Introduction
and
Review of literature
1. Introduction and Review of Literature

Meat industry is a classical example for paradoxical situation prevalent in our country. Although India is bestowed with largest livestock population in the world meat production, consumption level is one of the lowest in the world. India has livestock population of 1558.4 millions, as shown in Table.1, with cattle (189.4 millions), buffalo (105 millions), goat (140 millions) and sheep (57 millions), poultry (1049 millions) and pig (18 millions). Further, the country has 16.49% of world’s cattle population and 56.78% of world’s buffalo population. Out of the total livestock in the country, around 12.15% are cattle, 6.73% are buffaloes, 3.65% are sheep, 67.31% are poultry, and 8.98% are goat (DAHD, 2010).

In spite of big potential, the meat industry in India has not taken its due share. The major constraint for the industry are lack of scientific approach to rearing of meat animals, unorganized nature of meat production and marketing, socio-religious taboos associated with meat eating, inadequate infrastructure facilities and poor post harvest management. The situation is further compounded by insistence of domestic consumers to buy fresh cut meat than processed or frozen. Hence, our meat industry still remains unorganized and unscientific. At present we have 3600 registered slaughter houses in our country. A majority of these abattoirs have out dated, primitive slaughtering facilities, use unhygienic practices and lacks basic facilities for the production of wholesome meat. Further, most of the domestic meat consumption comes from poultry, sheep, goat that are slaughtered in unregistered roadside meat shops. Thus domestic consumers are exposed to great risk due to laxity in meat inspection and quality control.

Due to increased urbanization, rising income, changing food habit and consumer awareness, domestic consumers too demanding hygienically produced meat and meat products. Therefore, there is an urgent need to reorganize and strengthen the meat sector based on scientific approach to provide wholesome meat to domestic consumers. India can be food basket of world in change in economic scenario in globalized world (Viswanadham, 2005). To play a major role in international meat trade, Indian meat industry need urgent interventions to make it more scientific, organized to meet the changing consumer demand and meet global standard on health, safety and quality. Reduction in transport costs and expansion of cold chain
infrastructure will go a long way in making our meat products internationally competitive.

The healthy disease free animals are procured from the livestock markets/farmers/feedlots/farms and are rested for 24 hour to produce quality meat. During production of meat veterinarians subject the animals to ante-mortem examination during the rest period. After their approval, they are slaughtered either under Halal/Jhatka procedure depending upon consumers choice. Thereafter, the Veterinarian subjects the carcass to post-mortem examination. After its approval for safety, it is sold in the retail market as fresh meat (FAO, 1990).

Meat is defined by the Codex Alimentarius (2003) as “All parts of an animal that are intended for, or have been judged as safe and suitable for, human consumption”. Meat is composed of water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components, and small quantities of carbohydrates.

Meat consumption in developing countries has been continuously increasing from a modest average annual per capita consumption of 10 kg in the 1960s to 26 kg in 2000 and will reach 37 kg around the year 2030 according to FAO STAT (2012) projections. This forecast suggests that in a few decades, in developing countries consumption of meat will move towards that of developed countries where meat consumption remains stagnant at a high level. Current trends indicate that by the end of the century, 80% of the world's population will be living in the under-developed countries and a significant number of these will have large food deficits. An increased production of animal protein would make an important contribution towards filling this deficit (FAO, 1984; FAO, 1985; FAO, 1990). India ranks top in animal population. The meat and meat processing industry is still to come up. Some top players in the meat processing industry like Venkateswara Hatcheries, Godrej Agrovet, Vista Processed Food, Al Kabeer, Allanasons etc., with modern state-of-the-art slaughter and processing plants, have changed the entire scenario, making the industry grow at almost 10%. There is a huge scope for expanding exports, especially in buffalo and poultry meat, eggs and dairy products. India ranks first in world buffalo population, with 56.5% i.e. 94.1 million of buffalo population and one-sixth of goat population in the world (MOFPI, 2011).
1.1. Sources of meat

In India, the most common sources of meat are sheep and poultry and to a lesser extent cattle, buffaloes and pigs. In some regions, other animal species such as camels, yaks, horses, ostriches and game animals are also eaten as meat. To a limited extent, meat is also derived from exotic animals such as crocodiles, snakes and lizards (FAO STAT, 2012). For thousands of years, poultry supplied meat and eggs, cattle, sheep and goats provided meat and milk, and pigs provided a source of meat. These species are the main sources of animal protein for humans. The meat derived from cattle is known as beef, meat derived from pigs as pork and from chickens as poultry. Pork is the most widely eaten meat in the world accounting for over 36% of the world meat intake. It is followed by poultry and beef with about 33% and 24% respectively (FAO STAT, 2012). The world livestock population for different species has been shown in Table.2.

Table.1. Livestock Production and Meat Production in India-2010

<table>
<thead>
<tr>
<th>Livestock Species</th>
<th>Population (in millions)</th>
<th>Meat Production (million tonnes)</th>
<th>Share in total meat production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>189.4</td>
<td>1.49</td>
<td>31.1</td>
</tr>
<tr>
<td>Buffalo</td>
<td>105</td>
<td>1.58</td>
<td>30.5</td>
</tr>
<tr>
<td>Sheep</td>
<td>57</td>
<td>0.25</td>
<td>4.9</td>
</tr>
<tr>
<td>Goats</td>
<td>140</td>
<td>0.57</td>
<td>10</td>
</tr>
<tr>
<td>Pigs</td>
<td>18</td>
<td>0.60</td>
<td>10</td>
</tr>
<tr>
<td>Poultry</td>
<td>1049</td>
<td>1.60</td>
<td>13.4</td>
</tr>
</tbody>
</table>

(Source: DAHD, 2010)

Table.2. World livestock numbers (million head)

<table>
<thead>
<tr>
<th>Livestock Species</th>
<th>1990</th>
<th>2000</th>
<th>2010</th>
<th>% Change 1990-2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>1447</td>
<td>1479</td>
<td>1623</td>
<td>12</td>
</tr>
<tr>
<td>Porcine</td>
<td>856</td>
<td>899</td>
<td>966</td>
<td>13</td>
</tr>
<tr>
<td>Poultry</td>
<td>11792</td>
<td>16078</td>
<td>21489</td>
<td>82</td>
</tr>
<tr>
<td>Ovine</td>
<td>1799</td>
<td>1811</td>
<td>2000</td>
<td>11</td>
</tr>
</tbody>
</table>
1.2 Production of meat and meat products

The amount of meat consumption per person and meat preference of consumers in different countries is influenced by the differences in social and economic state, religious beliefs and geographical region (Bender, 1992). Table.3. shows India’s meat export statistics from 2008-2009 to 2010-2011. India's export of buffalo meat products has been increased from 460 031 MT in 2008-09 to 513 668 MT in 2010-11. The export of sheep/goat meat products has also been increased from 37113 MT in 2008-09 to 52 868 MT in 2010-11.

Meat dominated the exports with a contribution of over 97%. The demand for bovine meat in international market has sparked a sudden increase in the meat exports from India. The main markets for Indian bovine meat are Malaysia, Philippines, Mauritius, and Gulf countries (APEDA, 2012).India’s exports of Processed Meat attained 1,366.17 MT with the value of Rs. 2,104.86 crores in 2010-11. India's livestock population includes, 88 million buffaloes, which is 58 per cent of the world’s buffalo population. Animals, which are generally used for production of meat comprise of sheep and goats, pigs and poultry. Besides about 3600 slaughter houses, there are nine modern abattoirs and one integrated abattoir meat processing plant for slaughtering buffaloes for exports and domestic consumption (APEDA, 2012).

Table.3. Export of meat products from India

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>4,62,749.65</td>
<td>4,83,971.0</td>
<td>4,95,019.70</td>
<td>5,48,060.09</td>
<td>7,09,437.49</td>
<td>8,41,268.61</td>
</tr>
<tr>
<td>Sheep/Goat</td>
<td>37,790.64</td>
<td>49,336.97</td>
<td>52,868.00</td>
<td>74,720.08</td>
<td>11,908.39</td>
<td>25,318.88</td>
</tr>
<tr>
<td>Poultry</td>
<td>10,57,016.4</td>
<td>42,205.80</td>
<td>10,16,783.08</td>
<td>37,211.88</td>
<td>6,19,150.77</td>
<td>30,132.73</td>
</tr>
<tr>
<td>Processed products</td>
<td>857.66</td>
<td>1,014.42</td>
<td>716.20</td>
<td>958.52</td>
<td>1,366.17</td>
<td>2,104.86</td>
</tr>
</tbody>
</table>

Source: DGCIS Annual Export (APEDA, 2012)
1.3. Composition of meat and nutritive value

In a broad sense the composition of the 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 behavior of meat and of its variability, cannot be based on such a simplification (Lawrie, 2006). The nutritional composition of various meats is given in Table.4.

On the contrary it must be recognized 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, et al., 2001).

Table.4.Nutritional composition of meats (100g)

<table>
<thead>
<tr>
<th>Product</th>
<th>Water</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>KJ*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef (lean)</td>
<td>75.0</td>
<td>22.3</td>
<td>1.8</td>
<td>1.2</td>
<td>116</td>
</tr>
<tr>
<td>Beef carcass</td>
<td>54.7</td>
<td>16.5</td>
<td>28.0</td>
<td>0.8</td>
<td>323</td>
</tr>
<tr>
<td>Pork (lean)</td>
<td>75.1</td>
<td>22.8</td>
<td>1.2</td>
<td>1.0</td>
<td>112</td>
</tr>
<tr>
<td>Pork carcass</td>
<td>41.1</td>
<td>11.2</td>
<td>47.0</td>
<td>0.6</td>
<td>472</td>
</tr>
<tr>
<td>Veal (lean)</td>
<td>76.4</td>
<td>21.3</td>
<td>0.8</td>
<td>1.2</td>
<td>98</td>
</tr>
<tr>
<td>Chicken</td>
<td>75.0</td>
<td>22.8</td>
<td>0.9</td>
<td>1.2</td>
<td>105</td>
</tr>
</tbody>
</table>

Composition is defined as the aggregate of ingredients, their arrangement, and the integrated inter relationship that forms a unified, harmonious whole. Meat is a valuable part of the human diet because: It is most concentrated and easily assimilable form of nitrogenous foods, and it is a good source of first class protein, i.e. it contains the amino acids essential for human life, It is stimulating to metabolism because of its high protein content, i.e. it assists the body in the production of heat and energy, It is satisfying, for the presence of fat in the diet delays emptying of the stomach (meat contains fat and therefore remains in the stomach for some hours and allays hunger), After suitable treatment, which includes
the processes of ripening and cooking, meat acquires a palatable flavor, acts as a stimulant to gastric secretion and is readily digested (Gracey et al, 1999).

### 1.3.1. Proteins

Meat is a good source of protein and contains all the essential amino acids which are far superior to the plant proteins due to a very high biological value. Among meat proteins sarcoplasmic and myofibrillar proteins are of high quality because they contain enough of essential amino acids. Sarcoplasmic protein constitute around 30-34% of total meat protein while myofibrillar accounts for 50-55% and remaining 10-15% is connective tissue proteins (Tornberg, 2005). Connective tissue proteins have low levels of tryptophan and sulphur containing amino acids while collagen is poor in lysine content (Sharma, 1999).

When meat is subjected to heating there occur several conformational changes in the protein known as denaturation while also there are protein-protein interactions that results in aggregation of proteins. Sarcoplasmic proteins tend to aggregate at temperature between 40-60\( ^\circ \text{C} \) (Hamm, 1977), while at low temperature long time heating has a tenderizing effect on meat due to release of certain enzymes (Laakkonen, et al., 1970). Similarly, freezing also affects the quality of meat by several changes in meat proteins including modifications in side chain formation of protein polymers, loss of solubility, increase in carbonyl groups, and change in amino acid composition (Soyer, et al., 2010).

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.

### 1.3.2. Fats

Animal fat consists of mainly neutral fats and phospholipids. Meat fat has high amount of free fatty acids. The most abundant fatty acid in meat is oleic acid followed by palmitic and stearic acids. Pork and organ meat are relatively good source of linoleic and linolenic acids. Fats, or more correctly lipids, form essential parts of cell membranes and also act as a vehicle for energy storage. They also form the basis of steroid hormones. Fats are a very concentrated energy source (Warriss, 2000b). Meat contains a mixture of fatty acids both saturated and unsaturated. Phospholipids are
found as a major structural component of cell membranes but include the lecithins also found in blood plasma. Meat also contains some amount of cholesterol and organ meat has high cholesterol content than skeletal muscles. Cholesterol acts as precursor of steroid hormones such as estrogens and corticosteroids.

1.3.3 Vitamins & minerals

Meat is a good source of B-complex group of vitamins including thiamine, riboflavin, niacin, biotin, vitamin B6 and B12, pantothenic acid and folacin. Vitamin C is almost absent in meat but a minor amount is found in certain organ meats. Some losses of B-vitamins occur during cooking and the amount lost depends upon the duration and the temperature of the cooking method. Pork has a good amount of thiamine present compared to other meats.

Meat is an excellent source of all minerals except calcium. Potassium and phosphorus are abundantly present in meat. Iron is also present in good amount that helps in synthesis of myoglobin and haemoglobin along with certain enzymes. Muscle foods are rich source of zinc that provides resistance against infection caused by viruses, bacteria and other pathogens. Meat also provides useful amounts of selenium, copper, magnesium, potassium, iodine and chloride.

1.4. Structure of muscle

Animal carcass is composed of three major constituents i.e., muscle, bone and fat. Components of muscle have an important role in the final meat quality along with it several factors of both ante-mortem and post-mortem affects the tenderness and processing characteristics. Consumers always have some expectation regarding meat quality. These qualities depend on the breed, age, sex, feed of animals etc. the state of muscle contraction is known to influence the meat texture.

