American Journal of Agricultural and Biological Sciences 6 (4): 486-510, 2011
ISSN 1557-4989

Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review

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Abstract: Problem statement: Extremely perishable meat provides favorable growth condition for various microorganisms. Meat is also very much susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fat, protein and carbohydrates of meat results in the development of off-odors, off-flavor and slim formation which make the meat objectionable for human consumption. It is, therefore, necessary to control meat spoilage in order to increase its shelf life and maintain its nutritional value, texture and flavor. **Approach:** A comprehensive literature review was performed on the spoilage mechanisms of meat and meat products and preservation techniques. **Results:** Historical data reveals that salting, drying, smoking, fermentation and canning were the traditional methods used to prevent meat spoilage and extend its shelf life. However, in order to prevent wholesomeness, appearance, composition, tenderness, flavor, juiciness, and nutritive value, new methods were developed. These included: cooling, freezing and chemical preservation. Wide range of physical and chemical reactions and actions of microorganisms or enzymes are responsible for the meat spoilage. Microbial growth, oxidation and enzymatic autolysis are three basic mechanisms responsible for spoilage of meat. Microbial growth and metabolism depends on various factors including: pre-slaughter husbandry practices, age of the animal at the time of slaughtering, handling during slaughtering, evisceration and processing, temperature controls during slaughtering, processing and distribution, preservation methods, type of packaging and handling and storage by consumer. Microbial spoilage causes pH change, slime formation, structural components degradation, off odors and appearance change. Autoxidation of lipids and the production of free radicals are natural processes which affect fatty acids and lead to oxidative deterioration of meat and off-flavour development. Lipid hydrolysis can take place enzymatically or non-enzymatically in meat. In muscle cells of slaughtered animals, enzymatic actions are taken place naturally and they act as catalysts for chemical reactions that finally end up in meat self deterioration. Softening and greenish discoloration of the meat results due to tissues degradation of the complex compounds (carbohydrates, fats and protein) in the autolysis process. **Conclusion:** Microbial, chemical and enzymatic activities can be controlled by low temperature storage and chemical techniques in the industry. Proper handling, pretreatment and preservation techniques can improve the quality of meat and meat products and increase their shelf life. Combination of chemical additives (TBHQ and ascorbic acid) and low temperature storage (5°C) in darkness are well recognized techniques for controlling the spoilage (microbial, enzymatic and oxidative) of meat and meat products. Understanding of the intrinsic factors and extrinsic factors at every meat processing stage (from preslaughtering to meat product development) is necessary before developing proper handling, pretreatment and preservation techniques for meat.

Key words: Meat spolilage, Dark, Firm and Dry (DFD), Pale, Soft and Exudative (PSE), enzymatic actions, microbial spoilage, low temprature storage, chemical preservation

INTRODUCTION

Rich nutrient matrix meat is the first-choice source of animal protein for many people all over the world (Heinz and Hautzinger, 2007). In Canadian diet, the consumption of meat in 2008 was estimated at 36.6 kg capita^{-1} (beef and veal at 12.8 kg capita^{-1}, pork at 9.7 kg capita^{-1}, chicken meat at 11.2 kg capita^{-1}, turkey at 2.4 kg capita^{-1} and lamb was at 0.5 kg capita^{-1}) (SC, 2009). The total estimated consumption of meat (chicken, turkey, veal, lamb, beef, pork) in USA was 101 kg capita^{-1} in the year 2007 (THSUS, 2010). Consumption

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of meat is continuously increasing worldwide. The annual per capita consumption increased from 10 kg in the 1960s to 26 kg in 2000 and will reach 37 kg by the year 2030 (Heinz and Hautzinger, 2007). On the other hand, a significant portion of meat and meat products are spoiled every year. Kantor *et al*. (1997) reported that approximately 3.5 billion kg of poultry and meat were wasted at the consumer, retailer and foodservice levels which have a substantial economic and environmental impact. Significant portion of this loss is due to microbial spoilage. Cerveny *et al*. (2009) stated that if 5% of this meat loss is preserved it could satisfy the daily needs of approximately 320,000 people for meat and poultry.

The transformation of animals into meat involves several operations: (a) handling and loading of animals on the farm, (b) transporting animals to slaughterhouses, (c) off-loading and holding of animals and (d) slaughtering of animals (Chambers and Grandin, 2001). Poor operational techniques and facilities in any of these operations will result in unnecessary suffering and injuries to animals which can lead to loss of meat, reduced meat quality and spoilage of meat (Chambers and Grandin, 2001). Therefore, prevention of contamination after slaughtering during meat cutting and processing is essential (FAO, 1991). Storage time can be extend through hygienic slaughtering and clean handling of the carcass (FAO, 1990).

Different technical operations are involved in slaughtering: (a) stunning, (b) bleeding, (c) skinning, (d) evisceration and (e) carcass splitting. Inadequacy at one stage will result in a rigorous negative impact on the product and/or process in the following stage (FAO, 1991). In addition to the hygiene and storage temperature, the acidity of the meat and the structure of the muscular tissue also affect the rate of meat spoilage. For example, liver will spoil faster than the firm muscular tissue of beef (Berkel *et al*., 2004). After few hours of slaughtering of animals, muscles becomes firm and rigid, a condition known as rigor mortis. The process of rigor mortis depends on the stress induced on the animals during the slaughtering process (Miller *et al*., 2002). Raw meat quality is reported to be severely affected by the stress conditions during slaughtering process and the slaughtering methods (Miller *et al*., 2002; Chambers and Grandin, 2001).

Fat, protein, minerals, carbohydrate and water are the constituents of meat (Heinz and Hautzinger, 2007). The quality of meat and meat products degrade as a result of digestive enzymes, microbial spoilage and fat oxidation (Berkel *et al*., 2004). Lipid oxidation, protein degradation and the loss of other valuable molecules are the consequence of meat spoilage process. Table 1 shows the chemical composition of fresh raw and processed meat. Proteins and lipids can break down resulting in the production of new compounds causing changes in meat flavor, tenderness, juiciness, odour and texture. It is therefore, important to understand the causes of spoilage of meat and meat product in order to develop optimum preservation techniques to maintain the freshness of these food products.

CAUSES OF MEAT SPOILAGE

Preslaughter handling of livestock and postslaughter handling of meat play an important part in deterioration of meat quality. The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress which changes the pH of the meat, to higher or lower levels, depending on the production level of lactic acid (Miller, 2002; Chambers and Grandin, 2001; Rahman, 1999a). Lactic acid is produced due to the breakdown of glycogen content of animal muscles via an anaerobic glycolytic pathway as shown in Fig. 1 (Rahman, 1999a). Higher levels of pH (6.4-6.8) result in Dark, Firm and Dry (DFD) meat. Long term stress causes DFD meat which has a shorter shelf life (Miller, 2002; Chambers and Grandin, 2001). Sever short term stress results in a Pale, Soft and Exudative (PSE) meat. PSE meat has a pH lower than normal ultimate value of 6.2 which is responsible for the breakdown of proteins, providing a favorable medium for the growth of bacteria (Miller, 2002; Chambers and Grandin, 2001; Rahman, 1999a). Figure 2 shows the texture and color of the DFD, PSE and normal meat. The factors affecting the shelf life of meat and meat products are summarized in Table 2. There are three main mechanisms for meat and meat products spoilage after slaughtering and during processing and storage: (a) microbial spoilage, (b) lipid oxidation and (c) autolytic enzymatic spoilage.

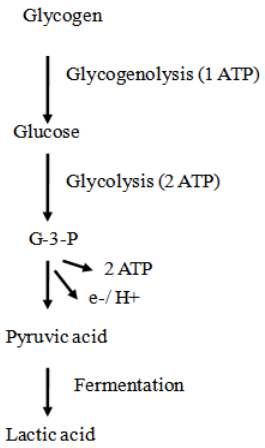
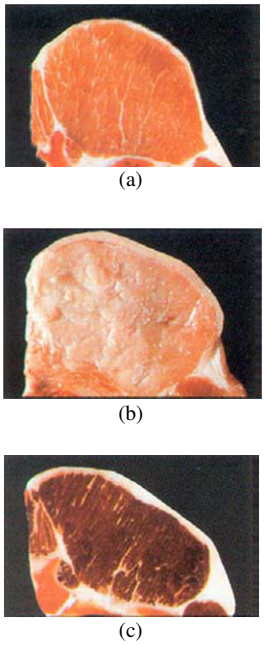


Fig. 1: Anaerobic glycolytic pathway (Diwan, 2007)

Table 1: Water, protein, fat, ash content and calories in fresh and processed meats (Heinz and Hautzinger, 2007)

Product	Water (%)	Protein (%)	Fat (%)	Ash (%)	Energy (Cal /100g)
Fresh					
Beef (lean)	75.0	22.30	1.80	1.2	116
Beef carcass	54.7	16.50	280.00	0.8	323
Pork (lean)	75.1	22.80	1.20	1.0	112
Pork carcass	41.1	11.20	470.00	0.6	472
Veal (lean)	76.4	21.30	0.80	1.2	98
Chicken	75.0	22.80	0.90	1.2	105
Venison (deer)	75.7	21.40	1.30	1.2	103
Beef fat (subcutaneous)	4.00	1.50	940.00	0.1	854
Pork fat (back fat)	7.70	2.90	88.70	0.7	812
Processed					
Beef, lean, fried	58.4	30.40	9.20	-	213
Pork, lean, fried	59.0	27.00	1300.00	-	233
Lamb, lean, fried	60.9	28.50	9.50	-	207
Veal, lean, fried	61.7	31.40	5.60		186
Raw-cooked sausage with coarse lean particles (ham sausage)	68.5	16.40	11.10		170
Raw-cooked sausage finely comminuted, no extender	57.4	13.30	22.80	3.7	277
Raw-cooked sausage (frankfurter type)	63.0	14.00	19.80	0.3	240
Precooked-cooked sausage (liver sausage)	45.8	12.10	38.10	-	395
Liver pate	53.9	16.20	25.60	1.8	307
Gelatinous meat mix (lean)	72.9	18.00	3.70	-	110
Raw-fermented sausage (Salami)	33.9	24.80	37.50	-	444



(a) (b) (c)

Fig. 2: Meat texture and colour (Chambers and Grandin, 2001) (a) Normal meat; (b) Pale Soft and Exudative (PSE) meat; (c) Dark Firm and Dry (DFD) meat

Table 2: Factors affecting shelf life of meat (Rahman, 1999a)

Type	Factors
Intrinsic	Type of animal (bovine, porcine)
	Breed and fed regime
	Age of animal at time of slaughter
	Initial microflora
	Chemical properties (peroxide value, pH, acidity, redox potential)
	Availability of oxygen
	Processing conditions and control
	Hygiene (standard of personnel and equipment cleaning)
Extrinsic	Quality- management system
	Temperature control
	Packing system (materials, equipment, gases)
	Storage types

Microbial spoilage: Meat and meat products provide excellent growth media for a variety of microflora (bacteria, yeasts and molds) some of which are pathogens (Jay *et al*., 2005).

