FRAUDULENT SUBSTITUTION OF MEAT AND ITS RECOGNITION

- In the handling of meats and preparation of meat food products attempts are sometimes made to substitute meat of lesser quality for that of higher quality with the object of deceiving public and gain more profit.
- The differentiation of the muscle and fat of animals is of importance in connection with the possible substitution of inferior and at times repugnant meat for that of good quality.
- The substitutions that may be practiced are, that of horseflesh for beef, chevon for mutton, mutton for venison, beef for mutton and occasionally the flesh of the cat for that of hare or rabbit.
- It is not difficult to differentiate the flesh and fat of these animals in the carcass form or in joints by means of anatomical confirmation.
- But, the recognition of horseflesh or other meats in minced or in sausage form depends on tests of chemical or biological nature.
- Horseflesh possesses high contents of glycogen than that of other food animals.
- Glycogen usually begins to disappear from the meat after slaughter.
- So, the estimation of glycogen from meat has to be conducted immediately after slaughter and dressing of the food animal.
- The liver of animals, particularly the pig and flesh of fetuses contain appreciable quantities of glycogen.
- Thus there is every chance of mixing liver to indicate high levels of glycogen.
- Hence, the interpretation of the result should be made with extreme caution.
- The level of the mixing may very from 1 to 99% - adulteration.
- If it is 100% it is substitution.

- We can differentiate or recognize the various types of meat being substituted as mentioned above, by three methods. They are
    - Physical or physiological methods.
    - Chemical tests and
    - Biological tests
- These methods are further classified as follows:
    - Physical methods
        - This is based on general appearance, colour, texture, odour and tenderness of different species of meat.
        - Besides the general characteristics of body fat, its colour, marbling, firmness of fat can be identified.
        - Dentition formula, vertebral formula and articulation pattern, rib number and degree of curvature, characteristics of long bones will also help to identify the species if the carcass is intact.
    - Chemical Methods
        - Linoleic acid content
        - Iodine value
        - Refractive index of fat
        - Melting point of fat
        - Myoglobin content
    - Biological Methods
        - Electrophoretic method
        - Immunological method

- Latest techniques

PHYSICAL METHODS

- Physical methods like anatomical differences of each species of the carcass and appearance of muscle and fat colour, odour, texture and taste have provided a general difference between species in earlier days for food analysis.
- So, this can be attempted, provided the meats are in the form of joints and in carcass form.

Carcasses of different species of food animals

- Horse
  - Neck and the bones of limbs are longer than the ox.
  - Sternum of horse is canoe (Paadle of boat or walnut)shaped.
  - No diarthrodial joint between the first and second sternal ribs.
  - There are 18 pairs of ribs and are narrower than those of ox.
- Bull
  - The outstanding characteristic in the bull carcass is the massive development of the muscles of the neck and shoulder and also in the hindquarters of the well-bred animals.
  - Neck is much thicker than that of the ox.
  - *Ligamentum nuchae* is thicker and stronger than in ox.

- Anterior part of the ischio pubic symphysis is well developed and forms a distinct tubercle.
- Inguinal canals are patent.

- Ox
  - Shows lesser muscular development than that of bull especially in the neck and shoulder region.
  - There is even covering of fat on the exterior.
  - The scrotal fat is prominent, nodular and more or less pointed. Pelvis is narrow and usually contains a relatively large quantity of fat.
  - Fat is usually plentiful over the kidneys and along the sublumbar region.
- Cow
  - Thigh is less rounded than that of ox.
  - This is very noticeable in the hind quarters (sunken round).
  - The pelvis is broader. Anterior tubercular pelvis is broader.
  - Udder is present, if removed triangular area of attachment is noticeable on each side of midline of the abdominal wall.
  - In heifers the udder is only slightly developed and consists chiefly of fat.
  - In old cows the udder is soft, spongy, round and pendulous.
- Sheep
  - The carcass of sheep (whether or ewe) is characterised by an abundant and even distribution of subcutaneous fat.
  - The carcass of ram is distinguished by great muscular development in the region of neck and shoulders; the *ligamentum nuchae* is large and strong.

- The neck is thick and the inguinal canals are patent.

- Goat
  - Goats are long and lean.
  - There is very little subcutaneous fat, kidney fat abundant even in poor carcasses.
  - Subcutaneous connective tissue is sticky in nature and during skinning loose hairs from the skin become adherent to the subcutaneous tissue and cannot be removed completely.
  - Pelvis of goat is long and narrow.
- Hog
  - Carcass of pig cannot easily be mistaken for that of any other animal.
  - In most countries the skin is left on the carcass.
  - But even when the skin is removed there should be no difficulty in identification.