The animal musculature can be classified into two major types such as striated and non striated muscles where striated muscles are either categorized as cardiac or skeletal muscles. Skeletal muscles have a complex composition because they contain, in addition to muscle fibers, large quantities of supportive connective tissue, a complete vascular supply, and a nerve supply controlling each of the billions of muscle fibers. Also, skeletal muscles serve as storage depots for lipids and contain considerable quantities of extracellular fluids, primarily consisting of water (Hui, et
All the muscles have the same basic structure that consists of muscle fibres that is further bound together into bundles. Muscles are made up of numerous tiny spindle shaped multinucleated cells or fibres covered in a thin membrane of sarcolemma. A group of muscle fibres are joined by a loose connective tissue, endomysium to form a bundle that is further embedded in another connective tissue perimysium and fat deposits (Warris, 2000b). Later epimysium covers the complete muscle. The muscle is supplied with blood vessels and innervated by nerve fibres. These nerve fibres originate from Central nervous system and ends at neuromuscular junction. Muscle fibres contain all the organelles and in young animals, it is found to be less in size compared to aged animals (Lawrie, 2006).

1.4.1. The microscopic structure of muscle fibres

The diameter of muscle fibres ranges from 10-100µ with either conical or tapering ends with length from 1-40mm. Myofibrils are considered responsible for the cross striated appearance of the muscle fibre that remains embedded in the cytoplasm of the muscle fibre called sarcoplasm. Fibres contain all the organelles found in living cells such as nuclei, mitochondria (contains enzyme responsible for aerobic metabolism), sarcoplasmic reticulum (store for calcium ions), lysosomes (reservoir for various proteolytic enzymes and glycogen granules) etc. (Warris, 2000b). Figures 1 and 2 show schematic representation of muscle fibre constituents and the banding pattern respectively.

At low magnification (2000 x) myofibrils show characteristic striated or banded pattern due to A-band or dark band and I-band or light band with a clear zone in between dark band called as H-zone i.e. bisected by a dark M-line while the light band is also bisected by a dark Z-line. The distance between two adjacent Z-lines is called as sarcomere (functional unit of myofibrils) (Sharma, 1999).

At 20,000 x, the thick filaments traverse the entire width of A-band whereas thin filaments extends from Z-line to the edge of H-zone consisting of contractile proteins myosin and actin.
1.5. Muscle to meat conversion

Muscle to meat conversion is a complex process, which is known to affect the meat qualities such as color and flavor that are both dependent on the oxidative changes that occur in meat. After the slaughter, meat muscle is stored at refrigerated condition for a required length of time to develop organoleptic quality of final product namely ‘meat’ (Ouali et al, 2006).

1.5.1. Post mortem changes in meat

1.5.1.1. Acidification of the muscles after animal death

After the death of the animal, oxygen supply ceases thus any further metabolisms undergoes through anaerobic pathway. The glycogen is broken down to lactic acid that is not removed by the blood system thus acidifies the muscle
gradually. In an unstressed and well-fed animal, the pH will fall from about 7.2 to 5.5. The final or ultimate pH varies between muscles (Warris, 2000a). The time required for acidification of muscle varies from animal to animal as reported by Dransfield (1994), the process of acidification in pigs takes 4-8 h, in sheep 12—24 h and in cattle 15-36 h. The ultimate pH is inversely proportional to the concentration of lactate and the initial glycogen concentration becomes limiting below about 10 mg/g muscle.

As the pH of meat falls, muscle protein gets denatured thus the water bound to protein is reduced and leads to reduction in water holding capacity of muscles. Reduction in water holding capacity further leads to increase in drip loss also increase in weight loss is observed. Meat pH has an impact on its physical characteristics as well as appearance such as light scattering properties are altered with the change in protein structure.

1.5.1.2. Rigor mortis

This is a Latin word meaning “stiffness”. As post mortem glycolysis proceeds the muscle becomes inextensible, this stiffening of muscle is termed as rigor mortis. As soon as the animal is, dead ATP reserve present in meat gets depleted. Muscle contraction occurs when ATP is hydrolyzed to ADP and rigor mortis eventually starts when the ATP level is low to a certain level that is required to maintain muscle relaxation (Figure 3.). As soon as the ATP in muscle is depleted actin and myosin present in muscle forms an actomyosin complex. This actomyosin complex is formed irreversibly and extensibility is lost. Firstly the actomyosin complex formation proceeds slowly in ‘the delay phase’ after that there is a rapid decrease in extensibility in ‘Rapid phase’. The ATP is slowly lowered with time due to surviving noncontractile muscle ATPase activity of myosin. ATP resynthesis in dead animal occurs when there is sufficient glycogen reserve but it cannot be maintained at a level that can prevent actomyosin complex formation. Figure 4. depicts different phases in the conversion of muscle to meat.

Before slaughter, animal is usually starved for certain period of time that leads to depletion of glycogen reserve thus lowers the pH. Excess of oxygen, by stimulating respiration of animal will delay the onset of rigor mortis. Rigor takes different time to develop in the different species. Onset of rigor mortis is dependent on the ATP and
creatinine phosphate reserve, temperature and initial store of glycogen. The loss of extensibility has a great effect on meat texture (Warris, 2000a).

![Image of ATP depletion and rigor mortis](image)

**Figure.3. The relation between ATP depletion and the onset of rigor mortis** (Warris, 2000)

1.5.1.3. Meat muscle structure related to tenderness

Meat eating qualities including tenderness, juiciness and flavour are considered the most important meat palatability traits by consumers (Lawrie & Ledward, 2006; Warriss, 2000b). Although tenderness is considered the most important trait, and consumers are willing to pay more for guaranteed tenderness, up to 20% of steaks sold to consumers are tough (Miller, *et al.*, 2001). Meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Kooohmarae & Geesink, 2006; Troy & Kerry, 2010). Myofibrillar (salt-soluble) and connective tissue (fibrous and insoluble) proteins are located intracellularly and extracellularly, respectively (Aberle, *et al.*, 2001).

The tenderness of meat varies widely among species of animals and among different muscles held for different times of post-mortem. Collagen is one of the most abundant proteins in the animal body and has a significant influence on meat tenderness. The relative proportion of connective tissues and muscle fibres differ, and as such contributes to the relative differences in meat tenderness (Kandeepan, *et al.*, 2009). There is general agreement that proteolysis of myofibrillar proteins, accelerated by the Calpains proteolytic system, is the major contributor to tenderization of beef during post-mortem storage (Bowker, *et al.*, 2008; Huang, *et al.*, 2011). Previous researchers reported that activation of μ-calpain and m-calpain was
responsible for post-mortem proteolysis and tenderization (Polidori, et al., 2000). In addition, l-calpain plays an important role in meat tenderization by weakening the structural integrity of the myofibrillar proteins (Geesink, et al, 2006; Koohmaraie & Geesink, 2006). Geesink, et al (2011) suggested that additional intervention methods, such as tenderizing enzymes are needed to improve the tenderness of pre-rigor cooked meat. Exogenous enzyme treatments have been shown to increase tenderness via myofibrillar and collagen protein degradation with no difference among high and low-connective tissue muscles in beef (Sullivan & Calkins, 2010).

1.6. Meat Tenderness

Consumers are willing to pay a premium for increased tenderness (Miller, et al., 2001). Hence it is important to develop safe tenderization methods to improve meat tenderness and consistency efficiently and economically for the meat industry. Tenderization of meat can be carried out chemically or physically. The technologies used to improve meat tenderness include electrical stimulation (Hope-Jones, et al., 2010; Hopkins & Thompson, 2002), post-mortem ageing (Jayasooriya, et al., 2007), mechanical tenderization (Anna, et al., 2007; Bowker, et al., 2007), ionic chemical solution (Hunt, et al., 2003) and injection of plant enzymes (Ashie, et al., 2002; Wada, et al., 2002).

Treatment by proteolytic enzymes is the most popular method for meat tenderization. Three common methods of introducing the proteolytic enzymes into meat cuts post-mortem include dipping the meat in a solution containing proteolytic enzymes, pumping enzyme solution into major blood vessels of the meat cut, and the rehydration of freeze-dried meat in a solution containing a proteolytic enzyme (Gerelt, et al., 2000). The first two methods are somewhat unsatisfactory, since they over-tenderize the surface and produce a mushy texture. As the enzymes are unable to penetrate within the meat, the interior is left unaffected (Lawrie & Ledward, 2006). The rehydration of the freeze-dried meat showed a much better distribution of enzymes than dipping or perfusion. However, this is not ideal, and requires the setting up of a freeze-dryer. Instead of introducing enzymes into meat post-mortem, pre-slaughter injection of the enzymes into live animals have been carried out (Beuk, et al., 1959) and has proved to be the most effective method of introducing the enzymes into meat (Christensen, et al., 2009; Gao, et al., 2011; Liu, et al., 2011).
Five exogenous proteases that have been classified as “Generally Recognized as Safe” (GRAS) by USDA’s Food Safety Inspection Service (FSIS) (Payne, 2009): papain, bromelain, ficin, and microbial enzymes sourced from *Bacillus* and *Aspergillus*. These enzymes are shown to have varying degrees of activity against myofibrillar and collagenous proteins. In addition to these GRAS enzymes, enzymes isolated from kiwi fruit (actinidin) and ginger showed potential for future inclusion in meat systems for tenderization (Han, *et al*., 2009; Naveena & Mendiratta, 2004; Wada, *et al*., 2002, 2004). Previous research investigating the effects meat tenderness using different treatments and proteases are summarized and presented in Table.5. However, to date there has been no research carried out on the volatile compounds generated.

**Table.5. Literature survey of meat tenderization using extracts and chemicals**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on meat texture</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate Fruit extract marination in goat meat</td>
<td>Pomegranate fruit protease was better for tenderization compared with 0.2% control papain</td>
<td>Narsaiah <em>et al</em>., 2011</td>
</tr>
<tr>
<td>Papain, Pineapple and Ginger injection on goose breast</td>
<td>Addition of 0.12% papain only slightly fractured the muscle fibre as compared to control. 6% pineapple juice and 4% ginger juice seriously ruptured the muscle fibre</td>
<td>Gao <em>et al</em>., 2011</td>
</tr>
<tr>
<td>Ginger extract marination on goat meat</td>
<td>Increased tenderness and protein solubility</td>
<td>Pawar <em>et al</em>., 2007</td>
</tr>
<tr>
<td>Ginger extract &amp; Papain marination on buffalo meat</td>
<td>Ginger was a better alternative to papain for tenderness. Ginger treated samples received better sensory scores</td>
<td>Naveena &amp; Mendiratta, 2004; Naveena <em>et al</em>., 2004</td>
</tr>
<tr>
<td>Ginger extract marination on spent hen</td>
<td>3% ginger extract was optimum for meat tenderization. The tenderization is achieved through its action on both myofibrillar and connective tissue components</td>
<td>Naveena &amp; Mendiratta, 2001</td>
</tr>
<tr>
<td>Ginger extract marination on sheep meat chunks</td>
<td>At the level of 3% ginger extract was found to be effective for improving the sensory and keeping qualities of mutton chunks</td>
<td>Mendiratta, <em>et al</em>., 2000</td>
</tr>
<tr>
<td>Calcium chloride injection treatment wether lamb</td>
<td>0.3M CacI$_2$ efficient in tenderizing lamb meat</td>
<td>Polidori, <em>et al</em>., 2000</td>
</tr>
</tbody>
</table>
1.6.1. Mechanism involved in tenderization of meat

Tenderization of meat is mainly caused due to the activity of proteolytic enzymes that are known to be present in muscle that continuously degrade and recycle proteins. Two enzymes Calpains and Cathepsins are mainly involved in process of meat tenderization. Cathepsins occur in lysozyme of sarcoplasm and are active at mild acidic condition whereas calpains are activated by calcium ions and shows maximum activity in neutral to alkaline medium.

Cathepsins when released during post mortem will degrade troponin T, some collagen cross links and muco-polysaccharides of connective tissue and under normal condition i.e., at pH below 5 it degrades actin and myosin in muscles.

Calpains are further of two forms i.e., m-calpains that are activated by high concentration of calcium ions (1-2 mM) and μ-calpains activated by low concentration of calcium ions (50-100 μM). calpains are also known as Calcium activated sarcoplasmic factors (CASF). Calpains are inhibited by enzyme calpastain. Calpain activity is enhanced by higher calcium levels, higher pH and temperature and reduced calpastatin activity. As soon as the ATP reserve present in animal gets exhausted, the membrane systems of the sarcoplasmic reticulum and mitochondria no longer takes up calcium ions. Thus, an increased calcium ion activates μ calpains and the proteolysis begins.

![Figure 4. Different phases of the conversion of muscle into meat. (Ouali, et al., 2006).](image-url)
1.7 Physico-chemical characteristics of meat

1.7.1 pH

The pH is a measure of the amount of hydrogen ions (H+) in a solution. Pure water dissociates to give equal numbers of hydrogen and hydroxyl (OH-) ions:

\[ H_2O = H^+ + OH^- \]

The pH is defined as the negative logarithm to the base 10 of hydrogen ion activity or concentration:

\[ pH = - \log_{10} [H^+] \]

pH of meat is important in predicting the stability, color, stage of rigor mortis as well as it also has an influence on water holding capacity and several other processing and quality characteristics. After slaughtering, some of the glycogen in the meat turns into lactic acid. As a result, the pH value is lowered. The increasing acidity of the maturing carcass varies in its speed, depending on a number of factors such as type of animal, breed, rearing characteristics and treatment of the animal prior to slaughter. Accumulation of lactic acid after animal death decrease the pH of muscle from about 7.2 to roughly around 5.5 depending on the species, condition of animal before slaughter, etc. and if glycogen reserve is low pH remains high leading DFD while if pH decline is rapid 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. pH is used in predicting the condition of meat and also plays an important role in ultimate meat quality.