The intestinal tract and the skin of the animal are the main sources of these microorganisms. The composition of microflora in meat depends on various factors: (a) pre-slaughter husbandry practices (free range Vs intensive rearing), (b) age of the animal at the time of slaughtering, (c) handling during slaughtering, evisceration and processing, (d) temperature controls during slaughtering, processing and distribution (e) preservation methods, (f)

type of packaging and (g) handling and storage by consumer (Cerveny *et al*., 2009). Table 3 and 4 present the major genera of bacteria, yeasts and molds found in meat and poultry products before spoilage. Mold species include *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Penicillium* and *Mucor* while yeasts species include *Candida* spp., *Cryptococcus* spp. and *Rhodotorula* spp. (Garcia-Lopez *et al*., 1998). Bacteria species include *Pseudomonas*, *Micrococcus*, *Streptococcus*, *Sarcina*, *Lactobacillus*, *Salmonella*, *Escherichia*, *Clostridium* and *Bacillus* (Lin *et al*., 2004; Arnaut-Rollier *et al*., 1999; Nychas and Tassou, 1997).

Hayes *et al*. (2003) found *Enterococcus* spp. to be the most dominant bacteria on 971 of the 981 samples (99%) of all meat (chicken, turkey, pork and beef) in the state of Iowa. About 97% of pork samples contained Enterococci with 54% of isolates identified as *Enterococcus faecalis* and 38% as *Enterococcus faecium*, 3.4% as *Enterococcus hirae*, 2.4% as *Enterococcus durans*, 0.8% as *Enterococcus casseliflavus*, 0.4% *Enterococcus gallinarum* and 1% as unindentified. All of beef samples contained enterococci with 65% of isolates identified as *Enterococcus faecium*, 17% as *Enterococcus faecalis*, 14% as *Enterococcus hirae*, 2% as *Enterococcus durans* 0.7%, as *Enterococcus casseliflavus*, 0.4% *Enterococcus gallinarum* and 0.9% as unindentified.

Cerveny *et al*. (2009) stated that storage conditions affect the type of microbes found in meat and meat products. They reported that Pseudomonas spp., *Moraxella* spp., *Psychrobacter* spp., *Acinetobacter* spp. and Gram-negative psychrotrophic members of the family. Enterobacteriaceae are frequently present on refrigerated meat product.

They also indicated that psychrotrophic lactic acid bacteria, Enterococci, Micrococci and yeasts are predominately found in raw, salted-cured products such as corned beef, uncooked hams and bacon due to their resistance to curing salts.

Table 3: Genera of bacteria most frequently found on meats and poultry (Jay *et al*., 2005)

Genus	Gram reaction	Fresh meats	Fresh livers	Poultry
Acinetobacter	−	xx	x	xx
Aeromonas	−	xx		x
Alcaligenes	−	x	x	x
Arcobacter	−	x		
Bacillus	+	x		x
Brochothrix	+	x	x	x
Campylobacter	−			xx
Carnobacterium	+	x		
Caseobacter	+	x		
Citrobacter	−	x		x
Clostridium	+	x		x
Corynebacterium	+	x	x	xx
Enterobacter	−	x		x
Enterococcus	+	xx	x	x
Erysipelothrix	+	x		x
Escherichia	−	x	x	
Flavobacterium	−	x	x	x
Hafnia	−	x		
Kocuria	+	x	x	x
Kurthia	+	x		
Lactobacillus	+	x		
Lactococcus	+	x		
Leuconostoc	+	x	x	
Listeria	+	x		xx
Microbacterium	+	x		x
Micrococcus	+	xx	xx	xx
Moraxella	−	xx	x	xx
Paenibacillus	+	x		x
Pantoea	−	x		x
Pediococcus	+	x		
Proteus	−	x		x
Pseudomonas	−	xx		xx
Psychrobacter	−	xx		x
Salmonella	−	x		x
Serratia	−	x		x
Shewanella	−	x		
Staphylococcus	+	x	x	x
Vagococcus	+			xx
Weissella	+	x	x	
Yersinia	−	x		

x = known to occur; xx = most frequently reported

Garcia-Lopez *et al*. (1998) reported that the growth of Enterobacteriaceae and Pseudomonas were more prevalent on modified atmosphere packed meat (especially on pork) than on vacuum packed meat, their growth being favoured by storage at 5°C. Sentence (1991) reported that *Pseudomonas* spp. growth rate was considerably slow at 0°C, but increased at 2°C and affected the shelf life of meat. He also noticed slow Salmonella growth below 7°C, which increased above 7°C and affected the shelf life of meat. Borch *et al*. (1996) reported that the growth of lactic acid bacteria on bologna-type sausage was retarded 2 and 4 fold with decreases in temperature from 7-2°C and from 7-0.6°C, respectively. Russell *et al*. (1996) stated that a favorable pH for the growth of spoilage bacteria for meat is in the range of 5.5-7.0. Slime formation, structural components degradation, off odors and appearance change were found in meat as a result of microbial growth within this pH range. Table 5 shows various compounds resulting from bacterial spoilage. The methylamine, dimethylamine and trimethylamine have been commonly detected during bacterial spoilage by Garcia-Lopez *et al*. (1998). Dainty (1996) stated that microbial metabolism produces fatty acids, ketones and alcohols, which exhibit a variety of fruity and sweety odours. Generation of hydrogen sulphide, methylsulphide and dimethylsulphide exhibit putrid and sulphury odours. The diamines, cadaverine and putrescine (the metabolic by-products of meat spoilage) have been studied as indicators of meat spoilage (Jay *et al*., 2005). The production of these diamines occurs in the following manner:

$$\text{Lysine} \xrightarrow{\text{decarboxylase}} \underset{\text{Cadaverine}}{H_2N(CH_2)_5NH_2} \quad (1)$$

$$\text{Ornithine or Arginine} \xrightarrow{\text{decarboxylase}} \underset{\text{Putrescine}}{H_2N(CH_2)_5NH_2} \quad (2)$$

Lipid oxidation: Autoxidation of lipids and the production of free radicals are natural processes which affect fatty acids and lead to oxidative deterioration of meat and off-flavours development (Gray, 1978; Pearson *et al*., 1983; Simitzis and Deligeorgis, 2010).

After slaughtering of animals, the fatty acids in tissues undergo oxidation when the blood circulation stops and metabolic processes are blocked (Gray and Pearson, 1994; Linares *et al*., 2007). Lipid oxidation is the reaction of oxygen with double bonds of fatty acids (Hultin, 1994). It involves three stage free radical mechanisms: initiation, propagation and termination (Frankel, 1985; Khayat and Schwall, 1983; Fernindez *et al*., 1997).

Table 4: Genera of mold and yeast most often found on meats and poultry (Jay *et al*., 2005)

Genus	Fresh and refrigerated meats	Poultry
Molds		
Alternaria	x	x
Aspergillus	x	x
Aureobasidium	x	
Cladosporium	xx	x
Eurotium	x	
Fusarium	x	
Geotrichum	xx	x
Monascus	x	
Monilia	x	
Mucor	xx	x
Neurospora	x	
Penicillium	x	x
Rhizopus	xx	x
Sporotrichum	xx	
Thamnidium	xx	
Yeasts		
Candida	xx	xx
Cryptococcus	x	x
Debaryomyces	x	xx
Hansenula	x	
Pichia	x	x
Rhodotorula	x	xx
Saccharomyces		x
Torulopsis	xx	x
Trichosporon	x	x
Yarrowia		xx

Table 5: Bacterial spoilage compounds (Garcia-Lopez *et al*., 1998; Church, 1998; Borch *et al*., 1996)

Spoilage bacteria	Spoilage compounds
Pseudomonas spp. and *Enterobacteriaceae*	Cysteine, cystine, methionine, Hydrogen sulphide, methylsulphide and dimethylsulphide
Pseudomonas fluorescens	Methylamine, dimethylamine and trimethylamine ethyl esters
Enterobacteriaceae fragi	
Clostridium spp.	Oxygen and carbon dioxide
Lactobacillus sake, Hafnia alvei	hydrogen sulphide
Shewanella putrifaciens	trimethylamine, hydrogen sulphide, methylmercarptan,
Enterobacteriuceue,	Dimethylsulphide, hypoxanthine
Brochothrix thermosphacta and homofermentative	Acetoindiacetyl and 3-methylbutanol
Luctobucilhls spp.	
Brochothrix thermosphucta	Acetoin and acetic acid
Photobacterium phosphoreum	Trimethylamine, hypoxanthine
Vibrionacaea	Trimethylamine, hydrogen sulphide
Aerobic spoilers	Ammonia, acetic, butyric and propionic acid

