Speciation of meat

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Meat speciation and species identification.

The origin (species) of meat in a product.

Consumption of meat products containing mislabeled flesh not only causes **allergic reactions** in the sensitized individuals.

The religious sentiments of various communities. Muslims, Hindus, Christians and Jews have prejudices against specific type of meats.

Above all, the consumer stands cheated because he does not get the product for which he is paying

Adulteration in meat can be defined as “the fraudulent practice which involves substitution or mixing of flesh of cheaper variety which is objectionable for the reasons of health, religion and economics”.

This act of cheating is punishable under the Prevention of Food Adulteration (PFA) Act, 1973 now FSSAI 2006.

Thus, identification of species of origin of flesh is an important aspect in the Veterinary Forensic Science.

Methods for the identification of meats.

(1) Anatomical : Colour, texture, odour, structure of bones, flesh and fat.

(2) Physical : Refractive index of fat, iodine value.

(3) Chemical : Glycogen content, lenolenic acid content.

(4) Biochemical : Iso-electric focusing (IEF), SDS-PAGE technique.

(5) Immunological : Agar gel precipitation test (AGPT), Counterimmuno electrophoresis (CIEP), Agarose gel electrophoresis (AGE), Enzyme linked immunosorbant assay (ELISA), Peroxidase anti-peroxidase technique (PAP), Radioimmuno assay (RIA), Starch agarose electrophoresis.

(6) Novel methods : DNA analysis, DNA based techniques such as polymerase chain reaction (PCR) and gene probes.

(1) *Anatomical methods - Species of meat can be differentiated on the basis of anatomical* peculiarities :

(i) Colour, texture and odour of flesh - Buffalo meat is darker and coarse when compared to cattle meat. Chevon has typical goaty odour especially in males.

(ii) *Osseous tissue like bones or cartilage - Bones or cartilages when present along with the* meat can be used for species differentiation based on the anatomical pecularities of the specific bones. Poultry meat can be identified from other species owing to smaller and soft nature of the bones.

(iii) *Colour, consistency and distribution of fat - Cattle fat is yellowish in colour while*
buffalo fat is creamy white. Fat in pigs is present subcutaneously and in abundant quantity but is not intermixed with flesh, whereas cattle/buffalo fat is uniformly distributed with muscle tissues termed as marbling.

(**2**) ***Physical methods -*** *Meat speciation based on certain physical parameters is sometimes* followed in absence of specific anatomical differences.

(i) *Measurement of refractive index of fat - Fat is liquefied and refractive index of oil is* measured by refractometer. R.I. values of some animals are : horse 53.5, cattle not above 40 and pig not above 51.9.

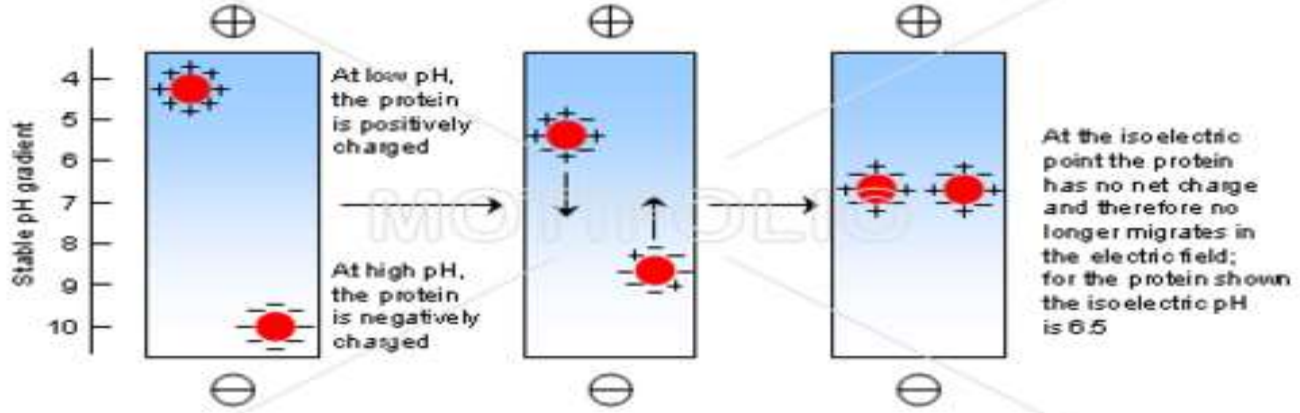
(ii) *Estimation of iodine value - This test measures the amount of iodine absorbed by* unsaturated fatty acids present in the fat and varies in different animals. Iodine value of fat in loin is 71 to 86, in cattle 38 to 46, in sheep 35 to 46 and in pig it is 50 to 70.