1.7.2 Meat color

The external appearance of meat is important for both consumer and manufacturer. Meat purchasing decisions are influenced more by product appearance than other quality parameters as color represents the perceived freshness of product (Mancini & Hunt, 2005). The meat color is highly responsible due to presence of pigments mainly myoglobin that has an iron porphyrin compound with globin moiety attached. Meat color is dependent on several factors such as: pigment content,
ultimate pH, rate of pH decline postmortem, physical characteristics of muscle and ingredients.

The defining factors of meat color are the oxidation (chemical) state of the iron and which compounds (oxygen, water or nitric oxide) are attached to the iron portion of the molecule. Most of the haemoglobin present in animal is removed during bleeding operation to improve the appearance of meat and only myoglobin is then left in the carcass as it is present in the muscle. Drip from raw meat is not blood rather it is the myoglobin dissolved in muscle exudates (Hunt and Hedrick, 1977). Some of the recent works on color characteristics were listed in the Table.6.

**Table.6. Literature survey of recent studies on meat colour**

<table>
<thead>
<tr>
<th>Study Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb colour affected by processing condition and storage temperature</td>
<td>Rosenvold and wiklund, 2011</td>
</tr>
<tr>
<td>Colour measurement by computer image analysis for assessing quality of pork</td>
<td>Chmiel, et al., 2011</td>
</tr>
<tr>
<td>Broiler breast meat colour on pH, moisture content, WHC and emulsification capacity.</td>
<td>Qiao, et al., 2001</td>
</tr>
<tr>
<td>How is the instrumental color of meat measured?</td>
<td>Tap III, et al., 2011</td>
</tr>
<tr>
<td>Measuring changes in internal meat colour, colour lightness and colour opacity as predictors of cooking time,</td>
<td>Pakula and Stamminger, 2012</td>
</tr>
<tr>
<td>The importance of chill rate when characterising colour change of lamb meat during retail display</td>
<td>Jacob and Thomson, 2012</td>
</tr>
<tr>
<td>The impact of new generation pre-dressing medium-voltage electrical stimulation on tenderness and colour stability in lamb meat</td>
<td>Toohey, et al., 2008</td>
</tr>
<tr>
<td>Initial chilling rate of pre-rigor beef muscles as an indicator of colour of thawed meat</td>
<td>Farouk and Lovatt, 2000</td>
</tr>
</tbody>
</table>

1.7.2.1 The chemistry of meat color

Immediately after slaughter the meat color is purple red due to myoglobin pigment which on exposure to air forms a bright red color as oxygen is absorbed and binds to the iron in myoglobin. Bright red color of oxymyoglobin is highly desirable in meat for consumers.

\[
\text{Mb}^{2+} + \text{O}_2 \rightarrow \text{Mb}^{2+}\text{O}_2
\]
Myoglobin and oxymyoglobin have the capacity to lose an electron (called oxidation) which turns the pigment to a brown color and yields metmyoglobin (Figure 5). The reaction of oxymyoglobin to form metmyoglobin is termed autoxidation because it occurs with oxymyoglobin as the only reacting species. Autoxidation is a slow reaction but is found to be important as meat must maintain a red color for several days in retail display (Hui, 2001). Meat color is affected by pH, temperature, reaction of myoglobin with NO and CO or metmyoglobin reductase, fat content or preservation and cooking treatments etc. (King & Whyte, 2006). Tornberg (2005) has reported that at temperature of about 35°C myosin gets denatured that contributes in the opacity of meat color whereas at 40°C the original myosin molecules change to monomers with merged myosin and at 50°C, the myosin molecules get completely coagulated that further leads to opacity in meat sample.

Figure 5. Visible myoglobin redox interconversion on the surface of meat (Mancini & Hunt, 2005; Livingston & Brown, 1982; Wallace et al, 1982).
1.7.3. Texture

During meat consumption, its acceptability is determined by the texture particularly juiciness and tenderness. Meat texture is an important character for both consumer as well as processors. It is highly dependent on three main factors such as sarcomere length, the amount of connective tissue and its degree of cross-linking, and the extent of the proteolytic changes that occur during conditioning post mortem (Warriss, 2000). Several other factors also affects the texture of meat such as water holding capacity, amount of intramuscular fat, ultimate pH, cooking temperature and time, other zootechnical characteristics such as breed, age, sex, type of muscle (Zamora, 1997), handling and feeding characteristics (Aalhus, et al., 1992), technological characteristics such as electrical stimulation etc. Texture is by definition a sensory parameter that only a human being can perceive, describe and quantify (Hyldig & Nielsen, 2001).

Multiple factors are considered responsible for the meat flavor and among them the most important of all is tenderness that influences the meat palatability (Miller, et al., 2001). Huffman, et al. (1996) shown that consumers can distinguish tough and tender meat and will pay for more tender cuts than tough cuts.

The three factors that determine meat tenderness are background toughness (exists at the time of slaughter and does not change during the storage period; the resistance to shearing of the unshortened muscle), the toughening phase (caused by sarcomere shortening during rigor development) and the tenderization phase (Koohmaraie & Geesink, 2006).

Three proteolytic systems present in muscle are found responsible for their possible role in postmortem proteolysis and tenderization: the calpain system, the lysosomal cathepsins and the multicatalytic proteinase complex (MCP) (Koohmaraie & Geesink, 2006). Low temperatures aging have a great impact on meat tenderness due to activity of proteolytic enzymes.

Cooking method has a large effect on tenderness (Sims & Bailey, 1992). Geesink, et al., (2011) studied the effect on tenderness for pre and post rigor meat and concluded that pre and at rigor the meat is very tough and the toughness of early postmortem meat is due to muscle shortening that occurs during the heating process.
thus if meat is cooked for a long period of time still tenderness improves but it is not to the extent as that aging of meat does.

1.7.4. Water holding capacity

WHC is defined as the ability of meat to hold all or part of its own or added water (Zhang, et al., 1995) or to retain its water during application of external forces such as cutting, heating, grinding or pressing. It is an important attribute in meat that has an effect on appearance before cooking and also determines the juiciness and other characteristics during cooking and mastication. In muscle water is present in three different forms i.e. free water, immobilized water and bound water (Sharma, 1999) but the majority of water remains within the myofibrils, between the myofibrils, between myofibrils and the cell membrane (sarcolemma), between muscle cells and between the muscle bundles (group of muscle cells) (Lonergan, et al., 2005). Water holding capacity is considered an important property in case of meat since it contributes to the juiciness of cooked meat besides influencing the color and texture. Honikel (1998) divided the procedures to measure water holding capacity into two categories based on the type of meat product and the process to which it has been subjected.

1.7.4.1. Drip loss in raw meat

Offer and Knight (1988) suggested that pH fall at pre rigor stage induces volume change in myofibrils due to attachment of myosin head to actin filaments at rigor leading, thus there is shrinkage of myofibrils. Denaturation of protein is also responsible for low WHC in meat mainly when there is rapid fall in pH at pre rigor stage. (Leygonie, et al., 2012) When a muscle is cut the accumulated fluids between fibre bundles, will drain out from the surface under gravity.

1.7.4.2. Water loss in cooked meat

Heating induces denaturation of proteins at different temperatures that is responsible for structural changes such as destruction of cell membranes, transverse cell membranes, transverse and longitudinal shrinkage of muscle fibres, the aggregation of sarcoplasmic proteins and shrinkage of the connective tissue that results in cooking losses (Honikel, 1998; Hamm, 1977; Offer, 1984). Water loss will take different time depending upon the storage and various other conditions to which
meat is subjected. Water in extracellular compartments will be lost more easily then that present in deeper compartments. There are several factors that determine the retention of water in muscle such as net charge of the myofibrillar proteins and the structure of muscle cells and its components as well as the amount of extracellular space within the muscle also (Lonergan, 2005).

1.7.4.3. Factors affecting water holding capacity

The factors affecting water holding capacity are listed below

1.7.4.3.1. Net charge

This has been reported that during conversion of muscle to meat there is accumulation of lactic acid as metabolism undergoes anaerobic pathway and stored glycogen is converted to lactic acid that lowers the pH of muscle (Offer and Trinick, 1983). As the pH reaches the isoelectric pH (pI) of major proteins present in muscle particularly myosin (pI= 5.4) the net charge becomes zero and as there is no charge on protein the interaction between polar water molecule and protein is not possible hence water is not held between muscle proteins. Also as the pH approaches pI there is diminished repulsion between the like charges and the structure becomes more closely packed with no or little space for water molecules. Partial denaturation of protein due to low pH is also found responsible for shrinkage in myofibrillar spacing (Offer & Knight, 1998).

1.7.4.3.2. Genetic factors

Fujii et al (1991) suggested that pigs that have inherited a mutation in the ryanodine receptor/calcium release channel (halothane gene) in the sarcoplasmic reticulum due to rapid decrease in pH while the muscle still remains warm that causes the protein denaturation resulting in a condition known as PSE (Pale, Soft, Exudate), results in release of calcium under stress. This calcium accelerates the rate of muscle metabolism and the rate of pH decline (Bendall & Wismer-Pedersen, 1962; Lundstrom, et al., 1989; Lonergan & Lonergan, 2005). Other metabolic state and existing conditions of muscle often direct the extent of pH decline in postmortem muscle such as lactate accumulation etc.
1.7.4.3.3. Post mortem proteolysis and rigor mortis

As muscle undergoes rigor, there is reduction in space for water to reside as cross bridges form between the thick and thin filaments (Offer & Trinick, 1983). This decrease in interfilamental space forces sarcoplasmic fluid to move out. Additionally, during rigor development sarcomeres can shorten; this also reduces the space available for water within the myofibril. In fact, it has been shown that drip loss can increase linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel, et al., 1986). Bendall & Swatland (1988) reported that there is loss in volume in the myofibrillar region as well as the pH induced lateral shrinkage of the myofibril leads to expulsion of water. Calpain and calpastain are the main two enzymes responsible for postmortem proteolysis.

The mechanism of water holding capacity is highly dependent on the muscle proteins particularly myofibrillar protein that has a direct effect of pH, ionic strength and protein oxidation and main mechanism behind is that there occurs alterations in protein structure that results in muscle cell shrinkage and movement of water in extracellular space (Longerman & Longerman, 2005).

1.8. Lipid oxidation

Food lipids are mainly triglycerides, phospholipids and sterols are a concentrated source of calories and also enhance the organoleptic perception to foods. Lipids are responsible for characteristic color, flavor, texture and mouthfeel hence highly desirable in foods by consumer. Oxidation of lipids occur in foods with substantial amount of fat such as milk, meat etc. that changes several quality attributes in food such as flavor change caused by production of hydroxyl acids, new volatile odorous compounds are formed that changes aroma, color change is due to formation of dark colored compounds due to condensation reaction between oxidation products of lipid and protein and finally due to protein cross links a new texture is observed in food (Kanner & Rosenthal, 1992). The mechanism of autoxidation of lipids as a free radical chain reaction involves the three stages of initiation, propagation and termination as shown below:

\[
\text{Initiation:} \quad RH + \text{initiator} \rightarrow R^- \\
RO_2\text{H} \rightarrow RO^-_2 \\
\]
The radical chain reaction has imparted several unique characteristics to lipid oxidation:

a. Lipid oxidation is autocatalytic.

b. Small amount of pro or antioxidants cause large rate changes.

c. The reaction produces multiple intermediates and products that change with reaction conditions and time.

Lipid oxidation is one of the major nonmicrobial factor involved in deterioration of muscle foods (Pradhan, et al., 2000). Control mechanism for lipid oxidation of food is important. In muscle lipid oxidation begins immediately after slaughter as after which there are a series of biochemical reactions involved in conversion of muscle to meat that leads to reduction in antioxidative capacity (Neill, et al., 1998). During meat processing the interaction of unsaturated fatty acids with cytosolic prooxidants, that accelerates the lipid oxidation process. Meat has various endogenous prooxidants and antioxidants (Decker & Xu, 1998) that has an important role in ultimate potential of lipid oxidation in meat. Major factor in lipid oxidation of stored meat is ferrylmyoglobin (activated metmyoglobin or H₂O₂ activated metmyoglobin) (Harel & Kanner, 1985; Kanner & Harel, 1985; Rhee, 1988). H₂O₂ in meat is produced either from the oxidation of oxymyoglobin or through other routes and the level of H₂O₂ is influenced by several factors that also determines the rate of lipid oxidation in meat (Pradhan, et al., 2000).

The extent of lipid oxidation in muscle foods is commonly determined by monitoring malonaldehyde (MDA) formation by means of the thiobarbituric acid (TBA) assay (Gray and Monahan, 1992). Reaction of MDA with TBA results in the formation of a red coloured complex, the intensity of which is related to the concentration of MDA.
1.9. Protein oxidation

Proteins are the major component in meat and serve as an important source of energy and essential amino acids. Protein oxidation is not much explored and an extensive description is also not available. Srinivasan & Xiong (1996) suggested that protein oxidation is responsible for protein fragmentation or aggregation, changes in hydrophobicity, and protein solubility, affecting technological properties such as gelation and also emulsification. Also many researchers reported that by controlling the protease enzyme activity protein oxidation is important in meat tenderness (Kristense, et al., 1997; Morzel, et al., 2006; Rowe, et al., 2004a).

Oxidation of amino acids generates oxidation products, protein-protein cross linkages and fragmentation (Stadtman, 1990, 2002; Filgueras, et al., 2011). This has been reported that the presence of non hydrolyzed proteins in digestive tract and their fermentation by colonic flora results in increased risk of colon cancer in humans (Evenepoel, et al., 1998; Geypens, et al., 1997).