Initiation: Heat, metal ions and irradiation act as catalyst and form lipid free radicals during the initiation stage. Reaction of these free radicals with oxygen form peroxyl radicals as follows:

$$R. + O_2 \longrightarrow R\cdot + \cdot OOH \quad (3)$$

Propagation: During the propagation stage, the peroxyl radicals react with other lipid molecules to form hydroperoxides and new free radicals as follows (Fraser and Sumar, 1998; Hultin, 1994):

$$R\cdot + O_2 \longrightarrow ROO\cdot \quad (4)$$

$$RH + ROO\cdot \longrightarrow ROOH + R\cdot \quad (5)$$

$$ROOH \longrightarrow RO\cdot + \cdot OH \quad (6)$$

Termination: Termination occurs when these free radicals interact to form non-radical products as follows (Hultin, 1994):

$$R\cdot + R\cdot \longrightarrow R - R \quad (7)$$

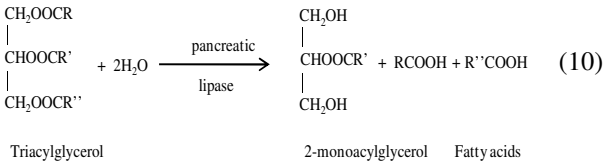
$$R\cdot + ROO\cdot \longrightarrow ROOR \quad (8)$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2 \quad (9)$$

Oxidation of lipids in meat depends on several factors including: fatty acid composition, the level of the antioxidant vitamin E (α tocopherol) and prooxidants such as the free iron presence in muscles. Polysaturated fatty acids are more susceptible to lipid oxidation. Hydroperoxides are produced due to the lipid oxidation of highly unsaturated fatty acid fractions of membrane phospholipids, which are susceptible to further oxidation/ decomposition (Enser, 2001; Simitzis and Deligeorgis, 2010). Their breakage causes secondary reaction products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) as well as other oxygenated compounds such as aldehydes, acids and ketones (Fernindez *et al*., 1997; Shahidi, 1994; Raharjo and Sofos, 1993). These secondary products can cause loss of colour and nutritive value due to sever effects on lipids, pigments, proteins, carbohydrates and vitamins (Simitzis and Deligeorgis, 2010) and are directly related to carcinogenic and mutagenic processes (Liu *et al*., 1995).

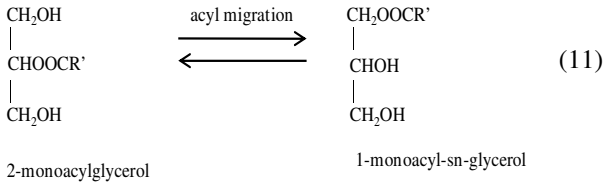
In meat, lipid hydrolysis can take place enzymatically or non-enzymatically. The enzymatic hydrolysis of fats is termed lipolysis or fat deterioration and is governed by specific enzymes such as lipases, estarases and phospholipase. Lipolytic enzymes could either be endogenous of the food product (such as milk) or derived from psychrotrophic microorganisms (Ghaly *et al*., 2010). Lipases enzymes are present in the skin, blood and tissue of animals. During lipolysis, lipases split the glycerides forming free fatty acids which are responsible for common off-flavour, frequently referred to as rancidity (Huis in't Veld, 1996; FAO, 1986). The main enzymes involved in meat lipid hydrolysis are phospholipase A1 and phospholipase A2 (Toldra, 2006). Lipid hydrolysis process is regiospecific and involved three steps of biosynthetic pathway: cleavage of triacylglycerol, acyl migration and cleavage of 1-monoacyl-sn-glycerol (Belitz *et al*., 2009; Christie, 2010).

*(a) cleavage of triacylglycerol***:** In this step, pancreatic lipase hydrolyses the 1(3) positions of the triacylglycerols and resulted in formation of 2-monoacylglycerols and fatty acids.



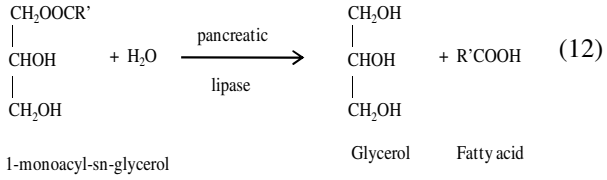
(10)

*(b) Acyl migration***:** In this step 2-monoacylglycerols isomerizes to 1-monoacyl-sn-glycerols through acyl migration as follows:



(11)

*(c) Cleavage of 1-monoacyl-sn-glycerol***:** In this step 1-monoacyl-sn-glycerols can be hydrolyses completely to glycerol and free acid in presence of pancreatic lipase as follows:



(12)

The non-enzymatic hydrolysis is caused by heme proteins such as hemoglobin, myoglobin and cytochrome which are susceptible to oxidation and produce hydroperoxides (Kanner, 1994; Love and Pearson, 1971). During heme catalysis, a Fe^{2+} protoporphyrin complex ($P–Fe^{2+}$), like myoglobin, will be oxidized to $P–Fe^{3+}$. The formed superoxide radical anion O_2^- reacts with H^+ and will yield H_2O_2. Hydrogen peroxide will then oxidize $P–Fe^{3+}$to the oxene species P–Fe = O (Belitz *et al*., 2009). The free iron redox cycle contributed by ascorbic acid is the main initiator of lipid peroxidation in fresh muscle foods and it

significantly affects the oxidation of oxymyoglobin (Cascone, 2005):

$$P - Fe^{+2} + O_2 \longrightarrow P - Fe^{+3} + O_2^- \quad (13)$$

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (14)$$

$$P\text{-}Fe^{+3} + H_2O_2 \rightarrow P^{+}\begin{matrix}H \\ Fe^{+3}\ldots O^{-}\text{-}O\text{-}H\end{matrix} \rightarrow \begin{matrix}P\text{-}Fe^{+3}=O \\ \Updownarrow \\ P\text{-}Fe^{+4}\text{-}\bar{O}\end{matrix} + H_2O \quad (15)$$

$$P - Fe^{+4} - \bar{O} + ROOH \longrightarrow P - Fe^{+4}\ldots OH^- + ROO \quad (16)$$

$$P - Fe^{+4}\ldots \bar{O} - H + ROOH \longrightarrow P - Fe^{+3} + ROO\cdot + H_2O \quad (17)$$

Autolytic enzymatic spoilage: Enzymatic actions are natural process in the muscle cells of the animals after they have been slaughtered and are the leading cause of meat deterioration. The enzymes have the ability to combine chemically with other organic compounds and work as catalysts for chemical reactions that finally end up in meat self deterioration (Tauro *et al*., 1986). In the autolysis process, the complex compounds (carbohydrates, fats and protein) of the tissues are broken down into simpler ones resulting in softening and greenish discoloration of the meat. These autolysis changes include proteolysis and fat hydrolysis which are prerequisite for microbial decomposition. Excessive autolysis is termed "souring" (Tauro *et al*., 1986).

Postmortem breakdown of polypeptides are the result of tissue proteases and is responsible for flavour and is textural changes in meat (Toldra and Flores, 2000). Post mortem aging of red meat results in the tenderization process (Huss, 1995). Post-mortem autolysis takes place in all animal tissues but at different rates in different organs, quicker in glandular tissue such as the liver and slower in striated muscle (Fearon and Foster, 1922). The enzymes calpains, cathepsins and aminopeptidases are found to be responsible for the post mortem autolysis of meat through digestion of the z- line proteins of the myofibril (O'Halloran *et al*., 1997; Huss, 1995). Among these enzymes, calpains has been described as a preliminary contributor to the proteolytic tenderization process of meat. Cathepsins were, also, found to contribute to tenderization at low pH. The mechanism of calpain catalyzed meat proteolysis is shown in Fig. 3 (O'Halloran *et al*., 1997). Proteolytic enzymes are active at low temperatures (5°C) which lead to deterioration of meat quality due to growth of microbes and biogenic amines production (Kuwahara and Osako, 2003).

PRESERVATION OF MEAT

Meat preservation became necessary for transporting meat for long distances without spoiling of texture, colour and nutritional value after the development and rapid growth of super markets (Nychas *et al*., 2008). The aims of preservation methods are: (a) to inhibit the microbial spoilage and (b) to minimize the oxidation and enzymatic spoilage. Traditional methods of meat preservation such as drying, smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, biopreservative and nonthermal techniques (Zhou *et al*., 2010). Current meat preservation methods are broadly categorized into three methods (a) controlling temperature (b) controlling water activity (c) use of chemical or biopreservatives (Zhou *et al*., 2010). A combination of these preservation techniques can be used to diminish the process of spoilage (Bagamboula *et al*., 2004).

Low temperature methods: The basic aim of cooling techniques is to slow or limit the spoilage rate as temperature below the optimal range can inhibit the microbial growth (Cassens, 1994). Low temperature methods of storage are used in three levels: (a) chilling (b) freezing and (c) superchilling. All these levels help to inhibit or completely stop bacterial growth (Zhou *et al*., 2010). However, the growth of psychrophilic group of bacteria, yeasts and molds is not prevented by all levels of refrigeration (Neumeyer *et al*., 1997) and both enzymatic and non enzymatic changes will continue at a much slower rate (Berkel *et al*. 2004).

