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ORIGINAL PAPER

Reverse line blot hybridization assay as a suitable method for the determination of food adulteration in example of sausage samples

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Abstract
Knowledge about the animal origin of food is of great importance for consumer due to the fraudulence, allergy factors or religious beliefs. The aim of the present study was to determine possible adulteration in popular meat product sausage. For this aim, reverse line blot assay was used for the simultaneously detection of DNA from cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, rat, chicken, goose, duck and turkey. As control probes, the catch all probe, catch all mammalian probe, catch all birds probe and as negative control *Homo sapiens* probe were used. DNA was extracted from 114 sausages with cattle meat in different percentage share (38 samples of each 40, 55, and 70%). These products were labeled with cattle meat, which were purchased from different companies in different batches. No declaration about the use of other prepared animal tissues was deciphered from the labels. The results showed that DNA from bovine and chicken was detected in 108 samples, whereas chicken DNA could be detected in 6 samples. The sausages containing only chicken meat could be detected in all three percentage shares (40, 55, and 70%). In conclusion, our results showed that the reverse line blot can be used for the analysis of food adulteration.

Keywords DNA · Meat products · Sausage · Reverse line blot

Introduction

Meat products such as hamburger and sausages are usually vulnerable to food adulteration, such as the scandals of using the horse meat in Europe and the rat meat in Asia [1]. The purpose of adulterations is mainly to get the financial benefit by the substitution of high-valued meats by cheaper one. The addition or substitution of cheaper animal meats is tempting for some meat industries. It was shown that sometimes food processors add prohibited materials in their products that may risk the consumer's health [2]. The consumers have special concerns about the type of meat that they consume; therefore, the labeling is an important step to give accurate information about consumer choice. Nowadays, counterfeit labels for the purpose of deceiving consumers are one of the most common types of food fraud in meat industries [3]. It is important to control the meat products regarding their animal origin which is of great interest for public health, economic, commercial and legal concerns [4]. Meat authenticity and traceability are an affair of primary importance in our modern community. In recent events, there is increasingly apparent food adulteration of meat products with non-declared species such as chicken, goat, donkey and old horse meat instead of cattle meat. Therefore, authenticity is an important standard for food safety and quality [5, 6]. There are various analytical methods and strategies that have been used to detect food adulteration in meat products. The methods enzyme-linked immunosorbent assays [7, 8], peptide mass fingerprinting (PMF) [9] and

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peptide fragmentation fingerprinting (PFF) [10] have been proposed for the identification of meat species in processed meat products. Protein-based immunological methods generally give accurate results with some limitations due to the protein denaturation and alterations of specific epitopes when applied to thermally processed foods, for animal species identification in raw meats [11, 12]. In recent years, significant attention has been turned toward DNA-based technologies, to detect food authentication. Among them, the polymerase chain reaction (PCR) and multiplex PCR are obviously the most common technique used to identify the origin of the meat adulteration [13, 14]. In the present study, we used the recently reported reverse line blot hybridization assay [15] to detect simultaneously different species-specific DNA in sausage.

Materials and methods

Samples

A total of 114 sausage samples labeled with cattle source of used meat containing 40% (38 samples), 55% (38 samples) and 70% (38 samples) of different production batch from 10 different most consumed brands were collected from supermarkets from 5 areas of Tehran/Iran (North, South, East, West and center city) and stored at − 20 °C until used.

DNA extraction

DNA was extracted from sausage samples using a DNA extraction kit (MBST, Tehran, Iran) according to the manufacturer's instructions and as described by Shayan et al. [15]. Briefly, 50 mg of each sample was lysed in 180 µl lysis buffer, mixed thoroughly and incubated for 10 min at 55 °C. Twenty microliter proteinase K was added to the solution and incubated for 20 min at 55 °C to degrade the proteins. A volume of 360 µl of the binding buffer was added and incubated for 10 min at 70 °C. A volume of 270 µl ethanol (100%, Merck, Germany) was added to the solution and mixed. The complete volume was transferred into the MBST column. MBST column was first centrifuged and washed twice with 500 µl washing buffer. Finally, DNA was eluted from the carrier with elution buffer and analyzed by electrophoresis on 0.8% agarose gel using ethidium bromide under ultraviolet light and stored at − 20 °C until the used.

