INTRODUCTION & REVIEW OF LITERATURE
1. INTRODUCTION AND REVIEW OF LITERATURE

Meat has long been a central component of the human diet both as a food in its own right and as an essential ingredient in many other food products for at least 2 million years. Human genetic make up and physical features have been adapted over 4-5 million years for a diet containing meat. The main function of food is to provide nutrients to meet the metabolic needs of an individual. Meat and meat products are an important part of diet in many parts of the world, especially in developed nations where consumption of animal protein per capita is at its highest (FAO, 2005). Meat is a highly nutritious and versatile food and has a special place in human diet. Meat is a good source of readily digestible protein and it contains all the essential amino acids in a balanced form (Lawrie, 1998). The primary importance of meat as a food lies in the fact that when digested, its protein is broken down releasing amino acids; these are assimilated and ultimately used for the repair and growth of cells. In the United Kingdom, meat and meat products supply 30% of dietary protein intakes (Ministry of Agriculture Fisheries and Food, 1999).

Meat is a nutrient dense food, providing valuable amounts of many essential micronutrients. It supplies fatty acids, vitamins, minerals, energy and water and is involved in the synthesis of proteins, fat and membranes in the body. With a limited range of foods available in primitive societies throughout history, meat provided a concentrated source of a wide range of nutrients (Sanders, 1999). Meat is also a good source of the vitamins of B group but usually low in fat-soluble vitamins (A, D, E, and K) and vitamin C. It is also a good source of minerals like Cu, Zn, Na, K, Fe and P. Meat contains Fe and Zn bound to heme protein, which is readily incorporated into the body (Newmann, et al., 2002). However, it is low in carbohydrates (USDA, 2000). All of the iron in our body comes from our diet, and meat is a rich dietary source. Concern about iron deficiency is one nutritional reason for recommending eating at least some meat (WHO, 1990; COMA, 1998).
1.1. Livestock scenario

The livestock wealth of India is one of the richest in the world. It is unique in number and diversity. The meat and meat products are treated as the by products of animal husbandry. The entire gamut of meat production, processing and marketing is a neglected one in the country. Marketing of livestock, meat, processed meat products and by products has remained more or less unorganised in India (Das et al., 2006).

In India the acceptance of meat as a part of diet is very often governed by the regional, cultural and religious bias. In spite of religious beliefs and socio-economic constraints, it still finds a prime place in the diet of nearly 70% Indians (Padda and Thind, 2002). However, due to low purchasing power and local food habits, the majority of Indian population consume meat occasionally. The per capita meat consumption is about 2.5 kg in India compared to the global average of 32 kg per annum and over 100 kg in many developed countries (Chatterjee, 1999). Consumer preference for goat or lamb meat is dictated by cultural and traditional background and the socio-economic status of the community. In India goat, lamb and chicken meat are widely preferred, while bovine meat and pork are consumed only by a small segment of the population. Meat produced in India is characterised by low yielding non-descript animals. Sheep, goat and pig are raised primarily by a large number of small shepherds and farmers, with little or no land holdings.

Livestock marketing as it relates to cattle, buffalo, sheep, goat and pig begins when the animal leaves the farm and enters the marketing channel. It includes transportation, marketing, organisation services, information services and even some aspects of processing. Meat processing includes the slaughter and sale of carcasses for wholesale and retail outlets. In the whole process large numbers of people are employed in handling, transportation and processing of the meat, involving huge transactions. Marketing also involves the distribution of products, which require huge work force, thereby creating vast employment opportunity. As a result much importance is given to livestock production sector, since there is an increasing trend in meat consumption and trade, the demand of which is being more or less matched
by enhancing production. Population of different livestock species and their contribution to meat production is depicted in Table-1.

Table 1: Population of different livestock species (in million) and meat production

<table>
<thead>
<tr>
<th>Species</th>
<th>Population</th>
<th>Meat production (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>India</td>
<td>World</td>
</tr>
<tr>
<td>Cattle</td>
<td>185</td>
<td>1355</td>
</tr>
<tr>
<td>Buffalo</td>
<td>98</td>
<td>174</td>
</tr>
<tr>
<td>Sheep</td>
<td>63</td>
<td>1080</td>
</tr>
<tr>
<td>Goat</td>
<td>120</td>
<td>807</td>
</tr>
<tr>
<td>Pig</td>
<td>14</td>
<td>960</td>
</tr>
<tr>
<td>Chicken</td>
<td>430</td>
<td>16725</td>
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</tbody>
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1.2. Production of meat

The marketing of meat and meat products is more complicated because of the highly perishable nature which may further affect the availability at short notice depending on fluctuations of demand, the unfavourable hot and humid climatic conditions particularly to India, lack of transportation facility as well as religious taboos of consumers (Das et al., 2006). The attitude of policy makers to meat is to treat it as a by-product and not as a commodity.

The meat meant for export has to pass through ante-mortem and post-mortem examination after 24 h resting period of the animals. The meat meant for local market is chilled for 24 h to bring down the pH below 6. Thereafter, it is deboned and deglanded. The meat is then packed into different cuts and frozen at -40°C for 12 h to bring down the deep bone temperature to -18°C (Qureshi and Ranjhan 2003). The frozen meat is stored in cold storage for export.
The meat produced for the domestic market is sold as hot meat. It is estimated that approximately 2% of meat is converted into processed meat products (Rao & Mahendrakar 2003). India's share in international trade is hardly 1% and can be increased substantially. Buffalo is the major contributor accounting for more than 95% of total meat exports, whereas goat and sheep meat contributed very little (5%) to the total meat export (Table-2). Poultry meat and meat products need to be promoted with better processing and marketing approaches. India has got several advantages in meat export like leanness of meat and low cholesterol content, lower cost of meat production, proximity to importing countries, good upcoming modern infrastructure, safer meat free from toxic residues etc. However, meeting the phyto sanitary standards through implementation of good manufacturing practices (GMP) and total quality management (TQM) has a greater significance under the changed world meat trade scenario (Salendra Kumar et al., 2002).

Table 2: Export of meat and meat products and casing during 2002-2005 (Value in crores)

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Quantity (MT)</td>
<td>Value (Rs)</td>
<td>Quantity (MT)</td>
<td>Value (Rs)</td>
<td>Quantity (MT)</td>
<td>Value (Rs)</td>
</tr>
<tr>
<td>Buffalo meat</td>
<td>297,897</td>
<td>1305.45</td>
<td>343,817</td>
<td>1536.77</td>
<td>300,971</td>
<td>1615.59</td>
</tr>
<tr>
<td>Goat &amp; Sheep meat</td>
<td>4,973</td>
<td>39.95</td>
<td>16,821</td>
<td>110.39</td>
<td>8,885</td>
<td>79.36</td>
</tr>
<tr>
<td>Poultry products</td>
<td>26,450</td>
<td>156.47</td>
<td>415,228</td>
<td>202.40</td>
<td>264,607</td>
<td>154.11</td>
</tr>
<tr>
<td>Animal casing</td>
<td>8,296</td>
<td>140.27</td>
<td>732</td>
<td>12.43</td>
<td>552</td>
<td>12.57</td>
</tr>
<tr>
<td>Processed meat products</td>
<td>669</td>
<td>4.8</td>
<td>986</td>
<td>7.63</td>
<td>107</td>
<td>1.56</td>
</tr>
</tbody>
</table>

1.3. Meat composition and nutritive value

In a broad sense the composition of meat can be approximated to 75% of water, 19% of protein, 3.5% of soluble non protein substances and 2.5% of fat, but an understanding of the nature and behaviour of meat and of its variability, can not be based on such a simplification (Lawrie, 1998). On the contrary it must be recognised that meat is the post mortem aspect of a complicated biological tissue, viz. muscle. Meat is composed of lean tissue or muscle fibre-cells, fat and connective tissue. Fat or adipose cells can be found in up to three depots or locations in meat. Fat can be deposited intramuscularly as marbling or contained between muscles or it can be found as external fat or subcutaneous fat. These three major components of meat, fat, lean or the myofibrillar components and connective tissue affect meat quality in different ways (Miller, 2002).

A comparison of the composition of meat from different animal sources is given in Table-3.

<table>
<thead>
<tr>
<th>Meat</th>
<th>Calories</th>
<th>Total fat g/100g</th>
<th>Saturated fat g/100g</th>
<th>Protein g/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>288</td>
<td>18.8</td>
<td>8.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Pork</td>
<td>364</td>
<td>28.2</td>
<td>10.2</td>
<td>24.7</td>
</tr>
<tr>
<td>Lamb</td>
<td>276</td>
<td>18.8</td>
<td>8.6</td>
<td>25.9</td>
</tr>
<tr>
<td>Chicken</td>
<td>141</td>
<td>4.1</td>
<td>1.3</td>
<td>24.7</td>
</tr>
</tbody>
</table>

1.3.1. Lipids in muscle system

Animal fat is composed chiefly of neutral fats and phospholipids. The neutral lipids are principally glycerol esters of straight chain carboxylic acid of triglycerides, which typically contains 16-18 carbon atoms (Dugan, 1971). Phospholipids are found in animal fats in small percentage. They play a key role as structural and functional components of cells and membranes. Phospholipids constitutes a major portion of intra-muscular lipids of muscle.
foods. They normally comprise about 0.5 to 1% of lean muscle. As the total lipid in a muscle decreases from 5 to 1%, the percentage of phospholipid to total lipid increases from less than 10% to nearly 70% (Lawrie, 1998). Polyunsaturation of phospholipids fraction is about 15 times greater than that of the triacylglycerol fraction (Igene and Pearson, 1979; Igene et al., 1985). There is also significant variation in total phospholipids content among species from muscle to muscle location in the same animal. Poultry and fish muscle is known to be higher in phospholipids than red meat (Igene et al., 1979).

Meat contains a mixture of fatty acids both saturated and unsaturated. The predominant saturated fatty acids in meat are stearic acid (C_{18:0}) and palmitic acid (C_{16:0}). In general terms saturated fats are known as the "bad" fats as they tend to raise blood cholesterol and cause atherosclerosis. However, not all saturated fats are equal in their effects on blood cholesterol. For instance stearic acid does not appear to raise blood cholesterol (Bonanome and Grundy, 1988) or other thrombotic risk factors (Kelly et al., 1999, 2001). Stearic acid is a prominent saturated fat in meat, for example it accounts for approximately one third of the saturated fat in beef. Similarly palmitic acid, another major saturated fat in meat does not consistently raise blood lipids. On the other hand myristic acid (C_{14:0}) is the most atherogenic fatty acid, it has four times the cholesterol raising potential of palmitic acid (Ulbricht and Southgate, 1991). Myristic acid is found only in minor quantities in meat.

Meat contains a mixture of unsaturated fatty acids, polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). MUFAs are the dominant unsaturated fatty acid in meat and they account for approximately 40% of the total fat in meat. Meat and, meat products are the main contributors to MUFAs in the British diet, supplying 27% of total MUFA intake (Ministry of Agriculture Fisheries and Food, 1999). MUFA in meat is oleic acid (cis C_{18:1}).

The PUFAs have a structural role as they are found in the membrane phospholipids and they are also involved in eicosanoid synthesis. There are
two types of PUFAs, the omega-3 (n-3) and the omega-6 (n-6). Meat and meat products supply 17% n-6 and 19% n-3 PUFA intake (Gregory et al., 1990). Linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) are essential fatty acids as it cannot be synthesised in the human body. In the body these are further elongated and desaturated to longer chain derivatives, arachidonic acid (C20:4 n-6), decosa pentenoic acid (C22:5 n-6), eicosa pentaenoic acid (C20:5 n-3) and decosa hexaenoic acid (C22:6 n-3). These are found in useful quantities in meat. The positive effects of the consumption of n-3 fatty acids are indicted by GISSI trials for the reduction in the coronary heart disease deaths (GISSI, 1999) in addition to anti-inflammatory and anti-tumour urogenic properties. Meat and fish are the only significant sources of preformed very long chain n-3 PUFAs in the diet. Species difference in the fatty acid composition was reported by Enser et al., (1996).

An emerging dietary benefit for meat is conjugated linoleic acid (CLA). The best natural dietary sources of CLA are ruminant products such as beef and lamb (Ma et al., 1999). Factors affecting the CLA content of meat include the breed age and diet of the animal (O’Shea et al., 1998, Mulvihill, 2001). Since CLA is formed predominantly in the rumen the CLA content of ruminant meat, beef and lamb is much higher than non-ruminant meat such as pork, chicken and game (Chin et al., 1992). Meat and meat products supply approximately a quarter of dietary CLA in Germany (Fritsche and Steinhart, 1998). CLA appears to have a variety of potential health benefits. It has been shown to have tumour-reducing (Belury, 1995, IP et al., 1999) and atherosclerotic-reducing properties (Gavino et al., 2000, Nicolosi et al., 1997). CLA may also reduce adiposity (Park et al., 1997, West et al., 1998) and delay the onset of diabetes (House Knecht et al., 1998). It appears from the analysis of 14 European countries that the fat content of meat does not correlate with the percentage of trans fatty acid content (Hulshof et al., 1999). Trans fatty acids did not influence LDL and HDL cholesterol and a weak inverse association was found in total serum cholesterol (Van De Vijver et al., 2000).
The effect of including lean red meat (beef, veal and pork) and lean white meat (Poultry and fish) in the diet, on blood cholesterol of people with hypercholesterolaemia was studied by Davidson et al., 1999. It was effective in reducing both total cholesterol and LDL (bad) cholesterol. There is now a wealth of studies showing similar results (Scott et al., 1990; Mann et al., 1997; Davidson et al., 1999).

1.3.2. Protein in meat

Meat is a good source of protein and it contains all the essential amino acids. Muscle food proteins are characterised by high bio-availability (NPU value around 0.75 as against 0.5-0.6 for plant proteins), balanced amino acid profile and higher digestibility (Sharma, 2003). The positive effect of high protein intake were achieved without adverse effects on renal function (Skov et al., 1999 a,b). Some of the amino acids are limiting in plant protein like lysine in wheat, tryptophan in maize and sulphur containing amino acids in Soya bean. The damage to protein caused by cooking is of little practical significance and it can be argued that if there is meat in the diet it is likely that the quantity of protein would compensate for any shortfall in quality.

The nutritional quality of the proteins of meat rich in connective tissue is low since collagen and elastin are poor in the sulphur amino acids. There is only 0.8g of each per 100g of total protein compared with values of 2.6 and 1.3 of each respectively in “good meat”. Meat is tough to eat when it is high in connective tissue and such meat is often used for canning since the relatively high temperature involved in the sterilisation process partly hydrolyses the collagen so making the product more palatable. However, it still result in a product with NPU as low as 0.5 compared with a value of 0.75-0.8 for good quality meat (Bender and Zia, 1976).

1.3.3. Meat as a source of vitamins and minerals

Meat and meat products are important sources of all the B-complex vitamins including thiamine, riboflavin, niacin, biotin, vitamin B6 and B12, pantothenic acid and folacin. The last two are especially abundant in liver which with certain other organs is rich in vitamin A and supplies appreciable
amount of vitamins D, E and K. Some losses of B-vitamins occur during cooking and the amount lost depends upon the duration and the temperature of the cooking method.

Pork and its products including bacon and ham are one of the richest sources of thiamine. Pork contains approximately 5-10 times as much thiamine as beef or lamb (Higgs et al., 2002). Meat is the richest source of niacin. Meat and meat products supply more than a third of niacin intakes in Britain. Vit B12 is exclusively of animal origin as it is a product of bacterial fermentations, which occurs in the intestine of ruminant animals such as cattle, sheep and goats. Vit B12 is required to produce red blood cells and acts as a cofactor for many enzyme reactions. Deficiency of B12, B6 and D vitamins lead to the development of many diseases and physiological disorders. (Krajcovicova-Kudlackova et al., 2000; Baik and Russell, 1999; Deluca and Zierold, 1998).

