Review Article

Identification of Different Animal Species in Meat and Meat Products: Trends and Advances

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Abstract | Identification of animal species of origin in meat and meat products is a matter of great concerns such as religious, economical, legal as well as medical aspects. Thus, several analytical techniques have been suggested for the identification of meat species either in individual or in mixed samples to protect consumers from the fraudulent and bad habits of marketing. DNA-based techniques especially the techniques based on polymerase chain reaction (PCR) are recognized as the most appropriate methods employed for species identification in raw and processed meat. PCR techniques including randomly amplified polymorphic DNA (PCR-RAPD), restriction fragment length polymorphism (PCR-RFLP), PCR with species-specific primers, real-time PCR and PCR-nucleotide sequencing allow identification of meat species under different processing conditions. But the variability of DNA content on the level of species as well as target tissue make the DNA-based methods somewhat unsuitable for the quantification of exact percentages of different species in meat and meat products. For these reasons the proteomic approaches depending on identification of different peptide biomarkers has been developed and employed to give information on the different composition of food. To broad the knowledge about these technologies, this review is compiled in an attempt to provide an overview of the possible PCR-based analytical techniques that could help in identifying the meat species of origin in meat and meat products and threw the light on the identification of species specific peptide biomarkers by proteomic technologies as a new and attractive alternative that could overcome some of the limitations that faced DNA-based methods especially when used for meat exposed to intensive heating of processing as well as for meat mixtures.

Keywords | Species identification, animal, meat, meat products, DNA, PCR, proteomics

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INTRODUCTION

Meat identification in various feedstuffs and foods including processed meat products deserves an increasing interest owing to many considerations. Rapid examination of adulteration are very critical issues for healthical requirements, specific food allergies, religious affairs, fraud and malicious marketing practices in addition to economic and legal concerns (Koh et al., 1998; Arslan et al., 2006; Mane et al., 2009). Furthermore, identifying the meat authenticity in meat products is an important issue in food regulatory control for determination of fraudulent replacement of higher commercial valued meat species by inferior, cheaper or undesirable alternatives, the presence of undeclared species, and replacement of animal meat by plant proteins, accurate food labelling (Ballin et al., 2009) and for the evaluation of food composition and providing consumer needed information to achieve food safety (Sta-

moulis et al., 2010).

Protection of consumers and producers from mislabelled meat products, fraudulent actions, and bad practices of meat adulterations through processing and marketing and the prevention of illegal sale of protected species were always critical concerns that enforce legal authorities as well as many researchers to develop different techniques and analytical methods for species identification present in meat or their products including a wide range of degraded and processed materials that were broadly based on measuring either DNA or protein (Matsunaga et al., 1999; Calvo et al., 2001; Herman, 2001; Myers et al., 2003; Peter et al., 2004; Aida et al., 2005).

The species-specific protein biomarkers have been identified using electrophoretic and chromatographic techniques (Vallejo-Cordoba et al., 2005; Chou et al., 2007), or enzyme-linked immunosorbent assay (ELISA) (Berger et al., 1988; Andrews et al., 1992; Chen and Hsieh, 2000) and isoelectric focusing (IEF) (King, 1984; Kim and Shelef, 1986; Scarpeid et al., 1998). These methods have been suggested to resolve proteins of skeletal muscle based on the differences in their isoelectric point or molecular weight (Bauer and Hofmann, 1989; Käuffer et al., 1990; Di Lucciaet al., 1992; Hsieh, 2006) and could be used for mapping of the skeletal muscle proteins of different animal species such as cattle (Bouley et al., 2004; Chaze et al., 2006), swine (Kim et al., 2004; Hollung et al., 2009; Xu et al., 2009), poultry (Doherty et al., 2004) and sheep (Hamelin, 2001). The protein based methods has been reported to be non-suitable for species identification in heated meat products due to denaturation of protein by intensive heating during food processing which in turn lead to modifications in the antigenic activity of molecules and their mobility after electrophoresis (Jemmi and Schlosser, 1991; Guoli et al., 1999; Giovannacci et al., 2004) consequently, change the ability of antibody to identify its target protein (Owusu-Apenten, 2002), moreover, the possible cross-reaction between closely related species (Hsieh et al., 1998). For these reasons protein-based methods have been replaced by DNA-based ones. DNA characterized by more stability under intensive heating, pressures, and chemical processing, has conserved structure in whole body cells, has a great identification power since they are rely on the recognition of specific DNA segments sequence of a particular tissue or animal (Calvo et al., 2001; Frezza et al., 2003; Girish et al., 2004; Lanzilao et al., 2005; Akasaki et al., 2006; Arslan et al., 2006; Rashid et al., 2014). From DNA-based techniques, polymerase chain reaction (PCR) is the most employed, simple, time saving, sensitive and specific method that could identify the species of origin exposed to different processing conditions (Mafra et al., 2008; Bottero and Dalmasso, 2011; Floren et al., 2015). In addition, the use of PCR in food analysis has provided various analytical methods for rapid detection and identification at species and intra-species level; however DNA-based methods still face some important limitations especially for quantitative measurements of food composition (Woolfe and Primrose, 2004). To overcome these limitations attention has been paid to the development of new technologies that could be successfully used when quantitation assessments are required. Among the attractive newly developed analytical techniques that used for quantitative determination for different composition present in meat processed under high temperature or complex mixes is the proteomic technology that depends on analysis of protein and peptide biomarkers as described by many researchers (Jorfi et al., 2012; Giaretta et al., 2013; Montowska and Pospiech, 2013; Boyaci et al.; 2014; Zhao et al., 2014).

Within this context, the aim of this review is to provide an overview of the main PCR-based techniques that are published concerning the species identification of meat and meat products with special reference to the advantages and disadvantages of each method and the mitochondrial genes that have been reported to be used for species identification in meat and meat products. PCR-based techniques most frequently used for meat species identification include randomly amplified polymorphic DNA (PCR-RAPD), restriction fragment length polymorphism (PCR-RFLP), PCR with species-specific primers, real-time PCR and PCR-nucleotide sequencing. Besides, the advances in proteomic technology for species identification have also been covered.

POLYMERASE CHAIN REACTION (PCR)-BASED TECHNIQUES

Randomly Amplified Polymorphic DNA (PCR-RAPD)

The PCR-RADP depends on the use of a single arbitrary primer to initiate and activate the reaction of elongation of strands of the amplified fragment and give a species-specific "fingerprints" followed by isolation of amplified fragments based on size of fragments by gel electrophoresis. So, there is no need for DNA sequencing, restriction enzymes or hybridization (Wu et al., 2006) it is simple, cheap, makes it possible to reveal genetic variability without previous knowledge of the sequence of the tested DNA. But, it requires a known standard for species identification and could not be used to identify composition of meat mixtures or severely (autoclaved) heat treated meat (Koh et al., 1998) and the obtained results were non-reproducible (Wolko et al., 2004).

Koh et al. (1998) could identify buffalo, wild boar, kangaroo and red deer meats by RAPD technique. Meats from buffalo, Elk, reindeer, kangaroo, ostrich and some domestic species could be identified by RAPD under dif-

ferent conditions including fresh, freezing and canning (Martı´nez and Yman, 1998). Martı´nez and Danielsdottir (2000) designed a primer based on cyt b gene that help in identifying different types of meat products of seal and whale under different processing situations by RAPD and PCR-SSCP. RAPD technology was also employed by Huang et al. (2003) for authentication of quail, ostrich, pheasant, emu and dove meats. Saez et al. (2004) generated species-specific finger printings by RAPD- and AP-PCR methods to identify meat species. These methods help in identification of beef, pork, lamb and poultry. Arslan et al. (2005) discriminated meats from certain domestic animals as goat, cattle, camel, sheep, pork and rabbit as well as meat from wild swine, donkey, dog and cat by PCR-RAPD using 10 base primer on both individual and mixed meat samples. Also, this method was used to identify meats from different fish species (Jin et al., 2006).

Restriction Fragment Length Polymorphism (PCR-RFLP)

PCR-RFLP is one of the main genetic techniques conducted by many researchers for species identification in meat and meat products obtained from mammals, poultry or fish. This technique based on amplification of a DNA fragment of various sequences followed by its digestion with an appropriately selected restriction enzyme allowing species differentiation of even closely related species (Pascoal et al., 2004). The PCR-RFLP method characterized by its simplicity and non-expensive costs and easy application in the inspection purposes (Pfeiffer et al., 2004). But unfortunately it could not be employed for identify composition of meat mixtures or severely heat treated meat due to degradation of DNA and the data recorded after digestion of the PCR products might show a combination of diverse restriction types representing all the possible kinds included in the adulterated sample (Girish et al., 2005; Girish et al., 2007). Another defect of the PCR-RFLP method is the possibility of developing erroneous results due to the possible incomplete digestion of the restriction site or occurrence of intra-specific differences which may result in removal or development of restriction locations (Gil, 2007), where, relatively large amplicons are commonly need to perform enzymatic restriction of DNAs (Fajardo et al., 2006). RFLP were developed and applied on the PCR products of certain mitochondrial DNA like mitochondrial displacement (D-loop) region and cytochrome b (cyt b) as well as 12S and 16S rRNA genes as reviewed in our work.