Polymerase chain reaction

The PCR was performed as described by Shayan et al. [15] with 100 µl total volume, including approximately 100 ng DNA, 2 µl of each common forward primer and common reverse biotin-linked primer (20 mM, MWG, Germany, Table 1), 1 × PCR buffer, 2.5 U Taq Polymerase (Cina clone, Iran), 2 µl of dNTPs (each 200 mM, Fermenta), and 1.5 mM $MgCl_2$ in an T100™ Bio-Rad thermal cycler (USA) with

Table 1 The primers 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	Rat	5′ AATTAAATTAACACATACTT 3′	NC_014864
15	Human	5′ AGTATACTTCAAAGGACATT 3′	FJ800808
16	Pig	5′ ATGTAGT AATAAAAATAACCT 3′	AP003428.1
17	Chicken	5′ GCCATCAACATCAATAAATATATA 3′	AY235570
18	Turkey	5′ ATACCCAACCCTAGCTAAAG 3′	NC_010195
19	Wild duck	5′ TACCACGTAAATGCCAAA 3′	NC_009684
20	Buffalo	5′AAGTAAATATAATGCATCCA 3′	NC_006295

the following program: 5 min incubation at 95 °C as initial step for the denaturation of double-stranded DNA, 38 cycles of 45 s at 94 °C (denaturation step), 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 common forward primer and common reverse biotin-linked primer were derived from the mitochondrial genome as listed in Table 1. The PCR products were loaded on 1.5% agarose gel in 0.5 × 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 UV Illuminator.

Reverse line blot assay

Reverse line blot assay was used as described previously by Shayan et al. [15]. Briefly, a Biodyne C blotting membrane was first activated in 16% EDAC solution at room temperature for 10 min. The activated membrane was shortly washed in Aqua Bidest and placed in a Mini-Blotter (MBST, Iran). The oligonucleotide probes specific for cattle, buffalo, sheep, goat, horse, donkey, camel, dog, cat, pig, rabbit, rat, chicken, goose, duck, turkey, for all animals (catch all), for all mammalian (catch all mammalian) and all birds (catch all bird) with N-terminal (TFA)–C6 amino linker (MWG, Germany) at concentration of 1000 pmol in 250 µl 500 mM sodium hydrogen carbonate (pH 8.4) (Table 1) was transferred into the slots of Mini-Blotter and incubated for 1 min at RT. The unbound oligonucleotides were aspirated and the membrane was incubated for 10 min in 100 mM NaOH at RT. Subsequently, the membrane was washed for 5 min twice in 2 × saline–sodium phosphate–EDTA (20 × SSPE = 175.3 g NaCl, 27.6 g NaH2PO4, 9.4 g EDTA, pH 7.4), 0.1% sodium dodecyl sulfate (SDS), once at 60 °C, and once at 42 °C. The membrane was then placed into the Mini-Blotter with the slots perpendicular to the line pattern of the applied probes. As described by Shayan et al. [15], 45 µl of PCR products were diluted with 200 µl 2 × SSPE, 0.1% SDS, heated to 100 °C for 10 min, and then cooled on ice. The slots were aspirated from remaining probe solutions and then filled with the heat-treated and on ice-cooled PCR products. Subsequently, the 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 for each 10 min. The membrane was then incubated with 25 ml 2 × SSPE, 0.5% 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 in 2 × SSPE for each 5 min. Finally, chemoluminescence detection was performed on X-ray film, according to the standard procedure and according to the manufacturer's instructions (Amersham, UK). Subsequently, the bound PCR products to the corresponding probes on the membrane were stripped by washing the membrane twice in 1% SDS for 30 min each time at 80 °C. The membrane was then washed for a short time in 20 mM EDTA (pH 8.0) and stored in fresh EDTA solution at 4 °C for reuse. The membrane can be used five times after washing the membrane in 2 × SSPE, 0.1% SDS for 5 min at 37 °C.