Meats are excellent sources of some of the minerals such as iron, zinc, selenium, copper and manganese, and play an important role in the prevention of Zn deficiency, and iron deficiency (Scrimshaw, 1991). Iron deficiency anaemia (Walker, 1998) remains the most common nutritional disorders in the world today. It affects between 20-50% of the world’s population. Meat and meat products provide 14% of iron intake (MAFF, 1999). Meat has an important influence on iron bioavailability due to its enhancing properties and overall greater absorption capacity. Studies have shown that despite the fact that vegetarians have either a similar or a higher iron intake than their omnivore counterparts, their iron status is lower (Nathan et al., 1996; Ball and Bartlett, 1999; Wilson and Base, 1999).

Muscle foods are rich source of zinc that provides resistance against infection caused by viruses, bacteria and other pathogens. It plays an important role in immunity, reproduction (Aggett and Comerford, 1995) and cognitive development (Sandsted, 2000). Selenium, a trace element, is an important component of the enzyme glutathione peroxidase and acts as an antioxidant. It is considered to protect against coronary heart disease and
certain cancers, such as prostate. Meat contains about 10mg selenium per 100g, which is approximately 25% of our daily requirement. Beef and pork contain more selenium than lamb. Bioavailability of selenium from plant foods was thought to be greater than that from animal foods, but recent data demonstrate that meat, raw and cooked are higher bio available source (Shi and Spallholz, 1994).

Meat also contains phosphorous, a typical serving provides roughly 20-25% of an adult’s requirement. Phosphorous has important biochemical functions in carbohydrate, fat and protein metabolism. Meat also provides useful amounts of copper, magnesium, potassium, iodine and chloride.

1.4. Meat quality

Quality can be defined as a degree of excellence. Meat quality in broad terms can be described as that include overall nutritive value, edibility, wholesomeness and freedom from disease. In other words quality is that which the public likes the best (St. Angelo, 1996). The approach to the definition of quality could be viewed by distinguishing between two extremes. One view is that quality may be regarded as a construct in the mind of the customer which is highly subjective and which cannot be measured consistently and objectively. The other extreme view is that quality is objectively defined and exists only to the extent it is scientifically measurable. The subjective view is taken to the extreme in the following statement “Quality cannot be defined it can only be recognised”. The objective view is taken to the extreme in this statement. ‘Quality exists only to the extent it can be measured with laboratory methods’ (Becker, 2002).

1.4.1. Consumer perceptions of quality

Quality attributes of a food product is shown in Fig-1. Sensory studies are frequently used to evaluate the quality of meat and meat products. According to these studies preferences for meat seem to be strongly affected by colour / appearance and texture and to a lesser extent by changes in flavour. Texture may be understood with juiciness and tenderness as different dimensions of textural quality (Risvik, 1994). Flavour may be
regarded as consisting of taste and smell. However, eating or sensory quality is the only one dimension of consumer perceived quality. Many consumer surveys in several countries of the European union clearly demonstrated that consumers not only care about eating quality but also other quality attributes such as product safety (in the light of out breaks such as bovine spongiform encephalitis (BSE) and foot-and-mouth disease), animal welfare, ecological production methods, or the presence of residues or additives such as hormones or antibiotics used in animal production (Becker, 2002).

![Quality attributes of a food product](Erdstieck, 1989)

Quality standards for meat, as laid down in mandatory public quality schemes are predominantly targeted towards food safety and hygiene, though they do cover eating quality, animal welfare and ethical issues. Food hygiene is defined "as all measures necessary to ensure the safety and
wholesomeness of food stuff". The HACCP system is made mandatory, but the introduction in the agricultural sector is still limited.

Eating quality could be improved further by integrating sensory research with research in meat science. In an ideal world from the quality management perspective, research should start with consumer perceptions of important attributes and investigate profitable ways of producing the product and process characteristics linked to these quality attributes.

1.4.2. Raw meat quality

The understanding of raw meat eating quality and consistency is an important component of meat production systems. Meat quality encompasses the visual appearance and eating quality. Both of these quality factors can be influenced by ante-mortem and post-mortem production factors (Miller, 2002).

1.5. Fat component

Intra muscular fat content has been shown to affect flavour, juiciness, tenderness and visual characteristics of meat. In general as fat content increases, palatability increases. As intra muscular fat increases, consumers perceive that the meat is juicier. Savell and Cross (1988) stated that fat may affect juiciness by enhancing the water holding capacity of meat by lubricating muscle fibres during cooking, by increasing the tenderness of meat and thus the apparent sensation of juiciness or by stimulating salivary flow during mastication.

Intra muscular fat also has an indirect relationship to meat tenderness. As animals grow and develop, fat is deposited sequentially and marbling is the last fat depot to fill. Marbling therefore is an indication of growth and nutritional status of animals. Marbling has been shown to affect consumer and trained sensory panel meat flavour attributes (Miller et al., 2000). As fat level increases consumers tend to like the flavour of beef and pork. Fat has a characteristic flavour and has been identified as one of the major components of the meat flavour (Johnsen and Civille, 1986). When meat contains very low levels of fat, the predominant flavours are those associated with the lean such
as cooked beef lean, serumy, bloody, grainy, metallic, livery/ organy, and brothy (Johnsen and Civille, 1986; Lyon, 1987). As the level of fat marbling increases, the cooked fat aroma increases in meat and this aroma can assist in decreasing or masking flavour attributes associated with lean, providing a balance of flavours.

1.6. Lean or muscle fibre component

The major component of meat is lean and lean is mainly composed of muscle fibres. Muscle proteins are the components in the muscle fibre that binds water or interacts with water to hold it in the muscle fibre. The structural integrity and the ability of the muscle proteins to bind water affect meat tenderness and juiciness. In living tissue muscle fibres are elastic and have the ability to contract and relax. Through the conversion of muscle to meat, muscle proceeds through rigor mortis where muscle fibres lose their ability to relax and results in loss of much of their elasticity. Strength of the structural components within the muscle fibre also has been related to meat tenderness. Degradative enzymes work to break apart the muscle fibre structural apparatus. The major enzyme system shown to affect post-mortem muscle fibre degradation is the Calpain proteolytic system (Koohmaraie, 1988, 1992; Goll et al., 1995). Actin and myosin, the most abundant proteins in the muscle fibre bind the majority of water within the muscle fibre. As the net charge of protein becomes either more positively or negatively charged, ionic forces increase and water is bound or held more tightly to the protein. An increase or decrease in meat pH will change the ratio of positive and negative charges on protein side chains and will alter the ability of muscle proteins to bind water. Therefore meat pH is an important component of meat quality as it relates to the ability of muscle proteins to bind water and the subsequent juiciness and tenderness of the meat.

1.7. Meat colour

Myoglobin is the major pigment-containing compound in meat. The level of myoglobin, the oxidative state of the heme-ring within myoglobin and what is bound to the myoglobin ligand affects meat colour. The level of myoglobin within a muscle is influenced by species, muscle function within the
animal and age of the animal. The state of iron within the pherforin ring of myoglobin (Fe$^{2+}$ or ferrous; Fe$^{3+}$ or ferric) and what compound is bound to the myoglobin ligand is mainly affected by storage conditions of the meat. As the myoglobin content increases, colour intensity of the meat increases from white or pink to very dark red (Miller, 1994). Muscle colour has been used as an indication of maturity and quality within meat species.

While myoglobin is the major pigment in meat, accounting for 50 to 80% of the total pigment, hemeoglobin, the major colour pigment in blood, can also contribute to meat colour. Conditions during slaughter that influence proper blood removal can influence hemeoglobin content. Higher hemeoglobin content results in darker meat. Other meat pigments, cytochrome catalase and flavins, exist within muscle and influence meat colour, but only to a very minor extent (Miller, 2002).

The three major components of meat, fat, lean and connective tissue contribute to meat quality with each uniquely contributing to meat juiciness, tenderness and flavour. These are not independent components, but they are interconnected and interact biologically within the muscle or meat system. Therefore, ante-mortem and post-mortem factors that affect meat quality may affect any of the three components and subsequently affect meat quality.

As meat quality is affected by the lipid, muscle fibre and connective tissue components within an animal, it is not surprising that animal genetics can play a major role in meat quality. It has long been understood that the unique genetic code for each animal regulates the production of protein and that genetic variation exists within meat animal species for important meat quality attributes.

Storage of meat can strongly affect quality positively and negatively. The positive effect of meat storage influences meat tenderness, also referred to as meat ageing. During refrigerated post-mortem storage, meat tenderness improves. The major factor responsible for post-mortem improvement in meat tenderness is degradation or proteolysis of muscle
proteins. The negative effect of meat storage on meat quality is due to microbial growth and/or lipid oxidation. Both of these processes result in reducing the shelf life of meat.

1.8. Meat microbiology

The microbial population of fresh meat is affected by a number of factors such as species, health and handling of the live animal, slaughtering practices, chilling of the carcass, sanitation during fabrication, type of packaging used and handling through distribution and storage (Young et al., 1988). The predominant organisms on the surface of fresh carcasses are gram-negative bacteria such as Acinetobacter, Aeromonas, Pseudomonas and Moraxella. Enterobacter and Eschrechia are also found. Gram-positive organisms are less abundant but commonly include Brochothrix, other lactic acid bacteria and Micrococcaceae.

Many factors influence the nature of the micro flora that develop in processed meat products during chill storage. The main factors are nitrite concentration, salt concentration (which affects the $a_w$), and presence of oxygen and permeability of the packaging film. The overall pH of the product may also play a part (Zeuthen and Mead, 1996). The most frequently observed types of bacterial spoilage in vacuum packed, sliced, cooked, cured meats are sweet/sour odour caused by lactobacilli, leuconostocs and streptococci (Mol et al., 1971); a cheesy odour caused by Brochothrix thermosphacta (Egan et al., 1980); a sulphide odour caused by Enterobacteriaceae and greening caused by hydrogen peroxide producing lactobacilli. Boerema et al., (1993) reported that the advantage of shelf life extension afforded fresh meats by carbon dioxide controlled atmosphere packaging, over that attainable in vacuum packaging, does not apply for sliced cooked ham. The presence of yeasts and moulds can be a problem for some processed meat products, especially on dry meat surfaces. The most common species of yeasts encountered on meat products are Candida and Rhodotorula. The presence of mould growth on packaged meats is usually an indication of a defective packaging system. Yeasts can cause spoilage when counts are as low as $10^5$ /g meat, whereas bacterial spoilage does not usually
occur below populations of $10^7/g - 10^9/g$ meat (Walsh and Kerry, 2002). Mc Daniel et al. (1984) reported that vacuum packed cooked beef steaks were organoleptically acceptable after 21 days of storage at $4^\circ C$, while modified atmosphere packaged steaks were not fit for consumption after 14 days of storage. Hintilian and Hotchkiss (1987) found that high CO$_2$ MAP was effective in inhibiting the growth of *Pseudomonas fragi*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Clostridium perfringens* on cooked, sliced roast beef.

1.9. Pale, soft, exudative (PSE) condition

Pale, soft, exudative (PSE) condition is a quality problem most commonly affecting pork but which also affects beef, lamb and poultry. PSE meat is characterised by its pale colour, lack of firmness and fluid (exudate) and dripping from its cut surfaces. When cooked, this meat lacks the juiciness of normal meat. PSE meat is unsuitable for processing, as it results in products, which have an undesirable pale colour and are having extra fluid.

PSE condition results from an abnormally rapid drop in the pH of the carcass after slaughter. This condition is most often noted in carcass of pigs suffering from porcine stress syndrome (PSS), but can also affect carcasses of normal pigs, which have experienced pre-slaughter stress.

Much less is known about the red, soft, exudative (RSE) condition, which has characteristics similar to PSE except that the meat has a darker colour. RSE also appears to be related to abnormally low pH levels and can result from pre-slaughter animal stress.

1.10. Dark, firm, dry (DFD) condition

At the other extreme, dark, firm, dry (DFD) condition is a quality problem affecting beef, pork and lamb. It is less appealing to consumers due to its unappealing dark colour and less pronounced taste. An additional problem with this type of meat is that it is more susceptible to spoiling since it has a higher than normal pH which is favourable for the growth of microorganisms. This condition occurs in animals, which have survived stress
1.11. Importance of pH in meat quality

Knowledge of pH and its importance in the quality of meat is an essential element in meat quality measurements. Post-mortem glycolysis results in the accumulation of lactic acid and a decline in the pH of muscle from about 7.2, at death, to roughly 5.5 after rigor mortis onset (Geesink, 1993). If initial glycogen is limited, the pH stays high and the meat remains DFD (as it is in the live animal). If the pH decline is rapid (affecting muscle proteins while still warm) or extensive (giving a low ultimate pH), the meat becomes PSE. Thus the pH of meat has a profound effect on colour, firmness and water holding capacity, as well as subtle effects on taste, tenderness and rate of post-mortem conditioning.

A pH <6 is typically taken as the critical point below which commercially important PSE develops in pork (Bendall and Swatland, 1988). In pork pH measurements at 45 min post-mortem (pH45) are used to detect the presence of PSE conditions (Somers et al., 1985). Recently Kircheim et al., (2001) showed pH45 displayed a high degree of reliability in predicting PSE.

The relationship between pH and temperature upto 24 h post-mortem is an important factor when considering ultimate meat quality. Recent research has focussed on the manner in which even the temperature of the meat at the time of sampling will influence the pH (Jansen, 2001; Bruce et al., 2001).

1.12. Processing of meat and meat products

The production of hygienic, wholesome and quality meat and meat products has become imperative to satisfy the consumers demands within the country and more so for exports.

Meat was originally processed to preserve it, but since the various procedures cause so many changes in texture and flavour, it is also a means
of adding variety to the diet. Processing also provides scope to mix the less desirable parts of the carcass with lean meat and in addition is a means of extending meat supplies by including other foodstuffs.

Meat is a highly perishable product and soon becomes unfit to eat and possibly dangerous to health through microbial growth, chemical change and breakdown of endogenous enzymes.

These processes can be curtailed by reducing the temperature sufficiently to slowdown or inhibit the growth of microorganisms, by heating to destroy organisms and enzymes (cooking, canning) or by removal of water by drying or osmotic control (binding the water with salt or other substances so that it becomes unavailable to the organisms). It is also possible to use chemicals to inhibit growth and very recently ionising radiation is employed.

Traditional methods that have been used for thousands of years involve drying in wind and sun, salting and smoking. Canning, dates from early in the 19th century and allows food to be stored for many years since it is sterilised and protected from recontamination. Retort pouches replace now cans and thermal processing carried out in flexible retort pouches (Ravishankar et al., 2002; Srinivasagopal et al., 2001).

1.12.1. Processing – General aspects

Processed meats are products in which the properties of fresh meat have been modified by the use of procedures such as mincing, grinding or chopping, salting and curing, addition of seasonings and other food materials and in many instances heat treatment. Most of these processes extend the shelf life of meat.