Frozen storage causes several reactions between different meat components such as oxidative reactions that mainly occur due to the presence of oxidative catalyst in high concentration. Free radicals formed react with side chains of proteins that produce protein free radicals which on reaction with oxygen gives peroxy radicals. This has been suggested by several authors that hydroperoxides decomposes to form carbonyl derivatives (Decker, et al., 1993; Xiong, 2000) while sulphhydril group oxidation leads to formation of disulphide cross linkages or to the formation of mixed disulphide conjugates with glutathione, cysteine or other low molecular weight mercaptans. These disulphydryl cross linkages are responsible for production of high molecular weight polymers that are mainly derived from actin and myosin. When a free radical reacts with muscle protein there is a loss of sulphhydril groups and generation of carbonyl compounds (Soyer, et al., 2010), which ultimately results in decrease in muscle protein functionality, leading to increasing water losses, weaker protein gels or less stable emulsions (Xiong, 2000). Stadtman (1990) suggested that protein oxidation can be checked by measuring the formation of carbonyls and the decrease in sulphhydril groups.

Sante-Lhoutellier et al (2008a) studied the effect of meat storage on myofibrillar protein oxidation and concluded that protein oxidation, during
refrigerated storage of meat, is probably lower than that obtained in conditions generating high levels of free radicals such as cooking, irradiation, freezing/unfreezing cycles, and high oxygen packaging.

Protein oxidation is responsible for decreased protein digestibility (Gatellier & Santé-Lhoutellier, 2009; Santé-Lhoutellier, et al., 2008b), decreased amino acid bioavailability and less efficient amino acid assimilation. On oxidation of amino acids carbonyl groups are formed (Stadtman, 1990) and among this the basic amino acids and threonine oxidation leads to decrease in nutritional value of meat (Gatellier & Santé-Lhoutellier, 2009). Liu & Xiong (2000) said that there is an amide bond formation when carbonyls react with amino group of non oxidized amino acids of proteins that leads to aggregate formation with decrease in protein digestibility. Similar effects were seen on the aromatic amino acid oxidation i.e. decreased digestibility and formation of aggregates due to the formation of dityrosine bridges (Davies, 1987; Hanan & Shaklai, 1995; Morzel, et al., 2006; Gatellier, et al., 2009). By formation of disulfide bridges, cysteine oxidation can promote protein aggregation and decreased nutritional value of meat (Gatellier & Santé-Lhoutellier, 2009).

This can be concluded from various studies that frozen storage has strong susceptibility for both lipid and protein oxidation. Soyer et al (2010) from their study reported that decreasing the freezing temperature would reduce the oxidation of proteins meat during frozen storage.

1.10. Effect of breeding and pre slaughter stress

1.10.1. Dark Firm & Dry (DFD)

In meat industry DFD has been a problem as the cut becomes dark and unattractive on cutting. Muscles that maintains a consistent high pH during postmortem conversion to meat depicts a dark, firm and dry condition that is highly unacceptable among consumers (Sharma, 1999). Katsaras and Peetz (1990) reported that DFD meat is usually very tender although not in the same way as normal meat and that DFD meat had, compared with normal pH meat. Before slaughter, when animal undergoes chronic stress condition the glycogen reserves get depleted that results in less lactic acid formation thus the meat is not normally acidified and the ultimate pH remains high (Viljoen, 2002).
The muscle appears dark because of higher intracellular water, which reflects less light. The higher pH results in less denaturation of myoglobin and would facilitate a higher level of aerobic metabolism at the surface. In addition, the high pH actively holds iron in the reduced (ferrous) state. The muscle is firm due to the high water holding capacity, and the surface feels dry as the water is tightly held within the muscle.

Viljeon *et al* (2002) studied the consumer acceptability of DFD and normal pH beef steaks and concluded that normal pH beef steaks were more acceptable in comparison to DFD steaks in general appearance, color and overall acceptability. In a study, this has been reported that DFD muscles on irradiation and then vacuum packaged are stable and resistant to oxidative changes. DFD pork, highly susceptible to microbial spoilage due to high pH conditions, could benefit the most from irradiation because spoilage microorganisms along with pathogens will be dramatically reduced by irradiation (Ahn, *et al.*, 2001).

1.10.2. Pale, Soft and Exudate (PSE)

PSE in pigs is caused by severe, short-term stress just prior to slaughter, for example during off loading, handling, holding in pens and stunning. Here the animal is subjected to severe anxiety and fright caused by manhandling, fighting in the pens and bad stunning techniques. All this may result in biochemical processes in the muscle in particular in rapid breakdown of muscle glycogen and the meat becoming very pale with pronounced acidity (pH values of 5.4-5.6 immediately after slaughter) and poor flavour. This type of meat is difficult to use or cannot be used at all by butchers or meat processors and is wasted in extreme cases. Allowing pigs to rest for one hour prior to slaughter and quiet handling will considerably reduce the risk of PSE (FAO, 1990).

PSE is said to have occurred when the pH of meat is < 6 at 45 minutes after slaughter. White muscle fibres have relatively high glycogen and are prone to PSE. Example is the muscles in the loin region. When animal is exposed to stress that include the beating of animals prior to slaughter, overcrowding of the lairage and fighting among one another before sticking etc. the rate of acidification is stimulated faster than normal and low pH is reached in the muscle when the temperature of the carcass is still high. This causes denaturation in muscle proteins that is responsible for
reduction in water holding capacity as the fluid is expelled into extracellular space (Warriss, 2000). A large amount of exudates reflects poor water holding capacity as found in PSE meats. Warriss (2000) explained that, light scattering from meat surface is probably due to differences in refractive indices of the sarcoplasm and myofibrils. The larger the difference, the higher the scattering and the paler the meat appears. The shrinkage of the myofilament lattice increases the amount of light reflected from the meat. At high scattering the amount of absorbed light is low and the haem pigments selectively absorbed green light, thus reducing the normal red color. This makes PSE meat less red and more yellow. The low pH in PSE also promotes the oxidation of haem pigments from purple or red myoglobin (Mb) and oxymyoglobin (MbO₂) to brown metmyoglobin (met Mb) (Adzitey & Nurul, 2011).

1.11. Meat microbiology

Meat is a highly perishable food product that is a good source of nutrition for micro-organisms. Safety of meat is highly desirable among the consumers with an increase in awareness on the increased number and severity of food-borne outbreaks (Maurice, 1994). Bacteria, yeasts & mold can grow on meat and can potentially spoil meat and poultry products. A healthy animal is protected against infection by various physical barriers and the activity of immune system (Adam & Moss, 2008).

1.11.1. Sources of contamination

The sources of meat contamination as enumerated by Jay, 1987 are,

1.11.1.1. The stick knife

After being stunned and hoisted up by the hind legs, animals such as steers are exsanguinated by slitting the jugular vein with what is referred to as a "stick knife." If the knife is not sterile, organisms are swept into the bloodstream, where they may be deposited throughout the carcass.

1.11.1.2. Animal hide

Organisms from the hide are among those that enter the carcass via the stick knife. Others from the hide may be deposited onto the dehaired carcass or onto freshly cut surfaces. Some hide biota becomes airborne and can contaminate dressed out carcasses as noted below.
1.11.1.3. Gastro-intestinal tract

By way of punctures, intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of freshly dressed carcasses. Especially important in this regard is the paunch or rumen of ruminant animals, which typically contains $\sim 10^{10}$ bacteria per gram.

1.11.1.4. Hands of handlers

This is a source of human pathogens to freshly slaughtered meats. Even when gloves are worn, organisms from one carcass can be passed on to other carcasses.

1.11.1.5. Containers

Meat cuts that are placed in non-sterile containers may be expected to become contaminated with the organisms in the container. This tends to be a primary source of microorganisms to ground or minced meats.

1.11.1.6. Handling and storage environment

Circulating air is not an insignificant source of organisms to the surfaces of all slaughtered animals.

1.11.1.7. Lymph nodes

In the case of red meats, lymph nodes that are usually embedded in fat often contain large numbers of organisms, especially bacteria. If they are cut through or added to portions that are ground, one may expect this biota to become prominent.

Upon slaughter the meat is kept at 2-5°C for a required period of time such that a series of reactions could take place and the muscle can be converted to meat. But this has been observed that on prolonged storage the microbial spoilage begins in the product. It is well known that meat spoilage can be caused due to a fraction of microorganisms (Mataragas, 2007). There are varieties of sources hence the microorganisms are also in a large variety responsible for the contamination (Frazier & Westhoff, 1995).

Micro organisms isolated from fresh and processed meat have been listed in Table.7. The predominant organisms on the surface of fresh carcasses are gram-
negative bacteria such as *Acinetobacter*, *Aeromonas*, *Pseudomonas* and *Moraxella*. *Enterobacter* and *Escherichia* 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 develops 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. Meat spoilage causes discoloration, off-odors and off-flavors, slime or carbon dioxide formation etc. (Table.8.) (Nychas, *et al.*, 2007). Slime, souring, putrefaction etc are some of the common defects caused by bacteria in meat products (Table.9.)

**Table.7. Frequently isolated microorganisms from meats**

<table>
<thead>
<tr>
<th>Frequently isolated microorganisms from meats</th>
<th>Micro organisms Isolated</th>
</tr>
</thead>
</table>
| **Fresh and refrigerated meat**              | Bacteria: *Acinetobacter*, *Moraxella*, *Pseudomonas*, *Aeromonas*, *Alcaligenes* & *Micrococcus*.  
Molds: *Cladosporium*, *Geotrichum*, *Sporotrichum*, *Mucor* & *Thamnidium*.  
Yeast: *Candida*, *Torulopsis* & *Rhodotorula* |
| **Processed and cured meat**                 | Bacteria: *Lactobacillus*, *Acinetobacter*, *Bacillus*, *Micrococcus*, *Serratia* & *Staphylococcus*.  
Molds: *Aspergillus*, *Penicillium*, *Rhizopus* & *Thamnidium*.  
Yeast: *Torulopsis*, *Trichosporon*, *Candida* & *Torula*. |


**Table.8. General type of Meat Spoilage**

<table>
<thead>
<tr>
<th>General type of spoilage of meat:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Conditions</td>
</tr>
<tr>
<td><strong>Surface slime</strong></td>
</tr>
</tbody>
</table>
| **Color changes**                             | *Leuconostoc*, *Lactobacillus sp.* etc. causes meat pigment color change.  
Surface color changes due to pigmented bacteria such as:  
Red- *Serratia marcescens*, Blue- *Pseudomonas syncyanae*,  
Yellow- *Micrococcus* or *Flavobacterium* etc. |
| **Changes in fat**                            | *Pseudomonas*, *Achromobacter* or by yeasts |
| **Phosphorescence**                           | *Photobacterium* spp. |
| **Whiskers**                                  | *Thamnidium chaetocladiodes*, *T.elegans*, *M. mucedo*, |

<table>
<thead>
<tr>
<th>Defect</th>
<th>Meat product</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slime</td>
<td>Meats</td>
<td>Pseudomonas, Lactobacillus, Enterococcus, Weissella, Brochothrix</td>
</tr>
<tr>
<td>H₂O₂ greening</td>
<td>Meats</td>
<td>Weisella, Leuconostoc, Enterococcus, Lactobacillus</td>
</tr>
<tr>
<td>H₂S greening</td>
<td>Vacuum packaged meats</td>
<td>Shewanella</td>
</tr>
<tr>
<td>H₂S production</td>
<td>Cured meats</td>
<td>Vibrio, Enterobacteriaceae</td>
</tr>
<tr>
<td>Sulfide odor</td>
<td>Vacuum packaged meats</td>
<td>Clostridium, Hafnia</td>
</tr>
<tr>
<td>Cabbage odor</td>
<td>Bacon</td>
<td>Providencia</td>
</tr>
<tr>
<td>Putrefaction</td>
<td>Ham</td>
<td>Enterobacteriaceae, Proteus</td>
</tr>
<tr>
<td>Bone taint</td>
<td>Whole meats</td>
<td>Clostridium, Enterococcus</td>
</tr>
<tr>
<td>Souring</td>
<td>Ham</td>
<td>Lactic acid bacteria, Enterococcus, Micrococcus, Bacillus, Clostridium</td>
</tr>
</tbody>
</table>

Table 9. Common defects in meat products caused by bacteria

Meat deterioration can occur even in the absence of micro-organisms also (e.g., proteolysis, lipolysis and oxidation), but it has been seen that microbial growth is by far the most important factor in relation to the keeping quality of fresh meat (Lambert, et al., 1991). Spoilage is a subjective judgment by the consumer, which may be influenced by sensory acuity and the intensity of changes (Nychas, et al., 2008).

After animal death enzyme activity ceases that also prevents glycolysis and ultimately the pH reaches 5.4-5.5. Hence the indigenous enzymes contribute negligibly in spoilage compared to the microbial action of the microbial flora (Nychas & Tassou, 1997; Tsigarida & Nychas, 2001). Nychas et al (2008) said that indigenous enzyme (proteolytic and lipolytic) activity is not sufficient for the meat conditioning. Hence required tenderization in meat is achieved by artificial means such as enzymes, mechanical means or chemicals are applied (Koohmaraie, 1994; Lawrence, et al.,
2003). It has been suggested that microbial activity rather than the activity of microbial enzymes and as a consequence, it is the accumulation of metabolic by-products that characterizes food spoilage (Nychas, et al., 2007).