Meyer et al. (1995) amplified the 359-bp fragment of the cyt b gene followed by digestion with *RsaI*, TaqI, *AluI* and *HinfI* to identify cattle, swine, buffalo, wild boar, goat, sheep, horse, turkey and chicken meat. On this context, the PCR-RFLP technique allowed identify of 25 animal species in frozen meat or freeze-dried protein samples using tRNAGlu/cyt b and 11 various restriction enzymes (Wolf et al., 1999). In another report, Partis et al. (2000) stated that PCR-RFLP on the basis of CYT b1 and CYT b2 using C1 and C2 primers which amplify the gene coding cytochrome b that yield products of 359 bp and 464 bp after digestion with HaeIII and *HinfI* can be applied to analyse both raw and cooked meat species as it could differentiate all the tested species except buffalo and kangaroo, but they do not recommend this method to determine species composition of mixed meats.

The discrimination power of cyt b gene has been proved by various studies for example, the study of Bellagamba et al. (2001) in which PCR- RFLP products of cyt b gene conducted to identify species in meat meal and animal feed stuffs. Bravi et al. (2004) amplified a fragment of cyt b to identify meat of cattle, horse, donkey, pig, sheep, dog, cat, rabbit, chicken, and human using universal primers and 3 restriction enzymes (*AluI*, *HaeIII*, and *HinfI*). Ahmed et al. (2007) amplified a segment of cyt b gene (359 bp) followed by digestion with *TaqI* to differentiate between cattle's and buffalo's meat. The PCR products were 2 fragments (191 and 168 bp) in buffalo with no digestion for cattle. Ahmed et al. (2007) used PCR-RFLP to differentiate between horse and donkey meat by restriction enzyme *AluI* that digest the PCR product of cyt b gene amplification to give three fragments in horse's meat (189, 96 and 74 bp), while no digestion in donkey.

Doosti et al. (2014) investigated the PCR-RFLP analysis of the mitochondrial cyt b gene to differentiate between beef, sheep, pork, chicken, donkey, and horse meats in meat products (sausages, frankfurters, hamburgers, hams and cold cut meats) and suggested that this method provide a potential technique to rely on for authentication of halal (lawful or permitted) meat origin. Rahman et al. (2014) assessed the presence of dog meat in meatball by PCR assay for amplification of 100-bp region of canine mitochondrial cyt b gene in different circumstances (pure, raw, processed and mixed conditions). This assay tested with many other animal and plant species used in the formation of meatball and is found to be simple, stable, sensitive and specific to detect dog meat in processed food which is very important for halal authentication purposes.

Fei et al. (1996); Monteil-Sosa et al. (2000) and Mane et al. (2009) tried to differentiate the chicken from other meat species by designing a primer pair on the basis of mitochondrial D-loop gene to amplify 442bp of DNA fragments followed by subjecting the resulted fragments to digestion by HaeIII and Sau3AI enzymes where amplification of 442 bp DNA fragment was observed only in chicken even after cross testing with red meat species investigated (cattle, buffalo, sheep, goat, pig, duck, guinea fowl, turkey and quail) indicating the high specificity of this PCR assay for chicken meat that provide a useful tool

for detecting of meat species even in ad-mixed meat and meat products under different processing conditions.

The PCR-RFLP of 12S rRNA were used by several researchers to discriminate between various animal species (Prakash et al., 2000; Girish et al., 2004; Rodriguez et al., 2004 and 2005). More recent study by Girish et al. (2005) stated that the method of PCR amplification of 456-bp from the 12SrRNA gene followed by digestion with *AluI*, HhaI, ApoI and BspTI could differentiate between beef, buffalo meat, mutton and chevonin fresh and processed meat but not in meat mixtures. Girish et al. (2007) also amplified a DNA fragment of the same length (456 bp) from the 12SrRNA gene using universal primers followed by digestion with *HinfI*, Mph1103I, MvaI, Eco47I, that help in identification of duck, chicken, turkey, guinea fowl where the PCR-RFLP method identified all the poultry species in fresh meats, chicken meat detection was also possible in heated products. Similarly, Rajput et al. (2013) amplified a 440 bp length fragment from the 12SrRNA gene using universal primers to differentiate the meat of sambar and chital (wild animals) from meat of sheep and goat, where they used PCR-RFLP and sequencing to differentiate between these species. *AluI* and *RsaI* succeeded to differentiated between the meat samples from sambar and chital and the meat samples from sheep and goat. BsrI could differentiate chital from the all other species. DdeI helped in differentiation of chital and sambar from each other. Also, 16S rRNA was used for species identification by PCR-RFLP method (Borgo et al, 1996; Sawyer et al., 2003). Chikuni et al. (1994) discriminated goat and sheep meat by PCR-RFLP of satellite I DNA sequence using restriction enzyme of ApaI. Restriction profile of melanocortin gene was used as DNA marker for discrimination of Hanwoo meat from meats of Angus and Holstein (Chung et al., 2000).

PCR with the Use of Species-Specific Primers

The identification of species origin of meat by PCR using species-specific primers is precise, sensitive, cheap and not a time consuming method that help the novel identification of many mammalian and bird species in meat and meat products as compared to other PCR based assay (Mane et al., 2007). The PCR methods targets genomic and mitochondrial DNA for the purpose of the identification of meat species in large number of samples, even in cooked meat under different processing conditions without the need for further sequencing or digestion of the PCR products with restriction endonucleases (Di Pinto et al., 2005; Arslan et al., 2006; Mafra et al., 2008; Rojas et al., 2009b), but the most important requirement is that the nucleotide sequence of the gene used for species identification should be known for the purposes of primer designing (Spychaj et al., 2009).

The species specific PCR assay were also used by Hopwood et al. (1999) to identify chicken meat in fresh or cooked meat admixtures including meat of other species as beef, lamb, pork, horse, duck and pheasant. Calvo et al. (2002) designed specific primers for detection of pork meat in different meat products. Similarly, Kitpipit et al (2013) used this method to differentiate between pork, mutton and chicken meat.

Species-specific primers designing based on the mitochondrial cytochrome b gene has been reported by many researchers like Matsunaga et al. (1999) who could qualitatively identify fresh and thermally processed meats of pigs, cattle, sheep, goat, horse and chicken using seven primers, a forward primer designed for the conservative sequence of the cyt b gene in mitochondria and six reverse primers specific for each of tested kinds except the meat of horse exposed to intensive heating.

Colombo et al. (2002) designed species-specific primers based on the cyt b mitochondrial gene that help in the identification of goose (Anseranser) meat in salami meat product in presence or absence of pork or duck meat. Hird et al. (2003) designed species-specific primers depending on the cyt b gene for speciation of chicken and turkey meat under different manufacturing conditions where the products of amplification were of 120 bp for the chicken and 101 bp for the turkey. The species identification of chicken, turkey, duck, goose, pheasant, quail and guinea fowl in meat and meat products was also conducted by Schwägele et al. (2007) who used cyt b gene to design species specific primers where there was no cross-reactivity with any other species.

Haunshi et al. (2009) designed primers specific for pigeon identification based on cyt b and species-specific markers for chicken duck and pig D-loop mitochondrial genes that could strictly identify the mentioned species in fresh and processed meats. Barakat et al. (2014) amplified the mitochondrial cyt b and D-loop genes using porcine-specific primers followed by QIAxcel capillary electrophoresis system to detect and quantify the pork meat in "halal" meat products using raw and cooked sausages as models. This method proved rapidity and sensitivity as it gives specific DNA fragments for pork meat only.

The mitochondrial D loop genehas been employed for designing a pair of primers specific to the buffalo meat that used by Girish et al. (2013) to examine a different method for authentication of buffalo tissues based on DNA extraction by alkaline lysis from meat, liver, heart and kidney samples of buffalo and other related species like cattle, sheep and goat. This species specific PCR resulted in an amplicon of size 482 pb for buffalo and no amplification in the other species. Karabasanavar et al. (2014) designed a new species specific primers specific for the mitochondrial D-loop region of pigs that give a unique amplicon containing 712 pb providing a very sensitive and specific PCR

assay for detecting pork meat from many other species including meat of mammals, birds, rodents as well as fish. The developed assay also could detect the authenticity of pig tissues in different processing conditions (raw, cooked, autoclaved, micro-oven) helping in the purposes of forensic identification of pig species as well as adulteration of pig meat with other species meat.

Fajardo et al. (2007) used a PCR assay based on the amplification of DNA fragments of D-loop and 12SrRNA gene using species-specific primers to identify various cervid and wild ruminant meats including the meats from different deer species as red deer (Cervuselaphus), fallow deer (Damadama), and roe deer (Capreoluscapreolus). Species-specific primers depending on D-loop and 12S rRNA genes were similarly used by Rojas et al. (2009b); Rojas et al. (2010) for the identification of some species of game bird species. Similarly, Martı´n et al. (2007) used the specific primers based on 12S rRNA for identification of four duck species in meat mixtures and specific identification of Muscovy duck even if used on highly damaged DNA. Species-specific primers targeting 12S and 16 rRNA were applied for detection of some animal species like deer and some ruminant animals in meat products by Ha et al. (2006). Mule duck was identified by the primer sets of 12S and 5S ribosomal RNA, and a-actin genes (Rodriguez et al. 2001, 2003a, 2003b and 2004).

