Literature Review
CHAPTER-2
LITERATURE REVIEW

This chapter reports the review of available literature related to topic of investigation.

2.1 Meat.

Meat itself is not a living organism but it is subject to endogenic enzymatic activity, or proteolysis, which causes muscles tissue to become mature, tender and develop a typical taste.

Muscles, regardless of shape or functional properties, contain a continuum of connective tissues consisting predominantly of the fibrillar collagens which encompass and invest cellular and extra cellular elements. Collagen represents about 2-6% or more of the dry weight of muscle, depending on muscle type and animal’s age. A heavy sheath of connective tissue called the epimysium surrounds entire muscles. Myofibres are arranged longitudinally into bundles of fibres or fasciculi, which are enveloped, in thin sheets, or trabecula of connective tissue known as perimysium. The organelles of muscle cells are bathed in a cytosolic fluid known as the sarcoplasm. The sarcoplasm is a protein-rich, aqueous solution that occurs primarily in the interfilament spaces within each myofibril (Bailey and Light, 1989).

2.2 Pre-rigor Conditions in Meat.

The myofiber is unusual in that it contains many nuclei and has an elaborate cytoskeletal apparatus for contraction. It contains many of the cell components found in other tissue cell types and, in general, these organelles perform the same functions as they do in other cell types. Mitochondria utilize oxygen and other substrates to produce
energy in the form of adenosine triphosphate (ATP). When ATP splits into adenosine diphosphate (ADP) and inorganic phosphate (Pi), energy is released and is harnessed for contractile activity.

In the living state, ATP for muscle function may be obtained by aerobic or anaerobic metabolism. Aerobic metabolism includes the process of glycolysis and respiration (Greaser, 1986). In the myofibre, glucose is stored as the polysaccharide, glycogen (Schutt and Lindberg, 1993). Creatine phosphate (CP) is a chemical compound which serves as a storage for the high energy phosphate necessary to regenerate ATP from ADP. Creatine phosphate availability is especially crucial during times of high energy demand by the muscle. Creatine Kinase is an enzyme that catalyzes a reversible reaction whereby ATP may be regenerated from ADP utilizing CP (Hultin, 1985).

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ADP + CP \rightarrow ATP + \text{Creatine Phosphate}
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At death, the animal's ability to obtain and deliver oxygen to tissues is lost, but some aerobic metabolism can still occur at carcass tissue surface where oxygen is readily available. Although the capacity to produce ATP through aerobic processes in deeper location within the tissue has been compromised, all biochemical components necessary for an aerobic metabolism in cells are present and functional, and glycolysis proceeds. As a result, changes occur in the concentrations of glycolytic substrates and reaction products until a point is reached at which some reaction in the glycolytic process is arrested and metabolism ceases. When this occurs, the ability to product ATP is completely lost and rigor mortis is established (Newbold, 1966).

### 2.3 Postmortem changes.

During the post mortem pre-rigor process, several changes occur in the concentrations of glycolytic substrates and products. The
changes in concentration of H⁺ (pH), ATP, CP and extensibility from the point of death until the onset of rigor mortis (Ockerman, 1980).

*Rigor mortis* is a temporal process occurring during the time course of post mortem glycolysis and is characterized by progressive stiffening of the muscle (Greaser, 1986). The rate of post mortem glycolysis and the extend to which it occurs have implications for muscle food quality. As anaerobic glycolysis proceeds from the point of slaughter to rigor mortis, changes occur in the muscle. The production of H⁺ leads to more acidic condition i.e. decreased pH of muscle tissue (Love and Pearson, 1974). Degradation of specific myofibrillar proteins has been followed by many researchers. Hopkins and Thompson (2002) reported that protein such as titin, nebulin, troponin-T, desmin, filamin and vinculin are degraded at different rates during post-mortem storage of meat.

### 2.4 PSE and DFD.

Animals, which are exposed to stressful condition prior to slaughter, may produce meat that is pale, soft and exudative (PSE), or dark, firm and dry (DFD). In PSE condition, muscles are quite soft and tend to sag, meat surfaces are watery and light coloured. When cooked, PSE meat is very dry and unpalatable. Its utility for cooked / processed meats manufacture is limited due to poor water holding capacity. Research indicates that the loss of protein functionality in PSE is responsible for the inferior quality of PSE meat (Boles *et al*, 1992).

In PSE meat, an abnormally high rate of anaerobic glycolysis starts just after slaughter. The rapid biochemical reactions produce heat so quickly that muscle temperature increase immediately, may exceed the normal physiological level. The elevated glycolytic rate also means the muscle pH decrease rapidly (Abu Baker *et al*, 1982).
The dark, firm and dry (DFD) conditions in a meat quality are a defect. In this DFD, meat appears darker than normal appearance. Meat surfaces appear dry and the pH of the meat is higher than normal. The pH range of normal meat is 5.3–5.8. The pH of DFD meat generally exceeds 6.0–6.2 and may reach as high as 6.8 (Acton and Saffle, 1969). DFD meat is obtained from such animals which have been exposed to long term pre-slaughter stress. However, PSE meat has tremendously reduced palatability of meat due to higher moisture losses during cooking (Koohmaraie et al., 1984).

2.5 Biochemical Changes.

Various biochemical processes occur after rigor-mortis and some of these have significant implications for the quality of muscle as food. One of them is aging. Meat is aged to improve tenderness. Aging is necessary as meat is often unacceptably tough immediately following rigor onset. In Aging process carcasses are kept at 4°C (Perkin and Tauber, 1984). This has been found to be very effective in improving meat tenderness. During aging process, tenderization occurs as a result of protein degradation or proteolysis (Bonton et al., 1978).

2.6 Sensory Characteristics of meat.

People eat foods they like. The food may include muscle foods also. Sensory properties impact on these liking (Booth, 1990). A broad spectrum of sensory input, including appearance, aroma, flavour and texture is used by consumers to make purchasing and eating decisions related to muscle foods. In 1990, Cohen described the important sensory qualities of meat as flavour, colour, texture, nutritive value and wholesomeness.

For meat selection in a retail supermarket, consumers generally evaluate the sensory properties in terms of colour, surface characteristics, size and shape (Rhodes, 1979). The effect of meat colour
on acceptance of red meats is well established (Jeremiah et al., 1972). Size and shape also play roles in selection and purchase of muscle food. During storage, consumers continue to use visual characteristics as cues to quality and acceptance. Colour loss, especially the loss of red colour in meat, is perceived negatively by consumers, hence the practice of packaging meat in wraps that preserve the fresh colour.

2.6.1 Colour.

The red colour meat is due to the presence of heme protein, myoglobin. The degree of meat pigmentation is directly related to myoglobin content, (Elkoussy et al., 1977). Meat colour is an extremely important sensory characteristic by which consumers make judgements of meat quality. Meat colour determines the shelf-life of meat (Hamm, 1981). When a freshly cut piece of meat is exposed to air, both the reactions, oxygenation and oxidation take place. At a surface, where oxygen is freely available oxymyoglobin is formed and where oxygen has not penetrated, the pigment remains as purple, reduced to myoglobin. Oxygenation of myoglobin is rapid, on the other hand oxidation to metmyoglobin is slow. The importance of colour as marketing attribute of meat is well established, especially for retailing.

2.6.2 Flavour.

Flavour is complex sensation involving gustatory (taste) and olfactory (smell) stimulations (El-kady and Fahmy, 1984). It is one of the most important palatability attributes of food. The raw meat has a weak, salty and serum like flavour while the true meaty flavour develops during cooking. The lipids in meat contributes to flavour. The formation of lipid breakdown products leads to development of undesirable flavours and odours (Foad and Yourself, 1973).

2.6.3 Warmed over Flavour (WOF).
Meat is an excellent source of iron and this is a nutritional benefit. The iron can also serve to enhance lipid oxidation in meats. Cooking results in off-flavour known as warmed over flavour (WOF). WOF is a flavour defect that occurs in reheated meat products. This occurs in cooked meat that is stored under refrigerated conditions, the warm temperatures of the reheating process may also accelerate lipid oxidation. The outcome is that rancid flavour development, resulting in WOF (Wilding et al., 1986).

2.7. Textural Properties of meat.

Texture is the most important palatability factor that affects the acceptability of meat. The amount of connective tissue has a profound effect on the texture of meat (Tabilo et al., 1999). Texture, especially tenderness and juiciness, has a substantial effect on acceptability of intact cuts of meat. It has long been believed that tenderness is the most important attribute, because if meat is tough, other sensory properties become less important. Diamant et al., (1976) found that tenderness was the most important quality characteristics for cooked pork. Bouton and Harris (1972) indicates that, as tenderness increased, overall acceptability increased. Juiciness is most often discussed as essential in muscle foods. Juicy and moist were terms often mentioned by consumers in focus groups to describe an ideal piece of meat (Chamber et al., 1992 ‘a’). Texture characteristics such as flakiness are important in fish, especially for those people who do not like fish (Poste et al., 1988), but obviously would not be important in many other muscle foods. Focus groups (Chamber et al., 1992 ‘a’) indicated that consumers use their perceptions of meat to determine sensory acceptance, safety, healthfulness, quality and value. Appearance characteristics of both raw and cooked meat impact consumer’s perceptions of safety.
Texture characteristics of foods constitute one of the main sensory attributes perceived by consumers. Instrumental texture analysis is a good tool to assess texture of foods. The knowledge of physical sense and the correct calculation of the parameters allow a good interception of results (Pons and Fiszman, 1996). Proteolysis of muscle structural proteins is the main biochemical mechanism responsible for the progressive meat tenderization during dry-curing. The enzymes mainly involved in this process are proteinases (cathepsins, B, D, H and L and to a less extent calpains) (Toldra et al., 1993) and exopeptidases (peptidases and amino peptidases) (Toldra and Flores, 1998). Sensory characteristics of dry-cured ham are strongly affected by these enzymatic reactions although, its activity level depends on the properties of raw meat such as age, cross breeding, processing conditions (temperature, time and water activity) and curing agents (mainly salt) (Toldra et al., 1997, Toldra & Flores, 1998). Tenderness is an appreciated and desirable characteristic but an excessive degree of proteolysis can result in lower meat texture that can affect consumer acceptability.

Biochemical composition of muscle is an important factor that affects meat quality. Studies have revealed that the collagen content alone cannot be considered a reliable parameter for textural properties. The two collagen interacting proteoglycans, decorin and fibromodulin, in two muscles differing in texture, M. semitendinosus (ST) and M. psoas major (PM). The difference in texture was confirmed by Warner Bratzler shear force measurements. The proteoglycan expression level was determined both on protein and mRNA levels by SDS-gel electrophoresis and northern blots, and related to the collagen expression. The tougher muscle, ST contained more decorin than the tender muscle PM, but less decorin per collagen. However, the
difference in fibromodulin level per collagen was not significant between the two muscles, indicating that decorin is a better parameter to study in relation to textural properties in bovine muscle (Pedersen et al., 2001)

Consumers use textural attributes to determine both acceptability and quality. Many consumers define quality in terms of texture i.e. tenderness and juiciness, with more tender and juicy meat considered to have higher quality.

2.8 Physico-Chemical qualities.

2.8.1 pH.