DIFFERENTIATION OF CARCASSES OF ANIMALS

Differentiation of carcasses of horse and ox

- Carcass of the horse and ox may be differentiated by the following details
  - In the horse the unusual length of the sides is noticeable, together with the great muscular development of the hindquarters.
  - The thoracic cavity is longer in the horse; this animal possesses 18 pairs of ribs, whereas the ox has 13 pairs.
  - The ribs in the horse are narrower but more markedly curved.
  - The superior spinous processes of the first six dorsal vertebrae are more markedly developed in the horse and are less inclined posterior.

- In the forequarter, the ulna of horse extends only half the length of the radius; in the ox it is extended and articulates with the carpus.
- In the hindquarter, the femur of the ox possesses no third trochanter; the fibula is only a small pointed projection, but in the horse it extends two–third the length of the tibia.
- In the horse the last three lumbar transverse processes articulate with each other, the sixth articulating in a similar manner with the sacrum.
- They do not articulate in the ox.
- The horse carcass shows considerable development of soft yellow fat beneath the peritoneum, especially in the gelding and mare, but in the stallion the fat is generally of a lighter colour and almost white. In the ox the kidney fat is always firmer, whiter and more abundant than in the horse.
- Horseflesh is a dark red, initially brown or reddish brown on exposure to atmosphere the colour turns bluish.
- Marbling is absent in horsemeat; it is firm but sticky in nature due to high glycogen content.
- Horse meat has a pronounced sweet taste, repulsive odour and well defined muscle fibre.
- Beef lack the bluish tinge.

Differentiation of carcasses of sheep and goat

Features	Sheep	Goat

Back and withers	Round and well fleshed	Sharp, little fleshed
Thorax	Barrel shaped	Flattened laterally
Tail	Fairly broad	Thin
Radius	1.25 times length of metacarpus	Twice as long as metacarpus
Scapula	Short an broad, superior spine, bent back and thickened	Possess distinct neck. Spine straight and narrow, lateral border thin and sharp
Sacrum	Lateral borders thickened in the form of rolls	Sharp
Flesh	Pale red and fine in texture	Dark red and coarse with goaty odour. Sticky subcutaneous tissue, which may have adherent goat hairs.

Sheep, Goat and Deer

- Among these carcasses, in deer, the scapula's acromion is elongated into a sharp point, which is directed ventrally.
- The acromion is absent in the sheep and goat or is considerably smaller.
- The radio-ulna arch, which forms an oval opening in the sheep and goat, is very long in deer.

- In deer, the subcutaneous layer of fat is not as well developed as in sheep.
- The meat is poor in fat and possesses the odour of venison, which is easily distinguishable from the odour of sheep.

Hog and Dog

- The colour of dog meat is very darker than pork and is easily made out in cooked meat.
- The muscles of the dog are searier and the fat is oilier than hog fat.
- The odour of the dog meat is repulsive.

Cat and Rabbit

- The meat of the cat is paler than rabbit meat.
- The fat of the cat appears whitish in contrast to rabbit fat, which is honey yellow.

Meat and Fat of Sheep and Dog

- The meat and fat of sheep and dog are indistinguishable by the naked eye and the carcasses of large dogs are sometimes substituted for mutton.
- The ribs and sternum of the sheep are broad and flat, while those of the dogs are round in section.
- In the hind leg, the sheep has only one bone, the tibia articulating with the tarsal joint, while the dog has both tibia and fibula.
- The sheep has triangular scapula with a broad, prolonging cartilage and the radius and ulna lies close together for their whole length, while the scapula of the dog has a semi-circular posterior upper edge with practically no prolonging cartilage and the radius and ulna are widely separated along the greater part of their shafts.

- The xiphoid cartilage in sheep is firm and grisly, while in the dog, it is softer and florous and shaped like a dagger.

Cattle and Buffalo

- Generally fresh buffalo meat is darker (more reddish brown) and the fibres are coarser and looser in structure than beef.
- The odour of the buffalo meat and fat are always strikingly musky and if boiled in strong acidified (H_2SO_4) water, it develops a disagreeable odour similar to that of cattle manure.
- The cutaneous shoulder muscle of buffalo is only 3 to 4 finger broad, while that of cattle it is considerably broader.
- The fat of buffalo is strikingly white and drier and less sticky than in cattle.
- The confirmation of the bones of the buffalo is generally thinner and the bones are very brittle.
- The ischio pubic symphysis of the buffalo is strikingly plane.

