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Review Article

PCR Based Strategies to Identify and Differentiate Species Specific Meat

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ABSTRACT
Food authenticity and adulteration are the major issues where food industry and society is concerned. Due to various religious, economic and sanitary reasons specific and well founded techniques to distinguish the species of meat is required now a days as contamination of meat product with meat of cheaper or unwanted species is a common practice worldwide. Since last few years, researchers are trying to implement molecular genetic approaches for identification of meat species due to its advancements and sensitivity in addition to cost effectiveness and rapid. In the given review we will focus on PCR-based techniques for identification and differentiation of species specific meat.

KEYWORDS: Meat adulteration; molecular genetics; PCR; species specific meat

INTRODUCTION

Meat is a highly nutritious product liked by most of the consumers. The variety and quality of meat and its delicacy depends on the type of meat. According to the national and international regulations, in order to protect the integrity of food products, all the ingredients must be labeled and traceable [1]. Adulteration, unlisted ingredients and contamination in any food product, whether through deliberate actions or neglect, may breach both international regulations and religious laws [2]. Constraints are enforced by certain religions which forbid various meat species. Jews and Muslims don't like to eat pork and Hindus pass up from eating beef. Simultaneously vegetarians and vegans don't eat any type of meat and some people are allergic to unlisted ingredients and contaminations. Lard, made from pig fat is economical than butter and could be substituted for it in cakes and cookies [3].

Thus the authenticity of meat products and traceability of unidentified adulteration is always at stake and a major responsibility that concerns the consumers, industrialists and policy makers of all countries particularly in an ever expanding global meat market with increasing demand and inflation rates.

Identification of authenticity of meat to ensure desired quality of meat is very common practice in this time. Some other concern that has played main role in the process of identification are food safety issues, conservation of laws, consumer satisfaction, fair trade and sometime religious sentiments.

Due to the increased demand in the market of processed meat, there is an urgent need to incorporate some specific identification methods

for various feedstuffs. Strategies like molecular genetics, physical condition of meat are used for rapid examination of mixing of meat as adulteration is a very critical issue for health and ethical demands, specific food allergies, religious affairs, fraud and malicious marketing practices in addition to economic legal and economic concerns [4-5].

In addition to distinguish the meat credibility in processed meat is an critical issue in food regulatory authority for assurance of higher business esteemed meat species by second rate, less expensive or bothersome options of meat, the presence of undeclared species, and substitution of animal meat by plant proteins, accurate food labeling and for the assessments of food composition and giving purchaser required data to accomplish nourishment food safety.

Insurance of consumers and products from mislabeled meat items, false activities, and terrible practices of meat protection of consumers and producers from mislabeled meat products, fake actions, and poor practices of meat contaminations through processing and marketing and the blockage of illegal sale of conserved species have always been critical concerns that enforce legal authorities. Numerous researchers have propelled to create distinctive techniques and analytical methods for species identification of meat or their products including a wide range of degraded and prepared materials that were predominantly based on estimating either DNA or protein [6-7].

In past examinations the species-particular protein biomarkers have been recognized by scientists utilizing electrophoretic and chromatographic procedures [8], or enzyme-linked immunosorbent assay (ELISA) [9-11] and isoelectric focusing (IEF) [12-14]. These strategies have been recommended to determine proteins of skeletal muscle in their molecular weight or isoelectric point [15-18] and could be utilized for aligning of the skeletal muscle proteins of various animal species such as dairy cattle [19-23], poultry [24] and sheep [25].

The protein based strategies have been accounted for to be non-appropriate for species recognizable proof in heated meat products because of denaturation of protein by untensive warming during food processing which prompts to some alterations in the antigenic activity of molecules and their mobility after electrophoresis [26-28] and thusly, change the capacity of antibody to recognize its objective protein [29] and the possible cross-reaction between firmly related species [30].

Therefore protein-based techniques have been supplanted by DNA-based ones. DNA has the ability to be stable under high heating, pressures and chemical processing. It is conserved so the techniques depend on the identification of specific DNA segments sequence of a specific tissue or animal [31-35]. In the category of DNA based techniques, PCR is the most utilized, straightforward, efficient, sensitive and specific method that can identify the species of origin presented to various processing circumstances. [36-37].

In addition, the utilization of PCR in food examination has provided many type of analytical methods for quick detection and identification at species and intra-species level; however DNA-related approaches still face some significant limitations mainly for quantitative analysis of food contents [38]. To conquer these impediments considerations has been paid to the advancement of modern technologies that could be effectively utilized when quantitative assessments are required. The approach of proteomic innovation has turned out to be an extremely encouraging tool that relies upon examination of protein and peptide biomarkers. [39-43]

POLYMERASE CHAIN REACTION (PCR)-BASED TECHNIQUES

Since many years, PCR has been proved as the most promising method that has been used for identifying the species origin in meat, because of their high sensitivity and specificity, as well as expeditious processing time and low cost.