1.12.2. Convenience foods development

With rapid urbanisation and socio-economic changes, there has been an increase in demand for convenient ready to cook and ready to eat meat products. Various processing methods are generally employed which includes freezing, chilling, canning, curing, smoking, dehydration,
fermentation, emulsification, stabilisation etc. Meat processing refers to all processes utilised in altering fresh meat except simple grinding, cutting and mixing (Lawrie, 1998). In recent years several newer methods of processing meats like hurdle processing, irradiation, high pressure processing etc have also emerged. Processed meats offer variety, convenience, value addition, easy transportability and longer shelf stability (Pearson and Tauber, 1984). Since meat is a valuable and perishable commodity suitable processing and preservation methods have to be selected carefully to suit the changing needs of the consumers and also matching the availability of investment and energy, infrastructural facilities for storage, handling and distribution. Processed meats contribute significantly to the economy of meat industry all over the world. But in India for various reasons, it has not come up to the level in making meat industry a profitable venture. More than 95% of the meat produced is eaten as 'fresh meat'. A very small percentage is processed from mutton, chicken, pork and beef into limited range of products like canned meats, sausages, kababs, burgers, salami, corned beef, tikkas, fingers, lollipops and patties. These products are marketed frozen since they have limited shelf life at ambient temperatures (Kanatt et al., 2002). The market for these products is limited because of a lack of infrastructure in developing countries, such as India. Furthermore freezing does not eliminate pathogens and thus poses potential health hazards (Geraldine, 1992).

If meat industry has to thrive in our country and face stiff competition in the global market, all efforts should be directed to identify problems at all levels and find suitable solutions which need to be implemented sincerely and whole heartedly. The products can meet consumer expectations with regard to convenience, safety, quality and stability only if the manufacturers can produce the products with the cost reductions and maintaining the quality.

1.12.3. Chilling and freezing

Chilled food markets have shown enormous growth due to their ability to offer nutritive, high quality safe and tasty foods comparable to their frozen counterparts. Generally chilling is employed in combination with other methods of preservation. "Chilled" meat is usually stored at temperatures
around 1° C to 5° C where it keeps well for several days. Modern packaging techniques including storage under carbon di oxide or nitrogen or in vacuum can extend this period to about 10 weeks. Chilling at temperatures very close to the freezing point of meat, i.e., -15° C diminishes the dangers of most pathogens and slows the growth of spoilage organisms. Growth of some organisms, moulds, virtually ceases at -10° C. In precooked chilled comminuted meats, rapid onset of oxidation (warmed over flavour) could be a major problem and this requires special attention of the scientist.

1.12.4. Freezing

Major chunk of the meat products for human consumption in the world is frozen. Commercially at -29°C and domestically at -18° C is now a standard method of preservation. The quality depends on the control exercised during pre-freezing and strict temperature maintenance in the post freezing handling. Both slow freezing and rapid freezing are generally employed. But in recent years immersion freezing has gained popularity. The latter uses cryogens such as liquid nitrogen or carbon dioxide and considered to be expensive. Time-temperature indicators developed in recent years are helpful to monitor temperature abuses on frozen foods during handling.

1.12.5. Curing

Traditionally it was employed as a means of preserving meat using salt and nitrate or nitrite. The use of salt is one of the oldest methods of preserving meat since at concentrations greater than 4% in the aqueous phase it inhibits the growth of most spoilage organisms. To function as a complete preservative, the salt concentration would need to be around 17% at which levels the product would be unpalatable. In most cured meat products the salt concentration is between 2.5 and 5% and the nitrite inhibits the growth of other organisms. Nitrite the important ingredient in curing has a multiple role in extending shelf life by improving colour, flavour and stability. But use of nitrite is restricted to 200 ppm or less in the finished products since it is known to cause the formation of carcinogenic nitrosamines. Various substitutes for nitrite are also being examined, but it has not met much success.
1.12.6. Smoking

Meat has been treated with smoke from the earliest days—traditionally over a wood fire and more recently by producing smoke from wood sawdust in a generator and conducting the smoke over the meat. The substances deposited on the meat contribute to the flavour and appearance. Intensive smoking does prolong shelf life by heavier deposition of preservatives and by the drying effect of the hot air but it has a detrimental effecting on flavour. A modern development making use of the favouring effect is to use an aqueous solution of the constituents of smoke, which reduces the amount of strongly flavoured and other unwanted substances.

1.12.7. Meat emulsion

Meat emulsion includes sausages, burgers, pies, patties, kababs etc. The manufacture of the above products and other form of comminuted meats depend on the ability of lean meat to form stable emulsions in the presence of salt, sugar, water, vegetable, proteins etc. Sausages and comminuted meats offered in variety of forms, shapes, sizes, tastes have made a significance contribution to the success of meat industry as they are suitable for all purposes and occasions, as convenience food items rich in nutrients, easy to prepare and shelf stable products. Some varieties are fermented using natural flora or specific starter cultures to impart characteristic taste and flavour. There are some 800 types of sausages made comminuted or chopped meat of various kinds, seasoned with salt and spices, often mixed with cereal and packed into natural casings or made of cellulose, collagen or synthetic materials. There are six main types of sausages—fresh, smoked, cooked, smoked and cooked, semidry and dry. Comminuted products such as sausages and luncheon meat are based on lean meat, which technologically, provides water holding and meat binding capacity, with the addition of fatty meats and sometimes organ meats. The amount of these is limited otherwise the products have an unattractive soft texture and high shrinkage on cooking.
**1.12.8. Hurdle processing**

Hurdle processing uses a combination of factors (hurdles) that are beneficially used to impart the required functional qualities and suppress the spoilage due to microbes. A variety of factors such as temperature, water activity ($a_w$), oxidation-reduction potential, pH, available substrates, presence or absence of oxygen, concentration of major solutes present and preservatives are employed as hurdles (Gustavo et al., 1998). The use of the inhibiting factors in combination can be advantageous principally by allowing the less extreme use of any single treatment (Gould and Jones, 1989). Leistner (1985, 1995) introduced the hurdle effect, to illustrate that in most traditional and novel foods a combination of several preservation factors that should not be overcome by the micro organisms present.

Current consumer demand for foods that are less severely processed, additive-reduced, natural, and fresh, prompts food manufacturers to select milder preservation mechanisms. The hurdle technology concept can address these needs. Up to now about fifty different hurdles have been identified in food preservation (Girijspaardtivink, 1994).

An improved understanding of the mechanisms underlying the effectiveness of the non-thermal processes and the combinations with the traditional hurdles is therefore urgently required, so that new preservation possibilities can move forward with a sound scientific basis because, most likely, combining technologies is the future of food preservation.

The hurdle technology concept in food preservation was first introduced for meat products by Leistner (1985). He defined shelf-stable meat products as high moisture meats ($a_w > 0.90$) storable at room temperature after a mild heat treatment ($70-110^\circ C$ core temperature) in sealed containers. Crawford et al., (1996) evaluated the combination of high hydrostatic pressure, heat, and irradiation to eliminate *Clostridium sporegenes* spores in chicken breast. Using the hurdle concept, several high moisture fruit products (HMFP) were developed and their stability was established (Tapla de Daza et al., 1995; Welti et al., 1994; Alzamora et al., 1995; Argaiz et al., 1995).
There are few reports in the literature for the development of convenient meat products using hurdle approach. Various workers employed different factors for stabilisation. Kanatt et al., (2002) developed shelf stable intermediate moisture (IM) ready-to-eat (RTE) meat products using a combination of hurdles, i.e., reduced water activity, vacuum packaging and γ-irradiation. Manufacture of a marinated meat product of pH 5.4–5.6 and \( a_w \) <0.97 (made from pork) with a shelf life of 5 week at room temperature was reported by Mella et al., (1995). Application of hurdle preservation techniques to extend the shelf life of coconut gratings using a combination of 3% NaCl, 0.3% citric acid, 0.009% sodium citrate and 0.02% BHA in laminated polythene packages at 5°C was reported by Gamlath et al., (2004). Chawla and Chander (2004) employed the hurdle technological approach on the microbiological safety of shelf stable meat products by applying reduced \( a_w \), irradiation and vacuum packaging. Improvement of shelf life by application of the hurdle concept to manufacture traditional Chinese meat products which may be stored without refrigeration, including cured products, dried products, raw ripened sausage products and ham products were discussed by Wei-Wang and Leistner, (1994).

The hurdle concept for preservation and the HACCP concept for shelf stable meat products achieved by means of heat treatment, \( a_w \), pH, or combination of these parameters were reported by Hechelmann and Leistner (1992). Effects of hurdle technology were investigated by randomly assigning restructured dried pork to 5 treatments: microwave heating, pasteurising, vacuum packaging, adding an oxygen scavenger and a control by Chow (1990). Safe and shelf stable natural casing were prepared using a combination of hurdles viz reduced \( a_w \) (0.80 ± 0.02), packing (polyethylene) and gamma irradiation (5-10 KGy). A dose of 5 KGy was sufficient to reduce total viable counts by three log cycles; spore counts by two log cycles and completely eliminate staphylococci and coliforms (Chawla et al., 2006). A combination of vinegar and sake improved the microbial stability of Korean seasoned beef product at 8 and 20°C, while it did not improve the sensory
quality of the product. Hurdle effect of vinegar and sake was studied by Jang et al., (2006). A method for preserving natural casings (beef and pork) that enables them to maintain their original properties during a 3 month storage period at 23-25°C was studied by Kudryashov et al., (2004) using different mixtures of citric and/or lactic and/or sorbic and/or acetic acid with kitchen salt as preservative agents. A process for preparation of mutton curry suited to Indian tastes was standardised using hurdle technology including hurdles such as a_w, pH, cooking pressure, temperature and oxido-redox potential. The product was stable for > 4 months at ambient temperature (27 ± 2°C) and > 6 months at refrigerated temperature (3 ± 2°C) (Das and Radhakrishna, 2001). A ground chevon meat product called keema was prepared by hurdle approach using goats meat mixed with various other additives at optimised levels (NaCl 2.5 %, sugar 1 %, skim milk powder 2 %, and 5% isolated soy protein) or preservatives, (2%spice mix, 500 ppm ascorbic acid, 500 ppm sorbic acid, 100 ppm sodium nitrite) and its stability at ambient temperature was established (Karthikeyan et al., 2000). Factors influencing microbiological quality, shelf life and sensory quality of cooked cured meat products are discussed in relation to the hurdle concept by Weber (2002). Effect of marination, packaging and storage period on quality and stability of hurdle-processed chevon at refrigeration was reported by Das (2002).

Hurdle technology enables production of safe, stable and nutritious foods, which are economical and cost effective. Hurdle processed foods are reported to have improved sensory qualities, high nutrient retention, besides providing convenience in preparation and storage (Alzamora et al., 1995). The primary aim of hurdle technology is to produce foods with good chemical and microbiological stability by applying strict control through the use of appropriate hurdles combined in an intelligent manner and in a sequential order. For each food a separate set of hurdles are required and their intensities also will be different depending on the natural micro flora, chemical composition, climatic conditions of handling and storage. By the application of suitable hurdles the homeostasis of micro organisms is disturbed, thus rendering them inactive. In hurdle processed meats, it is possible to obtain
semi ‘moist’, Ready to eat, stable, tasty, convenience items which can meet most of the requirements of civil and service sectors.

### 1.13. Drying

Preservation of foods by drying is one of the oldest methods practiced by man. The early man mainly depended on nature as means of drying foods to sufficiently lower moisture to preserve them. By the end of 19th century varieties of dryers were developed to dehydrate a variety of foods.

For the past 100 years the mechanism of drying has been the subject of many scientific studies. The outbreak of the First World War gave impetus to the studies on drying mechanism and since then a considerable amount of research has been conducted in this area as well as the production of dehydrated foods.

In the Second World War, dehydrated foods, particularly dehydrated vegetables, meat and meat products gained considerable popularity due to several logistic advantages like savings in storage space, transport, convenience and utility. After World War II food dehydration industry expanded greatly and reliable quicker dehydration methods were developed. Today dehydrated foods are being produced and marketed for the general public and still continue to be an important consideration in military food supplies and use.

Drying is a complex process involving simultaneous heat and mass transfer accompanied by various physical and structural changes. The heat raises the vapour pressure of the water present in food materials and helps in evaporation of moisture from the surface. Surface water thus removed is replaced with water from within by diffusion or capillary movement. In case of diffusion mechanism the driving force is concentration gradient as a result of either liquid or vapour movement.

Osmotic pressure is the main force for water movement in the liquid diffusion mechanism. In capillary mechanism the moisture moves due to
surface tension. Based on the above principle two types of dryers have been designed and fabricated, mainly direct and indirect heated ones. In direct heated dryers hot air is passed through or around the product to be dried. The heat in the air provides latent heat of evaporation for water present in the food material and cooled gas conveys the vapour away from the product. In indirect dryers either the steam heats the air or electrical energy is used to supply the required heat of evaporation by radiation, conduction, convection or a combination thereof. Water may also be removed from the solid food products by means of mechanical force through pressing/compressing or centrifugal force. This is known as mechanical dewatering. Normally this technique is used as a pre-processing step before thermal drying. In this technique the quality of the final product is affected due to the loss of soluble solids. Drying processes are broadly classified on the basis of water removal mechanism as thermal drying, osmotic dehydration and mechanical dewatering.

1.13.1. Thermal drying

In thermal drying a gaseous or void medium is used to remove water from the material. When hot air is blown over a wet food, heat is transferred to the surface and latent heat of vaporisation cause water to evaporate, water vapour diffuses through a peripheral film of air and is carried away by the moving air. This creates a region of lower water vapour pressure at the surface of the food and water vapour pressure gradient is established between the moist interior of the food and the dry air. This gradient provides the driving force for water movement from the food to the surface through liquid movement by capillary force or by diffusion. Thermal drying is divided further depending upon the mode of drying i.e., Air drying, low air environment drying and modified atmosphere drying.

1.13.2. Air-drying

Conventional air-drying is one of the most frequently used operations for food dehydration. The drying parameters usually examined for food quality mainly depend on the temperature, relative humidity as well as velocity
of air. Further the mechanism of moisture transfer depends mainly on the type or physical state of food material and drying process.

There are many types of air driers used in the dehydration of foods. The type selected being governed by the nature of the commodity to be dried, the desired form of the finished product and labour and operating conditions. These include cabinet dryers, solar dryers, bin dryers, conveyor dryers, fluidised bed dryers, kiln dryers, pneumatic dryers, rotary dryers, spray dryers and drum dryers.

1.13.3. Fluidised bed drying

The drier consists of a suitably housed porous refractory plate. The hot air is introduced from the bottom of a preloaded cylindrical bed of porous refractory plate, causing the food to become suspended and vigorously agitated. The air thus acts as both the drying and fluidising medium, exposing the maximum surface area of the food for drying. The direction of movement of food particles resembles the convection current observed when a container of water is heated from the bottom. The material thus behaves like a liquid and is discharged over the outlet of the dryer.

The main problems that were encountered in the conventional air-drying of meat are case hardening, oxidative breakdown of lipids leading to rancidity development and lower reconstitution characteristics and over all acceptability values. There is no literature available on the application of the fluidised bed-drying process in the development of meat products, however some reports are available on cereals, pulses and vegetables Patki et al., (2002) reported the application of freezing prior to fluidised bed drying (FT/FBD) in enhancing the overall product quality of whole legumes. Use of superheated-steam, fluidised bed dryer for the preparation of parboiled rice was investigated by Taechapairoj et al., (2003). Somchart et al., (2001) studied fluidised bed drying of soybean and the production of quick cooking potato cubes by osmotic pre-concentration and fluidised bed drying was reported by Ravindra and Chattopadhyay (2000).
1.13.4. Freeze-drying

Freeze drying is one of the most sophisticated methods used for drying biological components through sublimation. This technique is similar to ordinary vacuum distillation but with one very essential difference that the material to be dried must be frozen before being subjected to a very low absolute pressure and controlled heat input. Under these conditions the water (in the form of an ice matrix) is selectively removed via sublimation, i.e. ice transforms directly to vapour bypassing the intermediary liquid phase. Hence freeze drying is process of freezing a product and removing the water content while the product is still in frozen state. Four conditions found to be essential for proper freeze drying process are:

- Product must be frozen below its eutectic point.
- Condensing surface must be at a temperature lower than that of the product temperature (-40°C)
- The system must be capable of evacuating to an absolute pressure between 5-25 μ.
- Controlled heat supply for sublimation.