CHARACTERISTICS OF MEAT

Horse meat

- The meat of horse is dark red in colour, on exposure to air acquires a bluish tinge or shield on the surface and later become very dark.
- Odour is peculiar – sweet and to most people more or less repulsive. Horseflesh contains large quantities of glycogen (2%).

- Fat is yellow or brownish yellow in colour and owing to its high olein (ester of glycerol and oleic acid and or liquied portion of fat)content it is soft and greasy.

Beef

- The colour of beef varies from light red to dark red according to the age and the part of the carcass from which it was collected.
- The meat is moist, silky to the touch and is marbled with fat.
- Fat is fine usually creamy white or yellowish white in colour.
- In old cattle the fat tends to be more yellow and somewhat loosen in consistency.
- In Jersey and Guernsey fat is pronounced yellow colour.
- Meat of heifer closely resembles that of young ox.
- The meat of old cow is not marbled and tends to be lean, dry and somewhat coarse.
- However, the meat and fat of old dairy cows are often relatively moist.
- The veal is pale or grayish red in colour.
- Not very firm under pressure of fingers. Fibres are tough.

Mutton

- The meat of wether or ewe varies in colour from light red through brownish red to dark red.
- According to the age of the animal and to the part of the carcass – the fibres are fine, dense and firm..
- The fat is white, very firm and odourless.

Goat meat/Chevon

- Chevon is not marbled and bears a fairly close resemblance to that of sheep.

- The meat of uncastrated adult goat has a goaty odour.

Pig

- The meat of pig varies in colour according to the age and nutritive condition of the animal and also according to the body region from which it is derived.
- It may be pale red, reddish gray; rose red, dark red or in certain parts may be almost colourless.
- It is less firm to the touch than other food animals.
- The fibres are fine, fat is white, soft and greasy.

CHARACTERISTICS OF FAT

S. No	Species	Colour	Consistency
1.	Cow	Yellow	Fairly firm
2.	Bull; heifer	Yellowish white	Firm
3.	Calf	White or grayish white	Soft and gelatinous

4.	Buffalo	Strikingly white	Fairly firm
5.	Sheep ; Goat	Very white	Typically crispy in sheep. Very firm.
6.	Pig	Generously white	Fairly firm, greasy and not crispy
7.	Horse	Yellowish white	Soft and greasy

Table: Quality characteristics of meat of different animals

Meat	Colour	Consistency	Odour	Marbling
Beef	Dark red with slight brownish tinge	Firm and cut surfaces are shiny	-	Present
Buffalo meat	Dark red	Firm	-	Absent or poorly present
Veal	Pale grey to grayish red	Firm	-	Absent
Chevon	Light red and paler than mutton	Very firm	Goaty odour	Absent
Mutton	Dark red	Firm and dense	Ammonical	Absent to scanty
Pork	Grayish white to light red	Very soft	Urine like	Present
Poultry meat	White	Firm	-	Absent
Horse meat	Dark red with bluish tinge	Firm with prominent fascia	-	Absent
Camel meat	Red	Fairly firm	-	Absent
Dog meat	Dark red	Firm	Disagreeable and repulsive	Slightly present
Rabbit meat	Pale, grey to grey red	Firm	Pronounced	Absent
Venison	Dark red to brownish red	-	-	Absent or very less

Table: Quality characteristics of fat of different animal species

Fat	Colour	Consistency	Fat type	Bone marrow characteristics	Remark
Beef	Yellowish white	Firm	Intramuscular fat	Pure white to reddish yellow	-
Buffalo fat	Pure white	Slightly firm	No Intramuscular fat	-	-
Veal	Reddish yellow to white	Loose and greasy	No Intramuscular fat	Pink red	-
Chevon	Pure white	Hard , firm and brittle	No intermuscular fat	Firm and slightly red	-
Mutton	Pure white	Hard , firm and brittle	Abundant intermuscular fat	Firm and slightly red	-
Pork	White	Soft and greasy	Subcutaneous but	Pink red and soft intramuscular also	On boiling it turns to whitish grey
Poultry fat	Yellow	Loose	Mostly subcutaneous	-	-
Horse fat	In young-light gold to yellow In mature -white	Soft and greasy	No intramuscular fat	Waxy, yellow, greasy and soft	On exposure to air turns to blackish
Dog fat	White to whitish grey	Oily and greasy	Slight intramuscular	-	-
Rabbit fat	Whitish yellow	Loose	Fat is absent in muscle and confined to body cavity	-	-

Histological techniques: In this technique we generally measured muscle fiberlength, diameter, density and pattern of the muscle fibers in different meats of animal origin. Frequently encountered case of cow and buffalo meat mixing and illegal slaughtering may be identified by the histological techniques.