Thumber (2002) did meat speciation by molecular techniques in which meat sample from pig, buffalo, goat, sheep, cattle and chicken were used for molecular examination [44]. PCR has been approved as a potential procedure for meat speciation, consistent and effective tool for heat treated and purified meats and more informative than other techniques. The accompanying parameters are kept in mind while performing PCR. In recent time Researchers prepared a specific detection method for identification of pork adulteration in process meat products using pig specific mitochondrial D-loop target primers [45]

Primer Specificity

Primers specificity is the key to successful PCR reactions. Researchers are always trying to find new site for binding of the specific primers. Arbitrary oligonucleotide primers are used for the study of DNA fingerprinting of buffalo and cattle genome by polymerase chain reaction-random amplification of polymorphic DNA (PCR-RAPD) method [46]. Another a very useful finding produced in which PCR based assay was performed for determination of chicken in meat and meat products. In this experiment amplification of 442 bp DNA fragments from processed, freshed and autoclaved meat and meat products was performed by using primer based on mitochondrial D-loop gene. The detection limits in admixed meat and meat products were even less than 1%. [47] Very recently it was observed that mitochondrial gene targeting with common forward and specific reverse primer provides helpful information regarding meat quality detection [48]. A group of researchers detected 0.05 ng quantity of adulteration using specific primers for partial-length of cytochrome b (cyt b) gene of mitochondrial DNA (mtDNA) [49].

Physical condition of sample

A few analysts attempted heated and unheated samples of meat for PCR reactions [50]. Analysts created and assessed a PCR system to identify pork in heated and unheated meat, canned food, cured products, and pates using a DNA-specific procine repetitive element by nonspecific PCR amplification. Level of adulteration could be moderately evaluated by detecting up to 1 % pork contains in duck pate and 0.005% pork contain in beef [51].

In addition to the above findings a considerable work published by indicating a rapid identification of turkey and chicken in heated meat products using the PCR followed by amplicon visualization with vista green. DNA amplification in PCR was done by using species specific designed primers like, turkey forward (TF) and turkey reverse (TR) chicken forward (CF), chicken reverse (CR). The creation of an amplicon was detected after the end of the PCR in less than 5 min using vista green and a fluorescence plate reader. The presence of fluorescence denoted the presence of the target species in the sample [52].

Conserved Gene(s)

Gene specific identification strategies are especially successful in nowadays. Specific genes are targeted for identification of meat quality revealed that PCR amplification of the nuclear 5S rDNA gene could be used for the identification of mule duck and goose by Multiplex PCR using common forward primer and species-specific reverse primer [53]. The diverse sizes of the species-particular amplicon, isolated by agarose gel electrophoresis, allowed clear recognition of mule duck and goose samples. This hereditary marker was observed to be valuable for recognizing fake substitution of the mule duck liver for the more expensive goose liver.

A progression of trials led by for species identification and authentication of meat samples (beef, mutton, chevon, pork and chicken meat) by mitochondrial *12S rRNA* gene sequence analysis and gel electrophoresis [54]. By these methods, the authors could detect adulteration of meat at a level of 10% in heat treated products. Later on they utilized mitochondrial 16S rDNA gene, NADH dehydrogenase subunit 4 (ND4) genes and nuclear markers viz. the Actin gene, for authentication and identification of tissue of animal origin like chevon, mutton, buffalo meat etc. [55]. The outcomes proposed that mitochondrial markers were more effective than nuclear markers of the purpose of species identification and confirmation.

Mitochondrial genes are receiving much attention of researchers for PCR based identification of meat authenticity. Mitochondrial 12S r RNA gene PCR-RFLP to identify beef, chevon, mutton and buffalo meat [56]. PCR amplification yielded 456-bp fragments in every one of these animal varieties. This technique did not yield attractive outcomes with meat blends/meats. However, steady outcome were obtained with both processed and fresh meat samples. Another method was studied for meat speciation of buffalo, beef meat, mutton and chevon by PCR-RFLP method using mitochondrial 12S rRNA gene. Application of this technique on contaminated meat samples could identify meats of any two animal species in proportion of 50:50 and 75:25 except in case of chevon and beef mixture [57]. The strategy, nonetheless, couldn't distinguish any of the two

species when proportion of mixture was 90:10 except in case of buffalo and cattle.

Karabasanavar *et al.*, (2010) evaluated meat samples of two species of birds, namely parakeet (*Psittacula krameri*) and black kite (*Milvus migrans*) using PCR assay sequence analysis of mitochondrial *12S rRNA* gene. They found that PCR amplification of the mitochondrial 12S rRNA gene and sequence analysis was useful to tackle the issue of distinguishing proof of an avian species unambiguously [58].