Probably, pH is the quality attribute most commonly measured in fresh meat, it affects technological ability, keeping ability and most sensory traits. The ultimate pH attained by the meat has important effects on other properties of the meat. Meat with a high pH is darker in colour than normal, slimy to touch and does not allow salt of curing pickle to penetrate readily. Meat that develops a relatively high pH is difficult to cure properly. It has been studied that the relation of the pH of meat to its electrical resistance and found that pH between 5.8 and 6.0, there is a rapid increase in resistance (Fogg and Harrison, 1975). Relatively high pH is desirable in meat, which is to be frozen since in this range of pH’s discoloration and the rate of fat oxidation are inhibited and the drip losses are minimized (Green, 1969). The rate of pH fall depends on temperature (Marsh and Thomson, 1958). Higher muscle pH values were associated with a more tender meat and the toughness of hot boned meat might be due to an accelerated pH fall (Peterson and Lilyblada, 1979). Higher pH values were resulted in greater bind in fresh pork patties, higher cooking yield and improved juiciness (Keeton, 1983) and decreased thiobarbituric acid (TBA)
values in fresh pork products (Judge and Aberle, 1980, Drerup et al., 1981).

2.8.2 Water Holding Capacity (WHC).

Water holding capacity means the ability of meat to hold water to its own or added water during application of any force (pressing, heating, grinding, centrifugation etc.). A high WHC in the lean meat is a decisive factor for producing a high quality sausage (Hamm, 1981). It also prevents rendering out of fat, which results in lower losses during smoking, cooking, storage, and canning of the sausage. The water liberated by such external forces is termed as “Loose water” and the water retained by tissue is called “bound water”. The term swelling means spontaneous uptake of water by meat from any surrounding water resulting in increased weight of muscle. WHC and swelling both are due to interaction between muscle tissues and water. This interaction is called “meat hydration”. The changes in WHC are determined by the extent to which the free water is immobilized within the microstructure of tissue (Hamm, 1960). Thermally induced meat protein gelation is the key element required to bind meat pieces together with optimal strength in processed meat products (Hedric et al., 1994 ‘b’). Heat induced gelation of the meat proteins i.e. myosin and actomyosin, plays an essential role in the development of binding properties of comminuted meat products such as sausages (Samejima et. al., 1969, Ishioroshi et. al., 1983). Thus, myosin and actomyosin are the most important factors for the development of the binding properties of thermally induced meat protein gels (Samejima et. al., 1969, Samejima, et. al., 1981,1982). Not only myosin and actomyosin contribute to the rigidity (firmness) of thermally induced meat, they are also critical to the water holding capacity (WHC) and high cooking yields (Haman, 1988, Smith, 1988). Water retention in processed meat
products is the major contributing factor to the sensation of juiciness (Hedrick et al., 1994 ‘c’).

2.8.3 Emulsifying Capacity (EC).

Emulsion theory holds that during chopping, salt soluble muscle proteins and water act as continuous phase of the emulsion, coating fat globules in a two-phase system, which is stabilizing upon the thermal processing. The emulsification of fats is mainly responsible for stability of comminuted meat products such as Frankfurters and Bologna. Recently following three physico-chemical events, which impart on protein’s functional properties in comminuted meat products have been recognized (Acton et al., 1983).

(i) Protein-water
(ii) Protein-fat association, and
(iii) Protein-protein aggregation.

These interactions may directly be related to the measurable functional properties of WHC, fat binding and textural strength respectively. Gelation of ingredients results in the stabilization of emulsion type sausage. Intracellular beef muscle proteins were ranked from greatest EC to least as follows-actin in the absence of salt, myosin, actomyosin, sarcoplasmic proteins and actin, in 0.3M salt (Hegarti et al., 1963). Myosin and actomyosin produced emulsions with superior stability, however at the pH of normal fresh meat (5.6-5.8); the sarcoplasmic fraction produced the most stable emulsions. Non-protein nitrogen compounds were reported to have no role in emulsion formation.

Fat globules in pre-rigor meat emulsions were more evenly distributed, rounder in shape and surrounded with heavy matrix of protein, where as with post-rigor emulsions fat globules were irregular
in shape and had a thin protein matrix around the fat globule (Froning and Neelakantan, 1971).

The majority of methods used to measure EC were based on a procedure developed by Swift et al., (1961). The EC was reported as ml of fat (oil) emulsified by unit weight of meat (weight basis) or as ml of oil emulsified by unit weight of salt soluble protein (Protein basis). Expression of EC data on weight basis was probably more pertinent for meat industry. The protein network physically and chemically enhances the water retention by their capillarity and non covalent bonding (Acton and Dick, 1984)

2.9 Microbiological Properties.

Food borne illness implicating meat and meat products as the vehicles of transmission for pathogenic microorganisms have been well documented in the literature. Fresh meat is ideal sources of nutrients for proliferation and growth of both spoilage and pathogenic bacteria.

In India, buffalo meat is one of the major item for export as well as domestic consumption. Reduction in microbial load by either keeping in refrigerated temperature or treating with different ways of preservation like curing, curing with antioxidants and smoking, can enhance the hygienic status of meat, provide extended shelf-life, increase consumer acceptability and further improve the export of buffalo meat.

Microbial safety of meat and meat products is of great public and industrial significance. However, some would claim that the numbers of disease out break caused by meat products have not decreased, even through a lot of research and various approaches had been evaluated to ensure the increased cleanliness of meat and meat products. The question then arises is why are more problems surfacing
in meat and meat products which are presumably cleaner than product more contaminated. Murphy (1996), in his article of “how clean is to clean”? reported that “Jay (1996 ‘a’) suggested that the current approach to food safety should be achieving the goal of safe meat, not just in reducing the number of microorganisms”. Jay, (1996 ‘a’) asked a thought provoking question “Is our meat too clean?” which is based on the hypothesis that lack of microbial interference and competition might be encouraging pathogens growth and causing food poisoning.

Beneficial attachments of non-pathogenic micro flora in fighting food borne disease of live poultry, called competitive exclusion, has been applied widely (Jay, 1996, Scanlan, 1997, Palmu and Camelin, 1997, Nuotio et. al., 1992, and Hume et. al., 1996,1997). The presence of other competitive micro flora have been known as an effective method in preserving food, and lactic acid bacteria have been suggested as suitable “barriers” against pathogenic organisms (Gombas, 1989). The most common microbial interferences used in meat and meat products are the use of LAB (Lactic acid bacteria) which are bacteria that have received a great attention in food. Abdel-Bar and Harris (1984) had reported that Lactobacillus bugaricus with levels of 104 to 106 CFU /ml has inhibitory effects on pathogens in ground beef.

Meat proteins are good source of carbon and nitrogen that are essential for microbial growth. Microorganisms degrade the muscle proteins by proteolysis and utilize the essential nutrients for their growth during spoilage process, which depends upon the composition of muscle and type of micro flora present on meat (Gill and Newton, 1982).

Microbiological analysis in the meat industry is mainly targeted to count total microorganisms or a specific past of the microflora, or to
detect the presence of selected genera or species of microorganisms. Investigations show that smaller butchers shops have poor record of hygiene, and that infection is entering the food chain through faecal contamination on slaughter lines (Pennington, 1988). Ground beef prepared from hot processed beef sides was equal or superior to that of beef from conventionally chilled sides through 45 days of storage at 0°C in terms of bacteriological quality (Emswiler and Kotula, 1979). However, greater microbiological counts in hot-boned cuts were reported (Kennedy et al., 1982) and lower counts in the latter case were attributed to greater dehydration of conventionally processed meat surfaces. It was recommended that hot boned meat must be cooled to 8°C or below within 4 hr of boning when the initial temperature of the boned meat is 40°C, 6 hr for 30°C and 9.5 hr for 20°C to maintain the microbiological integrity of meat (Herbert and Smith, 1980).

The process of conversion of live animals to meat for consumption invariably results in microbial contamination of meat. The increase in microbial load results in reduced shelf-life of meat (Surve et al., 1991, Ziauddin et al., 1992, 1995). The aerobic mesophiles count and psychrotrophic plate count (PPC)of ground buffalo meat were not significant affected by Tocopherol acetate treatment. The aerobic mesophiles count and psychotrophic plate count in control samples were log 5.46/g and log 5.2/g respectively while in treated samples it ranged from log 5.36/g to 5.33/g and log 4.94 to log 5.12/g respectively (Sahoo and Anjaneyulu, 1997). Agnihotri (1988), Anjaneyulu (1988) and Murthy (1988), reported that standard plate counts of ground buffalo meat ranged from log 5.46/g to log 7.06/g during refrigerated storage. They also observed that PPC increased up to log 6.62/g in ground buffalo meat after 10 days of refrigerated
storage. The samples in the present study were well below the level of incipient spoilage of meat, i.e. log 7.0/g (Hytiainen et al., 1975) at the end of the storage period.

Several simple methods have been investigated for reducing the initial microbial load on meat, such as use of water spray (Anderson et al., 1987), hot water dips (Hegarty and Allen, 1975), sprays (Charles, 1982; Hamm and Deathorage, 1960), organic acid dips (Smulders, 1995) and use of Chlorinated water for washing the carcass. Bachhil (1983) isolated Escherichia Coli from buffalo meat and reported that 25.6% of the isolates were pathogenic, means, E-Coli count/ g was $1.5 \times 10^2$ and 21 strains were enteropathogenic. Paranjape et al., (1985) compared the microbiological quality of minced and chunk buffalo meat. Mince meat spoiled more quickly than chunks and low ERV (Extract Release Volume) value were directly related to high TVC. Microbiological, chemical and sensory quality changes in vacuum packaging carabeef stored at various freezer temperatures were studied by Daulay et al. (1980). At -12°C and -6°C to -7°C the microbial population of both samples (consisting of micrococcus, lactobacillus, pseudomonas and bacillus sp.) decreased considerably. Micrococcus and lactobacillus and later lactobocacillus predominated throughout the storage period. Autolytic processes of microactivity resulted in a slight increase in pH while production of basic metabolites caused quality deterioration. All carabeef samples were still acceptable up to the end of storage period despite minor changes in the sensory properties. Sison et al. (1980) reported that based on the total microbial count, carabeef stored at 4°C C had a shelf-life of 5 days. Spoilage started on the sixth day and was manifested by off odour, slime formation and greenish discolouration by microbial
proteolysis predominantly caused by pseudomonas, micrococcus, alkaligenes bacillus and lactobacillus. Carabeef cuts stored at -7°C and -18°C had a shelf-life of six months. Whereas at fluctuating temperature of -18°C to 2.7°C, it had a shelf-life of 2.5 months. In India, total plate count of $3.85 \times 10^6$, coliform count of $6.0 \times 10^4$ and mould count of $2 \times 10^2$/ sq inch have been reported for sheep and goat carcasses (Kondaiah et al., 1986, 'b'). A higher total mesophilic count in minced meat to the order of $10^7$ to $10^8$ organism/g, Fecal streptococci upto 57000/g and total coliform up to $700 \times 10^3$ g have been reported by Sofos (1986).