Table : Differentiations of Beef and Buffalo meat on the basis of histological parameters

Characteristics	Beef	Buffa
Muscle fiber diameter	Larger	Small
Number of muscle fibers per mm^2	Less	More
Muscle striation	Less angular	More

CHEMICAL METHODS

- The chemical tests consist of the determination of
  - the content of glycogen in flesh
  - the percentage of linoleic acid in fat
  - the melting point of fat
  - the amount of iodine absorbed by unsaturated fatty acids in fat and
  - the refractive index of fat.

Test for Glycogen Content of Meat

- The horseflesh is richer than the flesh of other food animals in glycogen in horsemeat as compared with other kinds of meat, glycogen is found in large quantities irrespective of age.
  - Horse – 0.5 to 1.0 %
  - Beef - 0.0 to 0.5%
  - Pork and mutton - nil
- Disadvantages
  - The flesh should be tested for the content of glycogen soon after the slaughter as it disappears from the flesh quickly.
  - Liver of all food animals especially pig liver contains more glycogen when they are used in sausage making it gives a high percentage. So care must be taken in interpretation of results.

Linoleic acid content

- Horse fat contains 1-2% linoleic acid. Linoleic acid content in other animals' fat is not more than 0.1%.
- Thus adulteration of lard or beef and mutton fat with horse fat can be identified by estimation of the linoleic acid concentration.

Iodine value

- Estimation of iodine value is a valuable test for the detection of horse fat.
- Iodine value is the amount of iodine absorbed by the unsaturated fatty acid present in the fat.
- Good lard has an iodine value of 66.
- The iodine value of the fat from various food animals is:
  - Horse - 71-86
  - Ox (cattle) - 38-46
  - Sheep - 35-46
  - Pig - 50-70

Refractive index

- Refractive index is another valuable test for the detection of fat of different animal species.
- Fat is liquefied by heat and converted into oil for estimation of refractive index.
- All liquids including oils possess a specific refractive index.
  - Horse - 53.5
  - Ox - less than 40
  - Pig - not above 51.9

Melting Point

- The melting point of fat varies with the species of food animals and the kind of feed fed to the animal.
- The range of melting points of fat is

Myoglobin content

- The myoglobin content of different species is:
  - Beef - 0.30 to 1%
  - Pork - 0.06 to 0.40%
  - Poultry - 0.02 to 0.18%

IMMUNOLOGICAL METHODS

- Immunological methods include
  - Tube precipitin test
  - Haemagglutination test (HAT)
  - Complement fixation test (CFT)
  - Agar gel immuno diffusion test (AGID)
  - Dry disc immuno diffusion test
  - Enzyme linked immuno sorbent assay (ELISA)

Agar Gel Immuno Diffusion Test (AGID)

- Based on a simple double diffusion method (Ouchterlony, 1948). Species specific antiserum (antibody) and unidentified meat extract (antigen) are allowed to diffuse towards one another in an agar gel slab. If the antigen and antibody are homologous a precipitin band is formed along the line where the two meet.
- .
- The colour intensity is measured objectively at a specific wave length in a micro ELISA reader as absorbance value.

- Agar gel electrophoresis
- Starch gel electrophoresis
- Polyacrylamide gel electrophoresis
- Sodium dodecyl sulfate polyacrylamide gel electrophoresis
- Iso-electric focussing

- *Agar gel electrophoresis*
  - Electrophoretic separation of proteins on the basis of mobility of proteins in a supporting gel of agarose at a constant pH and electrical field is termed as Agar gel electrophoresis.
- *Counter Immuno Electrophoresis (CIE)*
  - The principle of this technique is very similar to agar gel immune diffusion.
  - The diffusion of antigen and antibody is facilitated by the application of a low voltage current. Further, the endosmosis created by the agar gel changes the charge of g -globulins and moves them towards cathode.
  - The meat proteins moving towards anode provide immune precipitate with homologous g -globulin at the point of equivalence.
- *Polyacrylamide gel electrophoresis (PAGE)*
  - Polyacrylamide gels are prepared using acrylamide and bis which provides cross linking between the polymerized long chains of acrylamide.
  - The separation of proteins on the basis of their mobility in a polyacrylamide gel is comparatively better since the resolution of different proteins are optimum. Polyacrylamide gel electrophoresis also requires a constant pH and electrical field to provide high resolution of protein.