1.12. Chemistry of spoilage

Glucose, lactic acid and certain amino acids followed by nucleotides, urea and water-soluble proteins are catabolized by almost all the bacteria of the meat microflora (Gill, 1986; McMeekin, 1982; Nychas, et al., 2007). These compounds are required for the microbial growth and concentration of these compounds can affect the type. (e.g., saccharolytic, proteolytic). The rate of spoilage considered as the principal precursors of those metabolites that is perceived as spoilage (Koutsoumanis & Nychas, 1999; Nychas et al., 1998; Skandamis & Nychas, 2002; Tsigarida & Nychas, 2001).

Three set of substances are involved in microbial spoilage of products in one or the other way i.e. (i) compounds contributing in the glycolytic pathway (e.g., glucogen, glucose, glucose-6-phosphate, lactate, etc.), (ii) metabolic products (e.g., gluconate, gluconate-6-phosphate, pyruvate, lactate, etc.) and (iii) nitrogen energy sources (e.g., aminoacids, proteins) (Gill, 1986; Nychas, et al., 1988, 1998).

1.13. Meat preservation techniques

1.13.1. Smoking

Smoking of meat belongs to the oldest methods of food preservation. Ages ago, meat hung above a fire was preserved by the combined action of drying and smoking, which was often preceded by pickling in brine. Smoking extended the shelf life and changed the sensory properties of the meats. The composition of the smoke is of major importance for the quality of smoked products. This has been reported by several researchers that pyrolysis of lignin certain phenolic substances are produced that are important in imparting organoleptic properties of smoked meat (Kjallstrand & Petersson, 2001) along with antimicrobial (Davidson & Branden, 1981) and antioxidative properties (Wittkowski, 1985; Pohlmann, et al., 2012).

Cold smoking takes place in the range of 12 – 25 ° C and warm smoking at 23 – 45 ° C (Sikorski & Kolakowski, 2010). Smoke is usually accompanied by curing
process. Wood smoke contains air, water vapor, CO₂, CO and at least several hundred organic compounds in different concentrations. (Toth and Potthast, 1984). The smoke compounds induce smoky color and flavor themselves and by interacting with the meat components, which results in the creation of other sensory-active substances. Interactions with the nitrogenous meat constituents may lead to some texture changes. The desirable intensity of sensory changes induced by smoking depends on the kind of meat products. Thermal decomposition of wood is as follows:

- **Drying**: up to about 170°C
- **Pyrolysis of hemicelluloses**: between 200°C to 260°C
- **Pyrolysis of cellulose**: between 260°C to 310°C
- **Pyrolysis of lignin**: between 310°C to 500°C

Certain undesirable products are also produced during the process. Smoke is produced in the smoke house or now a day’s liquid smoke is used that is generally prepared from hardwood wherein polycyclic hydrocarbons are removed by filtration and it also reduces the amount of strongly flavoured and other unwanted substances (Toth and Potthast, 1984).

### 1.13.2. Curing

Meat curing is also one of the oldest methods employed for preservation by addition of salt with or without nitrite or nitrate. Curing enhances the shelf life of product by preventing it from spoilage micro-organisms and it can be applied as dry process, can be dipped in brine (pickling), injection curing etc. Oxidative rancidity is prevented with reduction of nitrite or nitrous acid by microbial action (Honikel, 2008). Salt as the primary curing agent has several effects on meat. It lowers the water activity and retards or prevents microbial growth and spoilage. Salt enhances water binding in meat. Thus, it prevents the loss of water on heating and also covers fat particles, preventing their release on heating. By dissolving and swelling the meat protein structure, salt also tenderizes meat and leads to heat-stable structures in “emulsion type” sausages. The curing agent’s nitrite and nitrate react with meat ingredients due to the easily exchangeable oxidation status of nitrogen into many derivatives (Honikel, 2010). Nitrite gives the products stable red color, acts as an antioxidant by sequestering oxygen, prevents or retards microbial growth, and finally, adds a pleasant flavor. The positive effects are overwhelming compared to the small
possibility of the formation of nitrosamines. The intake of curing agents (nitrite plus nitrate) through meat products is small (a few percent) in comparison with other foods (EFSA, 2008). A number of constituents in muscle change their properties as a function of time and manufacturing operations (Vestergaard, et al., 2007). Nitrite is unique in that this single food additive can afford meat a characteristic cured color by means of a heat-stable nitrosyl protoheme pigment, a typical cured flavor, an extended period of refrigerated storage to cooked products without the worry of warmed-over flavor (WOF) development and bacteriostatic action against *C. botulinum* spores (Pegg & Shahidi, 2000).

### 1.13.3. Drying

Drying can be used both as a preservation technique as well as a method to reduce the surface area for easy transportation and also can be used in ready to eat products. High moisture content of meat makes it highly perishable. By dehydration or drying, water level in meat can be reduced to prevent it from microbial growth. Micro-organisms require high water content for growth i.e. available to them in meat at sufficiently high level. The water activity of meat ($a_w$) should be sufficiently low to avoid microbial growth hence drying helps to remove the free water and lowers $a_w$. Fat becomes rancid while drying hence the meat cut should be preferably lean and thin slices enhance the rate of moisture removal. The temperature should be nominal to prevent nutritional losses and the protein denaturation.

There are number of drying methods known such as air drying, vacuum drying, freeze drying etc. Each method has certain advantages and certain disadvantages. The ultimate aim of any process is to maintain the quality and should be acceptable by the consumers. Here in drying temperature and time are two important factors that need to be checked during the process. Freeze drying is the best method known, since the final product of highest quality are produced (Ratti, 2001; Wang, et al., 2010). Since freeze drying needs large capital investment and is a very costly method so combined drying processes are applied to reduce the cost (Laopoolkit & Suwannaporn, 2011).

The quality of conventionally dried product is reduced as compared to original food in terms of color change, nutritional values are altered, protein denaturation occurs etc. To minimize the losses suitable temperature and method should be
applied. Quality of finished dried product is judged on the basis of appearance, should be uniform and there should be no notches or wrinkles, color- should be uniform and dark and consistency of dried meat should be as that of frozen meat and must be hard. Taste and flavor- a slightly rancid flavor is usually observed due to chemical changes during drying hence fatty cuts should not be stored for long as it intensifies rancidity. (Virgili and Schivazappa, 2002)

1.13.4. Hurdle technology

Hurdle technology uses a combination of factors (hurdles), each at optimal level that is beneficially used to impart the required functional qualities and suppress the spoilage due to microorganisms. Leistner, (2000) reported that both microbial safety and stability along with sensory and nutritional quality of most of the foods is based on application of several preservative methods (hurdles) such that each impart certain level of preservation and not have any of the adverse effect the quality of food. The Hurdle technology is used in industrialized as well as in developing countries for the gentle but effective preservation of foods.

The most important hurdles used in food industry are acidity (pH), water activity ($a_w$), redox potential ($E_h$), preservatives (nitrites, sorbate, sulfite etc.) and competitive microorganisms (eg. lactic acid bacteria) (Leistner, 2000). Preservative method can have both positive and negative effect on food depending upon its intensity. 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. (Leistner and Gould, 2002)

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 consumers. Hurdle technology enables production of safe, stable and nutritious foods, which are economical and cost effective. 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 (Leistner and Gorris, 1995).
1.13.5. Freezing

Freezing foods has a historical origin in China where ice cellars were used to preserve foods as early as 1000 BC. Later, the Greeks and Romans stored food in cellars in which snow had been compressed. Foods intended to be consumed while frozen date back to the 1500s when flavored ices were made in France by master ice makers (Lund, 2000).

Freezing enhances the shelf life of product by lowering the temperature of food such there is no or little available water and the solute concentrates thus reducing the water activity. Proteins are unchanged in frozen storage and fat are susceptible to rancidity. Losses are also reported during thawing in the form of juices containing soluble proteins, vitamins and minerals.

Freezing for last many decades is known as one of the most effective method for preserving food however, some deterioration in quality is also observed in frozen food. The extent of quality loss depends on several factors such as rate of freezing and thawing, storage temperature, temperature fluctuations, freeze–thaw abuse during storage, transportation, retail display and consumption (Srinivasan, et al., 1997; Boonsumrej, et al., 2007). In freezing small ice crystals formation is critical as it minimizes drip losses and tissue damage. When food is subjected to thawing it undergoes physical, chemical and microbiological change. It has been seen that quick freezing at low temperature avoids rise in temperature and excessive dehydration of food is desirable to assure food quality (Li & Sun, 2002).

Meat being a perishable product needs to be preserved by one or the other way and freezing being an effective and cost effective method is usually preferred over other methods. In the literature researchers have evaluated that how freezing and thawing affects the quality of food as well as determined various methods for rapid freezing and thawing such that the quality is maintained (Li & Sun, 2002). Effect of freezing and thawing rates on meat quality such as drip losses, protein denaturation, cooking yield, color change, sensory, textural changes, consumer preference etc. have been investigated and reported by researchers in various studies (Ersoy, et al., 2008; Mortensen, et al., 2006; Ngapo, et al., 1999).
The freeze–thaw process is found to be detrimental to overall physicochemical and textural quality as it plays an important role in membrane disintegration and also affects the sensory attributes (Jeong, et al., 2011; Nilsson & Ekstrand, 1994). Oxidation in meat leads to deterioration in quality by the production of off flavor and off odour, it also has an effect on meat color as it has been assumed that lipid oxidation is related to myoglobin oxidation. Free radical formation also depends on temperature along with certain other important factors and it is greatly responsible for physicochemical and biochemical changes (Jeong, et al., 2011). Freeze thaw process for different animal products such as beef, pork, shrimps, fishes etc. has been studied for various changes that occurred during the time of storage and reported that the quality is greatly affected and losses are higher if the process is not carried out at right temperature (Jeong, 2011; Peitrasik & Janz, 2009; Boonsumrej, 2007; Mortensen, et al., 2006).

Freezing is a widely used preservation technique that helps enhance the storage life of meat but now preserving meat from micro-organisms is not the ultimate aim rather meat should be of desired texture, color, flavor and several other characters are required for its acceptance among consumers. This has been long back recognized that preserving meat at low temperature has higher shelf stability than when stored at higher or at ambient temperatures. Quality of frozen food is closely related to the freezing and thawing process. Freezing is not only a preservation technique but a well established frozen storage has helped in maintaining a constant supply of raw materials in off season and makes it possible to transport large quantities of food over geographical distances (Persson & Londahl, 1993). Freezing keeps food safe by slowing the movement of molecules, causing microbes to enter a dormant stage. Freezing preserves food for extended periods because it prevents the growth of microorganisms that cause both food spoilage and food borne illness. Frozen storage chart for different meat products has been shown in Table. 10. Freezing to 0°C inactivates any microbes, such as bacteria, yeasts and molds present in food. Once thawed, however, these microbes can again become active, multiplying under the right conditions to levels that can lead to food borne illness. Since they will then grow at about the same rate as microorganisms on fresh food (USDA, 2010).
### Table.10. Frozen Storage Chart for different meat and meat products

<table>
<thead>
<tr>
<th>Item</th>
<th>Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon and Sausage</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Casseroles</td>
<td>2 to 3</td>
</tr>
<tr>
<td>Egg whites or egg substitutes</td>
<td>12</td>
</tr>
<tr>
<td>Frozen Dinners and Entrees</td>
<td>3 to 4</td>
</tr>
<tr>
<td>Gravy, meat or poultry</td>
<td>2 to 3</td>
</tr>
<tr>
<td>Ham, Hotdogs and Lunchmeats</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Meat, uncooked roasts</td>
<td>4 to 12</td>
</tr>
<tr>
<td>Meat, uncooked steaks or chops</td>
<td>4 to 12</td>
</tr>
<tr>
<td>Meat, uncooked ground</td>
<td>3 to 4</td>
</tr>
<tr>
<td>Meat, cooked</td>
<td>2 to 3</td>
</tr>
<tr>
<td>Poultry, uncooked whole</td>
<td>12</td>
</tr>
<tr>
<td>Poultry, uncooked parts</td>
<td>9</td>
</tr>
<tr>
<td>Poultry, uncooked giblets</td>
<td>3 to 4</td>
</tr>
<tr>
<td>Poultry, cooked</td>
<td>4</td>
</tr>
</tbody>
</table>

(Source: FSIS, USDA)

Frozen storage will preserve food for months or even years if properly packaged and stored. Microbial spoilage is only possible in frozen food if unfrozen water is available. Below -9.5 °C, there is no significant growth of spoilage or pathogenic organisms in food and even a decrease in living organisms’ number is observed but as soon as food is subjected to thawing the food becomes highly susceptible to microbial spoilage.
Figure 6. Freezing curve (Fellows, 2000)

The different phases of freezing curve shown in Figure 6. have been explained below;

AS: The food is cooled to below its freezing point $\theta_f$ which, with the exception of pure water, is always below 0°C. At point S the water remains liquid, although the temperature is below the freezing point. This phenomenon is known as super-cooling and may be as much as 10°C below the freezing point.

SB: The temperature rises rapidly to the freezing point as ice crystals begin to form and latent heat of crystallisation is released.

BC: Heat is removed from the food at the same rate as before, but it is latent heat being removed as ice forms and the temperature therefore remains almost constant. The freezing point is gradually depressed by the increase in solute concentration in the unfrozen liquor, and the temperature therefore falls slightly. It is during this stage that the major part of the ice is formed.

CD: One of the solutes becomes supersaturated and crystallises out. The latent heat of crystallisation is released and the temperature rises to the eutectic temperature for that solute. DE: Crystallisation of water and solutes continues. The total time $t_f$ taken (the freezing plateau) is determined by the rate at which heat is removed.

EF: The temperature of the ice–water mixture falls to the temperature of the freezer. A proportion of the water remains unfrozen at the temperatures used in commercial
freezing; the amount depends on the type and composition of the food and the temperature of storage.