Multiplex PCR

Multiplex PCR was used as described by Kitpipit et al. [16]. We used this method previously for the identification of meat origin in some foods such as hamburgers and sausages. For this aim, 50–100 ng DNA was amplified using primers specific for cattle, sheep, horse, pig, chicken and ostrich (Table 2) [16]. The amplification was performed in a final volume of 50 µl with an initial denaturation step at 95 °C for 5 min, 35 cycles including denaturation at 94 °C for 30 s,

Table 2 The primers used for the multiples PCR reported by Kitpipit et al. [16]

Species	Primer	Sequences (50–30)	Gene	Product size (bp)	References
Pig	Sus-F1 Sus-R1	5′-GAA AAA TCA TCG TTG TAC TTC AAC TAC A-3′ 5′-GGT CAA TGA ATG CGT TGT TGA T-3′	*cyt b*	100	Lopez-Andreo et al. [30]
Sheep	Ovi-F2 Ovi-R2	5′-GAA AAA CCA TCG TTG TCA TTC AAC T-3′ 5′-AAA TAT TTG ATG GAG CTG GGA GA-3′	*t-Glu cyt b*	119	Lopez-Andreo et al. [30]
Chicken	Gal-F3 Gal-R3	5′-AGC AAT TCC CTA CAT TGG ACA CA-3′ 5′-GAT GAT AGT AAT ACC TGC GAT TGC A-3′	*cyt b*	133	Zhang et al. [31]
Ostrich	Str-F4 Str-R4	5′-CCC TTT AAA GAC ATC TGG TAT TGT GAG-3′ 5′-TAA ATT GTA GGC TCT CTG GGG TTC-3′	*12s rRNA*	155	Rojas et al. [32]
Horse	Equ-F5 Uni-R	5′-CGT TTG ATC TGT CCT TAT TAC GGC A-3′ 5′-CCG AAT GGT TCY TTT TTY CCY GAG TAG TA-3′	*COI*	253	Kitpipit et al. [16]
Cattle	Bos-F6 Uni-R	5′-CAT CAA CTT CAT TAC AAC AAT TAT CAA CAT AAA G-3′ 5′-CCG AAT GGT TCY TTT TTY CCY GAG TAG TA-3′	*COI*	311	Kitpipit et al. [16]

annealing at 60 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. Subsequently, the PCR products were analyzed on 2% agarose gel and visualized using ethidium bromide under UV condition.

Results

DNA was extracted from the sausage samples and analyzed on the agarose gel (Fig. 1a) and subsequently amplified using common primer pair derived from the mitochondrial DNA flanking hyper-variable region in the different animal species achieving a PCR product of 429 bp in length (Fig. 1b). After amplification, the PCR products were hybridized with the animal-specific oligonucleotides bound on the Biodyne C blotting membrane. With this membrane, it is possible to detect DNA from different animals (cattle, buffalo, sheep, goat, horse, camel, pig, dog, cat, rabbit, rat, chicken, goose, duck or turkey) simultaneously. The results showed that DNA from cattle and chicken could be detected in 108 sausage samples. Figure 2 shows the representative data for the mentioned sausages. All amplicons have hybridized with catch all, which was designed as a common probe for all mammals and birds (Fig. 2). Furthermore, the amplicons reacted with the catch all mammalian, which confirmed the presence of the DNA occurring in mammalian species. The reaction of amplicons with the catch all birds showed that at least one bird species must be within the sausages. Figure 2 shows that the amplicons could react with the cattle probe and chicken probe, assuming that meat from these two animals was used to prepare the sausages. Since all animal