Jain (2004) utilized *cyt b* gene variability in identifying meat of local market animals (buffalo, cattle, poultry, goat, sheep, horse and pig) by Multiplex PCR. Meat was cooked in microwave oven at 100° C and 120° C for 30 min [59]. The meat samples were allowable to rot in a characteristic condition at room temperature for 48 hours. PCR profiles of cyt b gene from fresh mat and putrefied meat was compared. Multiplex PCR, utilizing mitochondrial *cyt b* gene species specific primers, effectively gave amplification of DNA from rotted meat, demonstrating that putrefication did not hinder ability of amplification of cyt b gene region of various species in Multiplex PCR. Identification limits of mixed DNA templates were less than 1 ng. of single – nucleotide primer extension assay of mt DNA to authenticate buffalo and cattle meat [60]. The strategy was fast and dependable to distinguish and separate cattle and water buffalo meats focusing on the mitochondrial *cyt b* region using a Snapshot assay. Snapshot assay was found to identify 1% contamination in buffalo-cattle meat variety. Detection of contamination from degraded DNA obtained from putrefied and cooked samples was one of the very essential merits of this technique. The Snapshot gave an extremely delicate and specific assay to recognize and separate buffalo and cattle meat. This strategy was likewise fruitful with equal efficiency in fresh, cooked, and putrefied meat.

In some studies species of detected meat through PCR technique using actin gene as a marker. The species involved were cattle, sheep, pig, goat, buffalo and poultry [61]. Both cooked and raw meat was tested. The outcome showed clear cut identification of poultry and pig meat from the cattle, sheep, buffalo, and goat meat. But the approach failed to identify mutton from chevon and that of beef from buffalo meat.

Other PCR methods

Various modification of PCR techniques are fully utilized in the authenticity measurement of meat quality. An experiment based on Real time PCR was performed for identification and differentiation of meats of domestic animals particularly of cattle and buffalo meat [62]. DNA extractions were taken from meat samples of buffalo, cattle, goat, sheep, and chicken. It was achievable to identify and differentiate cattle meat mixed in buffalo meat in very minute amount (10-3 fraction), by running a duplex PCR followed by cattle specific Real time PCR.

RFLP Based Techniques

RFLF is the techniques which most attracted researchers for application due to its applicability. In the RFLP techniques different sequence of DNA amplified and digested with specific Restriction site for identification with suitable endonucleases. It is an easy and a very cost effective method which is gaining more acceptances now days. In recent time researchers have developed an advanced technique in which PCR method can be extend into further PCR-RFLP method for more specific detection of meat quality [63].

RAPD Based Techniques

Initiation in RAPD Methods based upon the application a specific single obituary primer for elongation. It has specific advantage over traditional methods like no need to sequence or DNA and also. Some previous studies have been done to identify buffalo meat [64].

Bar-coding

Barcodind is a very famous term in biological science as well as modern era of science and technology. This technology is also used in the PCR based detection techniques of various meat species. In this technique a specific sequence is selected and then PCR amplified for use as a barcode for matching of the various species samples. Many studies provide detail about barcoding techniques which is used most widely in these days to track down mislabeling and food piracy [65].

BY USING SPECIFIC DYE(S)

Researchers have construct and judged four assays based upon PCR amplification of short interspersed elements (SINEs) for species –

specific detection and quantization of bovine, chicken, porcine, and ruminant DNA [66]. Using SYBER Green (a nucleic acid dye) based detection, the minimum effective quantization levels were 0.01, 0.1,1 and 5 Pico gram of starting DNA template using chicken, porcine, bovine, and ruminant species – specific SINE – based PCR assays, respectively. Bovine, porcine DNA was detected at very low percentage (0.0005%) and chicken DNA at 0.05% in a 10 ng mixture of bovine, porcine, and chicken DNA templates. Previously it was classified a series of class specific (Aves), order – specific (Rodentia), and species – specific (equine, canine, rat, feline, guinea pig, hamster, and rabbit) PCR based assays for the quantification and identification of DNA using amplification of genome specific long and short interspersed elements [67]. Using SYBR Green (a nucleic acid dye) based detection, the minimum effective quantification levels of the assays ranged from 0.1 ng to 0.1 pg of starting DNA templates. The species-specificity of the PCR amplified fragments was further determined by the capability of the assay to exactly detect known quantities of species – specific DNA from mixed sources.

CONCLUSION

Techniques based on DNA, and especially those based on the DNA amplification, have special value for use in meat speciation. Although much of recent applications that have happened this zone have been basically directed to common domestic species, differential consumer inclination and globalization of meat industry have pushed the rise of molecular identification tools particularly designed for alternative meats like those from game animals. The PCR-based technologies discussed herein show sufficient advantages in terms of speed, reliability, specificity and sensitivity to find application on game meat authenticity testing. However, the feasibility of their use in the food industry is still today dependent on the bringing down of instrument and running costs. Considering the expanding number of research in recent times, it is likely that the potential of PCR-based methodologies for meat authentication will continue to grow as species coverage in the databases increases and, in time, perhaps these techniques can be adopted as complementary regulatory tools to allow identify and differentiate between meat species.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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