![Figure 7. Ice formation at different freezing temperatures. (Fellows, 2000)](image7)

![Figure 8. Temperature changes of food through the critical zone. (Fellows, 2000)](image8)

1.13.5.1. Rate of ice crystal formation

Rate of ice crystal formation depends upon the rate of heat transfer i.e. if the heat transfer is high it produces large number of small crystals while if heat transfer is slow small number of large crystals are formed that are considered to have an adverse effect on the quality of food as it damages the cell structure, separation of cell as well as also breaks the emulsions in food (Figures 7 and 8). It has been seen that large ice crystals can lead to puncture of frozen foam bubbles. The final quality of rapidly
frozen food is higher compared to slowly frozen product (Potter & Hotchkiss, 1995). Grujic et al (1993) also reported that freezing rate influences ice crystal size, location (intra - or extra - cellular), and morphology.

1.13.5.2. Effect of freezing and thawing on quality of food

1.13.5.2.1. Solute concentration

As the process of freezing proceeds there is formation of ice crystals which results in concentration of solutes present in food and as the temperature falls the solute tends to crystallize out and that temperature is known as eutectic temperature. Where the temperature of storage is below this temperature range, the formation of a glass protects the texture of the food and gives good storage stability. Solute concentration causes changes in the pH, viscosity, surface tension and redox potential of the unfrozen liquor (Fellows, 2000).

1.13.5.2.2. Volume changes

The volume of ice is more than water (9%) hence it leads to increase in volume on freezing but this expansion is highly dependent on moisture content, cell arrangement, concentration of solute, freezer temperature etc. Here there are more chances of tissues get cracked or shattered due to internal pressure (Alarcón-Rojo and Janacua-Vidales, 2010).

1.13.5.2.3. Tenderness

When meat is subjected to freezing immediately after slaughter, it can prevent formation of cold shortening i.e when the meat is subjected to chilling before the pH falls below 6.6 it will become extremely tough when it is subsequently cooked. Similarly, if no freezing is applied and the meat is at around 25°C even after rigor there is heat shortening that also causes adverse effect on meat quality (Dransfield, 1994).

When meat is stored at above freezing temperatures, it becomes progressively tender. This process, known as “ageing” (or, alternatively, as conditioning or maturation), is traditionally carried out by hanging meat carcasses for periods of 14 days or longer (in the case of beef) in a controlled environment at between – 1 and 5 °C.
1.13.6. Meat quality attributes affected by freezing and thawing

1.13.6.1. Moisture

Freezing and thawing alter both the content and the distribution of moisture in meat tissue. Moisture as a quality characteristic in meat can be evaluated in several ways, including drip loss; thaw loss; cooking loss; water binding capacity and total moisture content (Leygonie, et al., 2012a). Nonetheless, since the methods used to determine moisture loss and changes in meat are not set by an international standard, it is often difficult to directly compare and draw conclusions from studies in the literature that have employed different methods for such purposes. Moisture loss in meat is inevitable during post mortem due to the decrease in pH (closer to the isoelectric pH of proteins), the loss of adenosine triphosphate (ATP), and the steric effects due to shrinkage of the myofibrils as a result of rigor mortis and conditioning (Huff-Lonergan & Lonergan, 2005). These factors all act to release water that was previously immobilised and bound to proteins into the intrafibrillar spaces. The released water is then redistributed into the sarcoplasmic and extracellular spaces. Freezing and thawing are known to affect the amount of exudate (thaw loss and/or drip loss).

Research conducted to date has indicated that as the characteristic time to freeze increases above 19.5 min, the amount of exudate that forms becomes markedly higher than before freezing. The amount of exudate that forms, nonetheless, remains reasonably constant as the characteristic time of freezing increases beyond 19.5 min (Añón & Cavelo, 1980). This phenomenon has been associated with the size and distribution of the ice crystals that form along the freezing gradient (Añón & Cavelo, 1980). In terms of thawing, major differences in opinion exist regarding the correlation between the rate of thawing and the extent of exudates formation. Gonzalez-Sanguinetti et al (1985) concluded that a decrease in thawing time (time elapsed from −5 °C to −1 °C) to below 50 min resulted in decreased exudate. This was attributed to the melting of ice in the extracellular spaces causing an increase in water activity, resulting in the net flow of water into the intracellular spaces and its subsequent reabsorption by the dehydrated fibres. These authors suggested that at increased rates of thawing, the rate at which water becomes available exceeds the rate at which the fibres can reabsorb water, with the excess water being excreted as
exudate. Haugland (2002) also proposed that an increased rate (or decrease in time) of thawing caused less exudate to form. Ambrosiadis et al (1994) reported that rapid thawing of meat by submergence in water decreased the drip loss. On the other hand, it was found in the latter study that microwave thawing (35 min to reach 0 °C) increased the drip loss to within the same range as ambient air thawing (5–7 h), but this drip loss was still less marked than in the case of refrigerated thawing (28 h), which resulted in the highest drip loss.

In general, there is consensus in the scientific literature on the notion that freezing, frozen storage and thawing all contribute to a decrease in the water-holding capacity of meat (Añón & Cavelo, 1980; Ngapo, et al., 1999; Vieira, et al., 2009). It has been reported that the loss in water-holding capacity is related to the disruption of the muscle fibre structure, as well as the modification and/or denaturation of the proteins (Huff-Lonergan & Lonergan, 2005). The composition of the drip has been found to consist mostly of sarcoplasmic proteins (Savage, et al., 1990). Loss of moisture due to cooking has been reported not to differ significantly between fresh and frozen meat samples, as well as for samples frozen and thawed at different rates (Leygonie, et al., 2012b; Vieira, et al., 2009). This is believed to be due to the region in the muscle tissue from which cooking-loss water originates. During cooking, the melting of the fat and the denaturation of the proteins reportedly causes the release of chemically bound water (Vieira, et al., 2009).

1.13.6.2. Protein denaturation

It has been traditionally thought that protein denaturation could result during freezing due to an increased intracellular ionic strength following the migration of water to the extracellular spaces. This mechanism has been refuted by several authors. Añón and Cavelo (1980), Mietsch, et al., (1994) and Ngapo, et al., (1999) all suggested that protein denaturation does not contribute significantly to quality loss, as they found no significant differences in the amount and composition of proteins in the drip collected from fresh samples and those samples that had been frozen and immediately thawed. Some of these authors also used sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), capillary gel electrophoresis (CGE) and differential scanning calorimetry (DSC) to study the patterns of the protein exudate fraction and found no significant differences between the afore mentioned
samples. It was, however, noted by these authors that the time and temperature of the sample storage may have influenced the results obtained and no new explanations were offered with regard to the loss of meat quality during freezing. It would consequently be very beneficial to evaluate the drip composition of such samples using more modern techniques, such as proteomics.

After analysing meat samples for protein denaturation using DSC thermograms, Wagner and Añón (1985) reported that myosin was the protein most affected by freezing. The myofibrillar proteins were reportedly denatured irrespective of the freezing rate, causing unfolding of the protein and resulting in a lower enthalpy value. By comparing the data from the DSC thermograms, enthalpy change and ATPase activity, these researchers concluded that slow freezing causes more pronounced protein denaturation than rapid freezing. Benjakul, et al. (2003) found that freezing and frozen storage caused a marked decrease in Ca$^{2+}$-ATPase activity and an increase in Mg$^{2+}$-EGTA-ATPase activity, which translates into denaturation of myosin and the troponin–tropomyosin complex. They also reported strong interactions between protein oxidation (formation of carbonyls) and protein denaturation. The contradictory results reported in the various studies suggest that more research is required to establish the mechanisms involved in protein denaturation during freezing and frozen storage.

1.13.6.3. Oxidation of lipids and protein

The final temperature to which meat is frozen and stored determines the amount of unfrozen water that remains available for chemical reactions to proceed. Petrović (1982) showed that biochemical reactions could still take place in meat frozen and stored at temperatures higher than $-20$ °C, since sufficient unfrozen water remained available at these temperatures for such reactions to occur. The optimum temperature for the frozen storage of meat has been reported to be $-40$ °C, as only a very small percentage of water is unfrozen at this point (Estévez, 2011). This fraction of water is believed to be bound to other food constituents and thus is chemically inactive (Nesvadba, 2008; Singh & Heldman, 2001). The freezing of the water fraction also causes an increase in the solute concentration both intracellularly and extracellularly, which is thought to be the reason for the increased chemical reactivity during frozen storage (Fennema, 1975). The ice crystals, depending on their size and
location, will disrupt the muscle cells, resulting in the release of mitochondrial and lysosomal enzymes into the sarcoplasm (Hamm, 1979). The fraction of unfrozen water is also important in terms of oxidation, since chemical reactions can occur during frozen storage that initiate primary lipid oxidation (peroxidation) in the meat. This can lead to radical secondary lipid oxidation upon thawing (Owen & Lawrie, 1975) leading to adverse changes in colour, odour, flavour and healthfulness. This phenomenon has been demonstrated by Akamittath, et al., (1990) and Hansen et al. (2004), who reported accelerated lipid oxidation in frozen–thawed meat that was subjected to a refrigerated shelf-life study.

The quality of the secondary products of lipid oxidation is generally measured using the thiobarbituric acid reactive substances (TBARS) method. These secondary products cause rancid, fatty, pungent and other off-flavours. The development of these flavours was noted by Vieira et al (2009), who stated that TBARS of fresh meat were significantly lower than meat stored for 90 days at −20 °C. Such observations indicate that frozen storage is not necessarily sufficient to prevent oxidation from occurring. Although peroxidation was not measured in the aforementioned study, it would be expected that primary lipid oxidation would cease at such low temperatures by 90 days and secondary lipid oxidation would commence, which should be detected by the TBARS method. Benjakul and Bauer (2001) also found that freezing and thawing of muscle tissue resulted in accelerated TBARS accumulation and attributed this finding to the damage of cell membranes by ice crystals and the subsequent release of pro-oxidants, especially the haem iron. There is also increasing evidence to indicate that lipid oxidation takes place primarily at the cellular membrane level and not in the triglyceride fraction. Therefore, lipid oxidation has been reported in both lean and fatty meats (Thanonkaew, et al., 2006).

Protein oxidation can be linked to any of the pro-oxidative factors, such as oxidised lipids, free radicals, haem pigments and oxidative enzymes. Malonaldehyde is one of the substrates that react with protein derivatives to form carbonyls (ketones and aldehydes) (Xiong, 2000). Protein and lipid oxidation are, therefore, undoubtedly interlinked. Protein oxidation in meat may lead to decreased eating quality due to reduced tenderness and juiciness, flavour deterioration and discolouration (Rowe, et al., 2004a). These changes are partially due to the formation of protein aggregates through both non-covalent and covalent intermolecular bonds as reactive oxygen
species (ROS) attack the proteins. Other common changes in oxidised proteins include amino acid destruction; protein unfolding; increased surface hydrophobicity; fragmentation and protein crosslinking. These all lead to the formation of protein carbonyls (Benjakul, et al., 2003; Liu, et al., 2000; Xia, et al., 2009).

Freezing and thawing cause damage to the ultrastructure of the muscle cells with the ensuing release of mitochondrial and lysosomal enzymes, haem iron and other pro-oxidants. These increase the degree and rate of protein oxidation (Xiong, 2000). The amino acid residues that are mainly involved in these reactions are lysine, threonine and arginine, the oxidation of which leads to the polymerization of proteins as well as peptide scission (Liu, et al., 2000; Xia, et al., 2009; Xiong, 2000). These amino acids are mainly found in the myofibrillar proteins, which account for 55–65% of total muscle protein and are responsible for the majority of the physicochemical properties of muscle foods (Xia, et al., 2009).

Protein oxidation destabilises the protein matrix leading to increased toughness, loss of water-binding capacity and loss in protein solubility. The water-holding capacity of meat that has undergone protein oxidation decreases due to the shrinkage of the inter-filamental spaces, as the oxidation of the myofibrillar proteins leads to the aggregation and coagulation of myosin and actin. The shrinkage of the inter-filamental space results in an increase in the extracellular space, thus decreasing the capillary force that holds the water in the inter-filamental space. The other oxidative changes to the proteins also decrease their ability to hold water and hence the water leaches out of the meat as exudate (Liu, et al., 2010).

1.13.6.4. Colour (myoglobin proteins)

Myoglobin has been identified in exudate by gel-electrophoresis, accounting in part for the change in the colour stability of meat after freezing and thawing (Añón & Cavelo, 1980). It has also been reported that denaturation of the globin moiety of the myoglobin molecule takes place at some stage during freezing, frozen storage and thawing (Calvelo, 1981). The denaturation leads to an increased susceptibility of myoglobin to autoxidation and subsequent loss of optimum colour presentation. This theory has been verified by many authors by comparing the degree of bloom and the ability of the meat to resist oxidation to metmyoglobin during refrigerated storage post freeze/thaw (Abdallah, et al., 1999; Farouk & Swan, 1998; Lanari, et al., 1990;
The existence of an enzyme system capable of reducing metmyoglobin back to myoglobin was proposed by Livingston and Brown (1981) and was termed the metmyoglobin reducing activity (MRA). The theory is that in fresh muscle the enzyme is very active and the metmyoglobin formed is quickly reduced to deoxymyoglobin and oxygenated back to oxymyoglobin, thereby retaining the bloomed colour. However, as the meat ages or is frozen, the activity of the MRA is decreased and metmyoglobin begins to accumulate on the surface of the meat at a rapid rate (Abdallah, et al., 1999). Also, MRA and/or co-factors, such as NADH, could be ‘lost’ from the post mortem sarcoplasmic environment by leaching as exudate during thawing, and/or due to oxidation, and/or be used by reactions unrelated to MRA, which will all contribute to accelerated oxidation and loss of bloom (Abdallah, et al., 1999).