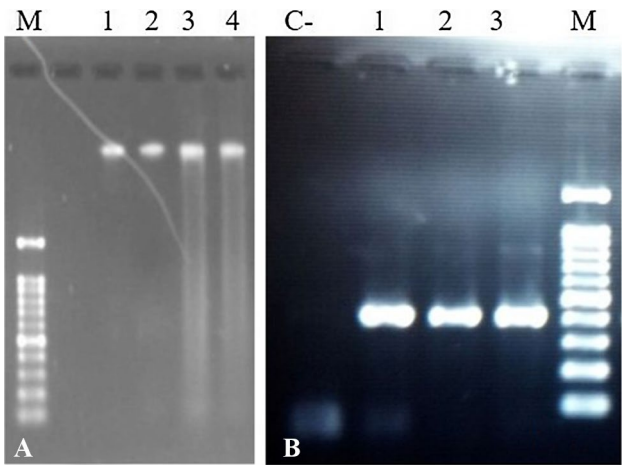

Fig. 1 DNA was extracted from sausages (lanes 1–4) and analyzed on 1% agarose gel. M is 100 bp DNA marker and C− is a negative control (**a**). The extracted DNA was amplified using common primer pair derived from mitochondrial genome resulting in a PCR product of approximately 420 bp in length (lanes 1, 2 and 3 are amplicons from DNA extracted from sausages). M is 100 bp DNA marker and C− is a negative control (**b**)

Fig. 2 The species-specific DNA probes for cattle, buffalo, sheep, goat, horse, camel, dog, cat, pig, rabbit, rat, chicken, goose, 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 sausages. The reaction was visualized by chemoluminescence assay

species-specific PCR products were generated with a common primer pair, and all chicken PCR products included also the common nucleotide sequences region for catch all bird DNA probe, we decided to replace the catch all bird DNA probe far from chicken DNA probe, to avoid the competition between the binding of the chicken-specific PCR products with catch all bird DNA and chicken DNA probes (Fig. 3). Interestingly, in six sausage samples (two with 70%, three with 40% and one with 55% red meat sausages), only the DNA from the chicken was detectable. Figure 3 shows that two samples marked with the white arrows reacted with the catch all representing the animal use in sausages and with the catch all birds and chicken probe demonstration, the presence of chicken meat in these sausages. The amplicons of these two samples as shown in Fig. 3 had not reacted with the catch all mammalian and also no other mammalian probes (white arrows). Figure 3 also shows two probes with most probably little share of mammalian meat (black arrows). These two probes showed gut color development with the catch all, catch all birds and chicken but very weak reaction with the catch all mammalian and no detectable reaction with cattle. We previously used multiplex PCR as reported by Al-Qassab et al. [17] for the detection of animal species in foods such as hamburger and sausage samples

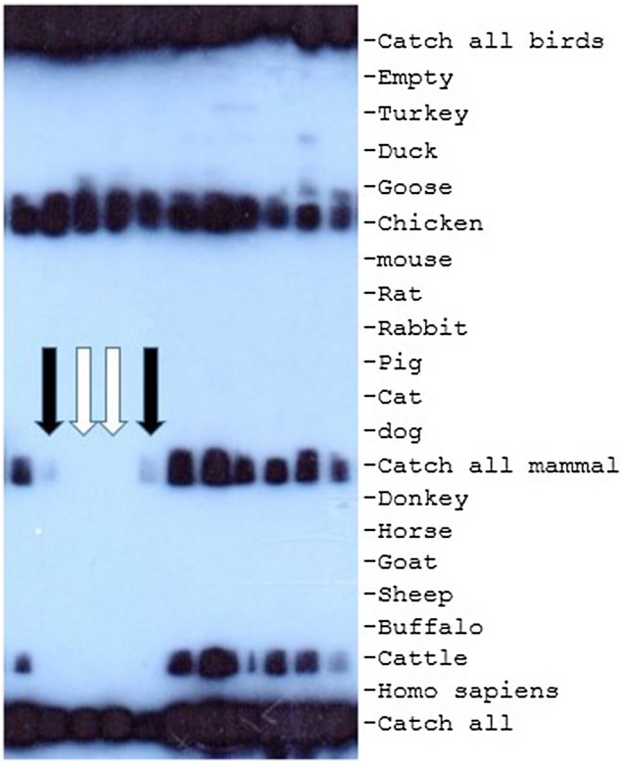

Fig. 3 The species-specific DNA probes for cattle, buffalo, sheep, goat, horse, camel, dog, cat, pig, rabbit, rat, chicken, goose, wild duck, turkey, human, catch all, catch all mammalian and catch all birds were bound to the membrane and the membrane was hybridized with the PCR products generated from sausages. The reaction was visualized by chemoluminescence assay. The white arrows showed that in two sausage samples only chicken DNA was detectable. The black arrows showed two sausage samples with little share of mammalian meat