Simplex and multiplex PCR using species specific primers based on the different mitochondrial genes were employed for identification of seven different animal species in meat broth samples instead of using meat directly where they used primers based on cyt b gene for bovine, goat and sheep, 12S RNA for poultry and pig , 16SRNA for ruminants, ND4 for cat and ND2 for donkey (Rashid et al., 2014).

Real-Time PCR

The real-time PCR is promising method used for detection of meat authenticity in very complicated mixtures even if the target species present in very small amounts (Koppel et al., 2009). Real-time PCR techniques help to achieve the quantitative determination of gene expression by detecting the received signals resulted from application of fluorescent pigments that help monitoring of PCR products generated in each PCR reaction cycle depending on the fluorescence intensity of these products so skipping of electrophoresis and gel staining that usually must carried out after completing the PCR reaction i.e. do not require additional detection steps. Furthermore, the possibility of contamination is rare (Rodriguez et al., 2005).

One of the chemical methods based on fluorescence detection of real-time PCR products is TaqMan probe technique. Species-specific primers and TaqMan fluorogenic probes reported to be useful in inspection procedures to ensure the prober labelling of raw and heat-processed meat and meat products. TaqMan probe method based on cyt b gene was employed to identify some closely related species by Chisholm et al. (2005) who developed a real time PCR to design species-specific primers to amplify cytochrome b gene, this assay could detect the meat of horse and donkey species in commercial products on the levels of 1 pg and 25 pg, respectively. Cytochrome b gene by this technique could identify beef, pork, lamb, chicken and turkey meats occurring in mixtures from raw (Dooley et al., 2004) and duck meat (Hird et al., 2005). Real-time TaqMan technology based on cyt b was used for identification of deer and some domestic species (Hird et al., 2004), and for quantification of DNA from ostrich and other meat species (Lopez-Andreo et al., 2006). Chisholm et al. (2008) used species-specific primers and TaqMan probes based on the mitochondrial cyt b gene to identify DNA from quail and pheasant in commercial food products. While, pork meat was identified by developed RT-PCR and TaqMan probe that based on amplification of the mitochondrial fragment of the 12S rRNA gene (Rodriguez et al., 2005). Similarly, Rojas et al. (2010b) used the same gene to identify pheasant, quail, pigeon, guinea fowl, partridge, Eurasian woodcock and song thrush. The real-time PCR technique was also succeeded to detect the different component of meat mixture congaing red deer, fallow deer, roe deer, chamois and pyrenean ibex as reported by Fajardo et al. (2008b and 2008c) using species-specific primers designed on D-loop genes and 12S rRNA. TaqMan probe method was also employed for identification of meat and meat products from different pigeon species common pigeon, woodpigeon, and stock pigeon (Columba oenas) by Rojas et al. (2012) depending mitochondrial 12S rRNA and the nuclear 18S rRNA gene from eukaryotic DNA.

Kesmen et al. (2009) in their study to identify meat species, designed sensitive and specific real-time PCR to design specific primers and TaqMan probes based on mitochondrial ND2 gene for donkey, ND5 gene for pork and ATP 6-8 gene for horse for differentiation and quantification of their meats in raw and cooked products. The used assay succeeded to detect very minute amounts of DNA (0.0001ng) of different tested species and meat mixtures and showed no cross-reaction was detected among the tested species and could differentiate them from chicken, turkey, ovine and bovine meats.

Druml et al. (2015) developed a TaqMan real-time PCR assay to quantify the roe deer content in different meat products. The percentage of roe deer content was detected depending on the myostin gene. The diluted DNA extracted from roe deer was analyzed serially and the efficiency of the amplification obtained was 93.9%, indicating the high specificity of this assay for roe deer and importance of it in detecting meat adulteration.

PCR-Sequencing

PCR nucleotide sequence might be of great value for identification on the species level. Sequencing usually involves part or all of the mitochondrial genome followed by its comparison with known sequences in Gene Bank (NCBI). PCR technique is suitable and accepted but it is expensive, and needs to more time and labour consuming due to the further step of sequencing products, mixtures cannot be separated, and the generated samples may not produce enough sequence results (Lenstra et al., 2001).

DNA Sequencing gives much information with no need for more steps like digestion with enzymes or analysis of the given data. With the help of a universal primer bands can be obtained for different animal species after PCR amplification that could help their differentiation (Kocher et al., 1989). The most appropriate mitochondrial genes used for species identification using sequencing technology are cyt b, 12S and 16S rRNA genes could give a considerable amount of mutations and there are also many information found on data bases concerning their sequences (Karlsson and Holmlund, 2007).

The cyt b gene has been completely partially or sequenced for identification of numerous different species of birds, mammals (Bravi et al., 2004; Andrzej and Kamila, 2005), fishes, amphibian and reptiles (Chow et al., 1993; Ram et al., 1996; Quinterio et al., 1998; Lindstrom, 1999; Parson et al., 2000) and also some invertebrates (Lee et al., 2009). In addition, Chikuni et al. (1994); Matsunaga et al. (1998); La Neve et al. (2008) used cyt b gene sequence to identify of meats and meat products of red deer, roe deer, song thrush, pyrenean ibex, chamois, quail and sparrow.

The variations in the sequences of mitochondrial 12S and 16S rRNA gene are suitable and sufficient for identification between different species from high number of vertebrates such as birds, fish, reptiles, mammals and amphibians (Kocher et al., 1989; Prakash et al., 2000; Kitano et al., 2007; Karlsson and Holmlund, 2007). Mitochondrial 12S rRNA gene sequenced to identify ostrich, emu, guinea fowl, and quail meats (Girish et al., 2009). Rostogi et al. (2004) assessed the use of the amplification and sequencing of 450 pb fragment of mitochondrial 12S rRNA gene universal primers to identify the species of origin of raw and cooked meat samples, viscera, blood and semen. This technique was found to identify all studied samples on species level, even if samples exposed to preservation at ambient temperature for long times. They then detected the meat adulteration using conformation-sensitive gel electrophoresis (CSGE) that was found to be of importance in authentication of meat in the field of forensic food analysis.

Abuzinadah et al. (2013) used sequencing of DNA fragment of the mitochondrial 12SrRNA gene followed by confirmation using species specific primer to detect the adulteration of chicken products including (Luncheon, burger, sausage and minced meat). They could detect the substitution of all samples used by inedible parts of turkey. Li et al. (2006) identify cervid species by sequence analysis of 12S rRNA and cyt b genes. Cawthorn et al. (2013) used a DNA-based LCD array followed by confirmation Species specific PCR and DNA and sequencing of Cyt b, 12SrRNA and ND2 genes to detect the fraud and mislabeling of meat products. 68% of samples used found to contain species that are not indicated on the product label. The diglycerideacyltransferase 1 (DGAT1) or 18S rRNA have been sequenced for the differentiation of meats from these species buffalo, crocodile and kangaroo (Matsunaga et al., 1998; Venkatachalapathy et al., 2008).

THE ROLE OF PROTEOMIC TECHNOLOGY AND PEPTIDE BIOMARKERS IN IDENTIFICATION OF MEAT ORIGIN

The great advances in the application of mass spectrometry in the peptides and proteins analysis make the proteomic technology gaining attention as alternative to the other methods used for species identification and meat authentication issues (Montowska and Pospiech 2011a and 2012a). Mass spectrometry has also been successfully employed to study the protein maps of muscles that differ in their composition of fiber (Hamelin et al., 2007), or in the identification of muscles derived from different genetic origins (McDonagh et al., 2006; Hollung et al., 2009) with a discriminating activity near to DNA- based analytical methods. However, the proteomic technology can overcome some of the limitations that face methods based on the analysis of DNA, in particularly the quantitative analysis of thermally processed food and meats that exposed to high degrees of temperature during processing as the peptide´s amino acid sequences are highly resistant to different processing conditions than DNA additionally the proteins and peptides are easily extracted compared to DNA (Ortea et al., 2009; Montowska and Pospiech 2011b; Montowska and Pospiech, 2013). Mass spectrometry method could help in identification of different meats depending on the mass differences between hemoglobins and myoglobins of the different meat species. This method was helpful in detection of horse hemoglobin that was present in a mixture with that of beef (Taylor et al., 1993).

