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Chapter · May 2018

DOI: 10.22271/ed.book10a01

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Chapter - 1

Methods for Detection of Adulteration in Meat

Abstract

Meat is defined as the dressed flesh, of certain animals, consumed as food. Most often this includes the skeletal muscles and associated fat and other tissues along with edible organs and offal. Meat and meat products have a great significance in human nutrition and thus for maintenance of consumer health. Meat is very rich source of proteins, containing all the essential amino acids Detection of species fraud in meat products is important for consumer protection and food industries. As meat and meat products represent an important and large component of human food, their quality is of concern to the consumers, the regulatory authorities, the processors and the retailers. The higher demand for meat and meat products accompanied by their escalating cost makes them prone to fraudulent adulteration, substitution and mislabelling. Adulteration literally means debasing something or rendering it impure by mixing it with some inferior or harmful substance. The determination of food authenticity and the detection of adulteration are major issues in the meat industry and are attracting increasing amount of attention. Identification of the species of origin in meat samples can be done using physical method, DNA based techniques and spectrophotometry method.

Keywords: Meat adulteration, species identity, DNA markers, physical method

Introduction

Meat is a primary source of protein, between 20% and 35%, along with fat and all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine and valine), as well as good amounts of various micronutrients (Aida et al. 2005). Fraudulent adulteration of costly meat with cheap meat is a common practice observed throughout the world. The ability to detect less desirable or objectionable species in meat products is important not only for economic, health, religious, and ethical reasons; but also to ensure fair trade and compliance with legislation (Spink, 2011). Authenticity testing of the animal species present in food is

important for economic, safety, legal, religious and health reasons. Product consumption containing non declared meat proteins can induce allergic reactions in predisposed individuals (Ong et al. 2007). Customers are gaining interest to know the origin of the foods because of quality related reasons, health and safety reasons and concerns about animal welfare. Due to some cost and religion concerns the meat producers have to comply with some rules issued by the legal institutions (Murphy et al., 2014). Hence, it is an important task for food control laboratories to be able to carry out species differentiation of raw materials to be used for industrial food preparation and the detection of animal species in food products (Luo et al. 2008).

Current available methods used to identify different species are electrophoresis, liquid chromatography, dot blot hybridization, randomly amplified polymorphic DNA PCR, RFLP analysis, and species-specific PCR (Aida et al., 2005; Syahariza et al., 2005; Che Man et al., 2007). However, molecular techniques are considered as reliable methods to identify meat types since there is a need to develop faster, cheaper and safe methods. Besides histological tests, immunoassays or DNA analysis, spectroscopic techniques are gaining interest due to being low cost and easily applied (Hashim et al., 2010; Lerma-Garcia et al., 2010; Zhang et al., 2012).

The power of the spectroscopy comes from giving chance to detect or quantify physical, chemical and biological attributes of the samples based on their spectral signature, and imaging transforms this information into chemical maps for spatial visualization. Thus, these methods can be used to determine what attributes, how much and where they are located in the sample (Kamruzzaman et al., 2013).

Physical Technique

In physical techniques, general appearance foe detection of different meat species is taken into consideration. It is a combined perception of colour, texture, odour and presence of other body parts along with meat. It gives the primary idea about the meat species on the basis of quality characterstics of meat and fat (Gracey et al., 1999; Sharma, 2008a, b, 2010; Singh, 1999) presented in the tables 1 and 2.

Table 1: Quality Characteristics of Meat of Different Species

Meat	Colour	Consistency	Odour	Marbling
Beef	Dark red with slight brownish tinge	Firm and cut surfaces are shiny	-	Present
Bufalo	Dark red	Firm	-	Absent or poorly present
Veal	Pale gray to graish red	Firm	-	Present
Chevon	Light red and paler than muton	Very firm	Goaty odour	Absent
Muton	Dark red	Firm and dense	Ammoniacal	Absent
Pork	Grayish white to light red	Vey soft	Urine like	Present
Poulty	White	Firm		Absent
Horse	Dark red with bluish tinge	Firm with prominent fascia		Absent
Camel	Red	Fairly firm		Absent
Dog	Dark red	Firm	Disagreeable and repulsive	Slightly Present
Rabbit	Pale, gray to gray red	Firm	Pronounced	Absent
Venison	Dark red to brownish red	-	-	Absent or very less

(Singh, 2008a, b, 2010, Sharma, 1999)

