4):257–262
7. Simontacchi C, Marinelli L, Gabai G, Bono G, Angeletti R (1999) Accuracy in naturally occurring anabolic steroid assays in cattle and first approach to quality control in Italy. Analyst 124(3):307–312
8. Giovannacci I, Guizard C, Carlier M, Duval V, Martin JL, Demeulemester C (2004) Species identification of meat products by ELISA. Int J Food Sci Technol 39(8):863–867
9. Calvo JH, Rodellar C, Zaragoza P, Osta R (2002) Beef-and bovine-derived material identification in processed and unprocessed food and feed by PCR amplification. J Agric Food Chem 50(19):5262–5264
10. Brodmann PD, Moor D (2003) Sensitive and semi-quantitative TaqMan™ real-time polymerase chain reaction systems for the detection of beef (*Bos taurus*) and the detection of the family Mammalia in food and feed. Meat Sci 65(1):599–607
11. Verkaar E, Nijman I, Boutaga K, Lenstra J (2002) Differentiation of cattle species in beef by PCR-RFLP of mitochondrial and satellite DNA. Meat Sci 60(4):365–369
12. Calvo J, Zaragoza P, Osta R (2001) A quick and more sensitive method to identify pork in processed and unprocessed food by PCR amplification of a new specific DNA fragment. J Anim Sci 79(8):2108–2112
13. Ranjbar-Bahadori S, Eckert B, Omidian Z, Shirazi NS, Shayan P (2012) Babesia ovis as the main causative agent of sheep babesiosis in Iran. Parasitol Res 110(4):1531–1536
14. Soltani M, Pirali E, Shayan P, Eckert B, Rouholahi S, Sadr Shirazi N (2012) Development of a reverse line blot hybridization method for detection of some streptococcal/lactococcal species, the causative agents of zoonotic streptococosis/lactococosis in farmed fish. Iran J Microbiol 4(2):70–74

15. Pascoal A, Prado M, Castro J, Cepeda A, Barros-Velázquez J (2004) 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
16. Cheng X, He W, Huang F, Huang M, Zhou G (2014) Multiplex real-time PCR for the identification and quantification of DNA from duck, pig and chicken in Chinese blood curds. Food Res Int 60:30–37
17. Johnson R (2014) Food fraud and economically motivated adulteration of food and food ingredients. Congressional Research Service, Washington, DC
18. Spink J, Moyer DC (2011) Defining the public health threat of food fraud. J Food Sci 76(9):R157–R163
19. Tafvizi F, Hashemzadegan M (2016) Specific identification of chicken and soybean fraud in premium burgers using multiplex-PCR method. J Food Sci Technol 53(1):816–823
20. Singh V, Neelam S (2011) Meat species specifications to ensure the quality of meat: a review. Int J Meat Sci 1(1):15–26
21. Ong S, Zuraini M, Jurin W, Cheah Y, Tunung R, Chai L, Haryani Y, Ghazali F, Son R (2007) Meat molecular detection: sensitivity of polymerase chain reaction-restriction fragment length polymorphism in species differentiation of meat from animal origin. ASEAN Food J 14(1):51–59
22. Lowenstein JM, Reuther JD, Hood DG, Scheuenstuhl G, Gerlach SC, Ubelaker DH (2006) Identification of animal species by protein radioimmunoassay of bone fragments and bloodstained stone tools. Forensic Sci Int 159(2–3):182–188
23. James D, Schmidt A-M, Wall E, Green M, Masri S (2003) Reliable detection and identification of genetically modified maize, soybean, and canola by multiplex PCR analysis. J Agric Food Chem 51(20):5829–5834
24. Wang RF, Cao WW, Cerniglia C (1997) A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. J Appl Microbiol 83(6):727–736
25. Scheu PM, Berghof K, Stahl U (1998) Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. Food Microbiol 15(1):13–31
26. Lockley A, Bardsley R (2000) DNA-based methods for food authentication. Trends Food Sci Technol 11(2):67–77
27. Pereira F, Carneiro J, Amorim A (2008) Identification of species with DNA-based technology: current progress and challenges. Recent Patents DNA Gene Seq 2(3):187–200
28. Beißbarth T, Fellenberg K, Brors B, Arribas-Prat R, Boer JM, Hauser NC, Scheideler M, Hoheisel JD, Schütz G, Poustka A (2000) Processing and quality control of DNA array hybridization data. Bioinformatics 16(11):1014–1022
29. Rasmussen RS, Morrissey MT (2008) DNA-based methods for the identification of commercial fish and seafood species. Compr Rev Food Sci Food Saf 7(3):280–295
30. Cottenet G, Sonnard V, Blancpain C, Ho HZ, Leong HL, Chuah PF (2016) A DNA macro-array to simultaneously identify 32 meat species in food samples. Food Control 67:135–143