The single most dominant microflora for refrigerated aerobic stored meat is Pseudomonas (Gill, 1983) with an average level of 104 CFU/g (Foegeding, et al., 1983), Pseudomonas had maintained 104 CFU/g from day 0 to 6 in beef samples. Enterobacteriaceae had also been reported as the refrigerated storage (Eribo and Jay, 1985; Gamage, et al., 1997). Giannuzzi et al. (1998) checked microbial flora at temperature of 0,4,7,9 and 10°C and the results of Enterobacteriaceae was 102 CFU/g to 103-105 CFU/g, LAB was 103 CFU/g to 104-106 CFU/g and Pseudomonas was 102 to 103 CFU/g to 104 to 107 CFU/g from day 0 to 7 by using gas permeable packaging. In the study of Palumbo et. al., (1997) they used ground beef with the background of bacteria of 103 CFU/g and 106 CFU/g and E.coli with 103 CFU/g.

**2.10. Nutritional quality of Meat with reference to Buffalo meat.**

The body must be nourished properly to be healthy and to stay healthy. Vast arrays of nutrients are needed to properly nourish our bodies. Many nutrients are considered essential because our body cannot manufacture them in great enough quantity to meet our needs. Meat provides an abundance of nutrients, generally at higher
concentrations than most other foods relative to caloric content. Most of the essential nutrients are present in muscle foods (Gray and Dugan, 1975). Meat provides nutrients in a form that potentiates greater bioavailability than nutrients from other food sources (Bodwell and Anderson, 1986).

Meat has been an important food item in human diet since time immemorial. According to Yudkin (1975) the human has evolved as an omnivorous animal with preference for meat and fruits. With increased modernization and rise in the standard of living our people are relaxing religious and social taboos against foods like meat. Among Indians meat eaters are not in a minority any longer. Meat use in India has great cultural and social prestige, which needs to be investigated and popularized amongst the public. Meat is an excellent source of high quality protein, B-complex vitamins and of certain minerals especially iron (Duston, 1974). The composition of meat depends upon the species from which the meat was obtained, its place of nutrition, the specific cut used, the extent of cutting and trimming, processing, packaging and storage. For meats with a covering of external fat layer about 1 cm thick proximate composition and energy values are protein 17%, fat 20%, moisture 62%, ash 1% and calories 250 Kcal/100 gram (Monin, 1998).

2.10.1 Meat Proteins.

Meat is a source of protein in concentrated form (Bake et al., 1969). The biological value and the nutritional quality of the meat proteins is high as they are easily digested and provide all the essential amino acids in good balance. Essential amino acids are metabolic necessities that cannot be synthesized in the human body. The amino acid composition of meat proteins is very much similar to those of the
human body proteins which is not so with the plant proteins (Guyton, 1981). Thus, muscle foods not only possess a nearly ideal amino acid profile but provide a significant portion of the daily requirement for protein in a relatively small serving (Wardlaw and Insel, 1990).

2.10.2 Lipids.

Meats have historically been considered high fat foods (Miller, 1991). Meats contain large amounts of lipids such as essential fatty acid, cholesterol, phospholipids and fat-soluble vitamins. About 95% of the total lipids exist as fat, the glycerol esters of fatty acids (Stiles, 1991). The quality and the quantity of the nutrition of the animals have a significant bearing on the fat depots of animals. There are slight differences between external and internal fat, the former being less saturated than the later (Chako and Perkins, 1962; Terrel et al., 1967). Although there are some quantitative variations in fatty acid distribution from animal to animal and from cut to cut within a carcass, the fat composition of each species is fairly constant. It is interesting that saturated fatty acids from less than half of the fats, despite the common reference to animals fats as saturated. Phospholipids are critical components of cell wall and help to regulate cell functions. These contain almost 50% poly unsaturated fatty acids (Hornstein et al., 1961). Major phospholipids are Lecithin and Cephalin.

Lean beef, pork and lamb muscles contain 70-75 mg/100g of Cholesterol (Tu et al., 1967), liver and brain have about 300 and 2000 mg/100g respectively. Fat also provides much of the flavour that we associate with food products. This is important from a nutritional standpoint because foods must be palatable for people to eat them. Muscle foods have always been considered highly palatable and desirable.
components of the diet, largely due to their flavourful fat content (Krause and Mahan, 1984).

Lipid containing polyunsaturated fatty acids and their esters are readily oxidized by molecular oxygen. Such an oxidation, called autooxidation, proceeds by a free radical chain mechanism. Various free radical scavengers can contribute to chain termination in this process (Ledward, 1985). It has been known that meat with fat containing unsaturated fatty acids undergoes deterioration during storage owing to auto oxidation of these fatty acids. Oxidative rancidity is a major cause of quality deterioration in raw meat during refrigerated and frozen storage (Walters, 1975). Cooked meat developed rancid flavour more rapidly than uncooked meat during storage resulting in warmed over flavour (Abd El-Alim et al., 1999).

2.10.3. Carbohydrate.

Muscle foods are low in carbohydrate. The only naturally occurring carbohydrate found in muscle food is Glycogen (Guyton, 1981). Glycogen serves as an energy reserve in muscle tissue and is used especially during anaerobic metabolism. The small energy reserve represented by Glycogen in muscle tissue is expanded during the slaughtering process when the muscle cells undergo glycolytic metabolism due to the loss of the blood supply. Therefore, little glycogen remains in most muscle food products (Okonkivo and Obanu, 1992).

2.10.4 Vitamins.

Muscle foods are a fair to good source of most water soluble vitamins (Beitz, 1992). Vitamin B_{12} is exceptional because it is found primarily in animal products. Muscle foods are not as good source of fat soluble vitamins as are vegetable source (Zapsalis and Beck, 1985).
2.10.5 Meat Minerals.

Muscle foods are a good source of minerals, and are known for its iron content. Biological availability of iron and zinc from meat is far superior to from other dietary sources. It has long been known that iron is required for survival of the human organism. It is of interest, given its relative abundance, that iron deficiency is the most common specific nutrient deficiency observed around the world today (Love, 1986). But Calcium, Sodium, Potassium and Magnesium are the other minerals found in reasonable amounts in meat. Iron levels in kidney and spleen are substantially higher than in other muscles and zinc are present in meats (Watts and Merrill, 1963). Although meat is a poor source of Calcium, it is usually a good source of Phosphorus (Wilson, 1961).

2.11 Effect of treatment on Shelf–life of Buffalo meat.

Long ago when man began to cook his food and to live in some kind of shelter, he soon learned methods of storing of meat. Probably he first discovered the method of smoking meat by hanging it near the roof of his cave or tent where it slowly dried and became smoked. Later he found that the addition of salt to the meat prevented its putrefication (Jensen, 1949).

2.11.1 Meat curing.

Meat curing was used originally almost entirely as means of preserving meat during times of plenty to carry over to times of scarcity. Meat curing is a classical example of food preservation (Urbain, 1978). Curing, through the incorporation of various cure adjunct (ingredients) contributes to the colour and flavour that consumers associate with the cured meats along with increasing the shelf-life (Cassens, 1990). There are numerous non-meat ingredients
that can be used in curing the meat. The most commonly used ingredients include water, nitrite, salt, phosphate, sugar and some vitamins (Leistner, 1992).

In Dry curing, the curing agents are rubbed on the surface of meat. For large cuts, the cure ingredients should be applied several times during the curing periods. The dry curing procedure is slow and requires a large amount of hand labour (Forrest et al., 1975). Researchers have attempted to reduce the dry cure processing time to a minimum while attempting to improve the standardize quality (Graham and Blumer, 1971 ‘a’). Nitrates have been used traditionally in dry and wet curing. Work by Kemp et al. (1974, 1975) has shown that intact meat cured either with nitrate, nitrite or a combination of these compounds, were similar in quality. Kemp et al., (1979) also reported that there were no differences in quality of boneless dry cured meat with or without nitrate. Tumbling a product has been reported to improve distribution of cure ingredients and to obtain a more desirable cured colour (Krause et al., 1978). In this research, the tumbling technique was applied to the dry-cured boneless and a “lard coating” technique was used to reduce internal mold growth and retard moisture loss during storage. Product was placed in a plastic freezer bag to keep the meat clean and to reduce moisture loss.

2.11.1.1 Salt.

It has three basic functions (i) add or enhance flavour, (ii) solubilise or extract proteins that are essential for improving moisture retention and in forming the necessary bind and texture in the finished product, (iii) extend the shelf-life (Barbut et al., 1986). The action of salt in the curing is to inhibit the growth of bacteria. Salt shows a selective action and does not inhibit the growth of all microorganism
(Jenson, 1949). When salt solution is brought in contact with fat, it actually retard oxidation (Gaddies, 1952). Salt favours the development of the bright red colour (Cassens, 1990). Use of salt as a curing agent increases the water holding capacity (Offer and Trinic, 1983). Salt can be both beneficial and detrimental effect on meat. Salt has undesirable oxidative effects on meat products, especially on fresh Sausages that are frozen and stored (Mc Mecking, 1982).

2.11.1.2 Nitrites.

It is the most important ingredient incorporated in the manufacture of cured meats without which most of these products would not exist as they are today (Mertens and Knorr, 1992). Nitrites serves as a vital bacteriostatic control, the out growth of spores produced form clostridium botulinum. Nitrite is involved in cured colour development and flavour protection (Breidenstein, 1983). Nitrite react with myoglobin and upon heat processing, forms a heat stable pink cured pigment. Nitrite contributes to flavour stability (prevention of warmed-over flavour) by complexing with heme iron, if free, could act as potent catalyst in lipid oxidation (Cassens, 1990). The development of proper colour during curing depends on nitrite and this depends on presence of certain groups of bacteria which slowly reduce the nitrate into nitrite (Paleari et.al., 1977).

The nitrous acid is reduced to nitric oxide ($\text{NO}$), which then reacts with myoglobin to form nitric oxide myoglobin (Meyer, 1973).

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \quad \text{(bacterial action)}
\]

\[
\text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO}_2
\]

\[
\text{HNO}_2 \rightarrow \text{NO} \quad \text{(reduction by compounds in meat.)}
\]

\[
\text{NO} + \text{Myoglobin} \rightarrow \text{NO}
\]
myoglobin bright red

The tolerance toward nitrite varies widely among different groups of bacteria. Several explanations have been offered for the bacteriostatic properties of nitrite, namely (i) interference with the Sulphur nutrition of the organism, (ii) reaction with the $\alpha$-amino groups of amino acids, (iii) reaction with monophenols such as tyrosin, and, (iv) reaction of the decomposition product nitric oxide with catalase and Cytochromes (Simon et al., 1973). The antibotulinal activity of nitrite may be due to its inhibition of non-heme, iron-sulphur enzymes (Birdsall, 1977). The bacteriostatic effect of nitrite is dependent on pH. As pH is lowered by one unit, the bacteriostatic effect increases approximately 10-folds (Eisenbrand, et al., 1974).

2.11.1.3 Sugar.

It contributes to the sweet taste of the product. Sugar has the ability to attract water and develop the surface colour of meat through browning reaction. Browning is a result of the sugar reaction with the amino group of protein during curing process (Challis et al., 1978). Sugar not only adds a sweet flavour, detectable in cured meats, but also produced conditions during curing and storage that ensure the best colour and conserve protein (Bouton et al., 1974). In the presence of many types of bacteria, sucrose is hydrolysed to glucose and fructose and then utilized by these organisms to form many different compo
manufacturers to heat process cured meats sooner by minimizing the
time necessary for the nitrate to react with myoglobin. Sodium
Erythorbate can be added upto 550 ppm. Sodium Ascorbate and
Sodium Citrate also have antioxidant properties that help to maintain
the colour and flavour of the product(Igene et.al.,1997 ‘a’).