Chilling: Chilling is employed at slaughtering plants immediately after slaughtering and during transport and storage. It is necessary to reduce the temperature of carcass immediately after evisceration to 4°C within 4 h of slaughtering (USDC, 1995). Chilling is critical for meat hygiene, safety, shelf life, appearance and nutritional quality (Cassens, 1994; Zhou *et al*., 2010).

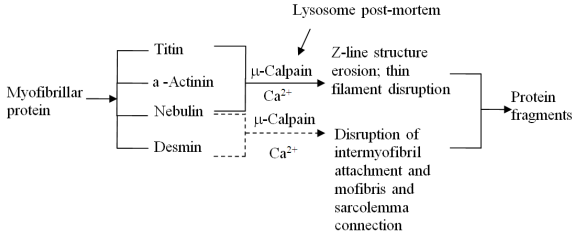


Fig. 3: Mechanism of calpain catalyzed meat proteolysis (Tarte and Amundson, 2006)

Table 6: Predominant spoilage microorganism found in frozen meat (Querol and Fleet, 2006; Gracey *et al*., 1999; Erickson and Huang, 1997)

Microorganisms	Genera
Bacteria	*Alcaligenes*
	Alternomonas
	Antrax bacilli
	Arthobactor
	Brochothrix
	Citrobacter
	Corynebacterium
	Cysticercus cellulosae
	Erwinia
	Escherichia
	Flavobacterium
	Klebsiella
	Kurthia
	Proteous
	Pseudomonas
	Salmonella
	Turbercle bacilli
Yeast	*Chrysosporium pannorum*
	Cladosporium cladosporoides
	Cladosporium herbarum
	Cryptococcus spp.
	D. hansenii
	Penicillium hirsutum
	Rhdotorula spp.
	Thamnidium elegans

It is employed by two methods: (a) immersion chilling, in which the product is immersed in chilled (0-4°C) water and (b) air chilling, in which the carcasses are misted with water in a room with circulating chilled air (Carroll and Alvarado, 2008). Carcass surface temperature is reduced at faster rate by air chilling which improves carcass drying and minimizes microbial spoilage (Ockerman and Basu, 2004). The microbial quality of the air-chilled product is better than that of a water-chilled product (Barbut, 2002; Sanchez *et al*., 2002).

Young and Smith (2004) reported that air-chilled carcasses lost 0.68% of their postslaughter weight in storage prior to cutting but lost no more during cutting or postcutting storage. On other hand water chilled carcasses absorbed 11.7% moisture in the chillers, of which 4.72% was lost within 24 h of intact carcass storage, 0.98% was lost during cutting and 2.10% was lost during storage resulting in 3.9% net water retention. Tuncer and Sireli (2008) studied microbial growth on broiler carcasses stored at 0, 4 and 7°C for 14 days after air- and water-chilling. Samples were taken on days 0, 4, 8, 10 and 14 of storage and analyzed for total bacterial count and *Pseudomonas* spp., Enterobacteriaceae, yeasts and molds. The results indicated that the air-chilling procedure was safer than the water-chilling procedure with respect to microbiological count. With regard to shelf-life, storage at 0°C was better than storage at 4 and 7°C in preventing spoilage. Zhou *et al*. (2010) stated that rapid chilling also helps to prevent denaturing of proteins which may lead to bacterial attack as they are more susceptible to denaturated protein than native protein. On the other hand, cold-shortening and toughening may result from ultra-rapid chilling of pre-rigour meat (Ockerman and Basu, 2004). Saide-Albornoz *et al*. (1995) found several foodborne pathogens in pork during processing at 3 slaughtering plants. They reported that *Salmonella* spp., *Yersinia enterocolitica* decreased, Staphylococcus and S. aureus increased while Listeria monocytogenes remained same during 24 h of chilled storage. Epling *et al*. (1993) examined pork carcasses immediately after slaughtering and then after 20 h of chilling at 4°C and found that *Campylobacter coli* caused contamination and 29% of Salmonella was not affected by chilling.

Freezing: Freezing is an excellent method of keeping the original characteristics of fresh meat. Meat contains about 50-75% by weight water, depending on the species, and the process of freezing converts most of water into ice (Heinz and Hautzinger, 2007). Meat freezing phenomenon is fast and almost 75% of tissue fluid freezes at -5°C. The freezing rate is increased with decreases in temperature, almost 98% of water freezes at -20°C and complete crystal formation occurs at -65°C (Rosmini *et al*., 2004). However, more than 10% of muscle bound water (chemically bound to specific sites such as carbonyl and amino group of proteins and hydrogen bonding) will not freeze (Rosmini *et al*., 2004; Garthwaite, 1997).

Freezing rate (slow and fast) affects the quality of frozen meat significantly. Fast freezing produce better quality meat than slow freezing. During slow freezing formation of large ice crystals damages the cell and results in protein denaturation. Concentration of enzymes and presence of other compounds govern the process of protein denaturation (Rahman, 1999b; Rahelic *et al*., 1985).

The preservation capacity of frozen meat is limited because the physical, chemical or biochemical reactions that take place in animal tissues after slaughtering do not stop absolutely after cold treatment (Rosmini *et al*., 2004). Microbial growth stops at -12°C and total inhibition of the cellular metabolism in animal tissues occurs below -18°C (Perez-Chabela and Mateo-Oyague, 2004). Complete quality changes of meat can be prevented at a temperature of-55°C (Hansen *et al*., 2004). However, enzymatic reactions, oxidative rancidity and ice crystallisation will still play an important part in spoilage (Zhao *et al*., 2010). During freezing, about 60% of the viable microbial population dies but the remaining population gradually increases during frozen storage (Rahman, 1999b).

Table 7: Food-poisoning pathogens associated with chilled and frozen raw meats, poultry and their products (Fernandes, 2009)

Organism	Gram reaction cell morphology	Oxygen requirement	Temperature requirement	Food poisoing
Aeromonas hydrophilla	G-ve rod	Facultative	Psychrotrophic	Infection
Bacillus cereus	G+ve sporing rod	Facultative	Mesophilic	Intoxication
Campylobacter spp.	G-ve spiral rod	Microaerophilic	Mesophilic	Infection
Clostridium botulinum	G+ve sporing rod	Anaerobe	Mesophilic	Intoxication
Clostridium perfringens	G+ve sporing rod	Anaerobe	Mesophilic	Intoxication
Escherichia coli	G-ve rod	Facultative	Mesophilic	Infection
Listeria monocytogenes	G+ve rod	Facultative	Psychrotrophic	Infection
Salmonella spp.	G-ve rod	Facultative	Mesophilic	Infection
Staphylococcus aureus	G+ve cocci	Facultative	Mesophilic	Intoxication
Yersinia enterocolitica	G-ve rod	Facultative	Psychrotrophic	Infection

Table 8: Storage life of meat at different temperatures (Berkel *et al*., 2004; FSA, 2002)

Product	Temperature (°C)	Storage life
Cooling		
Beef	-1	3-5 weeks
Pork	-1	1-2 weeks
Freezing		
Beef	-18	12 months
	-30	24 months
Ground beef (wrapped)	-18	6 months
	-24	8 months
Beef steaks (vac. packed)		18 months
		24 months
Lamb and mutton	-18	16 months
	-24	18 months
Pork	-18	6 months
	-30	15 months
Liver	-18	12 months
	-24	18 months

Predominant spoilage microorganisms of frozen meat are listed in Table 6. Food-poisoning pathogens associated with chilled and frozen raw meats, poultry and their products are listed in Table 7. Perez-Chabela and Mateo-Oyague (2004) reported that pathogenic microorganisms are commonly isolated from thawed frozen meat. Lowry and Gill (1984) reported that moulds grew at temperatures lower than -10°C. Perez-Chabela and Mateo-Oyague (2004) reported that larvae of *Taenia* spp. and *Trichinella spiralis* were killed after 1-3 weeks at-18°C or after ultra rapid freezing at-29°C.

Delmore (2009) stated that the shelf-life of vacuum-packaged fresh beef primals and subprimals is approximately 35-45 days; longer shelf-life of 70-80 days is possible with refrigeration of 0-2.3°C. Vacuum-packaged, frozen, whole-muscle beef has a recommended shelf-life of 12 months. Low (-18°C) and constant storage temperature substantially increase the shelf life of meat (Perez-Chabela and Mateo-Oyague, 2004). Shelf life of red meat stored at 15°C-30°C normally rages form 6 months to 24 months (Table 8). The shelf life of frozen chicken is also affected by storage temperature (Belitz *et al*., 2009).

Super chilling: Super chilling is a different concept than refrigeration and freezing and it has the potential to reduce storage and transport costs (Reynolds, 2007). Super-chilling refers to the temperature zone below its initial freezing point (1-2°C) but where ice crystals are not generated. In this process, instead of adding external ice to the food product, part of the internal water is frozen and works as a refrigeration reservoir, ensuring its refrigeration during distribution and transportation (Bahuaud *et al*., 2008) Respiratory metabolism and aging process are repressed but cell activity is maintained during the storage period of superchilling (Ando *et al*., 2005).

This method is mainly used for preservation of fish (Bahuaud *et al*., 2008; Ando *et al*., 2005; Hansen *et al*., 2004; Chang *et al*., 1998) and poultry (Frperc, 2004). The main advantage of this method of preservation over traditional methods is that it increases the shelf life of meat for upto 4 times (Magnussen *et al*., 2008). Although most microbial activities are stopped or inhibited, chemical and physical changes may progress and in some cases are even accelerate (Magnussen *et al*., 2008). James *et al*., (2006) reported that to eliminate the surface freezing of the chicken carcass during chilling, they were water chilled after eviscerated then kept at -15°C in an air freezer for approximately 30 min and stored and distributed at 1-2 °C.