Heat is supplied to the frozen product under vacuum and refrigeration to maintain the migration of water vapours from the product towards the condenser. The application of heat either by convection or conduction supplies the necessary energy (i.e., latent heat of sublimation) to drive off these vapours. Hence freeze drying is an operation involving both mass transfer and heat transfer and the rate of drying depends on the magnitude of the resistance of these transfers.

1.13.5. Effect of freeze-drying on the quality of food products

In freeze-drying process, material remains frozen and drying takes place at low temperature, hence heat induced alterations are minimum. In addition there is a little or no loss in quality of the product because the removal of ice crystals leaves a porous honeycomb type structure rendering the product to rehydrate rapidly. However, freeze-drying is slow and expensive process due to the requirement of additional energy to run
compressor and refrigeration units. Hence this technique of drying is highly suitable for high-value products (Cohen and Yang, 1994). The quality characteristics of the freeze-dried pineapple juice powder during storage was studied by Ammu et al., (1977) and Phanindra Kumar et al., (1991). Studies on the development of F.D breakfast and dessert foods and its shelf stability characteristics were reported by Vijaya Rao et al., (1994).

Literature is scanty on the area of FD of meat and poultry products. Radha Krishna et al., (1988) studied the effect of freeze-drying of rehydratable poultry product as large pieces. Attrey and Sharma (1979) evaluated the sorption behaviour and the importance of monolayer moisture content of raw freeze-dried mutton. Investigation were carried out on the effects of freeze drying on the quality of meat, pork and beef by analysing major chemical, physico-chemical and sensory aspects. (Kondratowicz and Kuldo, 2002). The effectiveness of using FD as a long term preservation technique and the lipid oxidative changes during storage of FD chicken and veal meat was discussed by Tsvetkova and Georgieva (1994).

There are a number of traditional dried products in various regions. For example biltong in South Africa, which is made from beef with the addition of nitrate or nitrite, spiced and dried in air for 1-2 weeks. Pemmican is dried meat that has been powdered or shredded and mixed with fat to form a solid product. Other traditional dried products include pastirma (Turkey, Egypt and Armenia) Odka (Somalia), qwanta (Ethiopia and East Africa) and Kilishi (Nigeria and West Africa). There is variable loss of vitamins and other quality changes from such products due to the long drying times which can be shortened by the use of modern drying techniques like freeze drying which causes little or no loss of vitamins and results in products which are readily rehydrated and much closer in texture and flavour to fresh meat than the traditional dried product. (FAO, 2005).

1.13.6. Partial dried / Intermediate-moisture foods

In dried meats the water activity is, below levels needed for microbial growth, so the product is shelf stable but still there will be chemical and
physical changes due to rancidity and discolouration which necessitate adequate packaging so it is necessary to combine an partial reduction in water activity with other methods.

In an attempt to avoid the relatively poor texture and flavour of most dried meat products, a recent development is, partial drying to a moisture content of 15 to 50% and reduction of free water to the required low levels by adding humectants such as glycerol, sorbitol or other polyhydric alcohols which combine with the free water, so that it cannot be used by the microorganisms.

1.14. Canning

The procedure is to seal the food into the container and then heat it under pressure in an autoclave (retort) to the required temperature for the required length of time and to cool rapidly to avoid overheating. This is achieved by heating in an airtight can or bottle, or more recently in a heat resistant or aluminium foil-laminated plastic pouch.

It is not always possible to destroy all the organisms without excessive heat which would spoil the product, so the objective is to destroy the greater proportion of the organisms when the remaining few pose no hazard so long as the container is cooled rapidly and stored below 20-25°C. This condition is termed "commercially sterile".

The intensity of heat treatment necessarily depends on the nature of the product, its pH and the amount of salt and other curing agents present as well as on the bacterial load. The time required at a given temperature will vary with the rate of heat penetration to the centre and with the size of the container. The intensity of heat treatment is defined in physical terms called F-value, which means that the product received heat treatment with the same effect on microorganisms as exposure to a temperature of 121°C for 1 min.

Virtually every type of meat product made from chopped, cured meat can be canned. Smaller size containers are most suitable for meat products
because heat penetration is mostly by conduction, so larger containers would require severe heat treatment involving over cooking. Heating to a higher temperature for a shorter time is an effective means of preservation and sterilization is achieved in a shorter time with less damage to the product. The process is termed high temperature short time heating (HTST) and has been particularly applied to milk, but can be applied to meat if there is sufficient liquid present to allow mixing of the contents by rotating the cans in the autoclave. The cans must be cooled immediately after the temperature of sterilization reached to avoid over heating.

1.15. Radiation processing

Radiation processing of food is an emerging, promising new food safety technology for improving hygiene and increasing storage and distribution life. Ionizing radiation can be used to bring about beneficial changes in food stuffs (Urbain, 1995) and it has been suggested as a method of ensuring the safety of meat products (Patterson and Stevenson, 1995). Ionising radiation interacts with an irradiated material by transferring energy to electrons and ionizing molecules by creating positive and negativities (Moseley, 1989).

The wholesomeness and acceptability of irradiated foods have been evaluated by various expert committees (JECFI / IAEA / WHO / FAO) and after reviewing all the data it has been recommended that the irradiation of any food commodity up to an over all average dose of 10 KGy presents no toxicological hazard as well as nutritional or microbiological problem. Radiation dosage greater than 10 KGy can lead to sterilized products as is the case with meat products prepared for the NASA space flight programme.

1.15.1. Meat irradiation

Radiation processing of meat is a novel alternative to traditional preservation methods such as salting, curing, smoking, drying, canning, cooking, refrigeration, freezing, modified atmosphere packaging and high-pressure. Some of the advantages of this technology are that it is a physical, cold and non-additive process that causes minimal changes in food. It is also
an ecofriendly process. It can be applied to pre-packaged food and is highly effective compared to chemicals and fumigants. It does not leave harmful residues in food. It is one of the best emerging technologies to ensure the microbiological safety of meat. In developed as well as developing countries an increase in the incidence of food-borne diseases especially of animal origin has been noticed (Kanatt et al., 2006). In the USA, the US FDA approved radiation processing of meat in 1997 and the USDA in 1999 (USDA, 1999). Radiation processed ground beef and poultry have appeared since on the market shelves of several states in the US. Meat and meat products pasteurised by radiation have been successfully marketed in Belgium, France, China, Indonesia, Netherlands, South Africa and Thailand for a number of years. In India, the Ministry of Health and Family Welfare approved meat and meat products including chicken for radiation preservation under prevention of Food Adulteration Rules in 1998.

There are several reports on the radiation processing of meat products like bacon, ham, sausages (Kiss et al., 1990) and beef burgers (Dempster et al., 1985). In addition to spoilage bacteria, meat products may contain parasites and pathogenic bacteria, which could be eliminated by irradiation. The radiation doses required to inactivate 90% of the colony forming unit of the common food borne pathogens associated with meat and meat products are in the range of 1-4 KGy (Thayer et al., 1993).

1.15.2. Beneficial effects of meat irradiation

Since the meat industry deals with a highly perishable commodity, its distribution lines are limited to areas, which have freezing/refrigeration facilities. Irradiation can help expand the available market to include a much wider clientele. It can also cut down losses incurred as a result of spoilage. Irradiation also offers as effective method to control pathogenic microorganisms in meat. Meat and meat products are irradiated at different doses for the following purposes.
1.15.3. Irradiation and meat quality

Oxidative lipid degradation is associated with many meat-processing technologies including meat irradiation. Radiation processing may affect the quality of meat and meat products. Irradiation may result in the formation of free radicals and possible development of off-odour. Hydroxyl radicals generated by the interaction of ionising energy with water molecules can cause initiation of lipid oxidation in muscle tissues or in meat products (Thakur and Singh, 1995). Oxygen dissolved in meat tissue or surrounding the product is subjected to activation by ionising radiation and may generate reactive oxygen species (Woods and Pikaev, 1994). Hydroxyl radicals and other reactive oxygen species interact with lipids in meat and form lipid hydroperoxides. Subsequent breakdown of such hydroperoxides generates volatiles, which may partially contribute to off odours in irradiated meat. Free radicals may interact with heme pigments in meat and change meat colour. Thus free radicals generated by irradiation can destroy antioxidants in muscle, reduce storage stability and increase off flavour production in meat (Thayer et al., 1993, Lakritz et al., 1995).

Ionizing radiation generates free radicals that may induce lipid peroxidation and other oxidative changes as well as influencing sensory quality of meat (Branka et al., 1992; Wong et al., 1995). The susceptibility of irradiated meat to oxidative rancidity is related to the nature, proportion, packaging, storage, and degree of saturation in fatty acids and the composition of phospholipids in cell membranes (Ahn et al., 2000; Kanatt et al., 2004). As lipids oxidize they form hydroperoxides, aldehydes, ketones and various other products that adversely affect flavour, taste, nutritional quality and overall acceptability.

Several factors affect consumer decisions to purchase meat, but an important one is the perception of quality. The perceived "healthiness" of a food is becoming the key issue for consumers and in the case of meat this is largely related to its fat content and its fatty acid composition. Fat content of meat is a primary factor that determines product shelf life/storage stability of meat and meat products (Kanatt et al., 2006). The class of lipid and fatty acid
composition of meat is also important for quality traits of meat such as nutritional value, flavour and textural properties. It varies widely depending on species, degree of cutting and trimming, nature of cooking, processing and on the meat preservation techniques employed (Rhee, 1992). There are some studies on lipid peroxidation of irradiated beef, pork and poultry (Ahn et al., 2000; Lescomo et al., 1991; Luchsinger et al., 1997).

1.15.4. Effect of radiation on lipids

On irradiation of lipids, the primary effect of incident energy leads to the formation of cation radicals and excited molecules.

\[
\text{RCH}_2\text{-O-} \text{CO-} \text{(CH}_2\text{)}^n\text{CH}_3 \xrightarrow{\text{excitation}} (\text{RCH}_2\text{-O-} \text{CO-}\text{(CH}_2\text{)}^n\text{CH}_3)^\cdot
\]

\[
\text{ionisation}
\]

The general mechanism of radiolysis of lipids is thought to involve primary ionisation, followed by migration of the positive charge either towards the carboxyl group or double bonds. Some sixteen free radicals have been postulated to be preferentially produced by cleavage of bonds in the vicinity of the carbonyl group (Stewart, 2001). These may then engage in a number of reactions involving abstraction, dissociation, recombination, disproportionate and radical molecule interactions leading to the formation of stable products. Products formed include hydrocarbons, aldehydes and ketones and these are considered important volatiles related to the off-odour production in irradiated meat. If oxygen is present during or after irradiation normal autoxidation is accelerated (Katusin-Razem et al., 1992). The formation of peroxides and volatile compounds and the development of rancidity and off-flavours have been reported by Merritt et al., (1975). The peroxide formed can also affect certain labile vitamins, such as vitamins E and K (Graham, 1980). Irradiation induced oxidative chemical changes are dose dependent.

Irradiation treatment is not effective in stopping the changes in meat that diversely affect consumer acceptance, such as oxidation of pigment to
yield brown or grey discolouration, drip loss from the cut surface of lean tissue, and oxidation of meat lipids which causes off-flavours, by atmospheric oxygen

1.15.5. Prevention of oxidative rancidity in irradiated meat

Oxidative rancidity in irradiated meat can be minimised / retarded by various means. The most obvious precaution to take against oxidative deterioration is removal of air. Vacuum packaging and modified atmosphere packaging (MAP) of meat is very effective in controlling oxidative rancidity (Lambert et al., 1992; Stapelfeldt et al., 1993). However, the usefulness of vacuum packing is limited by the product characteristics. Also meat packed in modified atmosphere increases the pack volume and different gas formulations are required for each product. Freezing of meat can considerably slow down the rate of oxidative rancidity. However freeze thawing, temperature abuse in handling and distribution and/or prolonged storage can accelerate lipid oxidation. Antioxidants are one of the principal ingredients that protect meat quality by preventing oxidative deterioration of lipids (Shahidi and Wanasundara, 1992; Pokorny et al., 2000). They can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers. There is an increasing demand for naturally occurring antioxidants because they are presumed to be safe since they occur naturally in food.

1.16. Factors influencing the quality of processed meat and meat products

During storage, processed meats deteriorate in the first instance because of discolouration, secondly because of oxidative rancidity of fat and thirdly on account of microbial changes (Pearson and Tauber, 1984).

1.16.1. Meat discolouration

The colour of fresh red meat is of almost importance in marketing since it is the first quality attribute seen by the consumer who uses it as an indication of freshness and wholesomeness (Faustman and Cassens, 1990). The colour of fresh meat is not well correlated with the eating quality, however
the consumer still demands that beef has a bright cherry red colour (Taylor, 1996), lamb a brick red colour and pork and chicken a uniform pink colour.

Meat colour depends on myoglobin, a pigment with several forms (Renerre and Labas, 1987). Myoglobin is concerned with the storage and transfer of oxygen within the muscle. Its concentrations are variable between species and between muscles. Light chicken meat, pork and beef contain 0.01, 1-3 and 3-6 mg/g myoglobin respectively (Warriss, 1996). Fresh meat colour depends on the relative amounts of three derivatives of myoglobin. Reduced myoglobin (Mb) is the purple pigment of deep muscle. On exposure to air, myoglobin combines with oxygen to form bright red oxy myoglobin (MbO2), which is synonymous with freshness and considered attractive by the consumer. However contact of Mb with oxygen also leads to the formation of the oxidised form, metmyoglobin (Met Mb) that is brown and unattractive (Lanari and Cassens, 1991). Development of such an undesirable brown colour is a common problem in marketing pre-packaged meat. During storage the rate of metmyoglobin accumulation is related to many intrinsic (pH, muscle type, animal age, breed, sex, diet) and extrinsic (Pre-slaughter treatment, chilling mode) factors. This is the pigment responsible for the brown discolouration associated with non-saleable meat (Kerry et al., 2000).

Deoxymyoglobin and oxymyoglobin are heme proteins in which the iron is present in the ferrous state (Fe²⁺) while metmyoglobin possesses the ferric (Fe³⁺) form. The conversion of the ferrous to ferric form is brought about by oxygenation.

1.16.2. Lipid oxidation

Lipid oxidation is one of the primary causes of deterioration in food system during cooking and storage leading to the development of off-flavour, loss of colour and texture, decrease in nutritive value and the production of potentially toxic compounds (Buckley et al., 1995; Gray et al., 1996; Morrissey et al., 1998). This is one of the primary causes of deterioration in quality of meat during storage, leading to development of off-flavour as well as reduced shelf stability and acceptability (Rhee et al., 1996). Oxidation of lipids is initiated during cooking which then continues during storage. Various factors
affect the extent of oxidation, e.g., availability of oxygen, temperature of cooking, method of cooking, species of the meat, packing and storage conditions and presence of various pro and antioxidants (Buckley et al., 1995; Ladikos and Longovois, 1990; Vasundhara and Honikel, 1992).

1.16.3. Process of oxidation

Lipid peroxidation can take place by enzymatic and/or non-enzymatic mechanisms. Oxidation of lipids in vivo and in muscle foods is believed to be initiated in the highly unsaturated phospholipid fraction in sub-cellular membranes (Morrissey et al., 2000). Lipoxygenases, the non-heme enzymes that contain iron in the active state can catalyse lipid peroxidation to produce hydroperoxides (Tomchick et al., 2001) but only if the enzyme is activated by preformed peroxides and the fatty acids are in the free form (Kanner, 1994).