2.11.2 Nitrosamines in meat products.

The conservation of meat by curing represents a very long
established and effective method of preservation, the products having
high consumer appeal and a very good safety record. Meat contains
amines and amides as potential precursors to nitrosamines and
nitrosamide in the presence of nitrite(Woolford and Cassens, 1977).
Nitrite can also be derived from sources other than its use as food
additives (Greenberg,1977). Of cured meat products, therefore, volatile
nitrosamines are to be found almost consistently at low concentrations
in fried bacon and far less frequently in most other products preserved
with nitrite (Groenen et. al, 1976). Addition of Sodium nitrite or
Sodium nitrate or in combination with Sodium Ascorbare or Potassium
Sorbate can effectively bring down nitrosamine formation and
Clostridium botulinum population hence “ Botulism hazard” (Schmidt
and Parrish,1971; Paquette et. al., 1980) The occurrence of
nitrosamines in cured products, which have been shown to be
carcinogenic to experimental animals (Jensen and Urbain,1986). In the
absence of any firm data quantifying the exposure to N-nitroso
compounds over long periods which is of concern in human
epidemiology, it is obviously essential to restrict their occurrence to a
minimum. However, this should not be accomplished at any loss of
safety so far as botulism or any other hazard is concerned and thus the
emphasis should be placed upon the most effective use of nitrate in
curing with the use of acceptable inhibitors of nitrosation such as Ascorbic Acid, or α-tocopherol (Gough and Walters, 1976).

### 2.11.3 Use of antioxidants in curing.

An antioxidant is defined as any substance which prolongs the shelf-life of a food by protecting it against deterioration caused by oxidation, including fat rancidity and colour changes (Mac Farlame, 1985).

Food lipids oxidation is considered to be a risk factors for human health. Some lipid oxidation products and few cholesterol oxides in particular, are considered atherogenic agents and appear to have mutagenic, carcinogenic and cytotoxic properties (Sahoo and Verma, 1999). Lipid oxidation is one of the primary cause of deterioration in the quality of meat and meat products during storage leading to the development of off flavour, loss of colour and texture and decrease in nutritive value (Buckley et al., 1995).

The mechanism of oxidation of poly unsaturated fatty acids, production of lipid oxidation, in vivo effects of peroxidation, factors affecting oxidation and control of lipid oxidation (Hsieh and Kinsella, 1989), oxidation rancidity and discolouration of meat (Watts, 1954), oxidative process in meat and meat products, quality implications (Kanner, 1994) had been overviewed. The products of lipid oxidation are responsible for unacceptable off flavours and limit the shelf-life of such meats. In raw meat, lipid oxidation occurs over days or weeks whereas in cooked meat the reaction proceeds rapidly (Tims and Watts, 1958; Mottram, 1987). Improving the natural antioxidant status of muscles by dietary means has also attracted considerable attention and it is established that vitamin E supplementation through diet increases the level of vitamin E and oxidation stability of muscles.
Both synthetic and natural antioxidants have been used in meat products to control lipid oxidation (Chastain et al., 1982). Colour of meat that determines meat quality depends on myoglobin, a pigment with several forms. Myoglobin oxidized to metmyoglobin is characterized by a change in pigment to a brown colour that is not acceptable to the consumers. There is the red to brown transition, which is responsible for the fading of meat (Sahoo and Verma, 1999).

The accumulation of metmyoglobin at the surface of fresh beef depends on several diffusion (Brook, 1929), oxygen consumption, auto-oxidation of the pigment in the presence of oxygen (George and Startman, 1952; Brown and Mebine, 1969) and the enzymatic reduction of metmyoglobin (Stewart et al., 1965; Watts et al., 1966).

The rate of metmyoglobin accumulation is muscle dependent. Differences in colour stability between muscles are of considerable importance to the meat industry (Paleari et al., 2000). Light, pH, temperature, bacterial contamination and lipid oxidation are known to influence the oxidation of myoglobin (Lawrie, 1979). According to Giddings (1974), the loss of anaerobic metmyoglobin reducing activity (mRA) in post rigor meat is due to factors such as decrease in tissue pH, depletion of substrates and co-factors such as co-enzymes, oxidative deteriorate changes, decreasing enzymes activities including disintegration of mitochondrial particles. DPN related enzymes system of raw meat are able to utilize oxygen and in the absence of oxygen to reduce metmyoglobin (Watts et al., 1966). There is an interaction between pigment and lipid oxidation. It has been found that development of WOF is related to the content of myoglobin in the muscles. Igene et al. (1979), found that heat treated chicken meat from dark muscles was more unstable towards oxidation than white muscles. Tappel (1962) proposed that haematin compounds catalyse the
decomposition of hydroperoxides, ineffect propagating further lipid oxidation. In contrast, Sato and Hegarty (1971) concluded from model experiments with cooked muscle tissue that non heme iron was responsible for the catalytic cleavage of hydroperoxides. This is in agreement with the result of Konopka et. al., (1995). Other studies, seems to indicate that metmyoglobin is responsible for the cleavage of hydroperoxide in muscle tissues. Harel and Kanner (1985) and Johns et al. (1989) thus found that following heat denaturation, haemoproteins appear to be more effective catalyst than inorganic iron. Many researchers have studied the use and effect of natural and synthetic antioxidants in preservation of meat.

2.11.4 Ascorbic Acid as antioxidant.

Ascorbic Acid is strong reducing agent. It is used as a biological antioxidant to stabilize meat colour by way of pre slaughter injection in beef (Hood, 1975), exogenous addition in ground beef (Greene et. al., 1971), dip treatment of surface application of beef steaks (Benedict et. al., 1975; Harbers et al., 1981; Mitsumoto et. al., 1991; Okayama et. al., 1987). In cured meat products such as cooked ham and smoked beef sausages, it reduces the requirement of nitrite, reduces nitrosamine formation (Counsell, 1971), and also reduces the time of processing (Pearson and Tauber, 1984). In dry fermented sausages Ascorbic Acid greatly enhances colour stability (Alley et al., 1992). Ascorbic Acid acts as an antioxidant by inhibiting radical formation at double bonds of mono or poly unsaturated fatty acid, quenching free radical scavenging oxygen and serving as a reductant (Cort, 1982; Cabelli and Bielsky, 1983). Ascorbic Acid is a good antioxidant for peraoxidations initiated in the aqueous phase, but, does not trap peroxy radicals in the lipid phase (Doba et. al., 1985). In non-
aqueous media, Ascorbic Acid and esters are not good antioxidant (Porter, 1980). The multiple effects of Ascorbic Acid and ascorbyl palmitate include (a) Hydrogen donation to regenerate the stable antioxidant radical,
(b) metal inactivation to reduce the rate of initiation by metal,
(c) Hydro peroxide reduction to produce stable alcohols by non-radical process, and,
(d) oxygen scavenging (Sahoo and Verma, 1999).

The pro-oxidant activity of vitamin C increased with ferric (Fe+++ ion concentration. Vitamin C incorporation maintained good colour in ground beef upto 5 days refrigeration display (Greene et. al., 1971; Shivas et. al., 1984). The colour stability of Sodium Ascorbate treated fresh beef muscles held at 5°C was significantly better than the control samples (Hood, 1975). A comparative study between Sodium Ascorbate and Sodium Erythorbate for the technological properties and processes of cooked cured pork loin was made. Residual Ascorbate or Erythorbate concentration, nitrate and nitrite concentration, colour properties and sensory qualities were assessed at fixed intervals during storage. No significant differences were observed between samples made with Ascorbate and those made with Erythorbate (Blunk, 1993). Ground buffalo meat containing 500 ppm Sodium Ascorbate had significantly higher colour score, Lovibond Tintometer red colour units and lower metmyoglobin content as compared to other levels of Sodium Ascorbate. The metmyoglobin was positively correlated with TBARS number (Sahoo and Anjaneyulu, 1996, 1997 ‘a’). Addition of reducing agents i.e. Ascorbic Acid and their salts improved color stability and extend storage shelf-life of the meat products (Ladikos and Longovois, 1990). Ascorbic Acid treatment showed inhibition of lipid oxidation in beef streaks stored in a cold room at 4°C for 13
days (Okayama et al., 1987). On the other hand, Benedict et al., (1975), reported that 50 ppm Vitamin C addition to ground beef caused greater TBA value than control. Exogenous addition of Ascorbate delayed lipid oxidation in ground beef (Shivas et al., 1984; Greene et al., 1971). Vitamin C treatment showed high lipid stability in ground beef, recording TBA value of 1.52 as compared to control samples (4.0) during display at 4°C for 7 days (Mitsumoto et al., 1991 ‘b’). In addition to colour stabilization and lowering nitrate levels, Ascorbic Acid can reduce cooking losses in both fat free and fat containing meat products (Reichest, 1994). Ground buffalo meat compared to other levels of Sodium Ascorbate (Sahoo and Anjaneyulu, 1997 ‘a’). Addition of Sodium Ascorbate to brine was shown to suppress N-nitrosodimethylamine (NDMA) from added DMA in experimental curing of pork middles. NDMA is formed due to canning or freezing of the cured meats. However, lowest amounts of NDMA were found in the bacon cured in the presence of Ascorbate (Carrol et al., 1978). Ascorbate addition proved beneficial in cured meat products, as it helped to reduce nitrite contents to less than 50% and the residual nitrite could be maintained at a lower level. Use of Sodium Ascorbate proved to be superior to Sodium Erythorbate (Counsell, 1971). Many workers studied the heat stability of Ascorbic Acid in the food during cooking. It was observed that ascorbic acid oxidized faster in utensil made up of standard aluminium than enameled cast iron or standard aluminium coated with aluminium (Evenshtein, 1971). Ubertalle and Faccini (1971) had reported that the salami type sausages, made from 74% lean pork and 26% fat, ripened for 45-90 days different temperature and ventilation conditions, the aroma and taste of sausages containing 25 mg NaNO3 and 100mg Ascorbic Acid were found
superior to those of sausages made with 250 mg Sodium Nitrate and 150 mg Sodium Nitrite / kg.

No significant effect was observed on the microbiological (aerobic mesophiles count, psychrotrophic plate counts) quality of ground buffalo meat treated with Sodium Ascorbate at different levels (Sahoo, 1995). Shivas et al., (1984) and Greene et al., (1971) also reported that microbial numbers were not different for Ascorbic Acid treatments in ground beef. Ascorbic acid did not appear to inhibit bacterial spoilage of beef (Sahoo and Verma, 1990).