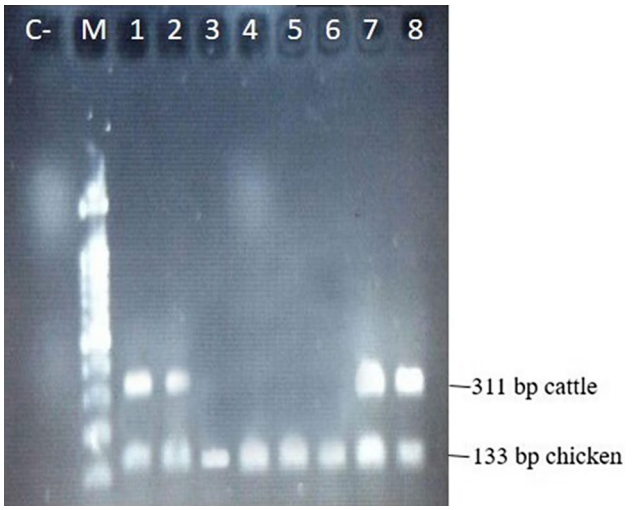

Fig. 4 DNA was extracted from sausages analyzed before with RLB assay resulting in the occurrence of DNA from cattle and chicken meat (lanes 1, 2, 7 and 8) or only chicken meat [lanes 3 and 4 (withe arrows in Fig. 3), lanes 5 and 6 (black arrows in Fig. 3)] in the sausage samples. Subsequently, the extracted DNA was analyzed using multiplex PCR technique. The C− is negative control and M is 100 bp DNA marker

Therefore, for more accurate analysis, the abovementioned samples, in which only chicken meat was detected, were analyzed again by multiplex PCR. In this experiment, sausage samples, in which cattle and chicken meat were detected, were also used. Figure 4 shows the comparable results achieved by RLB.

In all experiments, no hybridization could be recognized with specific oligonucleotide probes for buffalo, horse, donkey, camel, dog, cat, pig, rabbit, rat, goose, duck and turkey. The presence of only chicken meat in sausages labeled with cattle meat was clearly fraudulence.

Discussion

It is the consumer's right to be protected against unfair practices. But in contrast, it is often difficult for a consumer to know what they buy and they must have trust in the description labeled on the product packaging. Due to the economically motivated food fraud, some food operators find the adulteration tempting and substitute, for example, the cheaper meat instead of labeled meat. Therefore, the governmental authorities try to protect the consumer against fraudulent practices with the appropriate legislation. In Europe, the general principles and requirements of food law were described in EC Regulation no. 178/2002, which protect the consumers' interest in dealing with food adulteration. In the USA, food adulteration including mislabeling of the food products is regulated by the Food and Drug Administration (FDA). There are different methods reported for managing food hygiene and safety and protecting the consumers from the economically motivated adulteration. One of these methods is based on the histological examination, which is more suitable for the qualitative analysis and detection of illegal tissue in the food [18, 19]. The molecular methods, which can be divided at least into two categories, namely based on protein chemistry and DNA analysis, were reported as the methods of choice for detecting food fraud. Methods based on protein analysis include immunological assays [20], peptide mass fingerprinting (PMF) [9] or peptide fragmentation fingerprinting (PFF) [10]. Although these methods are to recommend for the detecting of food adulteration, they need expensive equipment and cannot be performed in every laboratory worldwide. DNA-based methods such as PCR and Multiplex PCR [16] were described as alternative methods for protein-based assays. Since DNA can be found in most all animal-derived tissues and liquids prepared in food, it can be also set for detecting the used animal origin. Currently, our group has reported a reverse line blot (RLB) as