(3) Chemical methods - Chemical methods are used to detect the presence or absence of a
particular chemical constituent and/or its quantity in meat. The tests, however, have not
gained popularity since the chemicals are not species specific and cannot differentiate
between the chemical agents with similar reactivity. Besides, the methods are time consuming
and exhaustive. Some of the chemical methods used are -
(i) *Glycogen content - Horse flesh has higher glycogen content than other animals, but when*
horse is exhausted and glycogen is depleted it may lead to misinterpret of the results.
(ii) *Lenolenic acid - Horse fat contains about 1-2% lenolenic acid, in other animals it is not*
present in proportions higher than 0.1%.

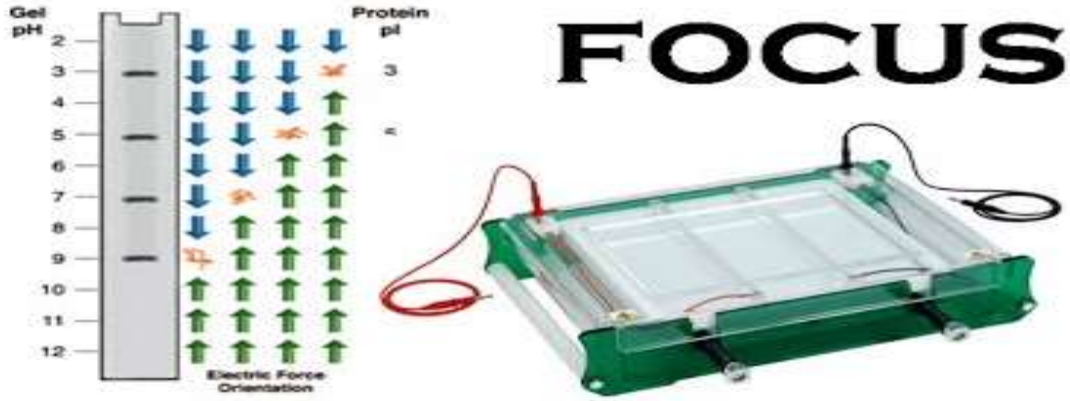
(4) **Biochemical methods** - *Electrophoresis of meat/ organ extract or any other biological* material results in separation of various proteins when migrate on the suitable medium under the influence of an electrical field. These separated protein fractions are species specific and thus help in identifying origin of biological materials.

(**i) *Iso-electric focusing (IEF)*** - *Proteins are low or high molecular weight compounds either* acidic or alkaline in reaction and negatively charged substances. Besides, they have a specific pH at which their overall electric charge becomes zero and they get precipitated. This precipitation point is termed as iso-electric point (PI). A suitable pH gradient is formed on the polyacrylamide or agarose gel with the help of amphoteric buffers (ampholytes) having specific pH range such as 3-9, 5-8 etc.

Separation of protein molecules by isoelectric focusing



ISOELECTRIC FOCUSING



When muscle proteins migrate on polyacrylamide or agarose gel having ampholyte bed under strong electric field, they precipitate at respective PI points forming a precipitation band on the gel. The precipitation band/bands of proteins are detected by staining and/or by densitometric scanning. The number of bands, their distribution pattern and isoelectric (PI) point at which they are precipitated are very specific to a species of animal which helps in identifying the species origin of meat even in mixtures. This technique can be used for identification of species of animal using many biological materials like blood, serum, milk, meat, organs, serum etc. The results specific, reliable and can be reproduced.

(**ii) *SDS-PAGE technique*** - *The overall charge on the proteins becomes zero when treated* with detergents like sodium dodecyl sulphate (SDS) due to denaturation. While migrating on suitable medium like polyacrylamide gel (PAGE), agarose gel etc. having a corresponding pore size such proteins precipitate on the basis of the molecular weights forming the characteristic bands. Staining and densitometric scanning of such bands provides an indication about the species of animal as the banding patterns of tissues from different animals vary. Identification of species is based on the molecular weight of proteins and not on the basis of their PI points as in IEF.