The lipid oxidation in muscle foods is generally non-enzymatic and mainly involves either free radicals and/or reactive oxygen species such as singlet oxygen (Gray et al., 1996) to react with substrates such as unsaturated fatty acids. When oxidised, they start an autocatalytic process which the oxidative products so formed can further catalyse the reaction, which causes the rate to increase with time.

1.16.4. Mechanism of lipid oxidation

The low oxidative stability of meat is a problem for all those involved in the meat production, including the primary producers, processors, distributors and retailers. Under normal physiological conditions, animal cells are continuously challenged by stresses arising from both internal and external sources. The most important of these are reduced derivatives of oxygen called the reactive oxygen species (ROS) (Table-4). These include free radicals having one or more unpaired electrons, which can exist independently for a brief period. Examples are hydroxyl radical (the most potent oxidant encountered in biological systems), super oxide anion radical and oxygen centred radicals of organic compounds (Peroxyl and alkoxy). Other ROS include hydrogen peroxide, hypochlorous acid and hydroperoxide
and epoxide metabolites of endogenous lipids. These are not free radicals but contain chemically reactive oxygen-containing functional groups.

Table 4: Examples of Reactive oxygen species (ROS)

<table>
<thead>
<tr>
<th>ROS</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radicals</td>
<td></td>
</tr>
<tr>
<td>Super oxide (O₂⁻)</td>
<td>Oxygen centred radical with selective reactivity. It is produced by a number of enzyme systems, by auto oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen.</td>
</tr>
<tr>
<td>Hydroxyl (OH')</td>
<td>A highly reactive oxygen-centred radical, which attacks all molecules.</td>
</tr>
<tr>
<td>Peroxyl, alkoxy (RO₂⁻, RO')</td>
<td>Typically organic radicals often encountered on intermediates during the break down of peroxides of lipids in the free radical reaction of peroxidation.</td>
</tr>
<tr>
<td>Oxides of nitrogen NO', NO₂⁻</td>
<td>Nitric oxide is formed in vivo from the amino acid L-arginine.</td>
</tr>
<tr>
<td>Non radicals</td>
<td></td>
</tr>
<tr>
<td>Hydrogen peroxide (H₂O₂)</td>
<td>Formed in vivo when O₂ dismutate by SOD and also by other oxidative enzymes. High levels of H₂O₂ can attack several cellular energy producing systems.</td>
</tr>
<tr>
<td>Hypochlorous acid (HOCl)</td>
<td>Formed in the human neutrophils at the sites of inflammation by the action of the enzyme myeloperoxidase.</td>
</tr>
<tr>
<td>Ozone (O₃)</td>
<td>This noxious gas has been shown to deplete plasma antioxidants, vitamin D, Vitamin E and Uric acid.</td>
</tr>
<tr>
<td>Singlet oxygen (¹O₂)</td>
<td>The spin of one of the electrons of the two outer orbitals is inverted removing the quantum mechanical spin restrictions of molecular oxygen.</td>
</tr>
</tbody>
</table>

ROS are produced naturally (Halliwell et al., 1995). During normal aerobic metabolism, mitochondria consume molecular oxygen and reduce it sequentially to produce H₂O. During this process O₂, H₂O₂ and OH' are
produced naturally, though at a low rate. In living tissue, control of oxidation is essential to prevent the oxidative destruction of lipid membranes, proteins and nucleic acids. Numerous systems exist to maintain the balance between the factors, which control oxidative reactions.

The oxidative stability of muscle is dependent on the composition, concentration and reactivity of three factors: oxidation substrates, oxidation catalysts and antioxidants (Table 5). In the living tissue control of oxidation is essential to prevent oxidative destruction of lipid membranes, proteins and nucleic acids. Numerous systems exist in the muscle to maintain the balance between the factors, which control oxidative reactions (Decker and Xu, 1998). However in the post slaughter period the balance is disrupted, control of oxidation is lost and oxidative modification of the chemical components of muscle occurs (Table 6) (Morrissey et al., 1994 a).

### Table 5: Factors influencing pro-oxidative/anti-oxidative balance of meat

<table>
<thead>
<tr>
<th>Oxidation substrate</th>
<th>Pro-oxidants</th>
<th>Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids</td>
<td>Transition metals</td>
<td>α-tocopherol</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Enzymes</td>
<td>Ubiquinone</td>
</tr>
<tr>
<td>Iron containing proteins</td>
<td>Carotenoids</td>
<td>Plant phenolics</td>
</tr>
<tr>
<td></td>
<td>Ascorbate</td>
<td>Chelators (polyphosphate, EDTA)</td>
</tr>
<tr>
<td>Carnosine/Anserine</td>
<td>Ascorbate</td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyamines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-oxidant enzymes</td>
<td>(SOD, CAT, GSH-Px)</td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Post-slaughter changes, which predispose meat to oxidation

<table>
<thead>
<tr>
<th>Post-slaughter changes</th>
<th>Oxidation Predisposing Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stunning and bleeding, circulation of blood ceases.</td>
<td>no essential note</td>
</tr>
<tr>
<td>Anaerobic metabolism—lactic acid accumulates, pH declines.</td>
<td>no essential note</td>
</tr>
<tr>
<td>Circulation of nutrients rapidly ceases.</td>
<td>no essential note</td>
</tr>
<tr>
<td>Preventative antioxidant enzyme system – superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase—become less effective.</td>
<td>- Essential note -</td>
</tr>
<tr>
<td>Acute phase proteins which scavenge iron—transferrin, haptoglobin—unlikely to be activated.</td>
<td>- Essential note -</td>
</tr>
<tr>
<td>Sarcoplasmic reticulum loses its Ca(^{2+}) accumulating ability.</td>
<td>- Essential note -</td>
</tr>
<tr>
<td>Ca(^{2+}) dependent proteinase degrades muscle proteins.</td>
<td>- Essential note -</td>
</tr>
<tr>
<td>Disruption of cell compartmentalisation</td>
<td>no essential note</td>
</tr>
<tr>
<td>Iron catalysed chain reactions.</td>
<td>no essential note</td>
</tr>
<tr>
<td>Initiation of membranal lipid oxidation</td>
<td>no essential note</td>
</tr>
</tbody>
</table>

Lipid peroxidation takes place through free radical mechanism i.e., three stages namely initiation, chain propagation and termination as shown below.

**Initiation:**

\[
\begin{align*}
\text{RH}^+ \text{ initiator} & \rightarrow R' \\
\text{RO}_2 \text{H} & \rightarrow \text{RO}'_2
\end{align*}
\]

**Propagation:**

\[
\begin{align*}
R. + \text{RH} & \rightarrow \text{RO}'_2 \\
\text{RO}'_2 + \text{RH} & \rightarrow \text{RO}_2 \text{H} + R'
\end{align*}
\]

**Termination:**

\[
\begin{align*}
R' + R' & \rightarrow R - R \\
\text{RO}'_2 + R' & \rightarrow \text{RO}_2 R
\end{align*}
\]

1.16.4.1. Initiation

In the initiation step, the free radical R' is formed from an unsaturated lipid molecule at an allylic methylene group, RH, or a lipohydroperoxide, RO\(_2\)H, by the action of an initiator (Halliwell and Chirico, 1993). The direct
action of unsaturated lipids with molecular oxygen is thermodynamically unfavourable. However the spin restriction that prohibits the interaction of ground state oxygen with unsaturated fatty acids can be overcome by a number of initiating mechanisms (Kanner, 1994). A large number of potential initiators and propagators of lipid oxidation in meat have been identified such as reactive oxygen species (ROS), mainly hydroxyl radicals, perferryl and ferryl radicals. Initiation of lipid oxidation necessarily occurs in the interior of the membrane where the unsaturated fatty acids are located. In meat Morrissey et al., (1998) indicated that iron released from high molecular weight molecules such as haemoglobin, myoglobin and ferritin after death is probably the main cause of lipid oxidation. After slaughter the pro-oxidant effect of iron is more potent because the protective anti-oxidant enzyme systems such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) are no longer efficient.

1.16.4.2. Propagation

In the propagation step, the free radical \( R' \) reacts with oxygen to form a peroxy radical \( RO_2' \), which can further react with another lipid molecule to generate a hydroperoxide, \( RO_2H \), and another lipid radical, \( R' \). Lipid hydroperoxide formed in the propagation reaction are both products of oxidation and substrates for further reaction with Fe\( ^{2+} \) and Cu\( ^+ \) (Morrissey et al, 1994 a).

1.16.4.3. Termination

In the termination phase, two radicals react to give products that do not sustain the propagation phase. Termination also occurs when antioxidants or free radical scavengers react with free radicals generated during propagation.

1.17. Warmed over flavour (WOF)/ Meat flavour deterioration (MFD)

The primary mechanism for the degradation of desirable flavour in stored meats is lipid autoxidation. Lipids in muscle foods particularly their phospholipid components, undergo degradation to produce a large number of volatile compounds. While hydroperoxides, the primary products of lipid oxidation are odourless and tasteless, their degradation leads to the formation
of an array of secondary products such as aldehydes, hydrocarbons, alcohols, ketones, acids, esters, furans, lactones and epoxy compounds as well as polymers. These latter classes of compounds are flavour-active, particularly aldehydes and possess low threshold values in the parts per million (ppm) or even parts per billion (ppb) levels, thus they are responsible for the development of warmed over flavours (WOF) as coined by Tims and Watts (1958), and meat flavours determination (MFD) (Drumm and Spanier, 1991). MFD is characterised by increased level of off flavour and a decline in desirable flavour attributes (Spanier et al, 1992). MFD long associated with the process of WOF development in meat products is the primary cause of rancidity during frozen storage of meat from all the species of meat products (Channon and Trout, 2002). Cooked meat is susceptible to lipid oxidation and phospholipids are the primary contributors to lipid oxidation and WOF development (Pearson and Gray 1983; Mottram, 1991; Gandemer, 1999). Autoxidation of membrane phospholipids is largely accepted as caused in the formation of WOF. It is thought that the polyunsaturated fatty acids from polar phospholipids rather than triglycerides are responsible for the initial development of off-flavour and off-odours in raw and cooked meats (Renerre and Ladabe, 1993).

Flavour, aroma and volatiles are of utmost importance in the quality attributes of muscle foods, because they influence the judgement of the consumer even before the food is consumed (Shahidi, 2005). The muscle foods in raw state have a mild flavour of their own, however upon heat processing their specific meaty aroma develops (Shahidi, 1989, Farmer, 1992). The shelf life and acceptability of processed ready to eat uncured meats is limited because of the rapid onset of rancidity denoted as WOF. This becomes evident during the refrigerated storage of cooked meat products within a few days (Vasundhara and Honikel, 1992).

A number of physical and chemical changes can initiate peroxidation that proceeds continuously in the presence of a suitable substrate until a blocking defence mechanism is available. Target substances include oxygen itself, PUFA, phospholipids, cholesterol and DNA. But it is the lipids as
constituents of cellular membrane, which are most susceptible because of the high probability of rapidly progressing destructive chain reactions, being initiated.

1.18. Factors affecting oxidative rancidity

1.18.1. Intra muscular lipids

Intra muscular lipids are present mainly in adipose tissue and also in muscle fibres. Intra muscular adipose tissue comprises of cells located along the fibres and in the inter-fascicular area. The fat cells are either isolated or are present in clusters. The distribution of fat cells within the muscles remains largely unknown. They contain almost exclusively triacylglycerols (TAG). The lipids of the fibres consist of cytosolic droplets of TAG, phospholipids (PL) and cholesterol.

1.18.2. Triacylglycerols (TAG)

The amount of TAG in the fibres varies from one muscle to another, but it accounts only for a small part of the total intra muscular TAG, the bulks of which are located in fat cells. TAG content may vary from 0.2% to 5% and depends on many factors, the most important of which are anatomical location, breed, age and sex (Pearson et al., 1977).

1.18.3. Phospholipids (PL)

PL content varies from 0.5% to 1% of wet weight irrespective of the total lipid content of muscles. PL concentrations are independent of lipid level, whereas, TAG increases as total lipid level increases. Muscle PL is mainly composed of phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE), which account for 45-60% and 20-30% respectively. The relative proportion of cardiolipin and phosphatidylinositol (PI) is 2-10% and 4-10% respectively (Kim and Gandemer, 1987; Rabot et al., 1995). Muscle PL also contains a small amount of Sphingomyelin and Phosphatidylserine (less than 2%). Cardiolipin and PE are the most unsaturated of the PLs. PL content and composition are related to the metabolic type of the muscle. Oxidative muscles contain more PL and a higher proportion of cardiolipin and PE than
glycolytic muscles. This is due to the higher amount of mitochondria in oxidative muscles than in glycolytic muscles. Furthermore mitochondria are the only organelles, which contain an appreciable amount of cardiolipin located in their inner membrane, and also exhibit a higher proportion of PE. Hence thigh muscle of chicken contains more PLs than breast muscle. Poultry lipids generally exhibit a higher degree of unsaturation compared to red meat. Thus poultry meat is more susceptible to oxidation. (Igene et al., 1985; Melton, 1983).

Lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipid fraction in the sub cellular bio membranes. Lipid hydroperoxides formed during the propagation phase of the peroxidation process are unstable and are reductively cleaved in the presence of trace elements to give a range of new free radicals and other non radical compounds including alkoxy and alkyl radicals, aldehydes, ketones and a range of carboxyl compounds which adversely affect texture, colour, flavour, nutritive value and safety of muscle foods.

The high sensitivity of PL to oxidation is mainly due to two causes. Firstly, PL contains long chain polyunsaturated fatty acids (PUFAs) that are very sensitive to oxidation. Secondly PLs are membrane components in close contact with catalysts of lipid oxidation located in the aqueous phase of the muscular cell. Within the PL fraction of the muscles PE is most sensitive to oxidation because it contains a large proportion of the long chain PUFAs, and as amino group as polar head. The degree of unsaturation of a fatty acid affects oxidation rate significantly. The relative reaction rate of linolenic acid (C\textsubscript{18:3}) with oxygen is much faster than that of linoleic acid (C\textsubscript{18:2}) and oleic acid (C\textsubscript{18:1}) (Frankel, 1991).

1.18.4. Cholesterol

Cholesterol is found in the cell membrane and is associated with polyunsaturated fatty acids of the membranal phospholipids. Cholesterol being an unsaturated lipid is readily susceptible to oxidation leading to chemically labile hydroperoxides, which are decomposed to form secondary cholesterol.
oxidation products (Kubow, 1992). Various food processing and storage treatments can lead to oxidation of cholesterol in the presence of oxygen, heat, light or radiation to yield COPs (Lee et al., 2001). Major COPs in food are 25-hydroxycholesterol, cholestane triol, hydroxycholesterol derivatives, $\alpha$- and $\beta$-epoxides and 7-ketocholesterol.

Lipid oxidation in muscle foods is affected by meat processing. Processing operations which disrupt the oxidative balance of skeletal muscle include, particle size reduction, which mixes oxidation catalysts with lipids and introduces oxygen into previously anaerobic tissue; cooking, which causes disruption of the cellular organisation of skeletal muscle tissue and causes protein denaturation resulting in loss of antioxidant enzyme activity and release of protein bound iron; and salting which increases the catalytic activity of iron and reduces the antioxidant enzymes activity (Decker et al., 1993). This processing operation can dramatically increase lipid oxidation in muscle foods.