Table 2: Quality Characteristics of Fat of Different Species

Meat	Colour	Consistency	Fat type
Beef	Yellowish white	Firm	Intramuscular fat
Buffalo	Pure white	Slightly firm	No Intramuscular fat
Veal	Raddish yellow to white	Loose and greasy	No Intramuscular fat
Chevon	Pure white	Hard and firm	No Intramuscular fat
Muton	Pure white	Hard and firm	abundant
Pork	White	Soft and greasy	Subcutaneous fat
Poulty	Yellow	Loose	Mostly subcutaneous
Horse	In young- light gold to yellow Mature- white	Soft and greasy	No Intramuscular fat
Dog	White to whitish gray	Oily and greasy	Slightly Intramuscular fat
Rabbit	Whitish gray	Loose	Fat is almost in muscle and confined to body cavity

DNA Based Methods to Identify the Meat Types

Meat and meat products are very susceptible to spoilage and also expensive as compared to other food types. Therefore, their composition and quality has always been interested.

Among the techniques used for species identification, PCR is a DNA based technique allowing the detection of very low amounts of nucleic acid probes and the determination of their sequence via the amplification of DNA or RNA individual strains. This method has some advantages such as high sensitivity and rapid performance with high sample numbers. Mitochondrial

Cytochromeb gene and 12Srna can be used for species specific PCR technique.

Table 3: Different primers for different species for cytochrome b gene

Species	Primer	Length of amplified product
Bovis	F-5′GCCATATACTCTCCTTGGTGACA-3′	271 bp
	R: 5′-GTAGGCTTGGGAATAGTACGA-3′	
Sheep	F: 5′-ATGCTGTGGCTATTGTC-3′	274 bp
	R: 5′-CCTAGGCATTTGCTTAATTTTA-3′	
Pork	F:5′-ATGAAACATTGGAGTAGTCCTACTATTTACC-3′	149 bp
	R: 5′-CTACGAGGTCTGTTCCGATATAAGG-3′	
Chicken	F: 5′-GGGACACCCTCCCCCTTAATGACA-3′	266 bp
	R: 5′-GGAGGGCTGGAAGAAGGAGTG-3′	
Donkey and horse	F: 5′-TTCTGCTCTGGGTGTGCTACTT-3′ R: 5′-CTACTTCAGCCAGATCAGGC-3′	221 bp

(Luo *et al.,* 2008, Abdel-Rahman *et al.,* 2009).

A rapid TaqMan-based duplex real-time PCR method was performed by Kesmen et al. (2014). This method based on simultaneous amplification of fragments of the mitochondrial ND2 and ND5 genes and used for the identification and quantification of pork and donkey meats in raw/cooked donkey-beef and pork-beef mixtures. As a result of this study 0.001% of the meat species in raw and oven-cooked mixtures and 0.01% of the meat species in autoclaved meat mixtures could be detected successfully.

RAPD (**Random Amplified Polymorphic DNA**) is a very fast, easy to perform, and does not require expensive equipment method. In this method, PCR allow the examination of genomic variation without prior knowledge of DNA sequences (Huang *et al*., 2003). RAPD PCR can be used to identify the meat species and produce good fingerprints from processed products in which DNA has been only slightly degraded, such as smoked or salted products (Martinez and Yman, 1998). RAPD is a modified PCR technique in which DNA fragments are generated in a very short period of time which can be visualized in a gel electrophoresis. In this technique DNA extraction is the first step, then it is amplified with specific primer. It is followed by denaturation of strand at 94°C for 1min, the primer annealing at 47°C for 1min. Then extension of primer is done at 72°C for 1min. Electrophoresis (10µL portion of amplification) is carried out at at 100V in a 3% agarose gel containing ethidium bromide (1µg/ml) in TBE buffer. At last DNA

fragments are visualized by UV transillumination (Calvo eta al, 2001).

DNA Barcoding puts forward the use of a sequence from a single genomic region (defined as *barcode*), as the base of a recognition system capable to identify all animal species (Hebert *et al.,* 2003). This biomolecular methodology consists in the identification of the belonging species through the sequencing of a fragment of mitochondrial genes, its alignment and comparison with the information available on the databases. In particular, the barcoding approach comprises the analysis of a 655 bp region located at the 5' end of the subunit I of the mithocondrial cytochrome *C oxidase* (COI) gene. This gene codes for one part of the terminal enzyme of the mithocondrial respiratory chain. The COI gene has been chosen as a universal molecular target since it allows the design of universal, robust and functional primers for almost all the members of the animal Phyla (Folmer *et al.*,1994). The effectiveness of this fragment for the identification of species has been demonstrated for several animal species, from vertebrates to invertebrates (Waugh, 2007; Ward and Holmes, 2007).