Proteomic technology could also successfully identify specific proteins of the different fish species in both fresh and processed fish products (Carrera et al., 2007; Mazzeo et al., 2008; Ortea et al., 2009) based on the use of MALDI–TOF MS and MALDI–TOF and LC–ESI–MS/MS as a rapid screening method. By the same spectrometry, Sentandreu et al. (2010) reported that the proteomic technology could

differentiate between turkey and chicken meats by the use of species-specific peptides derived from digestion of myosin light chain 3 (MLC-3) using in-solution trypsin digestion. On the same context, Montowska and Pospiech (2011b and 2012b) observed the inter-species differences in myosin light chain isoforms (MLC) in the raw and processed meat and meat products of some animal species including some poultry species (chicken, turkey, duck and goose) in addition to pig and cattle depending on the species-specific electrophoretic mobility. Zhao et al. (2014) reported that mid-infrared ATR spectroscopy could help in identifying the authentic higher and lower quality beef burger samples from other samples adultrated by beef offal under fresh and freezing conditions. Raman spectroscopy enables rapid determination of beef adulteration with horsemeat with high accuracy, few seconds for analysis and no sample preparation (Boyaci et al., 2014).

Proteomic approaches depending on specific peptide biomarkers were also investigated by Montowska and Pospiech (2013) who used some regulatory proteins, nmetabolic enzymes and myofibrillar proteins (troponin T and tropomodulin) to identify the different meat species. They observed inter-species differences in protein expression in raw meat, thermally processed meat and ready-made products. Especially in albumin and apolipoprotein B; the regulatory proteins (HSP27 and H-FABP) and the metabolic enzymes ATP synthase, cytochromebc-1 subunit 1 and alpha-ETF that were greatly differs in their species-specific electrophoretic mobility. The differences in the sequences of obtained fragments were species-spesific and very valuable in identification of poultry meat (chicken and turkey) as well as cattle and pig. The use of specific peptide sequences was highly valuable in differentiation between gelatin from bovine and porcine as described by Zhang et al. (2009) although the sequence of collagen in mammals are highly homogenous. Shibata et al. (2009) observed many species differences in the proteomes of Japanese Black Cattle that fed on grain than those fed on grass.

Moreover, the proteomic approaches depending on identification of different peptide biomarkers could also employed to give information on the different composition of food. The quantity of individual type of sarcoplasmic and myofibrillar proteins found to be different from one type of muscle to another of the same animal as described for white and red skeletal muscles of pig (Kim et al., 2004), sheep (Hamelin et al., 2007) and Bayonne ham (Théron et al., 2011).

Jorfi et al. (2012) used the amino acid content of meat as markers for halal meat authentication, their method succeeded to identify pork meat from other meat species including mutton , beef, chevon and chicken by the use of reverse phase-high performance liquid chromatography (RP-HPLC) followed by o-phthalaldehyde (OPA) derivatization and ultraviolet (UV) detection where histidine, alanine, serine, valine and arginine found to be the highly discriminative ones between porcine and other species meat. Giaretta et al. (2013) stated that myoglobin could be used as a marker in identifying the pork meat in the raw beef burger using ultra-performance liquid chromatography (UPLC). Where, the percentages of pork and beef meat can be quantified in premixed minced meat samples from different animal origin like beef, chicken, horse, ostrich, pig and water buffalo.

CONCLUSION

The problem of protein denaturation after exposure to high temperature during food processing makes protein based techniques not adequate for meat species identification because this denaturation of protein could lead to many problems such as changing the antigenicity of the molecules and the possible cross reaction between the closely related species. So the attention was directed towards applying of the DNA-based methods. Because DNA is more stable under different processing conditions and its conserved structure gives it a high discriminating properties. Both mitochondrial and nuclear genes have been broadly targeted for the identification of species of meat and meat products. The mitochondrial markers were proved to be more valuable than nuclear markers in species identification and authentication since the mitochondrial DNA is maternally inherited, mitochondrial genes have variable regions that are found in thousands per individual cell that facilitates PCR amplification improve the assay sensitivity allowing the achievement of positive result even if the DNA was severely fragmented or damaged under intense conditions of food processing. These properties give the probability to mitochondrial DNA to identify origin of meat in processed meat products and make mitochondrial markers highly efficient than nuclear ones in identification and authentication of meat species in fresh, cooked and autoclaved meat and meat products. Among the mitochondrial genes, the cyt b geneD-loop12S rRNA16S rRNA have been used for species identification. PCR is the most well developed DNA based methods until now that provides a wide range of analytical method which could be used for rapid detection and identification at species and intra-species level. PCR-based methods most frequently used for meat species identification include PCR-RFLP, PCR-RAPD Species specific primers, RT-PCR and PCR-nucleotide sequencing. Nevertheless, the PCR-RAPD is not suitable for identification of meat species in meat mixtures and intensively heated products. Also the PCR-RLFP technique could not give accurate information on the composition of meat present in mixtures. Sequencing of mitochondrial genes is costly, time and labour consuming and interpretation of the results is difficult. Additionally, DNA still facing certain limitations

in both quantitative analysis and degradation due to temperature and pressure used for food processing that may alter the results of PCR amplification especially with long DNA fragments. So many researchers directed toward developing new technologies that could overcome these limitations. One of the new developing technologies that could be successfully used to assess the meat authenticity is the proteomic technology that depends on analysis of protein and peptide biomarkers. This technique has been proved by many researchers to have a discriminating power comparable to that of DNA-based analysis and could be used for quantitative determination of the composition of thermally processed meats as the sequences of peptide's amino acids are easily extracted and highly resistant at high temperature than DNA.

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REFERENCES

- Abuzinadah OH, Yacoub HA, El Ashmaoui HM, Ramadan HA (2013). Moleculardetection of adulteration in chicken products based on mitochondrial 12S rRNA gene. Mitochondr. DNA. 1-4. http://dx.doi.org/10.3109/19401736.2013.840593
- Ahmed MMM, Abdel-Rahman SM, El-Hanafy AA (2007). Application of species specific polymerase chain reaction and cytochrome b gene for different meat species authentication. Biotechnology 6(3): 426-430. http://dx.doi.org/10.3923/biotech.2007.426.430
- Aida AA, Che Man YB, Wong CMVL, Raha AR, Son R (2005). Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat Sci. 69: 47-52. http://dx.doi.org/10.1016/j.meatsci.2004.06.020
- Akasaki T, Yanagimoro T, Yamakami K, Tomonaga H, Sato S (2006). Species identification and PCR-RFLP analysis of cytochrome b gene in cod fish (OrderGadiformes) products. J. Food Sci. 71(3): 190-195. http://dx.doi.org/10.1111/j.1365-2621.2006.tb15616.x
- Andrews CD, Berger RG, Mageau RP, Schwab B, Jhonston RW (1992). Detection of the beef, sheep, deer, and horse meat in cooked meat products byenzyme-linked immunosorbent assay. J. AOAC Int. 75: 572–576.
- Andrzej D, Kamila K (2005). Anew PCR-RFLP with in the domestic pigeon (Columba liviavar, domestica) cytochrome b (MTCYB) gene. J. Appl. Genet. 46(3): 315-317.
- Arslan A, Ilhak OI, Calicioglu M (2006). Effect of method of cooking on identification of heat processed beef using polymerase chain reaction (PCR) technique. Meat Sci. 72: 326-330. http://dx.doi.org/10.1016/j.meatsci.2005.08.001
- Arslan A, Ilhak I, Calicioglu M, Karahan M (2005). Identification of meats using random amplified polymorphic DNA (RAPD) technique. J. Muscle Foods. 16(1): 37-45. http://dx.doi.org/10.1111/j.1745-4573.2004.07504.x
- Ballin NZ, Vogensen FK, Karlsson AH (2009). Species determination-Can we detect and quantify meat adulteration? Meat Sci. 83(2): 165-174. http://dx.doi.org/10.1016/j.meatsci.2009.06.003
- Barakat H, El-Garhy HA, Moustafa MM (2014). Detection of pork adulteration in processed meat by species-specific PCR-QIAxcel procedure based on D-loop and cytb genes. Appl. Microbiol. Biotechnol. 98(23): 9805-9816. http://dx.doi.org/10.1007/s00253-014-6084-x
- Bauer F, Hofmann K (1989). Elektrophoretische Tierarten identifizierungbeierhitztem Fleisch und Fleischerzeugnissen. Fleischwirtsch, 69: 419–422.
- Bellagamba F, Moretti VM, Comincini S, Valfre F (2001). Identification of species in animal feeds us by polymerase chain reaction–restriction fragment length polymorphism analysis of mitochondrial DNA. J. Agric. Food Chem. 49: 3775–3781. http://dx.doi.org/10.1021/jf0010329
- Berger RG, Margeau, RP, Schwab B, Johnston RW (1988). J. AOAC 71: 406–409.
- Borgo R, Souty Grosset C, Bouchon Gomot, DL (1996). PCR-RFLP analysis of mitochondrial DNA for identification of quailmeat species. J. Food Sci. 61: 1–4. http://dx.doi.org/10.1111/j.1365-2621.1996.tb14712.x
- Bottero MT, Dalmasso A. (2011). Animal species identification in food products: evolution of biomolecular methods. Vet. J. 190: 34–38. http://dx.doi.org/10.1016/j.tvjl.2010.09.024
- Bouley J, Chambon C, Piccard B (2004). Mapping of bovine skeletal muscleproteins using two-dimensional gel electrophoresis and mass spectrometry. Proteomics. 4: 1811–1824. http://dx.doi.org/10.1002/pmic.200300688
- Boyacı İH,Temiz HT, Uysal RS, Velioğlu HM, Yadegari RJ, Rishkan MM. (2014). A novel method for discrimination of beef and horsemeat using Raman spectroscopy. Food Chem. 1(148): 37-41. http://dx.doi.org/10.1016/j.foodchem.2013.10.006
- Bravi CM, Liron JP, Mirol PM, Ripoli MV, Garcia PP, Giovambattista G (2004). A simple method for domestic animal identification in Argentina using PCR-RFLP analysis of cytochrome b gene. J. Legal Mad. 6(4): 246-251. http://dx.doi.org/10.1016/j.legalmed.2004.06.003
- Calvo JH, Rodellar C, Zaragoza P, Osta R (2002). Beef- and bovine-derivedmaterial identification in processed and unprocessed food and feed by PCRamplification. J. Agr. Food Chem. 50(19): 5262–5264. http://dx.doi.org/10.1021/jf020051a
- Calvo JH, Zaragoza P, Osta R (2001). Random amplified polymorphic DNA fingerprints for identification of species in poultry pate. Poult. Sci. 80(4): 522–524. http://dx.doi.org/10.1093/ps/80.4.522
- Carrera E, García T, Céspedes A, González I, Fernández A, Hernández PE, Martín R (1999a). PCR-RFLP of the mitochondraial cytochrome oxidase gene: a simple method for discrimination between Atlantic salmon (Salmosalar) and rainbow trout (Oncorhynchusmykiss). J. Sci. Food Agri. 79: 1654-1658. http://dx.doi.org/10.1002/(SICI)1097-0010(199909)79:12<1654::AID-JSFA414>3.0.CO;2-S
- Carrera E, García T, Céspedes A, González I, Fernández A, Hernández PE, Martín R (1999b). Salmon and trout analysis by PCR-RFLP for identity authentication. J. Food Sci. 64(3): 410-413. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15053.x
- Carrera M, Cañas B, Piñeiro C, Vázquez J, Gallardo JM (2007). De novo mass spectrometry sequencing and characterization of species-specific peptides from nucleoside diphosphate kinase B for the classification of commercial fish species