1.19. Effect of transition metal ions

Transition metals such as iron, copper and cobalt may catalyse the initiation and enhance the propagation steps involved in lipid autoxidation (Shahidi, 2005). For example, Fe$^{2+}$ will reductively cleave hydroperoxide to highly reactive alkoxy radicals, which in turn abstract a hydrogen atom from other lipid molecules to form new lipid radicals. This reaction is known as hydroperoxide-dependant lipid peroxidation (Svingen et al., 1979). Morrissey and Tichivangama (1985) and Tichivangama and Morrissey (1985) have reported that ferrous ion at 1-10 ppm levels acts as a strong prooxidant in cooked fish muscle. Similarly, copper (II) and Cobalt (II) were effective prooxidants. These observations are in agreement with the findings of Igene et al., (1979) who reported that iron ions were the major catalysts responsible for enhancement of autoxidation in muscle foods. Furthermore, Shahidi and Hong (1991) demonstrated that the prooxidant effect of metal ions was more pronounced at their lower oxidation state and found that in the presence of chelators such as disodium salt of ethylenediamine tetra acetic acid (Na$_2$EDTA).
EDTA) and sodium tripolyphosphate, the pro-oxidant effect of metal ions was circumvented. Cassens et al., (1979) have shown that 1-3% of the total amount of nitrite added to meat during curing was recovered in the lipid extracts using the Folch method (Folch et al., 1957). Thus stabilisation of meat lipids by nitrite may also be influenced by direct coupling of nitric oxide with lipid radicals, but most of the nitrite was in the protein bound form as nitrosothiol, nitrite/nitrate and nitrosylheme complex, among others (Kanner et al., 1984).

1.19.1. Catalytic activity of iron (heme/non-heme) on lipid oxidation of meat

Hemoproteins in meats are generally known for their prooxidant activity (Pearson et al., 1977; Igene et al., 1979; Rhee, 1988; Shahidi et al., 1988; Johns et al., 1989; Shahidi and Hong, 1991; Wettasinghe and Shahidi, 1997). The prooxidant activity of heme compounds arises, at least in part from their decomposition upon cooking of meat and liberation of free iron. Meanwhile, nitric oxide derivatives of heme pigments, namely nitrosyl myoglobin and nitrosyl ferrohemochrome (or cooked, cured-meat pigment.CCMP), are reported to have antioxidant effect (Wettasinghe and Shahidi, 1997).

Cooked meat will develop off flavour faster than its uncooked counterpart during refrigerated storage (Shahidi, 2005). It has been generally accepted that iron in some form promotes the oxidization of meat lipids and quenching of such prooxidant catalytic activity is considered to be a key factor for the oxidative stability of meat (Zanardi et al., 2000; Schricker and Miller, 1983). Non-heme iron and heme iron, non-heme being the major could promote lipid oxidation in cooked meat (Chem. et al., 1984). The catalytic effect of heme proteins and inorganic iron on lipid oxidation in a muscle model system was investigated by Monahan et al., (1993). Igene et al., (1979) concluded that level of ferrous iron greatly increased during cooking and accelerated lipid oxidation in cooked meat. The release of non-heme iron in the presence of salt and its role in enhancing lipid oxidation in pork was studied by Hsing-Feng-Lin (1997). Heme pigments serve as a source of free iron being readily broken down during the cooking process and catalyse auto
oxidation leading to rancidity in cooked or dehydrated meat, more so if the meat has a high degree of unsaturation.

The non-heme-iron values, taking as a marker to evaluate the extent of lipid peroxidation was reported by Estevez and Cava (2004), for the refrigerated storage of liver pate. Effects of released Fe, on the lipid oxidation of washed cod flesh was investigated during storage at 2°C by Richards and Rong-Li (2004). Pro and antioxidative activity of protein fractions from pork (longissimus dorsi) were analysed for their effect on radical formation and oxidation and also to see the enhancement in non-heme Fe absorption (Carlson et al., 2003). The effects of iron compounds in muscle foods on the lipid oxidation was studied by Karabudak, (2003) and reported as heme Fe in raw red meat and non-heme Fe in cooked meats. The major pro oxidant effect of Fe in foods and its lipid hydroperoxide decomposition leading to the formation of low molecular weight volatile fatty acid break down products, perceived as rancidity was reported by Cho et al., (2003).

Role of ferritin as a lipid oxidation catalyst in muscle food was studied by Decker and Welch (1990). Iron was released from ferritin at the pH found in muscle foods (5.5-6.9). The rate of Fe release from ferritin was influenced by temp and reducing agent concentration. Effects of low concentrations of H₂O₂ on lipid oxidation and of storage and pH on non-heme iron content of raw beef muscle was reported by Rhee et al., (1989). Influence of myoglobin, ferrous and ferric iron, nitrite, EDTA and ascorbate on lipid oxidation in cooked water-extracted and non-extracted mackerel meat were investigated, to elucidate the mechanism involved in oxidative rancidity of cooked mackerel meat during refrigerated storage (Ohshima et al., 1988). Both myoglobin and ferrous iron accelerated lipid oxidation of cooked water extracted mackerel meat. EDTA inhibited the lipid oxidation accelerated by ferrous iron, but not that accelerated by myoglobin. Addition of nitrite in combination with ascorbate resulted in a marked inhibition of lipid oxidation in the cooked mackerel meat. The prooxidant action of haemoglobin and FeSO₄ was studied in model emulsions by Johns et al., (1989) and concluded that inorganic Fe had a little prooxidant activity but haemoglobin exhibited
considerable prooxidant activity. Same pattern was observed in cooked muscle system also. An assay for relative catalytic effect of myoglobin derivatives on lipid oxidation was developed for prediction of oxidative stability of processed meat and meat products by Mikkelsen et al., (1992).

1.20. Effect of salt

Sodium chloride, or table salt is an important ingredient in the meat industry. It acts generally as a prooxidant, but sometimes also as an antioxidant (Kanner and Kinsella, 1983). In comminuted meat samples, under different processing conditions, sodium chloride did not act as an antioxidant, but its neutral or prooxidant effects were clearly demonstrated (St. Angelo et al., 1992; Wettasinghe and Shahidi, 1996). Takiguchi (1989) and Kanner et al., (1991) have demonstrated the prooxidant effect of NaCl in a comminuted muscle system and suggested that it may promote the displacement of iron from binding sites of heme compounds by interfering with iron-protein interactions. The free iron ions so formed may catalyse lipid peroxidation. Recently Wettasinghe and Shahidi (1996) reported that LiCl, KCl, CsCl, MgCl₂ and CaCl₂ exhibited pro-oxidant effects in a cooked meat model system. Thus the overriding pro-oxidant activity was thought to be due to the chloride ion of salts. The results of Rhee et al., (1983 a,b) and Cho and Rhee (1995) for the effect of NaCl, KCl and MgCl₂ in ground pork samples are in agreement with these findings. Further studies on the effect of replacing chloride with their fluoride and iodide counter parts showed inhibition of lipid oxidation in meats but bromide salt behaved very similarly to their chloride counter parts. However, the situation was somewhat different when alkali earth halides were used. Thus it was concluded that pro or antioxidative effects of salts are primarily dictated by their anions, but mediated by their cations because of existing differences in their ability to participate in iron-pairing interactions with the anion counter parts.

1.21. Effect of nitrite and nitrite alternatives

Nitrite is a key ingredient of the cure and is responsible for producing the characteristic pink colour in cooked-cured products and contributes to the typical flavour associated with cured meats and prevents the formation of

The role of nitrite in modifying flavour of cooked meat (Hadden *et al.*, 1975; Macdonald *et al.*, 1980) and suppressing lipid oxidation and MFD in cooked meat (Fooladi *et al.*, 1979) is well documented. Sato and Hegarty (1971) reported that nitrite at 50 ppm was capable of suppressing oxidation of meat lipids. However, Shahidi *et al.*, (1987) demonstrated that presence of a reductant such as sodium ascorbate was essential to eliminate lipid oxidation in meats when less than 150 ppm of nitrite was used. Meat flavour deterioration therefore does not develop in cured meat. This observation might be attributed to any or a combination of the effects related to

a) Stabilisation of heme pigments (Zipser *et al.*, 1964)
b) Stabilisation of membrane lipids (Zubillaga *et al.*, 1994)
c) Chelation of free metal ions and pro-oxidant catalysis (Shahidi and Hong, 1991) and

Heme proteins and their related products as well as transition metal ions have been implicated in meat lipid oxidation (Shahidi and Hong, 1991). As a result of heat processing heme compounds in untreated meats are rapidly oxidised and produce ferrous and ferric ions. In cured meats, nitric oxide produced from nitrite reacts with myoglobin and also combines with Fe$^{2+}$ ions and thus suppresses MFD. The antioxidant activity of some nitrosylheme compounds has been demonstrated by Wettasinghe and Shahidi (1997).

1.22. Inherent antioxidant enzymes

The antioxidant enzymes, CAT, GSH-Px and SOD are believed to provide an important line of defence in protecting biological systems from oxidative reactions. Antioxidant enzymes control endogenous sources of superoxide anions, lipid peroxides and hydrogen peroxide, which interact with
transition metals leading to the production of hydroxyl and alkoxyl radicals (Halliwell and Chirico, 1993). Mei et al., (1994) have suggested that the temperature dependant acceleration of lipid oxidation in cooked muscle foods could be partially due to heat inactivation of antioxidant enzymes.

1.22.1. Glutathione peroxidase (GSH-Px)

It is a selenium containing enzyme that catalyses the reduction of hydrogen or lipid peroxides (ROOH) with reduced glutathione (GSH).

\[ 2\text{GSH} + \text{ROOH} \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{GSSG} \]

Selenium that required for GSH-Px activity is obtained from the diet. GSH-Px is located in the mitochondria and cytosol of skeletal muscle cells. Differences were reported in the GSH-Px activity of red and white muscle. Red muscle of mackerel contains 34-fold higher GSH-Px activity than white muscle, while GSH-Px activity of chicken red muscle is 1.4 fold higher than white muscle.

GSH-Px is the only antioxidant enzyme in muscle foods that has been reported to be influenced by animal diet. Supplementation of chicken diets with 0.25 ppm selenium increased GSH-Px activity in both breast and leg muscle. Selenium supplementation also decreased TBARS formation during the storage of minced muscle suggesting that dietary selenium could increase oxidative stability of muscle foods.

1.22.2. Superoxide dismutase (SOD)

Atmospheric or triplet oxygen can be converted to superoxide anion by the addition of an electron. Superoxide anions promote lipid oxidation by participating in the redox cycling of prooxidant metals and by forming its conjugated acid, the perhydroxyl radical which directly catalyses hydrogen obstruction from unsaturated fatty acids (Kanner et al., 1984). The reactivity of superoxide anion and perhydroxyl radicals is controlled by SOD. It is a metalloenzyme that catalyses dismutation of the superoxide anion radical.
SOD is present in all oxygen-consuming organisms, where it has been suggested that it plays a role in protecting against the damaging effect of superoxide anion radical. Two types of SODs are found in eukaryotic organisms: one containing Cu and Zn is located in the cytosolic fraction and is inhibited by cyanide. SOD could potentially delay the onset of oxidation rancidity in stored meat (Hernandez et al., 2004).

1.22.3. Catalase (CAT)

CAT is located in peroxisome and peroxisome-like micro bodies of cells and is an enzyme that destroys H₂O₂. It is a heme-containing enzyme, which inactivates hydrogen peroxide by converting it to water and oxygen. H₂O₂ is rapidly decomposed by transition metals to form hydroxyl radicals. The hydroxyl radical is an extremely reactive free radical, which can oxidise most biological molecules at diffusion limited reaction rates.

Several researchers have reported SOD, CAT and GSH-Px activities in muscle. The level of enzyme activity varies with the species and muscle type (Pradhan et al., 2000; Hernandez et al., 2002). In addition, antioxidant

\[
\text{SOD} - \text{Cu}^{++} + \text{O}_2^- \rightarrow \text{SOD} - \text{Cu}^+ + \text{O}_2
\]

\[
\text{SOD} - \text{Cu}^+ + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{SOD} - \text{Cu}^{++} + \text{H}_2\text{O}_2
\]

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

\[
\text{Catalase} - \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Compound 1}
\]

\[
\text{Compound 1} + \text{H}_2\text{O}_2 \rightarrow \text{Catalase} - \text{Fe}^{3+} + 2\text{H}_2\text{O} + \text{O}_2
\]

\[
2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}
\]
enzyme activity can vary within the animals of a single species. Animal to animal variations in antioxidant enzyme activity suggests that genetic selection of animals, which have naturally high concentration in muscle enzymes, may be useful in improving oxidative stability of meat. Cooking of meat results in inactivation of these enzymes.

1.23. Inhibition of lipid oxidation by antioxidants

Living tissues do not autoxidise because of the presence of antioxidants in tissue cells (Burton and Ingold 1989). When animal's are slaughtered, the cells become filled with prooxidative compounds, peroxidised lipids and oxygen radicals because the cellular antioxidant capacity is no longer viable (Harrel and Kanner, 1985; Kanner et al., 1991). Reactive oxygen species (ROS) such as superoxide radical (O$_2^-$), hydroxyl radical (OH$^-$), peroxyl radical (ROO$^-$) during lipid oxidation can cause lot of adverse health effects (Fisch et al., 2003; Nakamura et al., 2003; Shon et al., 2003; Valentao et al., 2002). Products of lipid oxidation also interfere with the absorption of protein or folic acid and it has also been found that they can cause pathological changes in the mucus membranes of the digestive tract (Karpinska et al., 2001). Lipid peroxidation and DNA damage caused by reactive oxygen species are associated with various diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, malaria, arthritis and ageing (Beckman and Ames, 1998; Huang et al., 1999). Therefore it is possible that intake of antioxidant may protect against occurrence of these diseases. The positive effects of antioxidants like phenolic compounds, ascorbic acid etc against these diseases were reported by Fritz et al., (2003) and Packer et al., (1999).

In foods including those made from muscle, there is a need to preserve the shelf life of the product until it has been consumed (St. Angelo, 1996). To prevent oxidative deterioration of muscle foods, it is essential to develop technologies to maintain or improve the oxidative balance present in the muscle. Numerous studies have indicated that lipid oxidation may be controlled or at least minimised through the use of antioxidants (Gray et al., 1996). Antioxidants are classified as compounds capable of delaying, retarding, or
preventing autoxidation process (Pszezola, 2001). They vary widely in chemical structure and have diverse mechanisms of action (Table-7).

Table 7: Mechanisms of antioxidant activity

<table>
<thead>
<tr>
<th>Antioxidant class</th>
<th>Mechanism of antioxidant activity</th>
<th>Examples of antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>True antioxidants</td>
<td>Inactivating lipid free radicals</td>
<td>Phenolic compounds</td>
</tr>
<tr>
<td>Hydroperoxide stabilisers</td>
<td>Preventing decomposition of hydroperoxides into free radicals</td>
<td>Phenolic compounds</td>
</tr>
<tr>
<td>Synergists</td>
<td>Promoting activity of true antioxidants</td>
<td>Citric acid, ascorbic acid</td>
</tr>
<tr>
<td>Metal chelators</td>
<td>Chelating heavy metals</td>
<td>Phosphoric acid, Millard compounds, citric acid, ascorbic acid.</td>
</tr>
<tr>
<td>Singlet oxygen quenchers</td>
<td>Transforming singlet oxygen into triplet oxygen</td>
<td>Carotenes</td>
</tr>
<tr>
<td>Substances reducing</td>
<td>Reducing hydroperoxides in a non radical way</td>
<td>Proteins, amino acids</td>
</tr>
<tr>
<td>hydroperoxides</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antioxidants generally function as free radical inhibitors by interfering with free-radical mechanism fundamental to auto oxidation (Sherwin, 1972). They form stable low energy free radicals that will not further propagate the oxidation process. Any reaction that prevents propagation of peroxidation or removes free radicals from the system plays a key role in the termination mechanism (Simic and Taylor, 1987). Thus antioxidants are a very effective class of compounds that are able to inhibit chain peroxidation reactions (Simic et al., 1992). According to the type and concentration of antioxidant used in foods, autoxidation can be either inhibited or retarded. A chain-breaking antioxidant can interfere with either the initiation step or the propagation step as described below.

\[
\begin{align*}
\text{RO'}_2 + A' & \rightarrow \text{non-radical product} \\
\text{RO'}_2 + AH & \rightarrow \text{RO'}_2 + A''
\end{align*}
\]
Once reacted with a substrate molecule, antioxidant free radicals cannot initiate or propagate the oxidation reaction. It should be noted that substances containing polyunsaturated fatty acids are more susceptible to oxidation than those that contain fewer. It is also equally important to note that antioxidants do not prevent oxidation in toto, but they do delay the cascade of free radicals in the oxidative process. The self-life of the product will thus be increased.