- *Iso-electric focussing (IEF)*
    - It is an electrophoretic technique which utilizes the charge at the surface of the protein to drive it through a gradient gel.
    - The pH gradient is setup by polyacrylamide compounds, the ampholytes.
    - The proteins applied on to the gel reach a point where the surface charges becomes neutral at their iso electric point
-

Sodium Dodecyl Sulphate PAGE (SDS-PAGE): SDS-PAGE is a variant of PAGE is commonly used for separating protein subunits and determining their molecular weights. On heating, polypeptides dissociate and when these polypeptides bind with SDS in the presence of reducing agent 2-mercaptoethanol forms SDS-polypeptides. When this complex is subjected to a sieving polyacrylamide gel, migrate according to the molecular weights of the polypeptides. PAGE-SDS (pH 3-10) can be utilized for identification of beef, mutton, venison, rabbit meat and raw and cooked crustaceans. It is a suitable method for heated meat samples up to 100°C beyond which most of the protein bands disappears. This is a good technique for closely related meat species and has good resolution and reproducibility

RECENT TECHNIQUES IN MEAT SOECIES IDENTIFICATION

- Recent techniques in meat species identification include
    - Production of monoclonal antibodies and application of ELISA.
    - Use of DNA probes and application of DNA hybridization technique.
    - Use of Polymerase Chain Reaction (PCR) in species identification

Enzyme-linked immunosorbent assay (ELISA): It is a rapid, highly sensitive(able to detect 2% adulteration) and most suitable method for handling numerous samples at a time. There are several ELISA techniques are in use depending on the compound fixed, solid support used and concentrations of antigen and antibodies used such as Indirect ELISA, Competitive ELISA and Sandwich ELISA (Patterson and Spencer, 1985). In Indirect ELISA, species is detected by the antisera that are subsequently used and labeled with suitable conjugate. While, in Competitive ELISA a fixed amount of antigen antibodies are mixed with the meat extract and preincubated. These techniques are mainly based on the polyclonal antibodies against muscle or serum protein. These polyclonal antibodies are limited in production, have heterogeneous affinity and needs purification to avoid cross reactions. So, the use of monoclonal antibodies is most common in ELISA test because they are specific for a single antigenic site. These monoclonal antibodies can be produced from thermostable proteins of different species or by the hybridoma cell lines. It is a rapid test and results may be obtained within 2-3 h. By this technique we can detect closely related species with the capability of testing numerous samples at a time. This test is also able to detect pressure cooked meat at 133°C for 20 min.

The technique involves the application of species specific antibodies to the proteins (antigen) coated on the plastic surface of micro-titre plate.

The recognition of antigen results in the formation of antigen-antibody complex, which are detected by either enzyme linked immunoglobulin or protein A (antibody detector) producing visible colour reaction with added substrate

DNA hybridization technique: It is a qualitative or semi-quantitative technique of meat species speciation in which species-specific DNA sequence is detected. For this purpose probes prepared from DNA or cloned DNA are hybridized with target DNA and detected by colour development or audiography. During the early development of DNA sequence analysis, genomic DNA was used as a species specific probe and was hybridized to DNA extracted from meat samples. The subsequent development of probes derived from species-specific satellite repetitive DNA sequences has greatly improved the specificity of the assay, now making it possible to detect admixtures that contribute as little as 5% or less to a product (Wintero *et al.,* 1990). An alternative DNA detection system is based on the Polymerase Chain Reaction (PCR) amplification of a segment of the mitochondrial cytochrome b gene. Subsequent cleavage by a restriction enzyme gives rise to a species-specific pattern on an agarose gel. This method does not require the development of species-specific probes and, because it is PCR-based, is most suitable for critical samples in which DNA is largely degraded. This is a good technique for detection of adulteration as low as 0.1%.

Polymerase Chain Reaction (PCR): PCR is a rapid method because in this technique we can obtain multiple copies of specific piece of DNA sequence *in vitro* and it has high degree of selectivity and sensitivity. PCR amplifies a target DNA sequence in an exponential phase, being capable of detecting even a single copy sequence from a single cell sample. It is a qualitative test for meat species specification. There are two main techniques for amplification of genetic marker, i.e., mono-locus-specific primers for

amplification of a concrete DNA fragment and multi-locus amplification of non-targeted DNA. By this technique closely related meat species can be identified with the discrimination between male and female raw meat.