belonging to the family Merlucciidae. J. Proteome Res. 6: 3070–3080. http://dx.doi.org/10.1021/pr0701963

•Cawthorn DM, Harris A Steinman, Louwrens C Hoffman (2013). A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. Food Control. 32: 440- 449. http://dx.doi.org/10.1016/j.foodcont.2013.01.008

•Chaze T, Bouley J, Chambon C, Barboiron C, Picard B (2006). Mapping of alkaline proteins in bovine skeletal muscle. Proteomics. 6: 2571–2575. http://dx.doi.org/10.1002/pmic.200500452

•Chen FC, Hsieh YH (2000). Detection of pork in heat-processed meat products by monoclonal antibody-based ELISA. J. AOAC Int. 83: 79–85.

•Chikuni K, Tabata T, Kosugiyama M, Monma M (1994). Polymerase chain reaction assay for detection of sheep and goat meats. Meat Sci. 37: 337–345. http://dx.doi.org/10.1016/0309-1740(94)90051-5

•Chisholm J, Conyers C, Booth C, Lawley W, Hird H (2005). The detection of horse and donkey using real-time PCR. Meat Sci. 70: 727-732. http://dx.doi.org/10.1016/j.meatsci.2005.03.009

•Chou CC, Lin SP, Lee KM, Hsu CT, Vickroy TW, Zen JM (2007). Fast differentiation of meats from fifteen animal species by liquid chromatography with electrochemical detection using copper nanoparticle plated electrodes. J. Chromatogr. B. 846(1–2): 230–239. http://dx.doi.org/10.1016/j.jchromb.2006.09.006

•Chow S, Clarke ME, Walsh PJ (1993). PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): A simple method for species and stock identification. Fish Bull. 30:207–224.

•Chung ER, Kim, WT, Kim, YS, Han, SK (2000). Identification of Hanwoo meat using PCR-RFLP marker ofMC1R gene associated with bovine coat colour. J. Anim. Sci. Technol. 42: 379–380.

•Colombo F, Marchisio E, Pizzini A, Cantoni C (2002). Identification of the goose species (Anseranser) in Italian "Mortara" salami by DNA sequencing and a Polymerase Chain Reactionwith an original primer pair. Meat Sci. 61: 291-294.http://dx.doi.org/10.1016/S0309-1740(01)00195-4

•Di Luccia A, Santoro A, Anastasio A, Sarli T, Manniti F, Boffa F (1992). Differentiation of animal species by meat proteins. I. Detection of beef mixedwith pork meat by isoelectric focusing of the A2 myosin light chain. Ital. J. Food Sci. 3: 195–204.

•Di Pinto A, Forte VT, Conversano MC, Tantillo GM (2005). Duplex polymerase chain reaction for detection of pork meat in horsemeat fresh sausages from Italian retail sources. Food Control. 16: 391–394. http://dx.doi.org/10.1016/j.foodcont.2004.04.004

•Doherty MK, McLean L, Hayter JR, Pratt JM, Robertson DHL, El-Shafei A, Gaskell SJ, Beynon RJ (2004). The proteome of chicken skeletal muscle: changes in solubleprotein expression during growth in a layer strain. Proteomics. 4: 2082–2093. http://dx.doi.org/10.1002/pmic.200300716

•Dooley JJ, Paine KE, Garrett SD, Brown HM (2004). Detection of meat species using TaqMan real-time PCR assays. Meat Sci. 68: 431-438. http://dx.doi.org/10.1016/j.meatsci.2004.04.010

•Doosti A, Ghasemi Dehkordi P, Rahimi E (2014). Molecular assay to fraud identification of meat products. J. Food Sci. Technol. 51(1): 148-52. http://dx.doi.org/10.1007/s13197-011-0456-3

•Druml B, Mayer W, Cichna-Markl M, Hochegger R (2015). Development and validation of a TaqMan real-time PCR assay for the identification and quantification of roe deer (Capreoluscapreolus) in food to detect food adulteration. Food Chem. 1(178): 319-26. http://dx.doi.org/10.1016/j.foodchem.2015.01.003

•Fajardo V, González I, López-Calleja I, Martín I, Rojas M, Hernández PE, García T, Martín R (2007). Identification of meats from red deer (Cervuselaphus), fallow deer (Damadama), androe deer (Capreoluscapreolus) using polymerase chain reaction targeting specific sequencesfrom the mitochondrial 12S rRNA gene. Meat Sci. 76: 234-240. http://dx.doi.org/10.1016/j.meatsci.2006.11.004

•Fajardo V, Gonza ´ lez, I, Martı´n I, Rojas M, Herna ´ndez, PE, Garcı´a T (2008c). Real-time PCR for quantitative detection ofchamois (Rupicaprarupicapra) and pyrenean ibex (Capra pyrenaica) in meat mixtures. J. AOAC Int. 91(1): 103-111.

•Fajardo V, Gonza ´lez I, Lo ´pez-Calleja I, Martı ´n I, Herna ´ndez, PE,Garcı ´a T (2006). PCR-RFLPauthenticationofmeatsfromred deer(Cervuselaphus), fallow deer (Damadama), roe deer (Capreoluscapreolus), cattle (Bostaurus), sheep (Ovisaries)andgoat(Capra hircus). J. Agri. Food Chem. 54(4): 1144-1150. http://dx.doi.org/10.1021/jf051766r

•Fajardo V, Gonza ´lez, I, Martı´n, I, Rojas M, Herna ´ndez PE, Garcı´a, T (2008b). Real-time PCR for detection and quantification of red deer (Cervuselaphus), fallow deer (Damadama),and roe deer (Capreoluscapreolus) in meat mixtures. Meat Sci. 79(2): 289-298. http://dx.doi.org/10.1016/j.meatsci.2007.09.013

•Fei S, Okayama T, Yamanoue M, Nishikawa I, Mannen H, Tsuji S (1996). Species identification of meat and meat products by PCR. Anim. Sci. Technol. 67: 900–905. http://dx.doi.org/10.2508/chikusan.67.900

•Floren C, Wiedemann I, Brenig B, Schütz E, Beck J (2015). Species identification and quantification in meat and meat products using droplet digital PCR (ddPCR). Food Chem. 173: 1054–1058. http://dx.doi.org/10.1016/j.foodchem.2014.10.138

•Frezza D, Favaro M, Vaccari G, Von-Holst C, Giambra V, Anklam E (2003). A competitive polymerase chain reaction-based approach for the identification and semiquantification of mitochondrial DNA in different heat-treated bovine meat and bonemeal. J. Food Protect. 66: 103–109.