Vacinek and Toledo (1973) reported no quality problems with poultry meat when they were super chilled and then maintained at approximately 4°C. Jul (1986) reported that storage of chicken meat at 1-2°C (near to freezing point) maintained its quality and inhibited the microbial growth.

The freezing point of poultry meat is not very well documented in literature but it is generally accepted between -1.5 and -2°C (James *et al*., 2007). In USA, this technique is extensively in use and it increases the shelf-life of poultry meat five weeks (Mead, 2004).

Controlled water activity methods: Microbiological safety of food is directly influenced by the water activity (a_w). The term water activity (a_w) refers to water which is not bound to food molecules and can

support the growth of microorganisms. It represents the ratio of the water vapour pressure of the food to the water vapour pressure of pure water under the same conditions (Ghaly *et al*., 2010). Water activity in meat products is equivalent to the relative humidity of air in equilibrium with the product (Comaposada *et al*., 2000). Table 9 shows the water activity of different food categories in relation to preservation and shelf life. Most fresh meats, fruits and vegetables fall into moist food category, have a water activity more than 0.85 and require refrigeration or another barrier to control the growth of pathogens (Smith and Stratton, 2006).

Each microorganism has minimum, optimum and maximum water activities. Micro-organisms generally grow best between a_W values of 0.980-0.995 and growth ceases at a_W<0.900. Yeasts and molds can grow at a low a_W of 0.6. However, growth of pathogens is prevented at a_W of 0.85 (Ghaly *et al*., 2010). In processed and cured meats, the growth of gram-negative bacteria (that can tolerate an a_W of 0.94-0.97) can be suppressed with reducing water activity (Dillon, 1996). The minimum water activity for growth of the most common microorganisms associated with dried meat products are presented in Table 10. Water activity in meat is control by drying, refrigeration, adding chemicals or a combination of these methods. Sodium chloride and sugar have been used to control water activity as free water binds up in their presence which results in an osmotic imbalance and finally inhibition of cell growth (Ray, 2004).

Table 9: Water activity and foods (Smith and Stratton, 2006)

Water activity	Classification	Requirements for control
Above 0.85	Moist foods	Requires refrigeration or another barrier to control the growth of pathogens
0.60 - 0.85	Intermediate moisture foods	Does not require refrigeration to control pathogens. Limited shelf-life because of spoilage, primarily by yeast and mold
Below 0.60	Low moisture foods	Extended shelf-life, even without refrigeration

Table 10: The minimum water activity for growth of most common microorganisms associated with dried meat products (USDA, 2005)

Microorganisms	Water activity
Campylobacter	0.98
Pseudomonas	0.97
Clostridium botulinum (non-proteolytic)	0.96
Clostridium botulinum (proteolytic)	0.93
Salmonellae	0.94
Clostridium perfringens	0.93
Escherichia coli O157:H7	0.95
Listeria monocytogenes	0.92
Staphylococcus aureus (anaerobic)	0.90
Staphylococcus aureus	0.86
Aspergillus flavus	0.80

***Sodium chloride*:** NaCl in growth media or foods can be a source of osmotic stress by decreasing water activity (Doyle, 1999). Borch *et al*. (1996) stated that salt-sensitive microorganisms, such as Pseudomonas spp. and Eriterobacferiuceae, did not grew in meat when the water activity (a_W) was reduced from 0.99 to 0.97 with the addition of 4% sodium chloride. However, salt tolerant microorganisms such as lactic acid bacteria and yeasts could grow at that level of water activity. Chawla *et al*. (2006) reported a reduction in water activity of fresh lamb intestine from 0.95 to 0.80 with the addition of 10% (w/w) of sodium chloride. Bennani *et al*. (2000) reported that Enterobactereaceae species were eliminated in kaddid (dry-salted meat product) as a result of reduced water activity (a_w) below 0.9 after 3 days due to the subsequent actions of salting, spicing and drying. Domowe (2010) reported that adding 3% salt reduced initial water activity level to 0.97 in sausages which was further reduced to 0.95 through the 6 day drying process and as a result pathogenic bacteria (*Salmonella*, *Bacillus*) stopped multiplying. Wijnker *et al*. (2006) studied antimicrobial properties of salt (NaCl) for the preservation of natural sheep casings at different water activity (a_w) levels and found the activities of most spoilage and pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli O157:H7*) stopped when an a_w of 0.89 was reached.

However, sodium chloride prooxidant activity is a major hindrance for the use of sodium chloride which accelerates the development of lipid oxidation and thus the deterioration of value added products (Decker and Xu, 1998). In Canada, sodium salt is GMP (Good Manufacturing Practice)-listed with meats according to the Canadian Food and Drug Act, (DJC, 2009). In the United States, curing salts are GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009).

***Sugars*:** Sugars have the capabilities to bind with moisture and reduce water activity in foods. Dextrose, sucrose, brown sugar, corn syrup, lactose, honey, molasses, maltodextrins and starches are generally used in dried meat processing as a source of sugars or carbohydrates to enhance flavor, reduce harshness of salt and lower water activity (USDA, 2005). Chirife (1994) reported that sucrose restrained the growth of *Staphylococcus aureus* by lowering water activity. Gibbs and Gekas (2010) reported that the growth of xerophilic organisms ceased at an a_W of 0.96, adjusted with sucrose. Riemann (1968) observed that the addition of 5% sucrose to meal with a water activity of

0.9 increased the kill of Salmonella at 75°C by a factor of more than 10^4. Farber *et al*. (1992) investigated the ability of *Listeria monocytogenes* strain meat-2 to initiate growth at different temperatures in Brain Heart Infusion (BHI) broth adjusted to various water activity (a_w) with sucrose and found a_W minima of 0.93.

According to the Canadian Food and Drug Act, sugars are Good Manufacturing Practice-listed with meats (DJC, 2009). In the United States, sugars are GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009).

Chemical methods for controlling microbial spoilage: Energy intensive freezing operations are the greatest way to preserve carcass, meat and meat products for a longer time which inhibits bacterial growth, but not the psychrophiles and the spores. Most of these survive freezing and grow during thawing (Neumeyer *et al*., 1997). Traditional methods for preservation of meat by salting and picking are well accepted procedures. Other chemicals have been used as food additives for preservation of meat but every country has drawn its rules and regulations and established limits for the purpose of prevention of harmful effects to human (Cassens, 1994). In the United States, the additive must be GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009). In Canada, it must fall under GMP (Good Manufacturing Practice) in accordance with the Canadian Food and Drug Act (HC, 2006).

Antimicrobial preservatives are substances which are used to extend the shelf life of meat by reducing microbial proliferation during slaughtering, transportation, processing and storage (Rahman, 1999a). Growth of bacteria and spoilage of meat is depending on the species of bacteria, nutrients availability, pH, temperature, moisture and gaseous atmosphere (Cerveny *et al*., 2009). Antimicrobial compounds added during processing should not be used as a substitute for poor processing conditions or to cover up an already spoiled product (Ray, 2004). They offer a good protection for meat in combination with refrigeration (Cassen, 1994). Common antimicrobial compounds include: chlorides, nitrites, sulfides and organic acids (Chipley, 2005; Ray, 2004; Archer, 2002).

***Sodium chloride*:** Sodium chloride has a long history of use in food preservation in sufficiently high concentrations. It inhibits microbial growth by increasing osmotic pressure as well as decreasing the water activity in the micro-environment. Some bacteria can be inhibited by concentrations as low as 2% (Urbain, 1971). A concentration of 20% of sodium chloride is high enough to inhibit many food spoilage yeasts including Debaryomyces hansenii, Yarrowia lipolytica, Kloeckera apiculata, Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Kluyveromyces marxianus, Pichia membranaefaciens, Pichia anomala and Saccharomyces cerevisiae (Praphailong and Fleet, 1997). However, some microorganisms have shown ability to tolerant high concentrations of salt such as those from the genera Micrococci and Bacillus (Urbain, 1971).

The combination of sodium chloride with other antimicrobial agents may have an impact on the over all inhibitory effect. Casey and Condon (2002) found that NaCl reduced the inhibitory effect of acid pH on the growth of Escherichia coli O157: H45. Tan and Shelef (2002) reported that a combination of NaCl and sodium lactate was more effective than lactates alone in delaying the onset of meat spoilage and its effects on its color and fat stability. Sallam and Samejima (2004) reported the use of sodium chloride in combination with sodium lactate reduced the microbial growth, maintained the chemical quality and extended the shelf life of ground beef during refrigerated storage. Kenawi *et al*. (2009) reported that the use of sodium lactate with or without sodium chloride delayed the proliferation of aerobic bacterial plate count, psychrotrophic bacterial count and lactic acid bacterial count, and extended the shelf life for up to 24 days, compared to 8 days for the control samples.

***Nitrites*:** The nitrites used in meat preservation industry are always in the form of salts such as sodium nitrite or potassium nitrite. Nitrites provide stabilized red meat color, cured meat flavor and rancidity retardation (Jay, 2005). They are long known as antimicrobial compounds preventing the growth of the toxin producing *Clostridium botulinum*, *Staphylococcus aureus* and *Yersinia enterocolitica* which would grow under anaerobic environment in vacuum packages (Cassen, 1994; Ray, 2004; Roberts, 1975; de Giusti and de Vito 1992; Archer, 2002; Lövenklev *et al*., 2004; Sindelar and Houser, 2009). Nitrite salts are effective in controlling color, lipid oxidation and odour in addition to controlling the anaerobic bacteria (Sindelar and Houser, 2009; Lovenklev *et al*., 2004; Archer, 2002; de Giusti and de Vito 1992; Roberts, 1975).