Curing combined with Sodium Ascorbate have many positive effects on meat quality. It was found that there was improvement in pH, colour, texture and odour of meat samples. Though it did not show significant effects on microbial population initially but a marked effect of Sodium Ascorbate was observed during the later period of storage. Antioxidant property of Sodium Ascorbate effectively prevented lipid rancidity which otherwise would have led to the conversion of fat into simpler compounds rendering meat more prone to microbial growth (Desrosier and Tressler, 1997). Cured and Sodium Ascorbate treated samples had more shelf-life as compared to simply cured meat (Anzar, 2000). Prolonging the storage life of fresh meat is a very important consideration for both consumers and meat packers. The shelf-life of fresh meat can be prolonged by limiting the extend of discoloration, lipid oxidation and microbiological contamination (Okayama et. al., 1987). Greene et. al., (1971) have already reported that Ascorbic Acid plus propyl gallate or butylated hydroxyanisole effectively retards both pigment and lipid oxidation in ground beef. Govindarajan et. al., (1977) showed that Ascorbic Acid had no effect on the initial slow oxidation of myoglobin, but extended the time before initiation of rapid oxidation in ground beef.
2.11.5 Tocopherol as an antioxidant.

Chang and Watt (1949) reported that Ascorbic Acid markedly retarded rancidity in the presence of hemoglobin or nitrosohemoglobin and amounts of Tocopherol in artificial aqueous fat system. Tappel et al., (1961) studied the inhibition by Tocopherol of lipid peroxidation catalyzed by hematin compounds and demonstrated that synergism with Ascorbate greatly enhanced the oxidation activity of Tocopherol in animal tissues. Tocopherol as an antioxidant had received much attention for its ability to maintain meat colour, extend shelf-life, improve taste, reduce drip loss and offer health benefits (Armstrong, 1993). Oxymyoglobin and lipid oxidation were examined in low and high Vit. E (α-tocopherol) bovine M. longissimus thoracis et lumborum (LD) homogenates and in subcellular fractions of LD. Investigations of the effect of temperature (4, 22, 37°C) and pH (5.5, 6.4, 7.2) on lipid and oxymyoglobin oxidation indicated that oxymyoglobin oxidation increased with increasing temperature and was higher at the low pH. Lipid oxidation increased with temperature only at pH 5.5. The inclusion of 45μM FeCl3/Ascorbate (1:1) at pH 5.5 and 4°C, led to significant increase in lipid oxidation but oxymyoglobin oxidation occurred only when the extent of lipid oxidation exceeded a threshold level. Oxymyoglobin and lipid oxidation were significantly lower in LD fractions derived from high Vitamin E muscle compared to low Vitamin E muscle. α-tocopherol did not interact directly with oxymyoglobin to suppress its oxidation but positive unit affects the muscles α-tocopherol on metmyoglobin reductase activity was observed (O’grady et al., 2001). Sahoo and Anjaneyulu (1997) reported that the shelf-life of ground buffalo meat treated with 10 ppm α-tocopherol acetate could be extended to 8 days in refrigerated
storage conditions, without any undesirable changes in colour, odour or
microbial load, whereas, the control sample could be kept up to 5 days
only. Reduced heart attack risks in uses of Tocopherol supplements
(Stampfer et. al., 1993; Rimm et. al.,1993) and reducing susceptibility
of food lipoprotein to oxidation (Jailal and Grundy, 1992) had
encouraged many food scientists to have concerted research efforts in
the area of Tocopherol supplementation either through diet of meat
animals or by exogenous addition or by directly adding to the muscle
foods for increasing lipid stability storage and improving flavour and
taste of meat products. Tocopherol is very widely used in restructured
meat products. In the processed meats such as cooked rabbit meat
(Castellini et. al., 1988), minced beef (Formanek et. al., 1988), pork
chops (Asghar et. al., 1991), ground buffalo meat and buffalo meat
nuggets (Sahoo, 1995). α-Tocopherol Acetate i.e. Vitamin E had been
used successfully to enhance product quality and to extend shelf-life
during storage condition (Sahoo and Verma, 1999). α-Tocopherol
Acetate is associated with biomembranes and protects them by
neutralizing oxidative induced free radicals (Buttrish and Diplock,
1988). It acts as an antioxidant by donating a hydrogen atom to a free
radical (Tappel, 1962). A logical hypothesis is that α-Tocopherol
quenches free radical originating from lipid oxidation and in turn,
protects oxymyoglobin oxidation. Tocopherol behaves as a chain
breaking anti-oxidant by competing with the substrate for the chain
carrying peroxy radical, normally present in the highest concentration
in the meat system (Frankel,1996). The mixture of α-Tocopherol and
Ascorbic Acid exhibiting a strong synergistic effect was well
recognized (Uri, 1961; Tappel et. al., 1961). Pigment as well as the
lipid oxidation was inhibited by combined treatment of Tocopherol and

38
Ascorbate in oxymyoglobin liposome model (Yin et al., 1993) and in ground beef (Mitsumoto et al., 1991 ‘b’). Ascorbate alone enhanced lipid oxygen radical propagation but its pro-oxidant effects was reversed when Tocopherol was incorporated (Liebler et al., 1986). 10 ppm Vitamin E + 500 ppm Vitamin C showed the strongest synergism to inhibit pigment and lipid oxidation of beef (Mitsumato et al., 1991, ‘b’). Use of natural antioxidant and vacuum packaging extended shelf-life of Buffalo meat nuggets from 10 to 30 days under refrigerated storage (Sahoo and Anjaneyulu, 1997 ‘d’). Lipid oxidation, particularly oxidation of phospholipids limits the keeping quality of the meat and meat products during storage, especially of pre-cooked meats due to the development of unacceptable warmed over flavour (WOF) by auto oxidation process. The meat containing more myoglobin is more prone to such oxidative changes, because the oxidized meat pigment is the more potent catalyst than inorganic iron for the initiation reaction of the auto oxidation process.

2.11.6 Use of Lactic Acid as an antioxidant, and antimicrobial.

Meat traditionally sold at tropical open air market (local market) in third world countries especially in India also, is freshly killed, hot meat. Meat is sold from early morning until evening each day. The product is placed on a table exposing it to environment temperature and it is not refrigerated. This condition encourages a high level of microbial contamination. Surve et al., (1991) reported a number of organic acid are being used to decrease microorganisms on meat surface.

Woolthuis and Smulders (1985) reported that lactic acid is widely used as a sanitizer. Consumer trends would prefer a safer product without unusual chemical preservatives.
Lactic acid and trisodium phosphate were evaluated for the ability to reduce *Escherichia coli* and aerobic plate counts on lamb breasts that were inoculated with a lamb fecal paste. A 90-second water rinse was applied followed by either 2% lactic acid spray, 12% trisodium phosphate dip or a combined treatment of both lactic acid and trisodium phosphate treatments. Lactic acid reduced *E. coli* and aerobic plate counts by 1.6 log \(/\text{cm}^2\) and trisodium phosphate caused 1.8-log\(_{10}\)/cm\(^2\) reduction in *E. coli* and a 0.7-log\(_{10}\)/cm\(^2\) reduction in aerobic plate counts. Lactic acid and trisodium phosphate, used alone or in combination, were effective in reducing numbers of *E. coli* and could be useful as pathogens intervention steps in animal slaughter processing. (Ramirez *et. al.*, 2001).

Decontamination of meat carcasses with organic acids is gaining importance in recent years (Dickson and Anderson, 1992). Lactic acid, being a natural constituent of many foods, is often used for controlling the microbial growth and extending the shelf-life of foods (Smulders *et. al.*, 1986; Singh *et. al.*, 1989). The bactericidal effects of lactic acids were assessed on artificially inoculated beef (Gordon and Bryan, 1992). Since ancient times it has been known that spices prolong the shelf-life of certain foods, and it is now recognized that this antioxidant activity is due to mainly to phenolic compounds present in the plants. Application of antioxidants is one of the technically simplest ways of reducing fat oxidation. Recently, however, a certain reluctance has been observed to use synthetic additives including antioxidants for food products. At the same time natural substances are becoming more and more popular (Al-Jalay, *et. al.*, 1987). Spices are generally used in foods as flavouring agent and many of these had been found to have some antimicrobial activity (Davidson *et. al.*, 1983). Ginger rhizome had been shown to have antioxidant property
and it also contain powerful proteolytic enzyme, which can be useful in tenderizing tough meat (Lee et. al., 1986). Lactic acid and Sodium Chloride solution were shown to posses anti microbial property against some of the spoilage and pathogenic bacteria of meat (Ziauddin et. al., 1993).

2.11.7 Polyphosphate as an antioxidant.

The use of polyphosphate (PP) in meat and meat products of beef, pork and chicken has been increasing due to their beneficial effect in improving the functionality of meat and the palatability and storage of meat products. Food grade polyphosphates or their blends are incorporated in the formulations of meat curing brines, sausages and restructured meat products to enhance their quality in respect of water holding capacity (Hamm, 1960), meat particle–particle binding (Trout and Schmidt, 1984), emulsion stability (Knipe et. al., 1985), yield (Molins et. al., 1987), color, flavour and texture etc. and to inhibit oxidative rancidity (Vollmer and Melton, 1981; Smith et. al., 1984; Young et. al., 1987). Kondaiah et. al.(1985) reported the desirable effects of salt and polyphosphate on functional properties of buffalo meat.

All phosphates exhibit chemical structure in which each phosphorus atom is more or less tetrahedrally surrounded by four oxygen atoms. If they are shared in such a way so as to form P-O-P bonds, the resulting compounds are called condensed phosphates. Polyphosphate, though the term has been loosely applied to refer to a variety of phosphates that have been accepted as ingredients of foods, are the unbranched chain and unbranched ring structured condensed phosphates having generic formula $M_{n+2}P_nO_{3n+1}$, $M=$ Metal ion, when
n= 1, the resulting compound is orthophosphate Na₂PO₄, when n=2, it corresponds to pyrophosphate Na₄P₂O₇, when n=3, it corresponds to tripoly phosphate Na₅₃P₂O₁₀ (Hamm and Gram, 1955).

The Polymerization of polyphosphate can be expressed by the ratio of Na₂O : P₂O₅. The ratio being 2:1 for pyrophosphate, 5:3 for tripolyphosphate and 1:1 for hexametaphosphate (NaPO₃)₆. Polyphosphates are added to the cure to increase the water binding capacity and thereby the yield of finished product. The action of polyphosphates in improving water retention appears to be two fold, first raising the pH and second causing an unfolding of the muscle proteins, thereby making more site available for water binding. Water holding capacity means the ability of meat to hold water to its own or added water during application of any force (pressing, heating, grinding etc.). The main influence of polyphosphate addition in meat is improvement in its water holding capacity (WHC) (hydration). This effect is mainly due to following three factors, change in pH, increase in ionic strength and certain specific effects of phosphates (Bendall, 1954). Many researches have reported that polyphosphates increase the pH of meat. Sodium Hexa Meta Phosphate (SHMP) which caused greatest increase in pH also gives greatest WHC of meat (Shults el al., 1972). Increasing the phosphate concentration in chilled water used for chilling carcasses has been reported to cause a corresponding increase in pH of meat (Froning and Sackell, 1985). The increase in water holding capacity (WHC) of meats caused by polyphosphates is attributed to ionic strength by many workers.

There are many evidence to show that polyphosphates have specific effect on meat microstructure responsible for the increase in meat hydration. Hamm (1960) suggested that the action of polyphosphates is due to elimination of alkaline earth ions in muscles.
The effects of adding polyphosphates in salted meats are additive or synergistic. The reason for specific effect of polyphosphate on salted meats could be a direct union of polyphosphates anions by myofibrillar proteins. The natural actomyosin is bound by polyphosphate in the presence of NaCl also (Barbut et. al., 1986).