•Giaretta N, Di Giuseppe AM, Lippert M, Parente A, Di Maro A (2013). Myoglobin as marker in meat adulteration: a UPLC method for determining the presence of pork meat in raw beef burger. Food Chem. 141(3): 1814-20. http://dx.doi.org/10.1016/j.foodchem.2013.04.124

•Gil LA (2007). PCR – based methods for fish and fishery products authentication. Trends Food Sci. Technol. 18: 558-566. http://dx.doi.org/10.1016/j.tifs.2007.04.016

•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: 863–867. http://dx.doi.org/10.1111/j.1365-2621.2004.00859.x

•Girish PS, Anjaneyulu ASR, Viswas KN, Shivakumar BM, Anand M, Patel M, Sharma B (2005). Meat species identification by polymerase chain reaction-restrictionfragment length polymorphism (PCR-RFLP) of mitochondrial12S rRNA gene. Meat Sci. 70: 107–112. http://dx.doi.org/10.1016/j.

meatsci.2004.12.004
•Girish PS, Anjaneyulu ASR, Viswas KN, Santhosh FH, Bhilegaonkar KN, Agarwal RK,Kondaiah N, Nagappa K (2007). Polymerase chain reaction – restriction fragment length polymorphism of mitochondrial 12S gene: A simple method for identification of poultry meat species. Vet. Res. Commun. 31: 447-455. http://dx.doi.org/10.1007/s11259-006-3390-5
•Girish PS, Haunshi S, Vaithiyanathan S, Rajitha R, Ramakrishna C (2013). A rapid method for authentication of Buffalo (Bubalusbubalis) meat by Alkaline Lysis method of DNA extraction and species specific polymerase chain reaction. J. Food Sci. Technol. 50(1): 141-146. http://dx.doi.org/10.1007/s13197-011-0230-6
•Girish PS, Anjaneyulu ASR, Viswas KN, Anand M, Rajkumar N, Shivakumar BM (2004). Sequence analysis of mitochondrial 12S rRNA gene can identify meat species. Meat Sci. 66: 551–556. http://dx.doi.org/10.1016/S0309-1740(03)00158-X
•Girish PS, Anjaneyulu, ASR, Viswas KN, Haunsh S, Bhilegaonkar KN, Agarwal RK (2009). Poultry meat speciation by sequence analysis of mitochondrial 12S rRNAgene. Indian J. Anim. Sci. 79(2): 217-220.
•Guoli Z, Mingguang Z, Zhijiang Z, Hongsheng O, Qiang L (1999). Establishment and application of a polymerase chain reaction for theidentification of beef. Meat Sci. 51: 233–236. http://dx.doi.org/10.1016/S0309-1740(98)00116-8
•Ha JC, Jung WT, Nam YS, Moon TW (2006). PCR identification of ruminant tissue in raw and heat-treated meat meals. J. Food Protect. 69(9): 2241-2247.
•Hamelin M, Sayd T, Chambon C, Bouix J, Bibe B, Milenkovic D (2007). Differential expression of sarcoplasmic proteins in four heterogeneous ovineskeletal muscles. Proteomics. 7: 271–280. http://dx.doi.org/10.1002/pmic.200600309
•Haunshi S, Basumatary R, Girish, PS, Doley S, Bardoloi RK, Kumar A (2009). Identification of chicken, duck, pigeon and pig meat by species-specific markersof mitochondrial origin. Meat Sci. 83: 454-459. http://dx.doi.org/10.1016/j.meatsci.2009.06.026
•Herman BL (2001). Determination of the animal origin of raw food by-species-specific PCR. J. Dairy Res. 68: 429–436. http://dx.doi.org/10.1017/S0022029901004940
•Hird H, Goodier R, Hill M (2003). Rapid detection of chicken and turkey in heated meat products using the polymerase chain reaction followed by amplicon visualization with vistra green. Meat Sci. 65: 1117-1123. http://dx.doi.org/10.1016/S0309-1740(02)00341-8
•Hird H, Goodier R, Schneede K, Boltz C, Chisholm J, Lloyd J, Popping B (2004). Truncation of oligonucleotide primers confers specificity on real-time PCR assays for food authentication. Food Addit. Contam. 21: 1035-1040. http://dx.doi.org/10.1080/02652030400019398
•Hird H, Chisholm J, Brown J (2005). The detection of commercial duck species in food using a single probe-multiple species-specific primer real-time PCR assay. European Food Res. Technol. 221(3): 559–563. http://dx.doi.org/10.1007/s00217-005-1197-1
•Hollung K, Grove, H, Faergestad, EM, Sidhu, MS, Berg P (2009).Comparison ofmuscle proteome profiles in pure breeds of Norwegian Landrace and Duroc at threedifferent ages. Meat Sci. 81: 487–492. http://dx.doi.org/10.1016/j.meatsci.2008.10.003
•Hopwood AJ, Fairbrother KS, Lockley AK, Bardsley RG (1999). An actin gene-related polymerase chain reaction (PCR) test for identification of chicken in meatmixtures. Meat Sci. 53(4): 227-231. http://dx.doi.org/10.1016/S0309-1740(99)00060-1
•Hsieh YHP (2006). Meat species identification.In Y. H. Hui (Ed.). Handbook Food Sci. Technol. Engin. (Vol. 1, pp. 6). New York: CRC Press.
•Hsieh YHP, Sheu, SC, Bridgman RC (1998). Development of a monoclonalantibody specific to cooked mammalian meats. J. Food Protect. 61: 476–481.
•Huang MC, Horng YM, Huang HL, Sin YL, Chen MJ (2003).RAPD fingerprinting for the species identification of animals. Asian Austral. J. Anim. Sci. 16(10): 1406-1410. http://dx.doi.org/10.5713/ajas.2003.1406
•Jemmi T, Schlosser H (1991). Tierartbestimmungbeierhitzte FleischvoHaus-undWildwiederkäuernmittelsisoelektrischer Fokussierung. Fleischwirtschaft 71: 1191–1195.
•Jin LG, Cho JY, Seong KB, Park JY, Kong IS, Hong YK (2006). 18S rRNAgenesequences and random amplified polymorphic DNA used in discriminating Manchurian trout from other freshwater salmonids. Fish Sci. 72: 903-905. http://dx.doi.org/10.1111/j.1444-2906.2006.01234.x
•Jorfi R, Shuhaimi M, Yaakob BCM, Dzulkifly BMH, Awis QS, Abdoreza SF, Leila N, Pedram K (2012). Differentiation of pork from beef, chicken, mutton and chevon according to their primary amino acids content for halal authentication. African J. Biotechnol. 11(32): 8160-8166.
•Jung Wu Y, Hsieh CH, Chen HM, Hwang DF (2008). Identification of six common species of processed filefish using cutochrome b gene sequence and PCR-RFLP analysis. Rraffles B. Zool. 19: 151-158.
•Karabasanavar NS, Singh SP, Kumar D, Shebannavar SN (2014). Detection of pork adulteration by highly-specific PCR assay of mitochondrial D-loop. Food Chem. 15(145): 530-534. http://dx.doi.org/10.1016/j.foodchem.2013.08.084
•Karlsson AO, Holmlund G (2007). Identification of mammal species using species-specific DNA pyrosequencing. Forensic Sci. Int. 173(1): 16-20. http://dx.doi.org/10.1016/j.forsciint.2007.01.019
•Käuffer K, Beneke B, Bentler W (1990). Nachweis von zerkleinertemHühnerfleisch in nativen under hitzten Fleschmischungenmitderisoelektrischen Fokussierung in Polyacrylamidgelen. Fleischwirtsch. 70: 1188–1194.
•Kesmen Z, Gulluce A, Sahin F, Yetima H (2009). Identification of meat species by TaqMan-based real-time PCR assay. Meat Sci. 82 (2009): 444–449. http://dx.doi.org/10.1016/j.meatsci.2009.02.019
•Kim H, Shelef LA (1986). Characterization and identification of raw beef, pork, chicken and turkey meats by electrophoretic patterns of their sarcoplasmis proteins. J. Food Sci. 51: 731–741. http://dx.doi.org/10.1111/j.1365-2621.1986.tb13922.x
•Kim SH, Huang TS, Seymour TA,Wei C, Kempf SC, Bridgman CR, Clemens RA, An H (2004). Production of monoclonal antibody for the detection of meat and bone meal in animal feed. J. Agric. Food Chem. 52(25): 7580–7585. http://dx.doi.org/10.1021/jf048789a
•King NL (1984). Species identification of cooked meats by enzyme staining ofisoelectricfocussing gels. Meat Sci. 11: 59–64. http://dx.doi.org/10.1016/0309-1740(84)90017-2
•Kitano T, Umetsu K, Tian W, Osawa M (2007). Two universal primer sets for species identification among vertebrates. Int. J. Legal Med. 121(5): 423e427.