Isoelectric focussing and SDS-PAGE technique enable differentiation of raw and partially heated (cooked) meats. Simultaneous run of reference meat extracts along with sample in question is advocated since separation zones of different plates do not always coincide exactly. However, for differentiation of closely related species, the dissociation of bands is crucial and very slight differences in band intensity may lead to misinterpretation of results. Besides, it has been reported that the results vary from laboratory to laboratory, and person to person. In boiled or cooked meats, the bands produced are diffused, non reproducible and difficult to decifer. As such the technique is not of much use in differentiation of cooked/processed meats.

(5) ***Immunological/serological methods - On introduction in body, foreign proteins (antigen)***

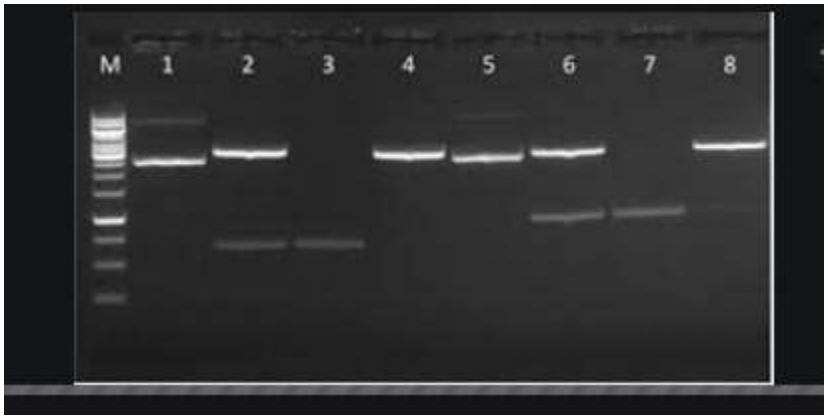
stimulate the host immune system to generate antibodies which can be demonstrated in its serum. In vitro combination of the antigen and antibody results in a variety of reactions such as flocculation, precipitation, agglutination, cytolysis and neutralisation. The response is very specific to the antigen or antibody types involved as the reactions occur only between homologous antigen and antibody.

The principle is employed for species identification of variety of biological materials such as milk, meat, organs, semen etc. In meat speciation, raw meat extract is commonly used as antigen for antibody formation in a suitable animal like rabbit or sheep. Though the antibodies can be used for species identification of raw meats, their greatest drawback is the occurrence of cross reactions with similar proteins which makes differentiation of phylogenetically related species (e.g. sheep and goat) difficult. Also, cooked meat is not identifiable. However, these limitations have been managed with the development of antibodies to heat stable antigens from adrenal glands.

Use of adrenal glands for antibody production has been advocated as they have two antigenic fractions, one adrenal specific and another present in other organs, blood and skeletal muscles. This widely distributed antigen is found to be species specific. Because of their resistance to boiling temperature and ethanol precipitability, these are widely known as BE antigens, wherein B stands for resistance to boiling temperature and E for ethanol precipitability

The antisera can be developed either in rabbits or sheep, and cross reacting antibodies can be removed by immunoabsorption to make them species specific. These monospecific antisera are useful for identification of tissues, organs, biological fluids (urine, milk, semen, etc.), semi-cooked and cooked meats, and processed meat products employing various immunological techniques viz. Ouchterlony's double gel diffusion technique, counterimmuno-electrophoresis and enzyme immunoassay.

(6)Novel methods - DNA based techniques such as gene probes and polymerase chain reaction (PCR) can be used for speciation of meat. Complimentary DNA or RNA strands can be used for the detection of DNA or RNA of suspected species by nucleic acid hybridization technique. A specific nucleic acid sequence can be exponentially amplified in-vitro in PCR resulting in large quantity of DNA sequence. Selected primers are used for amplification of searched genetic material and the amplicones can be detected by gel electrophoresis or detection methods (enzymatic label, antibody capture, etc.).



M: ladder\marker
Lane 1-8 different pattern from different meat extracts after PCR and Gelrun

Thankyou