Another factor contributing toward increase in WHC due to salt are increase in ionic strength of myofibrillar system (Bendall, 1954). Most of the studies on the effect of polyphosphates on meat have been done on pork and poultry and few on beef, but very few literature is available on mutton (intact or minced). The alkaline phosphate increase the water binding capacity (WBC) as well as the fat emulsifying capacity (EC) of the myofibrillar proteins. The increase in EC is the result of polyphosphate solubilising and dissociating actomyosin into actin and myocin, which in their dissociated form can emulsify more fat (Pearson and Tauber, 1984).

Pyro-, tripoly- and hexametaphosphates have effective anti oxidant effect (Tims and Watts, 1958). The phosphate provide protection against development of oxidative rancidity in cooked meat at concentrations as low as 0.01–0.05% (Watts, 1962). Sodium Chloride acts as a pro oxidant in processed meat products by accelerating oxidative reactions of unsaturated lipids causing increased rancidity (Gray, 1978). Use of polyphosphates decreased off flavour and rancidity development in many meat products and pro-oxidant effect of NaCl was marked by the anti-oxidant properties of polyphosphate (Sato and Hegarty, 1971; Lankey et.al., 1986; Huffman et. al., 1987). However, Schwarts and Mandigo (1976) reported synergistic effect between NaCl and SHMP in retarding oxidative rancidity and enhancing sensory traits of restructured pork. Polyphosphates offer protection against browning during storage and
acts synergistically with ascorbates to protect against rancidity in cured meat (Forrest et al., 1975). The SHMP and STPP had also protected myoglobin from oxidation both initially and over storage time (Chu et al., 1987).

Studies on foods and microbial media indicated that some polyphosphate such as SAPP, SATP and SHMP or blend of phosphates under certain conditions may have potential value for improving microbial or botulinial safety and stability of certain meat products (Madril and Sofos, 1986; Wagner, 1986). Microbial growth was delayed and short shelf-life of low salt (2-3%) meat products were extended with 0.5% SAPP in the formulation which was due to declining pH and presence of phosphates ion (Madril and Sofos, 1986). Some studies have shown that polyphosphates did not retard growth of micro-organisms (Ockerman and Dowiercial, 1980). Microbial growth and products spoilage were more rapid with decreasing brine level irrespective of presence of 0.36% STPP in comminuted meat products during storage at 20°C (Sahoo, 1995). Further STPP, SAPP and SHMP have no significant inhibitory effects on mesophilic and psychotropic organism and on S. aureus on uncooked meat during storage at 5°C but SAPP addition consistently lowered aerobic counts (Gram, 1993; Farber and Idziak, 1984).

Rao (1978) reported that minced prepared in lab under hygienic conditions had total mesophilic count (log/g) 2.2-4.5, coliform 0-1.0 and staphylococci 1.8-3.0, while market meat samples had total mesophilic count of 5.0-8.0, coliform 5.0-5.5 and staphylococci count 5.0-6.5. Padda et al., (1986) reported that though intact mutton had only 2-4.8 x 10^3 total count / sq. cm, it rose to 5.1 to 8.8 x 10^4 /g when minced. The anti microbial effects of polyphosphates have been known since 1864. (Matlock et al., 1984.) This action is due to the complex
formation by polyphosphates with bivalent metals essential to the microbial cell specially Mg$^{++}$ and Ca$^{++}$. This interferences with cell division reduces the stability of cell wall. Surve et. al., (1990) has suggested that the mode of anti microbial action of polyphosphate is through inhibition of spore germination. This anti microbial effect has also been attributed to higher pH caused due to these compounds (Steinhauer, 1983). Gram-positive bacteria are more sensitive to polyphosphate than Gram-negative bacteria (Poste et. al., 1986). Inorganic phosphates pose little health hazard to humans. However, Anderson et. al., (1990) reported both short term (abdominal distress and diarrhoea) and long term (increase calcium mobilization) possible health problem if used at the maximum permissible level of 0.5%. Polyphosphate at high concentrations may adversely affect meat texture (rubbery, flavour, metallic, astringent and soapy). Potential problems associated with high salt content in the processed meat products may be minimized by optimizing the salt level and phosphate concentration without acceptability of the product. Since SHMP is more effective anti microbial agent compared to other polyphosphate, it seems beneficial to include SHMP in phosphate blends to improve specific product functionality as well as to confer microbial stability to meat products.

Polyphosphate treatments of meat has been reported to improve the sensory attributes as tenderness, juiciness and flavour acceptability etc. Improvement of various sensory attributes of pork products due to polyphosphate have been reported by many worker (Barbut et. al., 1986; Brotsky and Everson, 1973; Dransfield et. al., 1984). The last group of workers reported a soapy or metallic flavour in polyphosphate injected pork due to which the scoring was less. Huffman et. al., (1984) reported a salt polyphosphate synergistic effect on sensory
attributes. Improved sensory characteristic colour of bologna sausages due to polyphosphates have been reported by Swift and Ellis (1957). Improvement in binding strength of various meat products as beef rolls and poultry waves has also been reported by (Pepper and Schmidt, 1975). Polyphosphate addition to meats reduces drip and thaw loss and moisture loss during storage (Jones et. al., 1986). Since polyphosphates improve water retention and cooking yield, the moisture content of cooked meat is increased 67.4% vs 61.2% of corresponding control (Fronning, 1965). Use of polyphosphate in Indian meat products will go a long way in improving their quality particularly in the utilization of low quality tough meats.

2.11.8 Smoking of Meat.

It seems probable that nomadic humans first discovered the preservative action and the desirable flavour imparted to meat that was hung near their fires. Regardless of its origin, smoking, like curing of meat has been practiced since the beginning of recorded history. Curing and smoking of meat are closely interrelated and are often practiced together, that is, cured meat is commonly smoked (Pearson and Gillet, 1997; Wisstreich, 1977).

The primary purposes of smoking meat are,

(i) development of aroma and flavour,
(ii) preservation,
(iii) creation of new products,
(iv) development of color,
(v) formation of a protective skin on emulsion-type sausages, and,
(vi) protection from oxidation.
The chemical components most commonly found in wood smoke include phenols, organic acids, alcohol, carbonyls, hydrocarbons, and some gaseous components, such as carbon dioxide (CO₂), carbon monoxide (CO), oxygen (O₂), nitrogen (N₂) and nitrogen oxide (NO₂) (Porter et. al., 1965). Phenols appear to play a three fold role in the smoking of meats and other foods as (i) they act as antioxidant, (ii) they contribute to the colour and flavour of smoked products, and, (iii) they have a bacteriostatic effect that contribute to preservation. The role of phenols in preventing oxidative changes in smoked meat is most important. The antioxidant activity of smoke is one of its most important attributes in smoke are due to the phenols with high boiling point especially 2, 6-dimethoxyphenol, 2-6, dimethoxy-4- methylphenol and 2,6 dimethoxy 4-etethylphenol (Gill, 1986). Colour and flavour are the important sensory attributes to the desirability of smoked meats. Colour development is caused by the interaction of the carbonyls in the vapour phase of the smoke with amino groups on the surface of the foods. Phenols also contribute to the colour development. Colour formation is due to the Maillard reaction (Howard et. al., 1966). Colour formation is directly related to the smoke concentration, temperature and the moisture content at the surface of the products, with 12-15% of moisture at the exterior surface of meat resulting in maximum colour development. The desired flavour of smoked meats is primarily due to the phenolic compounds in the vapour phase. The bactericidal action of smoking is due to the combined effects of heating, drying and the chemical components in the smoke. Smoke components such as acetic acid, formaldehyde and creosole prevent microbial growth. The phenols are known to possess strong bacteriostatic activity. The high boiling point phenols have the highest bactericidal activity. The bacteriostatic effect
is primarily on the surface, since the amount of smoke penetration is limited. A wide variety of alcohols are also found in wood smoke due to the destructive distillation of wood. Alcohol exert minor bactericidal effects on meat. Organic acids have little influence on the aroma and flavour of smoked meat. They have only a preservation effect. The gaseous components in smoke that is probably of the greatest significance is nitrous oxide, which has been linked to formation of nitrosoamines and nitrites in smoked meats (Schwartzberg, 1976).

Smoking can cause some destruction of thiamine, but has little effect on niacin and riboflavin. The antioxidant properties of wood smoke should help stabilize the fat soluble vitamins and would also be expected to prevent surface oxidation of smoked meat products.

The various smoke ingredients differ in their value. Those of the phenol fraction hinder the fat oxidant processes and improve its flavour. Dry wood of deciduous trees (beech, oak, aldar, maple and neem) is used for smoking. The smoke time depends on the type and different aspect of the products exclusively the storage periods. Researchers have reported smoking time and temperatures different for different products, as for hams, it is 18-35°C for 3-5 days, for bacon 30-45°C for 2-14 days. Optimal humidity in the smoke chamber is 30-40%. Higher humidity (70-80%) hamper dehydration and cause quick coagulation of the smoke on the surface of the meat.

As soon as the smoke is generated, numerous reactions and condensations occur. Aldehydes and phenols condense to form resins, which represent about 50% of the smoke components and are believed to provide most of the colour in smoked meats. Various biological characteristics influencing the quality of farmed Salmon and Smoked muscles were studied (Gomez-Guillen et. al., 2000).
Smoked meats are high calorie products of good eating properties and delicate juicy texture. The gloss formed on the surface of smoked meats is the result of two effects. Resins formed in smokes from the reaction of aldehydes particularly formaldehyde and phenols are deposited on the surface of the meat. Smoking produces a glossy finish on meat. When cured meat is smoked, the color brightens and becomes more stable. In India, the flavour called smoky or “Sondhapan” which is being found in smoked food is appreciated. Okonkive et. al., (1992) have also reported effects of smoking on beef samples. They observed that smoking caused increased darkening and hardness. Total viable aerobes, coliform and fungi were below the levels of detection while TBA value were low and all samples possessed no detectable rancidity. They, however, found the smoking led to a slight loss of some of the protein components due to denaturation. Smoking of cured meat improves its keeping properties further, as well as imparting an appetizing colour and flavour.

2.12 Refrigerated and Frozen Storage of Meat.

Our rural ancestors, before the advent of electricity and modern refrigeration have for centuries developed different methods of preserving the meat they obtained by hunting or from growing domesticated animals and fowls. The methods utilized for preservation include drying, smoking, sausage making, salting and, in northern region, using ice and cold temperatures. According to Stewart and Amerine (1973), canning of meat was developed about 1795 by Nicholas Appert for preserving for the French Army. These preservation methods are the forerunners of today’s modern processing, packaging and refrigerated storage methods, which make it
possible to have the meat of many species available to consumers the year around in any climate.