Nitrites affect the growth of microorganisms in food through several reactions including: (a) reacting with alpha-amino groups of the amino acids at low pH levels, (b) blocking sulfhydryl groups which interferes with sulfur nutrition of the organism, (c) reacting with iron-containing compounds which restricts the use of iron by bacteria, and (d) interfering with membrane

permeability which limits the transport across cells (Urbain, 1971; Cassen, 1994; Ray, 2004).

Woods *et al*. (1989) reported that sodium nitrite at 200 mg kg^{-1} and a pH of 6.0 retarded the growth of *Achromobacter*, *Aerobacter*, Escherichia, *Flavobacterium*, Micrococcus and Pseudomoman species in meat. Ducan and Foster (1968) found that germination of *Clostridium sporogenes* spores in meat was impended with sodium nitrite. Christiansen *et al*. (1973) reported that the toxin production by *Clostridium botulinum* was prevented at 200 μg nitrite/kg of meat. Sofos *et al*. (1979) reported that ciostridium botulinum toxin formation in chicken frankfurter-type emulsions was delayed fivefold when 156 μg nitrite/g of meat and 0.2% sorbic acid were combined.

The current limit for nitrite in food is 156 ppm in US, and 200 ppm in Canada for meat products (Ryser and Marth, 1999; DJC, 2009). On the other hand, the use of nirite as food additive may form carcinogenic nitrosamines with prolonged exposure. However there is no epidemiological evidence to support the relationship between nitrate consumption and a specific cancer or cancer risk (Ghaly *et al*., 2010).

***Sulphites*:** As antimicrobial agent, sodium sulfite is efficient against aerobic Gram-negative bacilli, molds and yeasts in meat and meat products (Ray, 2004). Dyett and Shelley (1966) stated that sulphites showed significant inhibitory effects on Gram-negative microbes including coli-aerogenes in meat sausages. Banks and Board (1982) reported that sulphites were used as antimicrobial agents in specified comminuted products such as fresh sausage because of their efficacy in controlling Enterobacteriaceae including pathogenic Salmonellae.

The antimicrobial activity is the result of the undissociated sulfurous acid which enters the cell and reacts with thiol groups of proteins, enzymes and cofactors. Yeast cells are attacked by sulfite because sulfite reacts with cellular Adenosine Triphosphate (ATP) and blocks the cystine disulfide linkages (Davidson *et al*., 2005). Table 11 shows the range of effective antimicrobial concentrations of sulfurous acid against various genera of yeast.

Currently, sulfites are not permitted in Canada as meat additives (DJC, 2009). Sulphur dioxide and the salts potassium bisulphite, potassium metabisulphite, sodium bisulphite, sodium metabisulphite and sodium sulphite collectively known as sulfites are removed from GRAS listing and they are not allowed for use as preservative in meat in the U.S. because the degradation of vitamin thiamine by sulphites (Walker, 1985; Cassen, 1994).

Table 11: Range of effective antimicrobial concentrations of sulfurous acid against various genera of yeast (Davidson *et al*., 2005)

Genus	Effective H_2SO_3 (mg/L)
Saccharomyces	0.10-20.20
Zygosaccharomyces	7.2-8.7
Pichia	0.20
Torulopsis	0.20
Hansenula	0.60
Candida	0.40-0.6

Table 12: Minimum inhibitory concentration of sodium lactate (Houtsma *et al*., 1993)

Bacteria	Minimum inhibitory concentration (mM)
Salmonella spp.	714-982
Lysteria monocytogenes and L. *innocua*	804-982
Pseudomonas spp. and *Yersinia* spp.	714-982
Campylobacter spp.	179
S. *aureus* and *Lactobacillus coryniformis*	268
Brochothrix	804

***Lactic acid*:** Lactic acid has shown antimicrobial activities against many pathogenic organisms such as Clostridium botulinum because of its abilities to reduce pH level, exert feedback inhibition and interfere with proton transfer across cell membranes (Doores, 2005; Davison *et al*., 2005; Cassen, 1994). The salt of lactic acid (lactate) is used in the meat industry as an antimicrobial agent (Davison *et al*., 2005).

Houtsma *et al*. (1993) reported on the minimum inhibitory concentration of sodium lactate under optimal growth conditions (pH 6.5, 20°C) for bacteria isolated from meat products (Table 12). Tan and Shelef (2002) reported that a combination of sodium chloride (1%, w/w) and sodium lactate (2%, w/w) enhanced microbial stability in refrigerated fresh ground pork.Mbandi and Shelef (2002) found the growth of strains of Listeria was delayed and the Salmonella numbers were declined during aerobic storage at 5-10°C for up to 60 days when sodium lactate (2.5%) and sodium diacetate (0.2%) were added to ready-to-eat meat. Greer and Dilts (1995) reported that pathogens (Listeria monocytogenes, Yersinia enterocolitica and Aeromonas hydrophila) were inhibited and did not grow on lean pork tissue during 15 days of storage at 4°C when immersed in 3% lactic acid solution.

The use of lactic acid bacteria as inoculums is a newly developed approach for food preservation (Davison *et al*., 2005; Hugas, 1998). Lactic acid bacteria are effective in inhabiting undesirable microorganisms in food by producing a wide range of substances (such as lactic acid, acetic acids, acetoin, diacetyl, hydrogen peroxide, reuterin and bacteriocins) which inhabit the growth of other microorganisms (Matamoros *et al*. 2009; Davison *et al*., 2005). Hugas

(1998) stated that competitions between lactic acid bacteria and pathogenic microorganisms for nutrients, oxygen, attachment/adhesion sites also exert antimicrobial effect. Natural strains of lactic acid bacteria in meats and meat products include *Carnobacterium piscicola*, *Carnobacterium divergens*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides subsp. mesenteroides*, *Leuconostoc gelidum,* and *Leuconostoc carnosum* (Hugas, 1998). Harding and Shaw (1990) found that Leuconostoc gelidum had rapid bactericidal effect on three Listeria monocytogenes strains. Park *et al*. (2005) found that the growth of S. enteritidis was significantly retarded by lactic acid producing bacterial culture.

Lactates have been permitted as a natural preservative up to 3 and 100 g meat by the USDA-Food Safety and Inspection Service (Kenawi, 2009). According to the Canadian Food and Drug Act, lactates are Good Manufacturing Practice-listed with unstandardized meats (DJC, 2009).

***Ascorbic acid*:** Ascorbic acid (vitamin C), sodium ascorbate and D-isoascorbate (erythorbate) have been used as meat preservatives. Their antioxidant properties can oxidize reactive oxygen species producing water. Ascorbic acid has been shown to enhance antimicrobial activity of sulfites and nitrites (Mirvish *et al*., 1972; Baird-Parker and Baillie, 1974; Raevuori, 1975). The enhanced activities include both the antioxidant properties and the sequestering of iron (Tompkin *et al*., 2007).

Jay (2005) reported that ascorbate and erythorbate reduced nitrosamine formation at a level of 550 ppm when they were used in combination with nitrite. Raevuori (1975) reported that the addition of 500 mg sodium erythorbate /kg of meat and 200 mg sodium nitrite /kg of meat had prevented the growth of *Bacillus cereus* spores in sausages kept at 20°C for 48 h. Giroux *et al*. (2001) evaluate the effect of ascorbic acid concentrations (0.03 - 0.5%) and irradiation doses (0.5 to 4 kGy) on microbial growth, color coordinates and sensory characteristics (taste and odor) of beef patties during storage at 4 ± 1°C and found significant reductions of Aerobic Plate Counts (APCs) and total coliforms and significant stability of color due to incorporation of ascorbic acid into the meat with irradiation. Schaefer *et al*. (1995) found greater stability of oxymyoglobin in skeletal muscle and less discoloration of meat with intravenous infusions of ascorbic acid.

According to the Canadian Food and Drug Act, ascorbic acid and erythorbic acid are Good Manufacturing Practice-listed with meats (DJC, 2009). In the United States, ascorbic acid and erythorbic acid are GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009).

***Benzoic acid*:** Benzoic acid and sodium benzoate are used as preservatives in the meat industry. The undissociated molecule of benzoic acid is responsible for its antibacterial activity (Krebs *et al*. 1983; Warth, 1991; Brul and Coote, 1999; Hazan *et al*. 2004; Feiner, 2006). The benzoic acid is generally used to inhibit yeasts and fungi rather than bacteria (Chipley, 2005; Feiner, 2006). The benzoic acid stays in their unassociated states under low pH (2.5-5.0) conditions which is the form that readily crosses the cell membrane. Once entered the cytosol, the acids dissociates because of neutral pH environment. The dissociated molecules (anion and cations) cannot diffuse back across the membrane and accumulated in the cytosol. The acidification of the cytosol and the depletion of ATP will cause physiological malfunction and finally inhibition of microbial growth (Krebs *et al*. 1983; Warth, 1991; Brul and Coote, 1999; Hazan *et al*. 2004; Feiner, 2006).

Seman *et al*. (2008) tested the effect of sodium benzoate (0.08-0.25%) on the growth of Listeria monocytogenes in ready-to-eat meat products over 18 weeks storage at 4°C and found that under low moisture content, concentration of 0.1% sodium benzoate was effective in inhibiting *Listeria monocytogenes*. Dąbrowski *et al*. (2002) found that sodium benzoate only reduced diversity of bacteria and yeasts in a tested product and exerted no influence on the total number of bacteria and yeasts. They suggested that an empty ecological niche was created after elimination of some species by the preservative and remainders substituted them.