- Kitpipit T, Kuangtiwa S, Phuvadol T (2013). Are these food products fraudulent? Rapid and novel triplex-direct PCR assay for meat identification. Forensic Sci. Int. Gen. 4: e33–e34. http://dx.doi.org/10.1016/j.fsigss.2013.10.016
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX (1989). Dynamics of mitochondrial DNAevolution in animals: amplification and sequencing of conservedprimers. Proceedings of the National Academy Sciences of the United States of America. 86. http://dx.doi.org/10.1073/pnas.86.16.6196
- Koh MC, Lim CH, Chua SB, Chew ST, Phang STW (1998). Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci. 48 (3-4): 275-285. http://dx.doi.org/10.1016/S0309-1740(97)00104-6
- Koppel R, Zimmerli F, Breitenmoser A (2009). Heptaplex real-time PCR for theidentification and quantification of DNA from beef, pork, chicken, turkey, horsemeat, sheep (mutton) and goat. European Food Res. Technol. 230: 125–133. http://dx.doi.org/10.1007/s00217-009-1154-5
- La Neve F, Civera, T, Mucci, N, Bottero MT (2008). Authentication of meat fromgame and domestic species by Sna P shot mini sequencing analysis. Meat Sci. 80: 216–224. http://dx.doi.org/10.1016/j.meatsci.2007.11.027
- Lanzilao I, Burgalassi F, Fancell S, Settimelli M, Fani R (2005). Polymerase chain reaction-restriction fragment length polymorphism of mitochondrial cyt b gene from species of dairy interest. J. AOAC Int. 88: 128–135.
- Lee JC, Tasi LC, Liao SP, Linacrre A, Hsieh HM (2009). Species identification using the cytochrome b gene of commercial turtle shells. J. Forensic Sci. Int. Gen. 3: 67-73. http://dx.doi.org/10.1016/j.fsigen.2008.10.005
- Lenestra JA, Bunjet JB, Janssen FW (2001). On the origin of meat-DNA techniques for species identification in meat products. Vet. Sci Tomorrow. 2: 1-15.
- Li B, Bai SY, Xu YC, Zhang W, Ma JZ (2006). Identification of sika deer and red deer using partial cytochrome b and12S ribosomal RNA genes. J. Forest. Res. 17(2): 160-162. http://dx.doi.org/10.1007/s11676-006-0038-9
- Lindstrom DP (1999). Molecular species identification of newly hatched Hawaiian *amphidromousgobioid* larvae. Mar. Biotech. 1: 167–174. http://dx.doi.org/10.1007/PL00011764
- Lopez-Andreo M, Garrido-Pertierra A, Puyet A (2006). Evaluation of post-polymerase chain reaction melting temperature analysis for meat speciesidentification in mixed DNA samples. J. Agri. Food Chem. 54(21): 7973–7978. http://dx.doi.org/10.1021/jf0615045
- Mafra I, Ferreira I, Oliveira M. (2008). Food authentication by PCR-based methods. Euro. Food Res. Technol. A. 227(3): 649–665. http://dx.doi.org/10.1007/s00217-007-0782-x
- Mane BG, Mendiratta SK, Tiwari AK (2009). Polymerase chain reaction assay for identification of chicken in meat and meat products. Food Chem. 116: 806–810. http://dx.doi.org/10.1016/j.foodchem.2009.03.030
- Mane BG, Mendiratta SK, Tiwari AK (2009). Polymerase chain reaction assay for identification of chicken in meat and meat products. Food Chem. 116: 806–810. http://dx.doi.org/10.1016/j.foodchem.2009.03.030
- Martı´nez I, Yman IM (1998).Species identification in meat products by RAPD analysis. Food Res. Int. 31(6e7): 459-466.
- Martın I, Garcı T´, Fajardo V, Ine ´sLo ´pez-Calleja, Rojas M, Herna ´ndez PE, Gonza ´lez I, Martı´n R (2007). Mitochondrial markers for the detection of four duck species and the specific identification of Muscovy duck in meat mixtures using the polymerase chain reaction. Meat Sci. 76: 721–729. http://dx.doi.org/10.1016/j.meatsci.2007.02.013
- Martinez I, Daníelsdóttir AK (2000). Identification of marine mammal species in food products. J. Sci. Food Agri. 80: 527-533. http://dx.doi.org/10.1002/(SICI)1097-0010(200003)80:4<527::AID-JSFA565>3.0.CO;2-7
- Matsunaga T, Chikuni K, Tanabe R, Muroya S, Nakai H, Shibata K, Yamada J, Shinmura Y (1998). Determination of mitochondrial cytochrome b gene sequence for red deer (Cervuseleaphus) and differentiation of closely related deer meats. J. Meat Sci. 49 (4): 379-385. http://dx.doi.org/10.1016/S0309-1740(97)00145-9
- Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata, Yamada J, Shinmura Y (1999). A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Sci. 51: 143-148. http://dx.doi.org/10.1016/S0309-1740(98)00112-0
- Mazzeo MF, De Giulio B, Guerriero G, Ciarcia G, Malorni A, Russo GL, Siciliano RA (2008). Fish authentication by MALDI–TOF mass spectrometry. J. Agri. Food Chem. 56: 11071–11076. http://dx.doi.org/10.1021/jf8021783
- McDonagh MB, Ferguson KL, Bacic A, Gardner GE, Hegarty RS (2006).Variation in protein abundance profiles in theM. Semitendinosus of lambs bred fromsires selected on the basis of growth and muscling potential. Aust J. Agri. Res. 57: 671–682. http://dx.doi.org/10.1071/AR04277
- Meyer W, Seger H, Hulmann G (1995). Remarks on specific adaptive scale structure of the hair cuticle in some European bats. Euro. J. Morphol. 33: 509-513.
- Montiel-Sosa JF, Ruiz-Pesini E, Montoya J, Rocale e´s, P, Lo´pezPe´rez, MJ, Pe´rez-Martos, A (2000). Direct and highly speciesspecific detection of pork meat and fat in meat products by PCR amplification of mitochondrial DNA. J. Agri. Food Chem. 48(7): 2829-2832. http://dx.doi.org/10.1021/jf9907438
- Montowska M, Pospiech E (2013). Species-specific expression of various proteins in meat tissue: Proteomic analysis of raw and cooked meat and meat products made from beef, pork andselected poultry species. Food Chem. 136: 1461–1469. http://dx.doi.org/10.1016/j.foodchem.2012.09.072
- Montowska M, Pospiech E (2011a). Authenticity determination of meat and meat products on the protein and DNA basis. Food Rev. Int. 2784–27100.
- Montowska M, Pospiech E (2011b). Differences in two-dimensional geelectrophoresis patterns of skeletal muscle myosin light chain isoform between Bostaurus, Susscrofa and selected poultry species. J. Sci. Food Agri. 91: 2449–2456. http://dx.doi.org/10.1002/jsfa.4486
- Montowska M, Pospiech E (2012a). Is authentication of regional and traditionafood made of meat possible? Crit. Rev. Food Sci. 52: 475–487. http://dx.doi.org/10.1080/10408398.2010.501408
- Montowska M, Pospiech E (2012b). Myosin light chain isoforms retain theispecies-specific electrophoretic mobility after processing, which enabledifferentiation between six species: 2-DE analysis of minced meat and meaproducts made from beef, pork and poultry. Proteomics. 12: 2879–2889. http://dx.doi.org/10.1002/pmic.201200043
- Myers MJ, Yancy HF, Farrell DE (2003). Characterization of a polymerase chain reaction-based approach for the simultaneous detection of multiple animal-derived materials