It is known that β-hydroxyacyl CoA-dehydrogenase (HADH) is released from the mitochondrion cytoplasm during freezing and thawing. This enzyme utilises NADH and would thus result in faster inactivation of MRA (Abdallah, et al., 1999; Lawrie, 2006). All forms of oxidation are considered to be associated with one another. Thus, when lipid oxidation is initiated it results in the formation of pro-oxidants capable of reacting with oxymyoglobin, which in turn leads to metmyoglobin formation. The same logic applies to protein oxidation (Farouke & Swan, 1998). Oxidation can consequently be compared to a chain reaction within meat, initiated by the lipid fraction and carried over to the myoglobin fraction. Hence, if lipid oxidation were accelerated by frozen storage, this would increase the quantity of free radicals present, leading to an increased rate of myoglobin oxidation. If the MRA has become less effective in combating oxidation, this would explain why a more rapid decrease in colour stability is observed post-freezing in meat subjected to chilled retail display (Xiong, 2000).

1.13.6.5. pH

The pH of meat that has been frozen and thawed tends to be lower than prior to freezing (Leygonie, et al., 2011). As pH is a measure of the amount of free hydrogen ions (H⁺) in a solution, it is possible that freezing with subsequent exudate production could cause denaturation of buffer proteins, the release of hydrogen ions
and a subsequent decrease in pH. Alternatively, the loss of fluid from the meat tissue may cause an increase in the concentration of the solutes, which results in a decrease in the pH. A further explanation for this finding may involve the deamination of proteins by microbial or enzymatic action, with the ensuing release of hydrogen atoms (Leygonie, et al., 2011).

pH is an important meat parameter, which varies with genetic origin, ante-mortem treatment, muscle and fibre type (Arnau, 1991; Monin, et al., 1987) and affects the water holding capacity of meat (Hamm, 1986) and water loss in dry cured sausages (Stiebing & Ro¨del, 1990) and hams (Arnau, et al., 1998). Yusop et al (2010) studied the effect of marinating time (30 min to 3 h) and marinade(citric acid) pH on the instrumental and sensory properties of cooked Chinese-style marinated chicken were investigated and reported that increased marinating times of 120–180 min were found to produce more acceptable end products with increased scores for colour, aroma and flavour attributes.

1.13.6.6. Tenderness (shear force)

There is general agreement in the literature that the tenderness of meat increases with freezing and thawing when measured with peak force (Farouke, et al., 2003; Lagerstedt, et al., 2008; Shanks, et al., 2002; Wheeler, et al., 1990). It has also been found that the increase in tenderness is correlated to the length of frozen storage and the degree to which the meat was aged prior to freezing. The tenderizing effect of freezing seems to be negated when the meat was sufficiently aged prior to freezing (Vieira, et al., 2009). The mechanism involved in the tenderization is thought to be a combination of the breakdown of the muscle fibres by enzymatic action during proteolysis, ageing, and the loss of structural integrity caused by ice crystal formation. The formation of large, extracellular ice crystals disrupts the physical structure, largely breaking myofibrils apart and resulting in tenderization. However, the formation of small intracellular ice crystals increases the rate of ageing probably by the release of protease enzymes (Vieira, et al., 2009), although many alternative postulations exist in the literature. Contradictory results have been obtained from sensory evaluation of tenderness (Lagersted, et al., 2008), where a lower peak forces was reported in freeze/thaw samples compared to chilled meat. In this case the trained sensory panel rated the freeze/thawed meat significantly less tender than the chilled
meat. This sensory result was attributed to the loss of fluid during thawing that resulted in less water available to hydrate the muscle fibres; thus, a greater quantity of fibres per surface area seemed to increase the toughness as perceived by the sensory panel. The decrease in the shear force was attributed to the loss in membrane strength due to the ice crystal formation thereby reducing the force needed to shear the meat (Liu, et al., 2010).

1.13.6.7. Microbial count

Neither freezing nor thawing appears to decrease the number of viable microbes present in meat. During freezing, however, microbial spoilage is effectively terminated as the microbes become dormant. Unfortunately, they regain their activity during thawing (Löndahl & Nilaaon, 1993). As thawing is a much slower process than freezing and is less uniform, certain areas of the meat will be exposed to more favorable temperature conditions for microbial growth. This is of particular concern when air thawing is employed. In addition to the risk of high temperature exposure, there is an increase in moisture and nutrients available to microbes post freeze/thaw due to exudates formation. The moisture lost during thawing is rich in proteins, vitamins and minerals derived from the structural disarray caused by the freezing process, which consequently provides an excellent medium for microbial growth. For this reason, good hygiene and handling practices are even more important for meat that is to be frozen and thawed compared to that which is to be sold fresh (Pham, 2004).

Vieira et al (2009) found in their study that beef frozen for up to 90 days, previously aged for 3 and 10 days, did not spoil due to microbial growth. They did, however, report an increase in the levels of psychrotrophic bacteria during the 90-day frozen storage, which were probably favoured above the other bacteria by the thawing process (48 h at 4 °C in a cooler). Greer and Murray (1991) found that the lag phase of bacterial growth in frozen/thawed pork was shorter than for fresh meat, but that the time to develop spoilage odours was not affected.

1.14. Structural changes in meat

Structural changes in meat can be studied using Scanning electron microscope (SEM). SEM gives images with great depth-of-field yielding a characteristic 3D
display that provides greater insight into the surface structure of a biological sample. For SEM, samples require preparation, such as cryofixation, dehydration, embedding (in resin), or staining (with heavy metal). SEM is a high-performance tool for investigating process-related changes in meat ultrastructure. In combination with histological analyses, Tornberg (2005) reviewed effects of heating on changes in secondary, tertiary and quaternary structure of proteins and then on cooked meat quality. Palka and Daun (1999) have also worked on structural changes during heating in bovine muscle. Structural changes in intramuscular connective tissue during tenderization of bovine meat by marinating in a solution containing proteolytic enzyme (Chen, et al., 2006) is also of interest in meat process control and can be accessed with SEM. Larrea et al (2007) outlined the comparisons and complementarities of SEM, cryo-SEM and transmission electron microscopy for the measurement of process-related ultrastructural changes in ham. Yang and Froning (1992) reported structural differences in washed or unwashed mechanically deboned chicken meat.

Nishimura et al (1999) focusing on the ante-mortem stage, studied structural changes in intramuscular connective tissue during fattening. Cryo-scanning electron microscopy consists in SEM observation after cryofixation, and is well adapted to biological tissues that are relatively unaffected by this specific sample preparation. More specifically, cryofixation is a solution for visualizing the electrolytes that are suppressed in a classical dehydration preparation. Garcia-Segovia et al (2007) studied the effects of cooking temperature and cooking time on losses, colour and texture of beef steaks using cryo-SEM to assess the endomysium and perimysium microstructure. Pig muscle cell ultrastructure versus freezing rate and storage time has also been investigated with this technique (Ngapo, et al., 1999). Cryofixation is also used in transmission electronic microscopy. Environmental scanning electron microscopy (ESEM) is a new development in the field of electron microscopy. It opens up the possibility of observing samples at almost normal atmospheric pressures (unlike classical SEM) without having to dehydrate or freeze them. Yarmand and Baumgartner (2000) used ESEM to study the structure of semi-membranous veal muscle. Even though ESEM offer promising possibilities for observing intact samples, contrast is often less good than in traditional SEM when the sample is stained with a heavy metal.
1.15. Cryoprotectants

Negative changes in meat during frozen storage can be identified with changes in the physicochemical properties of myofibrilar proteins Park & Lanier, (1993). This statement is true also because among all muscle proteins, myofibrilar proteins are characterised by the highest sensitivity to freeze damage. These changes can be reduced remarkably by adding the so-called “cryoprotectants” to meat before freezing. The favourable action of cryoprotectants was documented in reference to myofibrilar protein isolates and surimi-type preparations from fish and poultry meat, (Park & Lanier, 1993; Yoon & Lee, 1990; Yang, et al., 1990; Simpson & Morrisey, 1995; Kijowski & Richardson, 1996). There are only a few publications on cryoprotection of meat proteins of slaughter animals and poultry. The application of cryoprotectants in the technology of freeze protection of meat seems to be fully justified. Kijowski & Richardson (1996) in their studies into the effect of cryoprotectants on the mechanical properties of recovered broiler meat proved that the addition of saccharose and sorbitol mixture with STP reduced the negative changes in frozen meat functionality. Although results of these studies are very promising, the applicability of saccharose and sorbitol in the technology of frozen stored beef and pork is very unlikely since saccharose adds sweet taste to the preserved product, and sorbitol is several times more expensive than meat itself. Hence, the use of this additive would have an influence on the price of the final product. Park & Lanier (1993) showed that a similar effect was evoked by the application of much cheaper polydextrose. They proved that the addition of 8% polydextrose to minced beef inhibited a decrease in muscular protein solubility during storage at -28°C.

Recently, much interest of researchers dealing with cryoprotection of meat has been focused on hydrocolloids. They are an important group of texture-forming food components or food additives Makala, (2003); Adamczak et al., (2003a, b). It was observed that the stability of frozen products could be obtained by getting them to the glassy state.

1.16. Glass transition

Over the years water in food has been a great area of research for Food Scientists from simple physical properties like specific volume, density, viscosity, molecular mobility to recently discovered polymeric properties of water molecule in
food i.e. a crucial aspect of ‘Food polymer science’ or ‘Food material science’ (Farhat, 2000). There is a need to determine that how much availability of water in food will not pose any harm to quality and shelf life of food. Water content in food needs to be considered for safety, stability, technological performances, processing etc (Slade and Levin 1991). Water in food can be removed or its availability can be ceased by various methods such as drying, freezing etc. It has been seen that water in food has a plasticizing action on solutes. Glass transition is a recently discovered property that is used to determine the shelf life of food and its stability.

Glass transition is a process in which water is not crystallized but is in an amorphous form in the system. Such a system reaches the viscosity of 1012 Pa-s. In the glassy state, all physical and chemical processes are inhibited. Due to this, food products can be stored for a long time without the risk of deterioration. The glass transition, or strictly speaking temperature of this process Tg, is one of the basic parameters that characterize the quality of a dehydrated (frozen or dried) product. It has been proved recently that Tg has a major influence on the stability of food components and food products Li & Chien, (2001); Bell & White, (2000). An increase in Tg to the values applied in the technology of frozen preservation of water would allow meat to retain the technological utility for a long time. Table.11. shows glass transition temperature of different food items as reported by different researchers in the recent past.

Table.11. Glass transition temperature of different foods

<table>
<thead>
<tr>
<th>Product</th>
<th>Glass Transition Temperature(°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>-13</td>
<td>Akkose and Aktas, 2008</td>
</tr>
<tr>
<td>Chicken</td>
<td>-16.83</td>
<td>Delgado and Sun, 2002</td>
</tr>
<tr>
<td>Tuna</td>
<td>-54.2</td>
<td>Rahman et al, 2003</td>
</tr>
<tr>
<td>Tomato</td>
<td>20</td>
<td>Telis and Sobral (2002)</td>
</tr>
<tr>
<td>Tomato</td>
<td>20</td>
<td>Telis and Sobral (2002)</td>
</tr>
<tr>
<td>Apple</td>
<td>-18.5</td>
<td>Sa et al. (1999)</td>
</tr>
<tr>
<td>Carrot</td>
<td>25</td>
<td>Georget, et al, 1999</td>
</tr>
</tbody>
</table>
The first order phase transition includes such as melting, crystallization whereas the second order phase transition includes glass transition that occurs in amorphous materials. Glass transition is a phase transition from glassy to rubbery state and vice versa (White and Cakebread 1966; Slade and Levine 1994; Levine and Slade 1991). In polymer science when a crystalline material is melted it results in an amorphous melt which when super cooled results in a viscoelastic state i.e., a rubbery state and the transition between two states glassy and rubbery is known as glass transition. It occurs at a temperature at which the crystalline solid melts to form an amorphous glass like material i.e.in non equilibrium state.

The glass transition temperature depends on factors such as the cooling rate and the diagnostic used to locate the transition; it is not an intrinsic property of the system. Relaxation times in the super cooled liquid measured, for example, in dielectric or shear-stress relaxation experiments, increase dramatically with decreasing temperature and close to the glass transition become comparable with macroscopic time-scales. Below this temperature, the system exists in a metastable state having a disordered, liquid-like structure but with mechanical properties similar to those of a crystalline solid. The freezing-out of the translational and rotational degrees of freedom at the glass transition leads in many cases to anomalies in the temperature dependence of thermodynamic properties such as the specific heat. The change in behaviour at $T_g$ is therefore described as a “thermodynamic” or “calorimetric” phase transition, though its nature is very different from that of an equilibrium phase transition.

Levine and Slade (1994) postulated that $T_g$ is an important factor in stability of a food. Below $T_g$ there is low molecular mobility thus there is a significant delay in deteriorative changes in the food that includes enzymatic change, textural and flavor losses etc. Many physical and rheological properties can be described in relation to scaled ($T_g$) or shifted (T-$T_g$) where T is the temperature of interest i.e. may be processing or storage temperature that play an important role in shelf life studies whereas $T_g$ is the temperature at which a transition or change from glass to rubber or vice versa occurs (Farhat, 2000). Basically, glass transition occurs at a range of temperature. Quality of food is the ultimate criterion for food acceptability and as glass transition is related to food stability thus it is necessary to understand this aspect for further maintaining quality.
White and Cakebread (1966) studied glassy state in certain sugar containing food products and recognized the importance of glassy state and glass transition temperature in food. But their study was recognized only in early 1980s when many other workers contributed to the concept based on similar approach of the basic principle underlying the phase transition from glassy to rubbery state and its effect on food stability and quality (Slade and Levine, 1991).