The primary purpose of food preservation is to prevent food from spoilage. Whether food spoilage is mild or extreme, the primary cause is the action of microorganism, bacteria, moulds or yeasts aided by enzyme (Dessonki *et al*., 1981). The modern meat industry is based on efficient refrigeration. Carcasses of freshly slaughtered animals have surfaces that are both warm and wet and thus provide a perfect substrate for the growth of pathogenic and spoilage organism (Cortesi and Vaccaro, 1981). The combination of low temperatures and surface drying inhibits the growth of spoilage bacteria. To provide a long, safe, high quality shelf-life, the temperature of meat needs to be kept at a temperature close to its initial freezing point. Combination of high standard of hygiene and packaging with a refrigerated temperature during storage, transport and display can routinely extend shelf-life (Anon and Calvelo, 1980). If longer periods are required, then freezing the meat will extend the storage periods into years. Scientific studies show that freezing has little effect on the eating quality of meat. Overall the studies indicated that the meat may be slightly more tender after freezing. Freezing does increase the ultimate amount of drip from meat and this makes the meat less attractive (Miller *et al*., 1980). The preservative action of refrigeration is based on the prevention of multiplication of harmful bacteria, yeasts and moulds by the artificial lowering of the temperature. The failure of bacteria to grow at or below freezing depends mainly on the removal of the available water as ice, about 70% is removed at −3.5°C and 94% at −10°C. A further factor is the inhibition of the life processes of spoilage organisms at low temperature though the actual lethal effect is small. At a temperature of −8°C the multiplication of all
microorganisms stop and only resumes when the temperature is raised later to a suitable level. Neither fast (cryogenic) nor slow (blast) freezing completely destroys the bacteria commonly found in carcasses. Frozen meat which is thawed, yields and abundant supply of water and forms an excellent medium of bacterial growth. In addition, the pH of muscle, which remains constant while the meat is frozen, falls rapidly after thawing, but then again rises rapidly to create an environment which favours bacterial multiplication (Ockerman and Organisciak, 1979).

The surface growth of mould on meat is controlled not only by the temperature but by the relative humidity of the atmosphere. Some moulds are capable of growing on the surface of meat at several degrees below freezing point, but they require the presence of water in the surrounding atmospheres as otherwise they loose water by evaporation and wither (Singh and Wang, 1977). Chilling scarcely affects the flavour, appearance and nutritional value of meat and is particularly useful for short-term preserving. The meat is maintained at about ±1°C preferably in the dark, for light accelerates the oxidation of fat with the liberation of free fatty acids and the production of rancidity (Reid, 1987). In quick chilling, meat is said to have better keeping quality because lower air temperatures retard the rate of growth of bacteria on the surface of carcasses where their concentration is most pronounced. Shrinkage of meat is reduced substantially, through quick chilling, which is an important economic factor. The bloom is said to be enhanced by quick chilling. Quick chilling refers to a rapid lowering of carcass temperature starting not later than 1 hour after slaughter.

Toughness of meat i.e. "Cold shortening" occurs owing to extreme contraction of muscles subjected to temperature around 10°C
before the muscles were in normal rigor i.e. while the pH is still above 6.2 and adenosine triphosphate (ATP) is still present. Cold shortening can also occur with even part of the carcass e.g. the loin, with fairly slow chilling e.g. 10-12 hrs when the pH will be below 6.2 and rigor will have taken place with the complete disappearance of ATP from the muscle, or not chilling to below 10°C in less than 10 h. Cold shortening can also be prevented by the use of electrical stimulation, which advances the onset of rigor (Oscroft et. al., 1987).

The changes in free fatty acid amount and composition and in Thiobarbituric acid reactive substances (TBARS) were simultaneously determined in chicken breast and thigh muscles at intervals between 1 and 14 days of storage at 4°C. The rates of lipid hydrolysis were fast in the first 3 day and then slowed until day 14. Phospholipids showed the same pattern but hydrolysis of triacylglycerols was linear at least in thigh muscles. Oxidation increased linearly during storage. Thigh muscles contained 3 times more free fatty acids than breast muscles and 2 to 4 times less TBARS suggesting that lipolysis did not favour lipid oxidation although both increased concomitantly. (Alasnier et. al., 2000).

Although tissues of live animals may be sterile, meat becomes contaminated with a variety of microorganisms during slaughtering and subsequent handling. As a consequence, meat must be cooled rapidly to approximately 0°C. During storage at temperature near 0°C spoilage of meat is likely to be superficial unless it has been treated to distribute surface bacteria throughout the mass. If surface drying occurs during refrigerated storage, growth of bacteria will be minimized but certain moulds, primarily in the genera Thanamidium, Cladosporium and Sporotrichum may grow (Broughall and Brown, 1984). In contrast, if the surface remains moist during chilling storage,
a superficial slime of gram negative bacteria will form. These organisms are mainly in the genera *Pseudomonas* and *Achromobacter*, although this designation presently is not entirely correct (Kilsby and Pugh, 1981). As with many other foods, the microbial flora of meats is altered by the freezing process. The principal bacteria in meats are gram negative in character. Contamination is generally higher in ground meats than in intact cuts of meat. Freezing reduces the number of viable bacteria in meats and the thawed product is a suitable substrate for their growth (Ward and Baj, 1988). The textural attributes, juiciness and tenderness of cooked meat are related to the ultra structural and chemical characteristics of the uncooked meat. Diminution of water-holding capacity will lead to excessive exudation of fluid prior to and during cooking. Tenderness of cooked muscle is a reflection of the arrangement interaction and physical characteristics of fibres and fibrils (Morley, 1986). Frozen foods are very close to and sometimes better than the available fresh counterparts. From a sensory as well as from a nutritional point of view, the quality is generally higher than for any other preserved product. The need for longer shelf-life and improved taste and quality have given rise to the modern food freezing industry. Artificial freezing of food stuffs started in the second half of the 19\textsuperscript{th} century. One of the first shipments of frozen carcasses of meat veal and mutton was carried in good condition all the way from Buenos Aires to Rouen, France by the vessel *Frigorifique* (HR, 1986).

Post mortem changes of sardine muscle during 15 days storage at 0 °C were studied to evaluate its quality and functionality. No microbial deterioration was detected since trimethylamine and histamine concentration remained low with final values of 1.62 mg/100 g and 0.00018 ppm, respectively. A final proteolytic activity
20 μg Tyrosine /min/g protein was detected. Lipid oxidation from moderate to advanced was detected after day 5 with values of 31.8 to 33.9 mg/kg and 26 mg /kg for peroxide value and thiobarbutaric acid value respectively. Muscle protein showed no gel forming ability. Extraction of myofibrillar protein decreased 45% and 81% at day 5 and 15 respectively. Overall results indicated that good quality was maintained during the storage period with a final K value of 50.7% when proper handling practices were implemented. (Pacheco-Aguilar et. al., 2000).

All food preservation methods especially refrigerated or frozen storage are directed to inhibit or decrease the rate of the various reactions responsible for food deterioration. All of these reactions are among other factors influenced by the temperature. The rate of the juiciness is dependent on the water holding capacity of the uncooked meat reactions will decrease at lower temperature. Cooling and chilled storage, therefore, are well proven ways to enhance the storage life of most food products. Studies of effect of refrigerated and frozen storage on tenderness of animals muscle have led to conflicting results. Some investigators have reported that post rigor muscles of poultry, beef lamb and fish decrease in tenderness during frozen storage (Miles, 1974). Some investigators have found tenderness to increase or remain unchanged during frozen storage. Temperature of frozen storage is a major factor influencing the rate of textural deterioration. As the frozen storage temperature is lowered, the rate of tenderness loss is decreased (Tsuchiya et. al., 1975). The major chemical alterations of lipids in frozen muscle are auto oxidation and hydrolysis. The development of oxidative rancidity in frozen stored muscle is caused by the accumulation of carbonyl compound formed during auto-oxidation of muscle lipid. Changes in the pH of meat during refrigerated and frozen
storage may contribute to myofibrillar protein alteration. Changes in the pH of muscles during frozen storage are dependent on storage temperature, salt composition, physiological state, buffering capacity of protein and enzyme action. The pH changes taking place during frozen storage regardless of the temperature, it is unlikely that quality deterioration of these food products can be attributed to changes in pH alone (Miles, 1974). It is known that the faster the rate of breakdown of ATP in muscle the more rapid is the onset of rigor mortis and the greater the release of fluid from the muscles. Frozen meat stored too long becomes dry, rancid and less palatable, the most important change being the breaking down of the fat into glycerin and free fatty acids, with the production of rancidity. The better the quality of meat the less trouble one encounters in its storage. The storage temperature, the degree of fluctuation in the storage and the type of wrapping or packaging in which the meat is stored are generally thought to have the main influence on frozen storage life. Some bacteria are destroyed by freezing, but low temperatures merely inhibit the growth and multiplication of most until conditions favourable to their growth appear. Freezing is therefore no great value in rendering a carcass affected with pathogenic bacteria safe from human consumption, nor are the bacteria commonly found on beef carcasses destroyed by slow sharp freezing unless the curing smoking like treatments are provided before storage (Shared et. al., 1989). When frozen muscles are stored without an adequate moisture proof barrier an opaque dehydrated surface known as freezer burn is formed caused by the sublimation of ice on the surface region of muscle when the water vapour pressure of the ice is higher than the vapour pressure in the environmental air. The development of freezer burn can be minimized by aging beef muscle
for at least 24 hrs prior to freezing and by dipping muscles in curing solution (Hall and Alcock, 1987).

2.13 Packaging of Meat.

Packaging is an art and a science of delivering product to the consumer in an attractive manner with minimal cost and to protect the product from any physical, chemical or microbiological spoilage. (Dushyanthan et. al., 2000). Attractive display of fresh meat packed for retail sale requires the colour to be acceptable to the consumer and should compare favorably with that of the unwrapped product (Mills and Urbin, 1960).

The development of meat packing during the last 20 years has brought about major changes in the pattern of its distribution and marketing (Stanley et. al., 1972). At wholesale level, the traditional movement of carcasses or part-carcasses between abattoir and retail outlet has largely been replaced by the distribution from cutting and packing plants of boxed, boneless joints, vacuum packed to give long life without weight loss. Moreover, these partly prepared joints are convenient to use and economical in terms of labour and space. At retail level, in supermarkets and for self-service countries the selling of fresh and frozen meat is possible only with correctly used packaging. In-store packing of meat cuts is being supplemented, and may eventually be replaced completely by the distribution of pre-packed, the limitations of conventional methods of packing have become apparent. New techniques are being devised to extend the display life of fresh meat (Bakker and Marilyn, 1986). The most fundamental function is to contain and utilize the product in to sizes and cuts required for the market. This may mean single serving packages for retail or large packages for food service. Packaging must
have both high strength and integrity. Thermally processed, nitrite and antioxidants cured and treated meats often require more physical protection compared to fresh meats (Benning and Calvin, 1983). Products that are packaged at the same location as they are sold, such as fresh meat, do not require such a high degree of integrity or strength in their packaging. Sanitation and consumer appeal are of primary concern for these products. Meat packaging must be a barrier between the product and the environment in order to protect and preserve for a longer time. The degree of barrier required depends on the type of meat. Meats, which are stored at room temperature for months, must be protected from the effects of O₂ (Brody, 1989). Packaging for such products must be a high barrier to O₂ ingress and loss of water. Frozen meat must also be protected against water loss. Packaging must also provide a barrier against biological, chemical and physical agents that would detract from quality or safety (Brown, 1992). Components of the packaging material that might transfer from the package to the product during storage must not be toxic (Mead, 1983).