Some of the food spoilage yeasts have been reported to be resistant to benzoic acid and its salts. Hazan *et al*. (2004) reported that yeasts (such as *Saccharomyces* and *Zygosaccharomyces*) have intrinsic ability to resist benzoic acid under the tolerable toxicological limits. The combination of benzoic acid treatment and nitrogen starvation conditions was suggested by the author to enforce effective food preservation from yeast spoilage. Praphailong and Fleet (1997) found *Zygosaccharomyces bailii* and *Yarrowia lipolytica* were the most resistant yeast to benzoic acid at pH 5.0.

In Canada, benzoic acid is not permitted for unstandarized meat and the limit for benzoic acid is 1000 ppm for marinated or similarly cold-processed packaged meat (DJC, 2009). In the United States, the maximum permitted level for benzoic acid and sodium benzoate is 0.1% as GRAS preservatives (Davison *et al*., 2005).

***Sorbic acid*:** Sorbic acid (2, 4-hexadienoic) and its salts are widely used throughout the world as meat

preservatives for inhibiting bacteria and fungi (Urbain, 1971; Davison *et al*., 2005; Feiner, 2006). A concentration of 0.3% sorbates in food is high enough to inhibit the microorganisms. The sorbic acid has an inhibitory mechanism via depression of internal pH. Davison *et al*. (2005) stated that sorbates may interfere with the bacterial spore germination, inhibit the activity of several enzyme systems and interfere with substrate and electron transport mechanisms. Stratford and Anslow (2002) found some evidence that sorbic acid may act more like membrane-active substance rather than as a weak-acid preservative.

Tompkin *et al*. (1974) found that sorbate (0.1% wt/wt) has inhibitory effect on the growth of Salmonella aureus and *Clostridium botulinum* on cooked uncured sausage at 27°C. González-Fandos and Dominguez (2007) found that 5% potassium sorbate had a significant inhibitory effect on the growth of Listeria monocytogenes on poultry legs stored at 4°C for 7 days and on L. monocytogenes compared to the control (a decrease of about 1.3 log units after 7 days of storage). Osthold *et al*. (1981) found that the shelf-life of beef carcasses was increased upto 4 days at 15°C after sprayed with a solution containing potassium sorbate, sodium acetate and sodium chloride. Ahmed *et al*. (2003) sprayed meats from freshly slaughtered sheep and goat carcasses with a solution containing potassium sorbate (2.5%), sodium acetate (2.5%), sodium citrate (2.5%) (prepared w/v in potable water) and found the treatment inhibited Bacillus spp. to minimum and extended the lag phase of all organisms including psychrotrophes (pseudomonas) throughout the refrigerated storage at 5-7°C.

A solution of 10% potassium sorbate can be used in uncooked and dried sausages in U.S because they are effective inhibitor of mold, yeast and some highly aerobic bacterial in meat (Urbain, 1971; Cassen, 1994; Feiner, 2006). According to the Canadian Food and Drug Act, the allowable limit of potassium sorbate is 1000 ppm (DJC, 2009).

***Lactoferrin*:** Lactoferrin (LF) is a well-known natural antimicrobial protein that belongs to tranferrin family which can be isolated from various exocrine secretions and other humans and animals tissues (Levay and Viljoen, 1995; Naidu, 2000; Naidu, 2002; Farnaud and Evans, 2003). LF is a broad-spectrum antimicrobial which is active against bacteria, fungi, virus and protozoa (Davison *et al*., 2005; Elbarbary *et al*., 2010). LF shows a high affinity to iron and retains its bound to iron under acidic conditions (Farnaud and Evans, 2003; Davison *et al*., 2005).

Interaction between the protein and the bacterium was reported by Farnaud and Evans (2003). The antimicrobial activity of lactoferrin was demonstrated by Arnold *et al*. (1977) for *Streptococcus mutans* and *Vibrio cholerae*, but not for *Escherichia coli*. The anti-fungal activity of lactoferrin against many genera of fungi (such as Candida) and its antiviral activity were reported by Farnaud and Evans (2003). Naidu (2002) documented the lactoferrin activity against an array of bacterial pathogens including E. coli O157:H7, Listeria monocytogenes, Salmonella spp. and Campylobacter, as well as some meat spoilage organisms including *Pseudomonas* spp. and *Klebsiella* spp.

Chiu and Kuo (2007) found that the addition of 80-mg kg^{-1} lactoferrin to hot-boned ground pork resulted in lower Thiobarbituric Acid Reactive Substances (TBARS) values, total plate count, lactic acid bacterial counts than the controls. Al-Nabulsi and Holley (2007) reported that significant reduction of E. coli O157:H7 during sausage manufacture was achieved by the addition of lactoferrin.

U.S.A. permitted the use of lactoferrin at a level of 65.2 mg kg^{-1} in beef (Naidu, 2002). In Canada, there is no regulation for lactoferrin.

Chemical methods for controlling oxidative spoilage: Freeze storage cannot prevent oxidative spoilage and microbial/enzymatic spoilage (Jay *et al*., 2005). Thus, chemical preservation methods are quite beneficial in combination with refrigeration in order to optimize stability, product quality while maintain freshness and nutritional value (Cassens, 1994). Thorough understanding of lipid oxidation and its inhibition is necessary to prevent the development of rancidity, off flavour and discoloration in meat. Antioxidants and chelating agents can inhibit lipid oxidation by removal of the free radical catalysts (molecular oxygen and transition metals), Lipid oxidation is often determined by using a Thiobarbituric Acid Reactive Substances (TBARS) index.

Antioxidants can be classified as primary or long-term antioxidants and secondary or processing antioxidants. Primary antioxidants include phenolic compounds and secondary aryl amines while the secondary antioxidants include phosphates and thioesters. The primary antioxidants act as a radical scavengers or hydrogen donors or chain reaction breakers while the secondary act as peroxide decomposers (Andre *et al*., 2010). Among the widely used lipid oxidation inhibitory additives in meat are: phenolic antioxidants (primary antioxidants) and phosphates (secondary antioxidants).

***Phenolic antioxidants*:** Derivatives of phenol such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and Proply Gallates (PG) are referred to as synthetic phenolic antioxidants. Their use is extensive with the intention to delay, retard or prevent the negative effects of lipid peroxidation by scavenging chain-carrying peroxyl radicals or diminishing the formation of initiating lipid primary radicals (chain-breaking) and secondary radicals (preventive antioxidants) (Davidson, 1993; Simitzis and Deligeorgis, 2010). PG application in meat is combined with citric acid and commonly used in fresh pork sausages (Cassens, 1994). Besides the BHA, BHT, TBHQ and PG, tocopherols (vitamin E), which is also a phenolic substance is widely used in oxidation prevention of meat and meat products. Vitamin E is often treated as a natural additive. However, the tocopherol used in most studies is not derived from a natural source (Grun, 2009).

BHA, BHT and TBHQ have proven antimicrobial properties against bacteria (predominately gram-negative), fungi, viruses and protozoa (Branen *et al*., 1980). Cell membrane and enzymes are affected in presence of antimicrobial additives (Ray, 2004). As a stabilizer, blends of BHA and PG are most commonly used in edible and inedible lard and tallow. Hui (2006) reported that the antioxidative properties of TBHQ (at 0.01%) were double compare to BHA or BHT (at 0.02%). The efficacy of antioxidant may be in part due to the number of phenolic hydroxyl groups available for free radical scavenging (Rice-Evans, 1996).

Chastain *et al*. (1982) studied the effects of BHA, TBHQ and a combination of BHA and TBHQ on restructured combination of beef-pork steaks (50:50). Antioxidants were used at a 0.02% level (based on fat content of meat). Cooked steaks were evaluated for sensory properties and overall acceptability, initially and after 4, 8, 12, 16 and 20 week of freezer storage. BHA was more effective in protecting color while TBHQ was more effective in protecting flavor. All treated samples showed lower 2-thiobarbituric acid (TBA) values than control samples and samples treated with TBHQ and BHA + TBHQ had lowest TBA values in comparison to other treated samples.

Jayathilakan *et al*. (2007) studied the antioxidant potential of TBHQ, TBA and PG (0.02%) in sheep, beef and pork meat. After the addition of these antioxidative chemicals, samples were stored at 5°C and analyzed after 2, 4 and 6 days in terms of antioxidant activity. TBHQ showed higher (82-91%) antioxidant activity compared to TBA (30-60%) and PG (36-65%).

The Canadian Food and Drug Act limits the amount of phenolic antioxidants either individually or combined at 0.02% (DJC, 2009). According to the U.S Code of Federal Regulation Section 21, the amount of phenolic antioxidants either individually or combined is limited 0.02% (USFDA, 2009).

***Phosphates*:** Among the antioxidants in food additives, phosphates were one of the first investigated for their potential antioxidant activities in meat products (Trout and Dale 1990). A range of functionalities has been provided by phosphates to enhance meat, poultry and sea food products. Functionalities of phosphate salts vary with the type of phosphate salt or combination of them. Phosphates critical functions include: (a) optimizing the water binding capacity of the muscle proteins by influencing pH, (b) interacting with muscle fibers for improved emulsification of fats, (c) maintaining the stability of the protein-fat-water system, (d) chelating divalent cations and retaring rancidity which increase shelf life and (e) binding iron into the system and reducing oxidation (ICLPP, 2006).