Slade and Levine (1991), in their review ‘Beyond water activity’ put forward the comparison and the shift from known phenomenon of water activity to glass transition. They explained the concept and its advancement in food science. They said that their article is a critical overview of broadest range of moisture and the recent advances based on an alternative approach to the assessment of food quality and safety. Their work further strengthened the study of White and Cakebread (1966) and the concept was well known by that time.

The glass transition can be further understood with the concept of phase or state changes in water. Phase transition is change in physical state such as solid to fluid and then back to its original state. Phase transition has a significant effect on physical properties. Transfer or transition of physical properties is mainly observed due to temperature or pressure change. Biological materials are composed of various constituents that are in thermodynamically non equilibrium, amorphous state. These materials undergo time dependent changes and affect the shelf life of food. Temperature and pressure are two important parameters that result in phase changes; also chemical potential, molecular mobility and Gibb’s free energy are important. When a food is kept at lower temperature then first the water will get cooled and form ice crystals further as the temperature is lowered more ice crystal is formed and at a point no more crystals are formed and the viscosity of concentrated solutes increases. The glass transition of pure water is about -140°C

1.16.1. Factors affecting Glass Transition

In food material, there are certain factors that directly or indirectly affect the glass transition temperature. Some of the factors that affect $T_g$ are listed below:
1.16.1.1. Moisture content

$T_g$ is product specific and is a function of moisture content so also water activity ($a_w$) of the material (Rao, *et al.*, 2010). It has been observed by Slade and Levine (1991) that substantial adsorption of water increases with increase in relative humidity that also facilitates the crystallization and $T_g$ is depressed below ambient temperature. Rao *et al* (2010) reported that with an increase in moisture content of jaggery the $T_g$ values decreased that further supported the typical plasticization behaviour given by Slade and Levine (1991). Kawai *et al* (2005), observed a decrease in $T_g$ for inulin samples with an increase in moisture content.

1.16.1.2. Molecular weight

Molecular weight of solute has a direct influence on $T_g$. This has been repeatedly reported that low molecular weight molecules exhibit poor stability above $T_g$ (eg. such as for sugars by; Slade and Levine 1994). Low molecular weight molecules have a plasticizing effect (i.e. they increases the free volume) of polymers and thus lower $T_g$ of multi component system (Brake, *et al.*, 1999). Low molecular weight molecules that are present as monomer have lower $T_g$ compared to long chain molecules. Fox and Flory (1950) relationship has been in use to determine the effect molecular weight of a food polymer on $T_g$ (Slade and Levine 1994; Roos and Karel 1991; Bhandari and Howes, 1999)

$$T_g = T_{g\infty} - \frac{K_g}{M}$$

Where $T_{g\infty}$ is the limiting $T_g$ at infinite molecular weight, $K_g$ a constant (-25,000 K) and $M$ is the molecular weight. Here, $T_{g\infty}$ and $K_g$ are material dependent Bhandari and Howes (1999). Busin *et al* (1996), reported a linear relationship between $T_g$ and dextrose equivalent (DE) with a correlation coefficient ($r = 0.98$).

$$T_g = -14(\text{DE}) + 449.5$$

Here, $T_g$ measurement can be a tool to determine the degree of hydrolysis of starch. There is difficulty that occur during determination of food polymer $T_g$ since before reaching the transition temperature it gets decomposed that will ultimately
predict the $T_g$ of intercept (Busin, et al., 1996; Roos 1995; Bhandari and Howes, 1999).

1.16.1.3. Pressure

There is a direct relation between pressure and $T_g$, it gets affected by external pressure as there is a change in structural relaxation of liquid that is helpful in determining and understanding the glass transition. In thermodynamics with an increase in pressure on an amorphous system there will be a decrease in entropy (lack of order in structure). Pressure induces increase in density that further increases the relaxation time $\tau$ that results due to motion in terms of Vogel–Fulcher–Tamann (VFT) equation (Casalini, et al., 2001; Corezzi, et al., 1999) such as

$$\tau [T_g(\infty), P] = \tau_0 \exp\left[B_0 + [T_g(\infty, 0) - T_0(\infty, P)]\right]$$

Another VFT equation such as

$$T_b(V, P) = T_0(V, 0) + aP + bP^2$$

Here $\tau_0$ and $B_0$ are constants being insensitive of pressure, $a$ and $b$ are the experimental parameters and $T_0$ is the Vogel–Fulcher temperature. As the $P$ values increases i.e. pressure is increased there is decrease in temperature $T$ and also the molecular motion becomes slow thus increasing the $T_g$ (Lang et al 2006).

1.16.1.4. Heating/cooling rate

Both heating and cooling rate affects the glass transition. As the heating and cooling rate increases there is an increase in $T_g$ observed. $T_g$ decreases with increasing freezing rate (Fennema, 1996). This has been reported that due to rapid cooling there is less occurrence of freeze concentration and maximal amount of water remains in unfrozen state. After the solution is warmed, ice crystallization may occur at temperatures above $T_g$, a phenomenon known as devitrification or delayed crystallization (Hsu, et al., 2003). With slow cooling rate $T_g$ is higher as compared to fast cooling rate since, in slow cooling rate maximum water freezes. Hsu et al (2003) in their study on influence of cooling rate on $T_g$ of sucrose solution and rice starch gel and found that $T_g$ was lower at rapid cooling rate. Similar results were reported by Rahman, et al., (2007a) for spaghetti.
1.16.1.5. Aging

Aging is a function of storage time. It is a natural phenomenon that occurs when material is kept for a desired period of time at suitable temperature and it leads to structural and textural changes. Below $T_g$, physical aging is observed i.e. there is a structural transformation towards equilibrium (Chung and Lim., 2003). Hodge (1994) reported that quantitative measurement of physical aging can be determined with change in specific volume or relaxation enthalpy. Borde et al (2002) did a comparative study on physical aging of maize starch with polymers and concluded that it depends on cooling rate, storage temperature and chemical structure. There is a conformational change during aging that decreases the free volume and mobility of glassy starches chains thus raises the $T_g$ (Chung and Lim., 2003).

1.16.1.6. Annealing

Annealing is the process in which the product is kept for a particular temperature at a known interval of time. As the annealing temperature decreases there is a shift in $T_g$ towards lower temperature. While as the annealing time is increased there is lowering of $T_g$. Brake and Fennema (1999) reported that there is no literature available on standardized annealing temperature for determination glass transition temperature of meat.

1.16.1.7. Molecular mobility

Roudaut et al(2004) explained the concept of molecular mobility around the glass transition. The physical state and molecular mobility of food is governed by temperature of food is further related to stability and other quality parameters. Temperature is one of the factors that determine the physical state of food. In food polymer science the food may have any of the two forms i.e. may be amorphous (the structure is not defined) or crystalline (the structure is defined). Crystalline form has lower molecular mobility than the amorphous form. Molecular mobility is greatly influenced by the hydration and temperature of food and its constituents. The interaction of water present and the food constituents determines the properties in aqueous phase.

Higher the water content the more is the molecular mobility as the fluidity will increase also the lower the interaction between constituent and water will increase the
mobility as free water will increase. Hence more the interaction lower will be the free water present. Amount of water either in free or bound form will influence mobility although bound water has less influence compared to free water. Similarly temperature pose a great affect on molecular mobility as when the temperature increases there is transition from solid to liquid and further to gaseous state. It is a well known fact that in solid state the molecules are tightly bound to each other and there is less space between them thus the movement is confined whereas as there is transition to other state the molecular mobility increases as the intermolecular space is more and the molecule has more space to move thus mobility increases. Below T_g there is lowering of molecular mobility which in turn leads to delay in deteriorative changes thus increasing stability and shelf life while above T_g the stability is increased (Rahman, et al., 2003, Rahman, 2007a and Rahman, 2007b).

1.16.2. Application of glassy state and influence of T_g on physicochemical changes in food

The concept of glass transition is quite useful in predicting the intrinsic properties of food that helps determine the quality and relate to the quality control of product. Glass transition phenomenon is known to have a variety of applications as well as it is also responsible for several physicochemical changes in food. Nowadays, T_g is considered necessary to predict various properties of food. Glass transition has gained importance in last few years and is generally considered for predicting stability of food. The change in molecular mobility of food at T_g is reported responsible for various physical and chemical changes that occur in food during processing as well as storage.

1.16.2.1. Denaturation of protein

There are several factors responsible for protein denaturation such as pH, temperature, water content etc. Protein properties and functionality depend on its existing state i.e. native or denatured. Various studies show that glass transition temperature influences protein denaturation (Bell & Hageman, 1996; Bellavia, et al., 2011). A greater degree of protein destabilization was observed with lower T_g of component Bell & White, (2000). Depending upon the water content and temperature the hydrated proteins can be either in a glassy or rubbery state (Sochava, 1997). At lower hydration low T_g values were observed and a higher it promotes sugar-protein
interaction while high to intermediate hydration results in higher $T_g$ (Bellavia, *et al.*, 2011). A linear relationship was observed between glass transitions ($T_g$) and thermal denaturation ($T_{\text{Den}}$). In recent years a relationship between several plants/animal protein denaturation and glass transition has been investigated.

### 1.16.2.2. Microbial Stability

Water activity is considered an important intrinsic factor that is responsible for microbial growth on food since water is a major constituent in most of the foods. The microbial stability of food means that it should be free from any microbes’ at least up to certain period that is specified as the shelf life of food. The shift in use of $a_w$ to $T_g$ to predict microbial stability is mainly due to certain reasons such as: $a_w$ is a thermodynamic concept and it refers only to equilibrium, while in intermediate moisture food the measured water vapor pressure is not in equilibrium, thus it is not suitable for IMF and difference in microbial responses is found with different solutes as there is variation in $a_w$ also (Chirife and Buera., 1996). Water is one of the major factors that help microbes to grow in food hence the water activity of food should be sufficiently low so that it can inhibit the growth of microbes and maintain the safety and quality of food.

Slade and Levine (1987) came up with a hypothesis that microbial stability could be predicted in terms of water activity but it has certain limitations and then they put forward the idea of glass transition as an indicator. Food matrix is known to consist of components such as carbohydrates, fats, proteins, minerals, water and these components are found to be dissolved in water. Presence of water leads to high molecular mobility of compounds in water phase thus the food in its fresh form is considered highly susceptible to undergo various deteriorative changes such as chemical, enzymatic, microbial and various other physical changes. When food is dried or cooled the concentration of dissolved components takes place that forms glass when it is maximally concentrated or when cooled components would crystallize and may also remain in amorphous, glassy state (Nijhuis, *et al.*, 1998). Above glass transition temperature spores can be easily inactivated and at $T_g$ a high resistance to heat for spores is seen (Sapru and Labuza, 1993). Chirife and Buerz, (1994) in their study reported that glass transition and water activity are two different parameters with a very different significance to determine microbial stability of food.
Glass transition is a structural property while water activity is a solvent dependent property thus with only glass transition, the prediction of microbial stability in food is not possible.

1.16.2.3. Oxidation

Lipid oxidation is major cause of deterioration in food. And is mainly caused due to presence of oxygen and free radicals. The main phase transition of lipid in food is the change that occurs during processing, storage and consumption from solid fat to liquid oils. With a sufficient decrease in temperature, during freeze concentration occurs that finally lead to glassy state with low temperature. The amount of unfrozen water decreases and the characteristic glassy state formed is at glass transition temperature (Goff, 1992; Hansen, et al., 2004a). Above glass transition temperature the molecular mobility significantly increases and thus amorphous glasses are fairly stable below $T_g$ since there is low molecular mobility but with an increase in $T_g$ there is a loss of long term stability causing various deteriorative changes (Roos, 2002).

In glassy carbohydrates free radicals have sufficient mobility to diffuse through the matrix (Bowen, et al., 2006). Hansen et al. (2004b), studied the oxidative stability of frozen pork patties and reported that intermediate levels of oxidative change occurred at storage temperature fluctuations in comparison to high and low temperature fluctuations. Bowen et al (2006), concluded from their study on lipid oxidation of waxy maize that in spite of storing at glassy or rubbery state if lipid is present and gets rancid then the molecular weight of starch will go down. It is a known fact that $T_g$ is reduced and molecular mobility is increased with an increase in moisture content causing higher oxygen diffusion rate and mobility that is responsible for the off-flavor and odour that finally leads to oxidation of lipids.

1.16.2.4. Texture

Texture of a food depends on certain factors that further are responsible for the $T_g$ obtained. Every product has a specific characteristic texture such as crispy, hard, brittle etc. that are further governed by the water content, composition of food, type of processing it has undergone such as fried, freezed, canned etc., hence the texture cannot be defined by any single factor. The relationship between $T_g$ and texture depends on the specific texture attribute that is important for that particular food.
product. In glassy state the dried food usually have crispy texture. Deng et al (2008), studied the relationship of hardness or crispiness of six dried samples with $T_g$ and found that there exist no clear relationship between the two. They concluded that $T_g$ being a characteristic of water soluble phase may not contribute much in dried food while other phases were responsible for the mechanical behaviour of food. They also studied the relation between shrinkage with $T_g$ in which they found that for the given treatments such as for freeze drying pores were formed due to ice sublimation and shrinkage was there due to surface force however the increase in viscosity prevented much shrinkage. In case of hot air drying, when the temperature was above $T_g$ lower shrinkage occurred that finally led to a harder texture and compact structure. With a reduction in water during drying there is depression in $T_g$. This has been reported that in high sugar products, the dehydration temperature above $T_g$ the product will remain soft and when subjected to cooling it will become harder as $T_g$ lowers (Bhandari and Howes, 1999).