in animal feed. J. Food Protein. 66: 1085–1089.
- Natonek-Wiśniewska M, Ząbek T, Słota E (2007).Species identification of mammalian mtDNA using PCR-RFLP. Ann. Anim. Sci. 7 (2): 305-311.
- Ortea I, Cañas B, Calo-Mata P, Barros-Velázquez J, Gallardo JM (2009). Argininekinase peptide mass fingerprinting as a proteomic approach for species identification and taxonomic analysis of commercially relevant shrimp species. J. Agri. Food Chem. 57(13): 5665–5672. http://dx.doi.org/10.1021/jf900520h
- Owusu-Apenten RK (2002).Speciation of meat proteins by enzyme-linked immunosorbent assay. Food Protein Analysis (pp. 247–280). New York: CRC Press.
- Parson W, Pegoraro K, Niederstatter H, Foger M, Steinlechner M (2000). Species identification by means of the cytochrome b gene. Int. J. Legal Med. 114: 23–28. http://dx.doi.org/10.1007/s004140000134
- Partis L, Croan D, Guo Z, Clark R, Coldham T, Murby J (2000). Evaluation of a DNA fingerprinting method for determining the species origin of meats. Meat Sci. 54: 369-376. http://dx.doi.org/10.1016/S0309-1740(99)00112-6
- 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: 306-312. http://dx.doi.org/10.1007/s00217-003-0846-5
- Peter C, Brunen-Nieweler C, Cammann K, Borchers T (2004). Differentiation of animal species in food by oligonucleotide microarray hybridization. Euro. Food Res. Technol. 21: 286–293. http://dx.doi.org/10.1007/s00217-004-0958-6
- Pfeiffer I, Burger J, Brenig B (2004). Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and deer by PCR-RFLP. BMC Genet. 5(30): 1-5.
- Prakash S, Patole MS, Ghumatkar SV, Nandode SK, Yogesh S, Shouche (2000). Mitochondrial 12S rRNA sequence analysisin wild life forensics. Curr. Sci. 78: 1239–1241.
- Quinterio J, Sotelo CG, Rehbein H, Pryde SE, Medina I, Pérez-Martín RI, Rey-Mendez M, Mackie IM (1998). Use of mtDNA direct polimerase chain reaction (PCR) sequencing andPCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. J. Agri. Food Chem. 46: 1662-1669. http://dx.doi.org/10.1021/jf970552+
- Rahman MM, Ali ME, Hamid SB, Mustafa S, Hashim U, Hanapi UK (2014). Polymerase chain reaction assay targeting cytochrome b gene for the detection of dog meat adulteration in meatball formulation. Meat Sci. 97(4): 404-409. http://dx.doi.org/10.1016/j.meatsci.2014.03.011
- Rajput N, Shrivastav AB, Parmar SNS, Ranjan R, Singh S, Joseph E (2013). Characterization of 12S rRNA gene for meat identification of common wild and domestic small herbivores as an aid to wildlife forensic. Vet. World. 6(5): 254-259. http://dx.doi.org/10.5455/vetworld.2013.254-259
- Ram JL, Ram ML, Baidoun FF (1996). Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. J. Agri. Food Chem. 44: 2460–2467. http://dx.doi.org/10.1021/jf950822t
- Rashid PMA, Babashekh MO, Marouf AS, Amin KM (2014). Identification of Animal Species in Meat Broth by Simplex and Multiplex PCR. J. Zankoy Sulaimani- Part A (JZS-A). 16(1): 97-102.
- Rastogi G, Dharne M, Bharde A, Pandav VS, Ghumatkar, SV, Krishnamurthy R, Patole MS, Shouche YS (2004). Species determination and authentication of meat samples bymitochondrial 12S rRNA gene sequence analysis and conformation-sensitive gelelectrophoresis. Curr. Sci. 87(9): 1278–1281.
- Rodriguez MA, García T, González I, Asensio L, Fernández A, Lobo E, Hernández PE, Martín R (2001). Identification of goose (Anser anser) and mule duck (Anasplatyrhynchos x Cairina moschata) foie gras by multiplex polymerase chain reaction amplification of the 5S RDNA gene. J. Agri. Food Chem. 49(6): 2717-2721. http://dx.doi.org/10.1021/jf0014765
- Rodriguez MA, Garcı ´a, T, Gonza ´ lez, I, Asensio L, Mayoral B, Lo ´pez-Calleja I, Hernández PE, Martín R (2003b). Identification of goose, mule duck,chicken, turkey, and swine in foiegras by species-specific polymerasechain reaction. J. Agr. Food Chem. 51: 1524–1529. http://dx.doi.org/10.1021/jf025784+
- Rodriguez MA, Garcı ´a, T, Gonza ´lez I, Asensio L, Herna ´ndez PE, Martı ´n R (2004). Quantitation of mule duck in goose foiegrasusingtaqman real-time polymerase chain reaction. J. Agri. Food Chem. 52: 1478–1483. http://dx.doi.org/10.1021/jf035240n
- Rodriguez MA, Garcı ´a, T, Gonza ´lez I, Asensio L, Mayoral B, Lo ´pez-Calleja I, Pablo E Hernández, Rosario M (2003a). Development of a polymerase chainreaction assay for species identification of goose and mule duck in foiegras products. Meat Sci. 65: 1257–1263. http://dx.doi.org/10.1016/S0309-1740(03)00033-0
- Rodriguez MA, García T, Gonzalez I, Hernández PE, Martín R (2005). TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures. Meat Sci. 70: 113-120. http://dx.doi.org/10.1016/j.meatsci.2004.12.005
- Rojas M, González I, García T, E Hernández P, Martín R (2012). Authentication of meat and commercial meat products from common pigeon (Columba livia) woodpigeon (Columba palumbus) and stock pigeon (Columbaoenas) using a TaqMan real-time PCR assay. Food Control. 23(2012): 369-376. http://dx.doi.org/10.1016/j.foodcont.2011.07.034
- Rojas M, Gonza´ lez I, Fajardos V, Martı´n I, Herna ´ndez PE, Garcı´a T (2009b). Authentication of meats from quail (Coturnixcoturnix), pheasant (Phasianuscolchicus), partridge (Alectorisspp), and guinea fowl (Numidameleagris) using polymerasechain reaction targeting specific sequences from the mitochondrial12S rRNA gene. Food Control. 20(10): 896-902. http://dx.doi.org/10.1016/j.foodcont.2008.12.011
- Rojas M, Gonza ´ lez I, Pavo ´n MA, Pegels N, Lago A, Herna ´ndez PE, Garcı´a T (2010b). Novel TaqMan real-time polymerase chain reaction assay for verifying the authenticityof meat and commercial meat products from game birds. Food Addit. Cont. 27(6): 749-763.
- Russel VJ, Hold GL, Pryde SE, Rehbein H, Quinteiro J, Rey-Mendez M, Sotelo CG, Perez-Martin RI, Santos AT, Rosa C (2000). Use of restriction fragment length polymorphismto distinguish between salmon species. J. Agri. Food Chem. 48: 2184-2188.
- Saez R, Sanz Y, Toldrá F (2004). PCR-based fingerprinting techniques for rapid detection ofanimal species in meat products. Meat Sci. 66: 659-665.
- Sawyer J, Wood C, Shanahan D, Gout S, McDowell D (2003). Real-time PCR for quantitative meat species testing. Food Control. 14(8): 579-e583.

- Scarpeid HJ, Kvaal K, Hildrum KI (1998). Identification of animal species in ground meat mixtures by multivariate analysis ofisoelectrofocusing protein profiles. Electrophoresis. 19: 3103–3109.
- Schwägele F, Stirtzel S, Andree S (2007). Authentication of domestic poultry species in meat and meat products. Proceedings of the 53rd International Congress on Meat Science and Technology, Beijing, China, Pp. 617-618.
- Sentandreu MA, Fraser PD, Halket J, Patel R, Bramley PM (2010). A proteomic-based approach for detection of chicken inmeatmixes. J. Proteome Res. 9: 3374–3383.
- Shibata M, Matsumoto K, Oe M, Ohnishi-Kameyama M, Ojima K, Nakajima S, Muroya S, Chikuni K (2009). Differential expression of the skeletal muscle proteome in grazedcattle. J. Anim. Sci. 87: 2700–2708.
- Spychaj A, Paul EM, Edward P (2009). PCR methods in meat species identification as a tool for the verification of regional and traditional meat products. Acta Sci. Pol. Technol. Aliment. 8(2): 5-20.
- Stamoulis O, Stamatis C, Sarafidou T, Mamuris Z (2010). Development and application of molecular markers for poultry meat identification in food chain. Food Control. 21: 1061-1065.
- Taylor AJ, Linforth R, Weir O, Hutton T, Green B (1993). Potential of electrospray mass spectrometry for meat pigment identification. Meat Sci. 33(1): 75–83.
- Théron L, Sayd T, Pinguet J, Chambon C, Robert N, Santé-Lhoutellier V (2011). Proteomic analysis of semimembranosus and biceps femorismuscles from Bayonne dry-cured ham. Meat Sci. 88: 82–90.
- Vallejo-Cordoba B, González-Córdova AF, Mazorra-Manzano MA, Rodríguez-Ramírez R (2005). Capillary electrophoresis for the analysis of meat authenticity. J. Sep. Sci. 28: 826–836.
- Venkatachalapathy RT, Sharma A, Sukla S, Hattacharya TK (2008). Cloning and characterization of DGAT1 gene of Riverinebuffalo. DNA Seq. 19(3): 177-184.
- Wolf C, Rentsch J, Hubner P (1999). PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification. J. Agri. Food Chem. 47:1350-1355.
- Wolko Ł, Witucka-Wall H, Siemieniako B, Wolko B, Słomski R (2004). Poszukiwaniemarkerówcechużytkowychmetodą RAPD-PCR [Searching of markers of applied traits using the RAPD-PCR method]. In: Przykładyanaliz DNA. Ed. R. Słomski. Wyd. AR Poznań, 185-191 [in Polish].
- Woolfe M, Primrose S (2004). Food forensics: Using DNA technology to combat misdescription and fraud. Trend. Biotechnol. 22: 222–226.
- Wu XB, Liu H, Jiang ZG (2006). Identification primers forsika deer (Cervusnippon) from a sequence-characterised amplified region (SCAR). New Zealand J. Zool. 33(1): 65-71.
- Xu YJ, Jin ML, Wang LJ, Zhang AD, Zuo B, Xu DQ, Ren ZQ, Lei MG, Mo XY, Li FE, Zheng R, Deng CY, Xiong YZ (2009). Differential proteome analysis of porcine skeletal muscles between Meishan and Large White. J. Anim. Sci. 87: 2519–2527.
- Zhang G F, Liu T, Wang Q, Chen L, Lei JD, Luo J, Maa G, Su Z (2009). Mass spectrometric detection of marker peptides in tryptic digests of gelatin: A new method to differentiate between bovine and porcine gelatine. Food Hydrocolloid. 23: 2001–2007. http://dx.doi.org/10.1016/j.foodhyd.2009.03.010
- Zhao M, Downey G, O'Donnell CP (2014). Detection of adulteration in fresh and frozen beef burger products by beef offal using mid-infrared ATR spectroscopy and multivariate data analysis. Meat Sci. 96(2): 1003-1011. http://dx.doi.org/10.1016/j.meatsci.2013.10.015