an innovative method for the detection of animal species in food [15]. In this method, the DNA extracted from the food is amplified using common primer pair derived from mitochondrial DNA flanking a hyper-variable region in different animals. Subsequently, the amplicon will be hybridized with the animal species-specific DNA probes. Since the primer pair is common, the DNA of all animal species can be most probably amplified concentration dependent by PCR. The hybridization of the PCR product (s) with the species-specific DNA probes will show the animal origin. It is to mention that our preliminary study showed that the mixed meat DNA by the ratio 1/10 rather than 1/100 could be detected simultaneously [15]. Therefore, this method makes it possible to detect simultaneously a range of a few dozen samples of different relevant animal species in food [15]. In contrast, in the multiplex PCR, the primer pairs are not common for all species and the composition of their nucleotide sequences and the annealing temperature of each primer are critical for the correct multi-amplifications. In the present study, the amplicons generated from sausages were hybridized with the 16 specific animal species DNA probes bound on the Biodyne C blotting membrane and the results showed that the DNA from chicken could be demonstrated in all sausage samples, although the label of the packaging had only cattle meat to decipher. Interestingly, in six sausage samples, only chicken DNA was detectable, which clearly speaks for fraudulence. DNA from these sausage samples were also analyzed using multiplex PCR, which confirmed the results achieved with the RLB assay.

Due to the economic, religious, ethical and health concerns, the safety, quality and correct labeling of the food products must be guaranteed. But unfortunately, mislabeling and food fraud are very common worldwide [2, 21–26]. Naaum et al. [27] reported about the mislabeling rate of 20% in sausage samples collected in Canada. They showed that samples labeled with turkey meat had no turkey and one sample labeled with pork meat contained horse meat. Doosti et al. [28] reported the presence of donkey and horse in fermented sausages in Iran and Mehdizadeh et al. [29] reported that handmade hamburger and industrial hamburger samples collected in Iran contained undeclared chicken meat. In our previous study performed by multiplex PCR, the mislabeling rate of 94.6% in sausage samples could be detected [17], whereas in the present study performed by RLB, the mislabeling rate was 100%.

Since in RLB different controls (catch all, catch al mammalian, catch all birds) were used, we believe that RLB was more sensitive than multiplex PCR. The importance of the food fraud and the growing number of reports prompted Bouzembarak et al. [2] to develop a monitoring system with the ability to collect, process and present the published data dealing with the food adulteration in the media. Therefore, it is important to use a simple method to cover as much as possible animals in the analysis. Since in the multiplex PCR up to six animals can be tested, the reverse line blot with the capacity to analyze the presence of a few dozen different animal species simultaneously can be advantageous.

In conclusion, we believe that RLB, because of covering simultaneously a few dozen animals in the food fraud analysis, can be successfully used in the DNA-based authentication of the food.

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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. Ali ME, Razzak MA, Hamid SBA (2014) Multiplex PCR in species authentication: probability and prospects—a review. Food Anal Methods 7(10):1933–1949
2. Bouzembrak Y, Steen B, Neslo R, Linge J, Mojtahed V, Marvin H (2018) Development of food fraud media monitoring system based on text mining. Food Control 93:283
3. Ballin NZ (2010) Authentication of meat and meat products. Meat Sci 86(3):577–587
4. Bottero MT, Dalmasso A (2011) Animal species identification in food products: evolution of biomolecular methods. Vet J 190(1):34–38
5. Regenstein J, Chaudry M, Regenstein C (2003) The kosher and halal food laws. Compr Rev Food Sci Food Saf 2(3):111–127
6. Premanandh J (2013) Horse meat scandal—a wake-up call for regulatory authorities. Food Control 34(2):568–569
7. Chen F-C, Hsieh Y (2000) Detection of pork in heat-processed meat products by monoclonal antibody-based ELISA. J AOAC Int 83(1):79–85
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. Pappin DJ, Hojrup P, Bleasby AJ (1993) Rapid identification of proteins by peptide-mass fingerprinting. Curr Biol 3(6):327–332
10. 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
11. Rodríguez MA, García T, González I, Hernández PE, Martín R (2005) TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures. Meat Sci 70(1):113–120
12. Amaral JS, Santos G, Oliveira MBP, Mafra I (2017) Quantitative detection of pork meat by EvaGreen real-time PCR to assess the authenticity of processed meat products. Food Control 72:53–61
13. Nejad FP, Tafvizi F, Ebrahimi MT, Hosseni SE (2014) Optimization of multiplex PCR for the identification of animal species using mitochondrial genes in sausages. Eur Food Res Technol 239(3):533–541