Selected inorganic phosphates are approved for use in many whole muscle and sausage products at a level of 0.05% (Knipe, 2004). Watts *et al*. (1951) documented the efficacy of polyphosphates in artificial aqueous fat systems within the pH range of normal meat. Trout and Dale (1990) reported on the effectiveness of sodium tripolyphosphate in reducing rancidity. Sato and Hegarty (1971) reported that the development of rancidity in ground cooked beef was reduced with addition of all phosphates (tripolyphosphate, hexametaphosphate, and pyrophosphate) as antioxidants during storage at 4°C for 2 days. Craig *et al*. (1996) reported that ground turkey and beef treated with phosphates (0.3%) had less off-flavor when sensory evaluated after 3 days of refrigerated storage compared with control. Murphy *et al*. (1998) showed a most potentiality of tripolyphosphate among BHA/BHT, tripolyphosphate, rosemary oleoresin and sodium citrate antioxidants during refrigerated storage of precooked roast beef slices. Rosell and Toldra (1996) reported that polyphosphates at 0.1% (w/v) in presence of calcium chloride completely inhibited m-calpain activity. Cassen (1994) reported that phosphates have the ability to retard the microbial growth because they bind heavy metal ions.

The U.S Code of Federal Regulation section 9 (CFR 424.21 c) limits the use of phosphates in meat and poultry to 5000 ppm based on the total product weight (USFDA, 2009). The Canadian Food and Drug Act limits the amount of total added phosphate in meat and poultry to 0.5% (DJC, 2009).

Chemical methods for controlling autolytic enzymatic spoilage: Autolysis is a term used to describe a series of postmortem chemical changes in the tissues of animals after death due to the presence of the enzymes (lipolytic, amylolytic and proteolytic) responsible for the metabolic process during the life of animals which are responsible for degradation of fats, carbohydrates and proteins after the death of animal. Lypolytic enzymes are responsible for the fat deterioration or lypolysis (oxidation) while amylolytic enzymes are responsible for the change of glycogen to lactic acid. These changes occur during early stage of storage. Protein deterioration is the results of proteolytic enzymes which change the proteins to amino acids and then to the amino nitrogen, or the non-protein nitrogen causing the soluble nitrogen products of the meat to increases (Lowe, 1937).

O'Halloran *et al*. (1997) and Huss (1995) stated that calpains and cathepsins are responsible for the post mortem autolysis of meat. Barnoy *et al*. (2000) and Koohmaraie and Geesink, (2006) reported on the important contribution of the calpain system to the proteolytic tenderization process of meat during storage. Pomponio *et al*. (2008) indicated that the calpain system consists of at least three proteases (μ-calpain, m-calpain and skeletal-muscle-specific calpain p94) in skeletal-muscle. Among the three proteases, μ – calpain is most responsible for post-mortem tenderization. Aminopeptidases are also important enzymes in the development of the characteristic flavour of meat products. They produce large generation of free amino acids during meat processing as are capable of hydrolysing amino acids from the N-terminus of peptides and proteins (Flores *et al*., 1997).

The enzymatic activities of calpain, cathepsins and aminopeptidases enzymes are affected by pH and temperature. The membrane of the lysosomes becomes leaky as the pH of the meat decreases post mortem and the enzymes are released (O'Halloran *et al*., 1997). Moderate rates of postmortem pH decline (from 6.9-6.2 after 3 h) allows for greater postmortem protein degradation and increased tenderization while rapid rates (from 6.9-5.8 in 3 h) and slow rates (from 6.9-6.6 in 3 h) produce less tender meat. Curing salts and acids have been used to inhibit the activity of such autolytic enzymes and prevent or slows degradation and spoilage (Maddock *et al*., 2005; O'Halloran *et al*., 1997).

***Salts*:** Curing salts have shown the ability to inactivate autolyzed calpain proteolytic activity. Tan *et al*. (1988) reported that the addition of either KCl or NH_4Cl up to 500 mM decreased the specific activity of unautolyzed m-calpain by 40%. Li *et al*. (2004) found that the specific activities of both autolyzed μ - calpain and autolyzed m-calpain decrease to 45-50% of their original activities after incubation for as little as 5 minutes in 500 mM KCl.

Geesink and Koohmaraie (2000) studied the inactivation of μ-calpain extracted from porcine spleen using 0, 100, 200, 300 mM NaCl and incubated for 5, 15, 30, 60, or 120 min at 25°C and found that as the ionic strength was increased proteolytic activity decreased and maximum reduction of 70% was obtained at 300 mM NaCl concentration.

Rosell and Toldra (1996) reported that NaCl at concentrations up to 0.5 M promoted a slight activation of m-calpain, while a further increase in NaC1 content inhibited the enzyme activity. Whipplea and Koohmaraie (1993) found that the activities of m-calpain and calpastatin decreased with calcium marination which improved tenderness of beef steak. Flores *et al* (1997) reported that NaCl reduced strongly inhibited alanyl aminopeptidase and pyroglutamyl aminopeptidase to about 40±50% of their original activities. Toldra and Flores (1998) reported that the cathepsins and aminopeptidases (except aminopeptidase B) were inhibited by salt, especially at high concentrations.

According to the Canadian Food and Drug Act, salts of sodium and potassium are Good Manufacturing Practice-listed with meats (DJC, 2009). In the United States, curing salts are GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009).

***Acids*:** The pH plays an important role on enzymatic activities and that depends on type of acid used. Rosell and Toldra (1996) reported that the addition of ascorbic acid inhibits enzymatic (m-calpain) activity by 40-45%. Flores *et al*. (1997) reported that arginyl aminopeptidase activity was inhibited only 10% at the 500 mg/l of ascorbic acid and pyroglutamil aminopeptidase was not affected by ascorbic acid but leucyl aminopeptidase was reduced to 30% of its initial activity. Toldra and Flores, (1998) reported that ascorbic acid exerts a slight inhibitory effect on cathepsin H. Kendall *et al*. (1993) reported on effect of pH and ionic strength on bovine m-calpain. Treatment pH values were 5.7, 6.2, and 7.0; which were adjusted with 1 N acetic acid. Ionic strengths were equivalent to 32, 100, 150, 200, 250, 300, 350 and 400 mM. They demonstrated that as the pH declined from 7.0-5.7 the m-calpain activity decreased markedly regardless of ionic strength. At pH 6.2, the activities decreased to 55 -66% of the pH 7.0 values, whereas at pH 5.7, activities decreased to 10 - 17% of that at the pH 7.0 activities.

Koohmaraie (1992) conducted experiments at various pH values (7.0, 6.2, and 5.8) adjusted with HCl and two temperature (25 and 5°C) to evaluate the autolysis and catalytic activity of bovine skeletal muscle μ-calpain and found that μ-calpain activity was significantly decreased by lowering temperature from 25-5°C and lowers the pH from 7.0-5.8.

According to the Canadian Food and Drug Act, organic acids are Good Manufacturing Practice-listed with meats (DJC, 2009). In the United States, organic acids are GRAS-listed (Generally Recognized as Safe) according to the American Food and Drug Administration (USFDA, 2009).

CONCLUSION

Meat is the first-choice of animal protein for human and consumption of meat is continuously increasing worldwide. The annual per capita consumption increased by 2.6 fold in 2000 and will increase by 3.7 fold by 2030 compared to that of 1960s. On the other hand, the rich nutrient matrix meat is subject to various types of spoilage depending on handling and storage conditions. Significant portions (3.5 billion kg) of meat and meat products are spoiled every year at the consumer, retailer and foodservice levels which have a substantial economic and environmental impact.

Meat spoilage leads to the development of off-flavours, off-odors and often slime formation due to the breakdown of valuable contents (fat, protein and carbohydrates) which make the product undesirable for human consumption. Because worldwide population growth and globalization of the food supply, the control of meat spoilage becomes essential in order to increase its shelf life and maintain its nutritional value, texture and flavor. Proper handling, pretreatment and preservation techniques can improve the quality of meat and meat products and increase their shelf life.

There are three main mechanisms for the spoilage of meat and meat products: (a) microbial spoilage, (b) lipid oxidation, (c) autolytic enzymatic spoilage. Microbial growth and metabolism depends upon the condition of the carcasses at the time of slaughter, the type of packaging and storage conditions. Microbial spoilage results in a sour taste, off-favours, discolouration, gas production, pH change, slime formation, structural components degradation, off odors and change in appearance. Fatty acids are affeted by the production of free radicals due to autoxidation of lipids and lead to oxidative deterioration of meat and off-flavours development. Lipid hydrolysis can take place enzymatically or non-enzymatically in meat. In muscle cells of slaughtered animals, enzymatic actions are taken place naturally and they act as catalysts for chemical reactions that finally end up in meat self deterioration. Softening and greenish discoloration of the meat results due to tissues degradation of the complex compounds (carbohydrates, fats and protein) in the autolysis process.

For controlling enzymatic, oxidative and microbial spoilage, low temperature storage and chemical techniques are the most common in the industry today. It is essential to store the meat at lower than 4°C immediately after slaughtering and during transport and storage as it is critical for meat hygiene, safety, shelf life, appearance and eating quality. Although, microbial and enzymatic spoilage can be stopped or minimized at lower temperature. However, oxidative spoilage cannot be prevented by freezing. A combination of chemical additives such as Tertiary Butyl Hydroxyl Quinine (TBHQ) and ascorbic acid can be most effective for controlling spoilage of meat and meat products. These chemical additives have both antimicrobial as well as antioxidation abilities. TBHQ (at 0.02% by weight of lipid content) has the ability to control lipid oxidation whereas ascorbic acid (at 0.2%) has ability to control microbial spoilage. The addition of preservative additives (TBHQ/ascorbic acid) and storage at refrigerated temperatures (5°C) in darkness is the most feasible combination to control spoilage and increase the self-life of meat and meat products. However, more efforts are required to understand the role of animal age, animal type, stress level before and during the slaughtering process, initial microbial load, type and nature of bacteria and their interactions in order to optimize the shelf-life of meat.

ACKNOWLEDGMENTS

This research was supported by the National Science and Engineering Research Council (NSERC) of Canada through a Strategic Research Grant.

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