Packaging must allow the meat product to be produced and distributed efficiently and economically (Brody, 1989). The effects of different packaging systems are best studied by first considering the behavioral properties of meat which are directly influenced as described below;

(i) Water loss- Uncovered meat losses weight by evaporation of moisture which dries and darkens the surface as during frozen storage water is lost from the meat surface by sublimation to colder surfaces in the vicinity,

(ii) Tissue respiration- Meat is a biological material containing respiratory enzyme system which continues to function after death. They consume oxygen and produce carbon dioxide at a
fast rate for 1-2 days, and thereafter at a slower rate. Respirations confined to the surface layer into which oxygen diffuses. The depth of penetration depends on a balance between oxygen concentration at the surface, driving inwards, and tissue respiration which consumes the oxygen as it becomes available. Aerobic bacteria on the surface of the meat may also consume oxygen and produce carbon dioxide. While the bacterial load is low, the gases involved may be negligible in quantity, but during storage, spoilage may increase to a level where microbial respiration is comparable with that of meat tissue,

(iii) Microbiology – After dressing, the surface of a carcass may carry between $10^2$ and $10^4$ bacteria/ cm$^2$ and after butchery, joints and pieces of meat for packing are likely to carry considerably higher numbers. They may comprise a large range of different bacteria some of which can grow between 0°C and 5°C,

(iv) Colour – Lean meat colour derives from the muscle pigment, myoglobin and its reaction with available oxygen. In meat which has been exposed to air for several hours the penetration depth may be 6-7 mm. In the presence of oxygen, myoglobin is oxygenated to oxymyoglobin, or oxidized to metmyoglobin. The relative amount of these two pigment forms depends on the partial pressure of oxygen. Oxymyoglobin, the bright-red ferrous form of the pigment is favoured by high oxygen concentration. Oxygenation of myoglobin is rapid, the surface of beef in air will appear red within half an hour at 5°C. On the other hand, oxidation to metmyoglobin is slow, and appears first as a thin brown layer at the limit of oxygen penetration. The
importance of colour as a marketing attribute of meat is well established, especially for self-service retailing customers, used to seeing bright-red meat prepared for sale, associate this colour with good eating quality, although there is little correlation between the two. The colour of frozen meat is initially dependent on freezing rate and the consequent size of ice crystals in the surface layer. The principal deterioration in frozen meat during storage is photo-oxidation of the pigments. Under direct illumination frozen meat oxidizes from the surface inwards, whereas chilled meat oxidation begins in the subsurface layer and progresses towards the surface. As with chilled meat, loss of redness is detrimental to the marketing of frozen meat (Takashi, 1990).

2.13.1 Influence of Packaging.

The primary function of meat package to prevent contamination, is easily accomplished with the range of plastic materials available today. However, packaging may also be used in a wider context to improve storage life, attract the customer and more recently, to extend the period of attractive display (Hotchkiss, 1990).

These functions are achieved by creating a new environment for the meat, the most important change being modification of the gaseous atmosphere. The composition of this atmosphere determines the colour of meat and the nature of spoilage, which develops. Gases dissolve in meat according to their partial pressures and reactions in the meat may consume or produce gases (Hotchkiss, 1990). The gases of primary importance are carbon dioxide and oxygen. Carbon dioxide has bacteriostatic properties and oxygen is consumed by respiration of tissues and aerobic bacteria (Hanlon, 1992). Because of the importance
of oxygen for meat colour, any restriction in supply to the muscle pigment will affect colour (Lawrie, 1991). All commercial packages of meat contain a certain amount of oxygen at some time, and a packaging system differ principally in the quantity available to the meat initially and thereafter during storage. The effects which environmental conditions have on meat microbiology have been reviewed in detail by Paine and Heather (1983).

2.13.2 Packaging material.

The growth of meat packaging has been accompanied by the development of plastic properties which are appropriate for a variety of packaging system (Brody, 1989). The choice of films for packaging meat is largely determined by their moisture and gas permeability (Dainty, 1983). The more important plastics now in use in meat packaging are:

(i) Polystyrene- It is colourless, transparent and hard, has a fairly high tensile strength and a high refractive index. It is resistant to acids and bases and is insoluble in aliphatic hydrocarbons and the lower alcohol.

(ii) Low Density Polyethylene (LDPE)- It is a tough, translucent, waxy solid with a density that can vary between 0.916 and 0.935 g/cm³. It has very high impact strength and in fact does not normally break under the usual impact strength test conditions. It retains its strength at temperatures down to about –60°C to 70°C. It is a good barrier to moisture vapour but it rather more permeable to gases and it is not normally suitable as a packaging material for oxygen-sensitive foods.

(iii) High Density Polyethylene (HDPE)- It is tough, slightly translucent material with a rather less waxy feel than that of
LDPE. It is rigid and hard but has a lower impact strength. Density range from $0.95 \text{ g/cm}^3$ to $1.25 \text{ g/cm}^3$. It has a lower permeability to water vapour and gases by a factor of about five to six. It suits for meat packaging.

(iv) Polypropylene (PP) – It has a lower density (0.90 g/cm$^3$) than either of the polyethylene, and harder and has a higher softening point. The permeability to oxygen, carbon dioxide and water vapour lies between LDPE and HDPE. The impact strength of PP is lower than that of HDPE although it is still adequate for most purposes.

(v) Polyvinyl Chloride (PVC) - PVC is hard, brittle material with a density of around 1.35-1.4 g/cm$^3$. Unplasticised PVC has excellent resistance to oil, fats and greases and is also resistant to acids and alkalis. The gas permeability is lower, however, so that good protection against rancidity can be given to oils and fats. The biggest usage for PVC in rigid packages for food is in bottles, for a wide range of food stuffs, including edible oils, fruits squashes etc.

(vi) Cellulose Acetate- It is a tough, hard material and is unlike celluloid (cellulose nitrate), non-flammable. Plasticisers are normally added to give suitable material. It is sensitive to water absorption and undergoes dimensional changes. It has a high permeability to water vapour and to gases but the triacetate is superior in this respect. Cellulose acetate sheet has very good clarity and it was this property that led to its early use for packaging sweets and chocolates.

(vii) Acrylics- Acrylics have many desirable properties including high strength, hardness, clarity and barrier properties.
(viii) Polyethylene Terephthalate (PETP)-PETP bottles are stretch below moulded and are strong and well able to resist the pressure generated by normal carbonated soft drinks. Gas barrier properties are also good. The softening point is high. It is posses excellent clarity.

(ix) Urea formaldehyde-It is resistant to solvents but are decomposed by strong acids and attacked by strong alkalis. Their main use in food packaging is for screw cap closures.

2.13.3 Packaging Methods.

2.13.3.1 Vacuum Packaging.

The introduction of vacuum packaging for the distribution and storage of chilled beef has been hailed as the greatest innovation in meat handling during the last 25 years (Gibbs and Patterson, 1977).

Vacuum packaging involves enclosing large boneless joints inflexible plastic containers to prevent moisture, loss and exclude oxygen from the meat’s surface. The plastic materials used for vacuum packaging must have low moisture and gas permeabilities and be strong enough to hold heavy joints (Gardner, 1983).

Use of vacuum packaging to increase shelf-life and to maintain quality of muscle foods has recently been reviewed (Sahoo and Anjaneyulu, 1995). During refrigerated storage of meat, vacuum packaging is most protected of various characters, viz, TBA value, visual colour, metmyoglobin, Hunter ‘a’ value etc. (Brewer and Wu, 1993).

When meat is vacuum packed the contaminating flora are exposed to an atmosphere containing 20-25% CO₂ and less than 1% O₂ (Zaemora, and Zaritzky, 1987). Both the high CO₂ and low O₂ tension depress the growth of *pseudomonas* and facultative anaerobes.
predominate: lactic acid bacteria, *B. thermosphacta* and *Enterobacteriaceae*. The antimicrobial activities of the *Lactobacilli*, coupled with the low storage temperature, combine in a synergistic manner to extend the shelf-life of vacuum packaged meat (Enfors and Molin, 1981).

### 2.13.3.2 Modified Atmosphere Packaging of Meat.

Vacuum packaging has the inherent disadvantage that both package and meat are subjected to mechanical strain. Mechanical pressure on the meat may increase drip loss and if bone is present and adequately covered with a suitable material, the pack may be ruptured. As an alternative to vacuum packaging, attempts have been made to store meat under various gaseous atmosphere, a process referred to as modified atmosphere packaging or MAP. The intention has generally been to preserve the fresh meat colour and to prevent an aerobic spoilage by using high concentration of O₂ (50 – 100%) along with 15-50% CO₂ to restrict the growth of *Pseudomonas* and related bacteria. Since containers for MA packaging are good gas barrier, the internal atmosphere will be modified by the meat during storage. The relative volumes of gas and meat are therefore important in determining the progress of the changes in concentration of gases during storage, and cognizance must also be taken of the high solubility of CO₂ compared to the relatively low solubility of O₂ and N₂ in meat (Finne, 1984). The antimicrobial effect of carbon dioxide CO₂ is well documented but comparison of the large number of often contradictory studies investigating the effect of CO₂ on chemical quality changes is lacking. The amount of absorbed CO₂ varies from 0-1.79 h CO₂/kg meat depending on the applied packaging and storage conditions, which clearly demonstrates the necessity of optimizing these...
conditions with respect to the required amount of CO₂. Absorption of large amounts of CO₂ in meat tissue cause a minor decrease in pH due to the dissociation of the produced carbonic acid to bicarbonate and hydrogen ions. A decrease in pH might affect other chemical quality parameters but this is not observed to be the case in the reviewed studies and general detrimental effects of CO₂ cannot be found for colour, weight loss or lipid oxidation. However, elevated CO₂ levels can cause pore formation in cooked meat (Jakobsen and Bertelsen, 2002).

Frozen meat is stored and displayed between −10°C and −30°C, at which temperatures microbiological growth is arrested (Taylor, 1985). Therefore the changes in meat most influenced by packaging are those associated with appearance, colour and the absence of frost inside the package. Being the two most important features relating to appearance, when frozen meat and meat products are stored without an adequate moisture vapour barrier, an opaque dehydrated surface known as “freezer burn” is formed (Waites, 1988). Oxidative changes are even more effectively reduced through exclusion of air by means of vacuum packaging (Hood, 1984). A large volume of meat are cured and therefore understanding of the changes which meat pigment undergo during the curing process is important (Parking and Brown, 1982). During storage, cured meats deteriorate, in the first instance because of discolouration, secondly because of oxidative rancidity in the fat, and thirdly on account of microbial changes. The latter having become of some what greater importance since the advent of prepackaged method of marketing. Smoke, traditionally produced by the slow combustion of hard woods, inhibit microbial growth, retards fat oxidation and imparts flavour to cured meats (Lundquist, 1987).
In any discussion of the shelf-life of packaged meats, it must be borne in mind that the comparison of published data is difficult and the making of generalizations fool hardly since there are large number of variables which interact to determine the actual shelf-life. The most important of these variables are temperature, treatments applied to the raw meat. Generally it can be said that laboratory scale trials control temperatures over a much smaller range compared to commercial scale trials, and this is the reason for the longer shelf-life obtained in laboratory scale trials over that obtained in commercial production. Other important variables include the microbiological status of the meat at the time of packing and the method used to determine the end of shelf-life of the meat. Clearly attempting to draw generally applicable conclusions from numerous published reports where the magnitude of these variables differ would only be misleading.