14. He H, Hong X, Feng Y, Wang Y, Ying J, Liu Q, Qian Y, Zhou X, Wang D (2015) Application of quadruple multiplex PCR detection for beef, duck, mutton and pork in mixed meat. J Food Nutr Res 3(6):392–398
15. Shayan P, Al-taghlubee D, Misaghi A, Shayan D, Gandomi H, Basti AA, Alghassab T, Kamkar A, Khanjari A, Eckert B (2018) An innovative reverse line blot for simultaneous detection of animal species in food. Eur Food Res Technol 244:1–7
16. Kitpipit T, Sittichan K, Thanakiatkrai P (2014) Direct-multiplex PCR assay for meat species identification in food products. Food Chem 163:77–82
17. Al-Qassab T, Kamkar A, Shayan P, Khanjari A (2019) Mislabeling in cooked sausage is a seriously increasingly problem in food safety. Iran J Vet Med 1:4. https://doi.org/10.22059/ijvm.2018.267894.1004935
18. Abd El-Aziz D (2018) Molecular and histological investigation of adulterated ready-to-eat heated meat products with chicken substances. Int Food Res J 25:5
19. Sadeghinezhad J, Izadi F, Latorre R (2016) Application of histomorphological method to assess meat products. Anat Sci J 13(2):73–78
20. Armstrong SG, Leach DN, Wyllie SG (1992) The use of HPLC protein profiles in fish species identification. Food Chem 44(2):147–155
21. Bansal S, Singh A, Mangal M, Mangal AK, Kumar S (2017) Food adulteration: sources, health risks, and detection methods. Crit Rev Food Sci Nutr 57(6):1174–1189
22. Elbialy ZI, Elkassas WM, Elkatatny NA (2016) Authentication and mislabeling detection in canned meat using multiplex PCR. Alex J Vet Sci 49(1):24–28
23. Ha J, Kim S, Lee J, Lee S, Lee H, Choi Y, Oh H, Yoon Y (2017) Identification of pork adulteration in processed meat products using the developed mitochondrial DNA-based primers. Korean J Food Sci Anim Resour 37(3):464
24. Fiorino GM, Garino C, Arlorio M, Logrieco AF, Losito I, Monaci L (2018) Overview on untargeted methods to combat food frauds: a focus on fishery products. J Food Qual 2018(3):1–13
25. Di Pinto A, Bottaro M, Bonerba E, Bozzo G, Ceci E, Marchetti P, Mottola A, Tantillo G (2015) Occurrence of mislabeling in meat products using DNA-based assay. J Food Sci Technol 52(4):2479–2484
26. 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
27. Naaum AM, Shehata HR, Shu Chen S, Li J, NicoleTabujara N, Awmack D, Lutze-Wallace C, Hanner R (2018) Complementary molecular methods detect undeclared species in sausage products at retail markets in Canada. Food Control 84:339–344
28. Doosti A, Ghasemi Dehkordi P, Rahimi E (2014) Molecular assay to fraud identification of meat products. J Food Sci Technol 51(1):148–152
29. Mehdizadeh M, Mousavi SM, Rabiei M, Moradian K, Eskandari S, Abbasi Fesarani M, Rastegar H, Alebouyeh M (2014) Detection of chicken meat adulteration in raw hamburger using polymerase chain reaction. J Food Qual Hazards Control 1:36–40
30. Lopez-Andreo M, Lugo L, Garrido-Pertierra A, Prieto MI, Puyet A (2005) Identification and quantitation of species in complex DNA mixtures by realtime polymerase chain reaction. Anal Biochem 339(1):73–82
31. Zhang CL, Fowler MR, Scott NW, 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(9):1149–1158
32. Rojas M, Gonzalez I, Pavon MA, Pegels N, Hernandez PE, Garcia T et al (2011) Application of a real-time PCR assay for the detection of ostrich (*Struthio camelus*) mislabelling in meat products from the retail market. Food Control 22(3–4):523–531