Usually antioxidants react with peroxy or alkoxy free radicals, formed by decomposition of lipid hydroperoxides. Others stabilise lipid hydroperoxides and prevent their decomposition into free radicals. Decomposition of hydroperoxides is catalysed by heavy metals, and consequently metal chelating agents can inhibit metal catalysed oxidation. Some substances, called synergists, demonstrate no antioxidant activity in them, but may increase the activity of true antioxidants. Another group decompose lipid hydroperoxides by a non-radical pathway, thereby reducing free radical content. Singlet oxygen oxides lipids many times faster than the common triplet oxygen, and consequently the singlet oxygen quenchers like carotenoids have an important inhibitory effect on lipid oxidation.

Depending on the mechanism of action, antioxidants are therefore classified as:

1.23.1. Primary antioxidants

They retard lipid peroxidation by scavenging free radicals and include phenolic compounds. These components are consumed during the induction period.

1.23.2. Secondary antioxidants

They do not directly scavenge the free radicals but act by a variety of mechanisms including binding of metal ions, scavenging oxygen, converting hydroperoxides to no radical species or deactivating singlet oxygen. Normally secondary antioxidants only show antioxidant activity when a second minor
component is present. Sequestering agents like citric acid are effective only in the presence of metal ions while reducing agents like ascorbic acid are effective in the presence of tocopherols or other primary antioxidants.

The production of ROS and the animal's antioxidant defences are roughly balanced in vivo. Animals have evolved several mechanisms, which limit inappropriate exposure to ROS. Enzymes including SOD, CAT and GSH-Px work together to convert $O_2^\cdot$ through $H_2O_2$ to $H_2O$, thereby minimising the production of $OH^\cdot$. Storage and transport proteins sequester transition metals in forms, which cannot catalyse the conversion of $O_2^\cdot$ and $H_2O_2$ to the more damaging $OH^\cdot$ (Halliwell et al., 1995). Tocopherol is the most important of these compounds in plasma lipids because it is present in concentration at least 15 times higher than the others. It is also an indispensable component of the cell membrane. Ascorbate is considered the most important antioxidant in the extra cellular fluids. The concentration of all these endogenous antioxidants plays an important role in the oxidative stability of meat.

1.23.3. Effect of synthetic antioxidants

Many types of compounds have been utilised as food grade antioxidants with the practice being carried out successfully over fifty years (Cuverlier, et al., 1994). They include that those function as free radical scavengers, oxygen absorbers and chelators. Some of the more popular synthetic antioxidants used are phenolic compounds such as Butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), mono-tertiary butyl hydro quinone (TBHQ) and propyl gallate (PG). These are commonly used as food preservatives (Verhagen et al., 1990, Mc Cathy et al., 2001) and are thus consumed in appreciable quantities by human beings (Nunn et al., 1991). They are primary antioxidants and are very effective in terminating lipid oxidation. BHA and BHT are often used in combination as they have synergistic effect, that is the combined effect from two antioxidants is greater than the sum of the individual effect obtained when used alone (Sherwin, 1972). There are reports about the successful application of BHA, BHT and
TBHQ to prevent the development of WOF in meat and meat products (St. Angelo, 1996; Morrissey, et al., 1998).

However the use of such synthetic antioxidants has been related to health risks resulting in strict regulations of their use in foods (Hettiarachchy et al., 1996; Namiki 1990). According to Ames (1993) and Baardseth (1989) they can have carcinogenic effects in living organisms. Because of the safety and toxicity problems of synthetic antioxidants like BHA, BHT TBHQ and PG that are commonly employed in lipid containing foods (Amarowicz et al., 2000; Van Esch, 1986; Ito et al., 1986), there is a renewed interest in the use of naturally occurring substances. Hence the emphasis is now on the use of natural antioxidants. Further more evidence is accumulating that natural antioxidants in food may have clear benefits because they have anti-carcinogenic effects and inhibit biologically harmful oxidation reactions in the body (Criqui and Ringel, 1994; Frankel et al., 1996). Since lipid peroxidation is implicated on being a cause of atherosclerosis and many degenerative diseases, it is anticipated that use of natural antioxidants not only protects food against rancidity developments; it might also augment the body for its antioxidant defence mechanism. Table-8 gives the advantages and disadvantages of synthetic and natural antioxidants.

Table 8: Advantages and disadvantages of natural and synthetic antioxidants

<table>
<thead>
<tr>
<th>Synthetic antioxidants</th>
<th>Natural antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Medium to high antioxidant activity</td>
<td>• Wide range of antioxidant activity</td>
</tr>
<tr>
<td>• Low water solubility</td>
<td>• Broad range of solubility</td>
</tr>
<tr>
<td>• Increasing safety concern</td>
<td>• Perceived as innocuous substances</td>
</tr>
<tr>
<td>• Use banned for some of them</td>
<td>• Increasing use and expanding applications</td>
</tr>
<tr>
<td>• Widely applied</td>
<td>• Use restricted to specific products</td>
</tr>
<tr>
<td>• Decreasing interest</td>
<td>• Increasing interest</td>
</tr>
<tr>
<td>• Inexpensive</td>
<td>• Expensive</td>
</tr>
</tbody>
</table>
There is a practical need for the screening and selection of natural antioxidants as effective alternatives in the prevention of meat deterioration. Proper and effective use of antioxidants is dependant on the basic understanding of the chemistry of fats, the mechanism of oxidation and function of antioxidant in counteracting this type of deterioration. Knowledge of the mechanism of antioxidant action reinforces several important aspects of antioxidant usage. The activity of an antioxidant is determined by:

- Its reactivity as a hydrogen or electron donating agent (which relates to its reduction potential)
- The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilise and delocalise the unpaired electron.
- Its reactivity with other antioxidants.
- The transition metal chelating potential.

1.23.4. The main classes of natural antioxidants

1.23.4.1. Tocopherols

Tocopherol (Vit-E) is the most important of the antioxidants present in muscle as it is present in concentrations at least 15 times higher than any of the other antioxidants. \(\alpha\) - tocopherol is the most effective antioxidant. It is also an indispensable component of the cell membrane.
Tocopherol inhibits lipid oxidation both in vitro and in vivo by scavenging free radicals. When it encounters a peroxyl radical (ROO'), Tocopherol (TOH) donates hydrogen from its chromonol phenolic group to form hydroperoxide and a tocopheroxyl radical (TO').

\[
\text{TOH} + \text{ROO}^\cdot \rightarrow \text{ROOH} + \text{TO}^\cdot
\]

TOH can scavenge ROO' about \(10^4\) times faster than they can react with RH, so that only relatively small amount of TOH need be present for it to be an effective antioxidant. Tocopherol might be regenerated by reaction at the aqueous interface with ascorbate or any other aqueous phase chain breaking antioxidant like reduced glutathione.

The antioxidant activity of Tocopherol is ascribed to its phenolic ring structure that is capable of stabilising the resulting tocopheroxyl radical. Tocopherol occurs in nature in eight different forms. These forms differ greatly in their biological activity. Tocopherol is very stable with respect to heat and has an excellent carry through effect. The dietary supplementation of Tocopherol or direct addition of it to meat during processing determines tocopherol content in meat products. The increased vitamin E concentration in meat can improve the storage stability of raw meat.

1.23.4.2. Carotenoids

Another group of lipid soluble endogenous antioxidants in muscle is carotenoids. Carotenoids are obtained from the diet. They are a group of lipid soluble pigments based around an isoprenoid carbon skeleton (Cooper et al., 1999). The most important of these is β-carotene. They are particularly efficient scavengers of singlet oxygen but can also trap peroxyl radicals at low oxygen pressure (Yanishleiva et al., 1998). β-carotene react with peroxyl radical to produce a resonance stabilised carbon centred radicals shown in the equation.
The other important role of certain carotenoids is as precursors of vitamin A which also has antioxidant properties. β-carotene has the highest pro-vitamin A activity of all carotenoids (Kotareddy and Devi, 1997). Ubiquinone or co enzyme Q has an isoprenoid side chain and is found in the mitochondria. Ubiquinones inhibit lipid oxidation by free radical scavenging. The reduced form of co enzyme Q is also an effective lipid soluble chain breaking antioxidant (Shi et al., 1999). Very little is known about the role of ubiquinones in muscle, but their presence in mitochondria suggests that they could be especially important in the oxidative stability of red muscle.

1.23.4.3. Ascorbic acid

One of the naturally occurring antioxidants that are used widely by the food industry is ascorbic acid, which has a varied chemistry. Depending on conditions ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger (Ghosh et al. 1996).
Ascorbic acid and its esterified derivatives function as antioxidants by protecting double bonds and scavenging oxygen. As an oxygen scavenger, ascorbic acid acts by being preferentially oxidised over other oxidisable components in the food system because the 2- and 3- positions are unsubstituted. In this situation oxygen is removed and converted into water at the expense of ascorbic acid, which is oxidised to form dehydro ascorbic acid. It efficiently scavenges H$_2$O$_2$, OH', ROO' and O$_2$$^{•-}$, is reactive enough to effectively intercept oxidants in the aqueous phase before they can attack and cause detectable oxidative damage to lipids. In the presence of a metal ion, such as iron, ascorbic acid is able to scavenge oxygen faster due to a chelated intermediate. In this scheme, a reducing agent such as reduced glutathione, is needed to convert the dehydro ascorbic acid back to ascorbic acid (Cort, 1982).

Some important chemical characteristics of ascorbic acid include its redox properties, which enable it to function as a reducing agent and as a free radical scavenger. In this role, ascorbic acid can donate a hydrogen atom, for free radical chain inhibition. Ascorbic acid can act as a synergist with tocopherol, by converting oxidised Tocopherol back to the reduced form. In these reactions, ascorbic acid can be regenerated by its action with reduced glutathione or NADH$^+$ (Cort, 1982). It should be noted that ascorbic acid can chelate metals (Martell, 1982) and can promote carbohydrate-amine browning reactions (Kamiya, 1960).

1.23.4.4. Effect of ascorbic acid in controlling oxidation of lipids in meat

There has been much interest in using derivatives of ascorbic acid as antioxidants. One such compound is ascorbyl palmitate. In the structure of
ascorbyl palmitate, the 2- and 3- positions are occupied by hydroxyl groups, the 6-position contains the fatty acid ester substituent. Other derivatives synthesised more recently are ascorbate-2-phosphate and ascorbate-2-triphasphate (Liao and Seib, 1990). Both of these compounds were reported to inhibit lipid oxidation as measured by chemical means in ground meat (St. Angelo et al., 1988). Ascorbate-2-phosphate was also found to inhibit WOF as measured by sensory means in beef (St. Angelo et al., 1988). Ascorbate-2-phosphate was used to inhibit off-flavour development in cooked frozen turkeys (Craig et al., 1991).

Many meat research scientists have recently been putting concerted effort to use L-ascorbic acid available in nature as antioxidant. Shivas et al., (1984) studied the effect of ascorbic acid on the display life of ground beef and found that the display life of ground beef was extended to at least 5 days when ascorbic acid was added. Use of ascorbic acid to control the lipid peroxidation has been reported in some species of meat (Okayama et al., 1987; Mitumoto et al., 1991; Sahoo and Anjaneyulu 1997; Kim et al., 1997). Effect of antioxidants (ascorbic acid (0-1% w/w.) sesamol (0.01% w/w) and alpa Tocopherol (0.01% w/w) ) on lipid oxidation and development of off odour volatiles in irradiated beef mince was studied by Nam et al., (2003) and reported that addition of ascorbic acid to beef mince before irradiation effectively reduced lipid oxidation and off odour. Effect of ascorbic acid and oxygen species on iron-related lipid oxidation in meat homogenates was studied by Kim et al., (1998). Moderate reduction of oxidative changes in ground beef and turkey by the addition of ascorbate salts was reported by Craig et al., (1996). Extension of shelf life of ground chevon during refrigerated storage by using ascorbic acid was reported by Verma and Sahoo (2000).

1.23.4.5. Carnosine, Anserine and Glutathione

Carnosine and Anserine are N – β – alanyl –L – histidine and N –β – alanyl –3 – methyl-L-histidine dipeptides, respectively, endogenous to skeletal muscle. White muscle fibres generally have higher anserine and carnosine
concentrations than red muscle fibres. It acts both as a buffering agent and as an antioxidant.

\[
\text{H}\text{COOH} \quad \text{CH}_2\text{CHNHCOCH}_2\text{CH}_2\text{NH}_2
\]

Carnosine

\[
\text{CH}_3\text{ COOH} \quad \text{CH}_2\text{CHNHCOCH}_2\text{CH}_2\text{NH}_2
\]

Anserine

The antioxidant mechanism of carnosine appears to be a combination of its ability to act as a chelator and a free radical scavenger. The hydrophilic nature of carnosine is of biological significance because it can provide protection in the cystolic environment where many lipid oxidation catalyst and free radicals are found.

\[
\text{H}_3\text{N}^+ - \text{CH} - \text{CH}_2 - \text{CH}_2 - \overset{\circ}{\text{C}} - \text{NH} - \text{CH} - \text{CH} - \overset{\circ}{\text{C}} - \text{NH} \text{CH}_2 - \text{COO}^-
\]

\[
\text{COO} \quad \text{CH}_2 \quad \text{SH}
\]

Glutathione
Glutathione is a tripeptide that inhibits lipid oxidation by inactivating free radicals and by providing a source of electrons which allows glutathione peroxidase to enzymatically decompose hydrogen and lipid peroxides. Carnosine, anserine and glutathione are largely unaffected by heat processing. The potential of these peptides as antioxidants in meat has been shown (Decker and Crum, 1993).

1.23.4.6. Phenolics

The term phenolics encompasses approximately 8000 naturally occurring compounds, all of which possess one common structural feature, a phenol (an aromatic ring bearing at least one hydroxyl substituent), (Table-9).