Spectroscopic Methods, including Fourier Transform Infrared Spectroscopy (FT-IR) have been taken into consideration due to the need for new and rapid analytical methods in the field of food adulteration. These methods are generally based on transmittance or reflectance readings, and they require none or very little sample pre-treatment (Reis *et al*., 2013). Infrared (IR) spectroscopy as a well accepted analytical technique because it is environment-friendly and does not need complicated sample preparation procedure (Al-Jowder *et al*., 1997; Hashim *et al*., 2010; Liu *et al*., 2012; Liu *et al*., 2013).

Conclusion

There is a great concern by consumers for good quality products of which origin is clear as well. The legal authorities need to find reliable ways to analyze the products and control the manufacturers by laws. Both the control mechanisms of the government and the producers need simply, low-cost and applicable methods in order to sustain auto-control. To date DNA based methods have always been in demand due to being dependable and certain. However, these techniques are too costly and need qualified-staff and support. In routine analyses while spectroscopic techniques appear to be advantageous, in lab scale, DNA based methods might provide more reliable results.

References

1. Aida AA, Che Man YB, Wong CMVL, Raha AR and Son R, 2005.

Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat Science, 69:47–52.
2. Spink, J. and Moyer and D.C, 2011. Defining the public health threat of food fraud. J. Food Sci., 76: 157-163.
3. Luo J, Wang J, Bu D, Li D, Wang L, Wei H and Zhou L, 2008. Development and application of a PCR approach for detection of beef, sheep, pig, and chicken derived materials in feedstuff. Agr. Sci. China, 7(10):1260–1266.
4. Ong SB, Zuraini MI, Jurin WG, Cheah YK, Tunung R, Chai LC, Haryani Y, Ghazali FM and Son R, 2007. Meat molecular detection: sensitivity of polymerase chain reaction-restriction fragment length polymorphism in species differentiation of meat from animal origin. ASEAN Food J., 14(1):51–59.
5. Calvo J.H and Osta R 2001. Random amplified polymorphic DNA fingerprints for identification of species in poultry meat. Poultry Science, 80:522-524.
6. Murphy KJ, Parker B, Dyer KA, Davis CR, Coates AM, Buckley JD and Howe PRC, 2014. A comparison of regular consumption of fresh lean pork, beef and chicken on body composition: a randomized cross-over trial. Nutrients, 6: 682-696.
7. Kesmen Z, Yetiman AE, Sahin F and Yetim H, 2012. Detection of chicken and turkey meat in meat mixtures by using real-time PCR assays. J Food Sci, 77(2): 167-173.
8. Huang MC, Horng YM, Huang HL, Sin YLand Chen MJ, 2003. RAPD Fingerprinting for the Species Identification of Animals. Asian-Aust J Anim Sci, 16(10): 1406-1410.
9. Martinez I and Yman IM, 1998. Species identification in meat products by RAPD analysis. Food Res Int, 31 (6-7): 459-466.
10. Al-Jowder O, Kemsley EK and Wilson RH, 1997. Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study. Food Chem, 59(2): 195-201.
11. Liu J, Wen Y, Dong N, Lai C and Zhao G, 2013. Authentication of lotus root powder adulterated with potato starch and/or sweet potato starch using fourier transform mid infrared spectroscopy. Food Chem, 141: 3103-3109.
12. Reis N, Franca AS and Oliveira LS, 2013. Discrimination between roasted coffee, roasted corn and coffee husks by Diffuse Reflectance Infrared Fourier Transform Spectroscopy. LWT-Food Sci Technol, 50: 715-722.
13. Hebert PDN, Cywinska A, Ball SL and de Waard JR, 2003. Biological identifications through DNA barcodes. Philos T Roy Soc B, 270: 313-22.
14. Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase sub-unit I from diverse metazoan invertebrates. Mol Mar Biol Biotech, 3: 294-9.
15. Ward RD and Holmes BH, 2007. An analysis of nucleotide and amino acid variability in the barcode region of cytochrome c oxidase I (cox1) in fishes. Mol Ecol, 7: 899-907.
16. Waugh, J., 2007. DNA barcoding in animal species: progress, potential and pitfalls. Bioassays, 29:188-97.
17. Sharma B.D., 1999. Fraudulent substitution of meat and its recognition: Meat and Meat products technology. 1st ed., Jape brothers medical publishers, New Delhi, pp. 88-94.
18. Singh V. P, 2008b. Training Booklets for Internship Students on Field Oriented issue of Livestock product Technology. DUVASU, Mathura, pp. 20-23.