Table 9: Main classes of phenolic compounds

<table>
<thead>
<tr>
<th>Class</th>
<th>Basic skeleton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple phenols</td>
<td>C₆</td>
</tr>
<tr>
<td>Benzoquinones</td>
<td>C₆</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>C₆–C₁</td>
</tr>
<tr>
<td>Acetophenones</td>
<td>C₆–C₂</td>
</tr>
<tr>
<td>Phenyl acetic acid</td>
<td>C₆–C₂</td>
</tr>
<tr>
<td>Hydroxy cinnamic acids</td>
<td>C₆–C₃</td>
</tr>
<tr>
<td>Phenyl propenes</td>
<td>C₆–C₃</td>
</tr>
<tr>
<td>Coumarins, isocoumarins</td>
<td>C₆–C₃</td>
</tr>
<tr>
<td>Chromones</td>
<td>C₆–C₃</td>
</tr>
<tr>
<td>Naftoquinones</td>
<td>C₆–C₄</td>
</tr>
<tr>
<td>Xanthones</td>
<td>C₆–C₁–C₆</td>
</tr>
<tr>
<td>Stilbenes</td>
<td>C₆–C₂–C₆</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>C₆–C₂–C₆</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>C₆–C₂–C₆</td>
</tr>
<tr>
<td>Lignans, neolignans</td>
<td>(C₆–C₃)₂</td>
</tr>
<tr>
<td>Lignins</td>
<td>(C₆–C₃)ₙ</td>
</tr>
</tbody>
</table>
Phenolic compounds are ubiquitous in plant foods and have been associated with the sensory and nutritional quality of fresh and processed plant foods (Ho et al., 1992; Robards et al., 1999). They form one of the main classes of secondary metabolites with a large range of structures and functions. Phenolic compounds have many biological activities such as chelation of metals, scavengers of active oxygen species and as antioxidants. Phenolics react with the ROS according to the following generalised scheme:

\[ \text{Ph} - \text{OH} + \text{ROO}' \rightarrow \text{Ph} - \text{O}' + \text{ROOH}. \]

The newly formed phenolic radical is much more stable due to mesomeric delocalisation of the odd electron and usually does not generate new ROS. Thus it acts as a chain breaker. The plant phenolics because of their diversity and extensive distribution are the most important group of natural antioxidants.

Current classification divides the broad category of phenolics into polyphenols and simple phenols, based solely on the number of phenol subunits present. Polyphenols possessing at least 2 phenol subunits include the flavonoids and those compounds possessing three or more phenol subunits are referred to as tannins.

1.23.4.7. Polyphenols

Flavonoids constitute a large group of naturally occurring plant phenolics (Heirn et al., 2002). They are important in the plant for normal growth, development and defence against infection and injury. The basic structures of these compounds consist of two aromatic rings linked by a three-carbon aliphatic chain, which normally gets condensed to form a pyran, or less commonly a furan ring. Some of the common flavonoids are apigenin, chrysin, luteolin, daidzein, quercetin, myricetin, morin and kaempferol. Approximately 90% of the flavonoids in plants occur as glycosides Glycosides are not antioxidants whereas their corresponding aglycons are antioxidants. Flavonoids can exert their antioxidant activity by inhibiting the activities of enzymes including xanthine oxidase, myeloperoxidase, lipoxygenase and...
cyclooxygenase, by chelating metal ions, by interacting with other antioxidants such as ascorbate and most importantly by scavenging free radicals. They possess a high reactivity for the hydroxyl radical, which is one of the most reactive compounds responsible for lipid peroxidation. Flavonoids with free hydroxyl groups act as free radical scavengers, and multiple hydroxyl groups, enhance their antioxidant activity (Jovanovik, 1994; Cook and Samman, 1996). Tannins are complex and poorly defined group of water-soluble phenolics with high molecular weights. They are highly hydroxylated molecules and can form insoluble complexes with carbohydrates and protein. In most polyphenolic antioxidants both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity. Polyphenols possess the ideal structural chemistry for free radical scavenging activities as they form low energy radicals through stable resonance hybrids. In addition the propensity for metal chelation, particularly iron and copper, supports the role of polyphenols as preventative antioxidants in terms of inhibiting transition metal catalysed free radical formation.

Natural antioxidants are primarily plant phenolics that may occur in all parts of the plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Plant phenolics are multi functional and can act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers (Mathew and Abraham, 2006). Many natural antioxidants have already been isolated from different kinds of plants, such as oilseeds, cereal crops, vegetables, leaves, roots, spices and herbs etc. (Ramarathnam et al., 1995; Shon et al., 2003; Wettasinghe and Shahidi, 1999). All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants (Bandoniene and Murkovic, 2002).

Spices and herb extracts were reported to contain compounds with antioxidant activity when used in food systems (Shahidi and Champaign, 1998). Antioxidant properties of natural plant extracts in meat were reported by Ahn et al., (2002). The effectiveness of spices in controlling the lipid oxidation and WOF in meat was reported by Jayathilakan et al., (1997). Further studies on the inhibitory effects of spices on the non-heme Fe release
and thus to control the lipid oxidation and enhancement of shelf life in fluidised bed dried mutton sample was carried out by Jayathilakan et al., (2007).

Oxidative stability of fermented goat meat sausage was studied by Nassu et al., (2003) and reported that addition of 0.05% of rosemary extract provided the most effective antioxidant activity. Effects of natural antioxidants like lycopene rich tomato pulp and pepper on the lipid oxidation and stability of beef patties was investigated by Escalante et al., (2003) and reported the significant antioxidative effect of both samples, pepper being the highest.

Antioxidant properties of cardamon, cinnamon and cloves for the preservation of cookies was reported by Badei et al., (2002), and concluded that all the spices increased oxidative stability of lipids in cookies and the greatest lipid-oxidation limited shelf life (7 months) was achieved with cloves. Effect of plant phenolics like rapeseed and pine bark phenols in inhibiting the oxidation of lipids was studied by Vuorela et al., (2005) and reported the potential for use in meat products as sources of antioxidants. Estevez and Cava (2006) reported that at 150 ppm level, rosemary essential oil exhibited an antioxidative effect significantly reducing the generation of lipid and protein oxidation products. At higher levels (300 and 600 ppm) the essential oil had in general no effect on lipid oxidation while significantly enhanced the oxidation of proteins and the release of iron from myoglobin. Examination of the suitability of potato peel extract for controlling lipid oxidation (Kanatt et al., 2005) revealed that addition at 0.04% to meat before gamma-irradiation retarded lipid peroxidation of irradiated meat during cold storage. Further studies by Kanatt et al., (2004) suggest that chitosan isolated from large quantities of shell fish waste can be added to gamma-irradiated meat to act as an antioxidant and substantially retard lipid peroxidation.

The effectiveness of mint leaves a common herb used in Indian cuisine, as a natural antioxidant for radiation processed lamb meat was investigated by Kanatt et al., (2005) and reported the beneficial effect of mint leaves in controlling the oxidation of lipids during irradiation and storage of lamb meat. Infusion of chicken meat with plant extracts like green tea at 3000
ppm was found to be effective for preventing and minimising major sensory changes in meat during irradiation (Rababah et al., 2004).

Effect of rosemary extract and onion juice on the oxidative stability of sardine mince was investigated by Serdaroglu and Felekoglu (2005) and reported the positive effects of these antioxidants in controlling the lipid oxidation and retaining the PUFA content during storage.

Antioxidant and antimicrobial effects of garlic in chicken sausage was studied by Sallam et al., (2004) and concluded that garlic powder and fresh garlic may be useful in preserving meat products due to their combined antimicrobial and antioxidative activities. Studies on the antioxidant activities of cinnamon bark extract was reported by Mathew and Abraham, (2006) and showed very good antioxidant activity. Addition of rosemary extract and grape seed extract in cooked ground beef had a significance effect in reducing TBARS and hexanal values during storage (Ahn et al., 2002). Antioxidant potential for aloe vera, fenugreek, ginseng, mustard, rosemary, sage, soy protein, tea catechins and whey protein concentrate was evaluated by Mc Carthy et al., (2001) in raw and cooked pork patties and observed that tea catechin was the most effective antioxidant in reducing lipid peroxidation similar to BHA, the synthetic antioxidant.

The importance of natural antioxidants found in plant has begun to receive much attention as non-toxic and safe antioxidants (Osada and Shibamoto, 2006). Recently volatile chemicals found in natural plants such as beans, herbs and spices have been reported to possess antioxidative properties (Lee et al., 2000; Lean and Mohammed, 1999).

1.23.4.8. Maillard reaction products (MRPs) as antioxidants

Compounds that are formed by non-enzymatic chemical reactions involving condensation of an amino group and reducing group and formation of intermediates that polymerise to form brown pigments are known as Maillard Reaction Products (MRPs). In food the reactants are commonly amino acids, both free and peptide bond and reducing sugars. The reaction
has been named after the French scientist Louis-Camille Maillard, who studied it in the period, 1912-1917.

The reaction involved in the formation of early Maillard reaction products can be explained as follows (Fig-2). The carbonyl carbon of the reducing sugar first undergoes nucleophilic attack by the amino nitrogen lone pair electrons. This is followed by loss of water and ring closure to form glycosyl amine. In the presence of excess reducing sugar diglycosyl amine may be formed. The glycosyl amine undergoes the Amadori rearrangement to produce a 1-amino-2-keto sugar.

Fig 2. Formation of early MRPs

If the initial sugar reactant is a ketose, a glycosylamine is formed by the same mechanism for aldoses but it can then undergo a reverse Amadori (Heyns) rearrangement to yield 2-amino aldose.

The Amadori compound formed may be degraded in at least two distinctive ways, one proceeding through a 3-deoxyseone intermediate and the
other through a methyl α-dicarboxyl compound. Both paths produce melanoidins pigments which have pyrazine and imidazole rings in addition to HMF and reductones.

Maillard first reported the reactions between sugars and aminoacids, polysaccharides and polypeptides or proteins. Over the years MRPs have been shown to produce a number of flavour precursors, flavourants, antioxidants and polymerised brown pigments (Bailey et al., 1987).

Hodge and Rist were the first to demonstrate that MRP had antioxidant activity when added to oils (Hodge and Rist, 1953). Later Evans et al., (1958) showed that reductones isolated from MRP could retard oxidation of vegetable oils. Zipser and Watts (1961) were the first to demonstrate that MRP was formed during excessive heating of beef and that these products contributed to decreasing lipid oxidation during storage. Sato et al., (1973) showed that MRP had strong antioxidant activity in preventing WOF in beef. Huang and Green (1978) later confirmed these finding and suggested that a temperature of 90°C was necessary to produce MRP that had antioxidant activity. Lingnert and Ericksson (1981) later demonstrated that MRP were also able to inhibit oxidation in emulsion-type sausages. More recently, MRP formed from reacting glucose with histidine were able to inhibit the development of WOF and to retard lipid oxidation (Hedrick et al., 1980).

Melanoidins or pre-melanoidins resulting from the Maillard reaction have strong antioxidant properties in certain lipid mixtures (Lingnert and Ericksson, 1981; Bailey et al., 1987). The Maillard reaction produces products with antioxidant activity that includes brown pigments. However, fluorescent Maillard reaction products may be precursors of the brown pigments and may also possess antioxidant activity. Morales and Perez (2001) suggested that fluorescence measurement has potential application during food processing for optimising formation of MRPs with antioxidative activity in foods, where browning is not acceptable. Maillard reaction products produced from whey suppressed the formation of hydroperoxides and Thio-
barbituric acid reactive substances (TBARS) and lowered oxygen uptake in the model system (containing copper catalysed soybean oil). Bedinghaus and Ockerman (1995) and Bedinghaus (1994) studied the effect of MRPs prepared from reducing sugars and free amino acids in cooked ground pork patties and reported significant difference in the lipid oxidation profile of control and MRP treated samples.

Oxidative reactions of model Maillard reaction products and α-tocopherol in a flour-lipid mixture was studied by Wijewickreme and Kitts (1998) and concluded that application of MRP in combination with α-tocopherol showed no synergistic or antagonistic effect. Conditions and type of reducing sugar and amino acid in synthesis of MRP can influence their anti- or pro-oxidant activity. Byrne et al., (2002) investigated the effects of MRP on WOF development in chicken meat and concluded the positive effects in controlling WOF.

MRPs have been shown to inhibit oxidation in model systems (Mastrocola and Munari, 2000; Mastrocola et al., 2000) as well as in storage experiments with food products (Nicoli et al., 1997). There have also been reports of volatile MRPs, which are responsible for flavours in food, possessing antioxidative activities, volatile compounds, in particular heterocyclic flavour chemicals, obtained from a sugar/amino acid model system have been reported to inhibit the oxidation of lipids (Elizalde et al., 1992; Shaker, et al., 1995). MRP obtained from Histidine-Glucose mixture possess peroxy radical scavenging activity (Yilmaz and Toledo, 2005). Chuyen, et al., (1990) reported that peptide-glucose reaction products, amadori rearrangement products, melanoidins, modified protein and its hydrolysate, brown pigment isolated from food stuffs showed strong scavenging activity against hydroxyl radical and superoxide anion. Yamaguchi, et al., (1981) and Chuyen et al., (1990) have studied the MRPs antioxidative effect generated from reaction of amino acids or peptides with reducing sugars. Metal chelating and antioxidant activity of model MRPs were reported by Wijewickerme and Kitts (1998). In many respects an
underlying cause or effect for the biological properties of MRPs reported by various investigators has been the antioxidant activity (Bedinghaus and Ockerman, 1995; Smith and Alfarvaz, 1995).

The Maillard reaction produces a variety of intermediate products and finally brown pigments (melanoidins) are formed (Van Bockel, 1998). The reaction is influenced by many factors, including reactant concentration, temperature, time, initial pH and water activity (Baxter, 1995; Ashoor and Zent, 1984, Naranjo et al., 1998; Tanaka et al., 1994; Wijewichreme and Kitts, 1997). The Maillard reaction produced from an amino acid-sugar model system has been associated with the formation of compounds with strong antioxidant activity (Tanaka et al., 1990; Yen and Hsieh, 1995; Yoshimura, et al., 1997). Antioxidant activity of MRPs derived from a protein-sugar system has been also studied (Jing and Kitts, 2002; Yeboab et al., 1999). However Lingnert and Eriksson (1980) found a lower antioxidant activity of MRPs derived from protein-sugar model system than amino acid-sugar model systems. Additionally, antioxidative activities of MRPs are affected by pH and temperature used (Alaiz et al., 1999; Mastrocola and Munari, 2000). MRPs have been used to prevent lipid oxidation in many products. MRPs exhibit as antioxidative activity in meat products. (Alfawaz, et al., 1994; Bedinghaus and Ockerman, 1995). Furthermore, MRPs derived from a fructose-tryptophan system also prevent the oxidation of sardine lipid (Chiu, et al., 1991).

Severini and Lerici (1995) studied the interaction between the Maillard reaction and lipid oxidation in model systems during high temperature treatment and reported the slow down of the rate of lipid oxidation due to the presence of MRPs. Maillard reaction products already known for their antioxidant properties were also shown to have a strong inhibiting effect on enzymic browning (Nicoli et al., 1991).

Maillard reaction products (MRPs) were synthesised from honey and lysine and their antioxidative activity was compared with that of honey alone, when added directly to meat. Antioxidative effects were monitored by measuring TBARS and hexanal. Addition of increasing amount of MRPs to
turkey meat increased the antioxidative activity (Antony et al., 2002). Guerard and Sumaya (2003) studied the effect of addition of the reaction mixture of protein hydrolysates with glucose in improving the antioxidative effect and antiradical activity. They reported the molecular rearrangements could be the cause of increasing these properties. Metal chelating behaviour of MRPs extracted from model and food system was reported by Wijewickreme and Kitts (1997). Metal binding ability of Maillard reaction products was investigated by spectrophotometric monitoring for complex/chelate formation. In addition, the antioxidative activity of MRPs in the presence of Cu (II) was also evaluated by Bersuder et al., (2001) and reported the reduction in prooxidant activity of Cu (II). Antioxidative potential of melanoidins isolated from roasted glucose-glycine model system was studied by Wagner et al., (2002) and found to increase the stability of oils during storage. The enzymic inhibition activity of MRPs were studied by Kyung and Park (2005) and reported the inhibition effect of polyphenol oxidase in potato. Ramirez et al., (2004) reported the antioxidative effect of MRPs during frying and its effect on lipid oxidation and colour in meat during frying and refrigerated storage. Antioxidant activity of Maillard reaction products from a porcine plasma protein – sugar system was studied by Benjakul et al., (2005) and concluded that antioxidant activity of PPP – sugar MRPs was coincidental with the browning development and the formation of intermediate products.