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Chung-ping Ho

Louisiana State University and Agricultural & Mechanical College

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BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF GROUND
BEEF IN MODIFIED ATMOSPHERE PACKAGING WITH GAS EXCHANGE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Animal Science

by

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ABSTRACT

The shelf-life of ground beef in modified atmosphere packaging (MAP) with exchange of gases before retail display was compared with conventional vacuum packaging (VP) and overwrapping with polyvinylchloride film (PVC) for display. Ground beef patties in MAP with distribution gases of 20% N₂:80% CO₂, 50% N₂:50% CO₂, or 80% N₂:20% CO₂ and display gases of 80% O₂:20% CO₂, 50% O₂:20% CO₂:30% N₂, or 20% O₂:20% CO₂:60% N₂ had decreased weight loss, microorganism growth rate, metmyoglobin formation, and color degradation compared with patties in VP-PVC. Increased CO₂ during distribution retarded microbial growth. Display gas mixtures with higher O₂ increased redness (HunterLab "a" values), decreased metmyoglobin formation, and minimally increased lipid oxidation during six days of retail display. Ground beef patties in 50% N₂:50% CO₂ for distribution and 80% O₂:20% CO₂ for display provided optimal shelf-life and color in this study.

An abusive display temperature (15°C) decreased shelf-life of ground beef patties in VP-PVC and MAP (50% N₂:50% CO₂ exchanged for 80% O₂:20% CO₂) to 1 and 3 days compared with 3 and more than 4 days with 7°C display. Abusive display temperatures increased incidences of pathogenic microorganism species in MAP samples.

Lipid peroxidation in ground beef and isolated microsomal fractions increased with increased time after
packaging in both VP-PVC and MAP with 50% N₂:50% O₂ exchanged for 80% O₂:20% CO₂. Enzymic lipid peroxidation activity of microsomes extracted from the ground beef at each sampling time increased with extended display time after gas exchange, but lipid oxidation in beef patties remained constant during retail display. High O₂ display gas increased HunterLab "a" values and reduced metmyoglobin formation in gas exchanged MAP samples without increasing lipid oxidation. MAP with gas exchange increased shelf-life compared with conventional VP for distribution and air permeable retail packaging for ground beef, but temperature is an important control of pathogenic microorganism growth.
CHAPTER I

INTRODUCTION
Fresh meat is a perishable food commodity. Traditionally, it is distributed and held at refrigeration temperatures and has limited shelf-life expectancy due to discoloration, lipid oxidation, and/or microbial growth. The preference for bright red color in fresh meat is a major factor in determining the way fresh red meat is packaged for retail sale. Newer forms of packaging systems have increased the possibilities for improving presentation and extending shelf-life and have added to a number of options available to the retailer (Hood and Mead, 1993). The systems that provide sufficient shelf-life generally are types of modified atmosphere packaging (MAP). With MAP technology, meat packers can implement centralized packaging processes and produce case-ready meat products for improved inventory management (Farris et al., 1991) as well as reduced retail store-handling costs (Bishop, 1988).

Case-ready packaging is defined as the fabrication and packaging of meat in consumer-sized packages. These packages are then displayed with minimal package manipulation after removal from the shipping carton (McMillin, 1994). Case-ready MAP meats with various gases of carbon dioxide (CO\textsubscript{2}), nitrogen (N\textsubscript{2}), or vacuum (VP) reduce costs due to spoilage when compared with conventionally overwrapped cuts (Starr, 1992). The choice of gas mixtures is influenced by the sensitivity of the product to oxygen and carbon dioxide, color-stabilizing
requirements, and the microbiological flora of the product. Along with packaging, temperature control has been an effective way to extend shelf-life and maintain quality of meat (Lioutas, 1988). Lower temperatures are known to keep pigments in the red oxymyoglobin state for longer periods of time in addition to inhibiting microbial growth. Freezing of meat minimizes microbial growth, but may cause appearance problems such as darkening or package frost accumulation, development of rancidity, and purge loss.

Microbial spoilage has been ranked as the most serious food safety threat to consumers (FMI, 1991). The decomposition and putrefaction of meat products have been linked to microbial growth and production of degradation enzymes (Hedrick et al., 1994). Offensive odor development, slime formation, and discoloration of meat are also attributed to microbial metabolism (Ayres et al., 1950). The microbiology of meats is important for determination of the potential safety and shelf-life quality. While the enumeration of food-borne disease-causing bacteria is usually related to potential food-poisoning risk, the relationship between microbial counts and spoilage is less well defined. It is important to recognize the imprecise nature of the traditional quality indicators of shelf-life properties when considering the loss of saleability or palatability of meat products (Brown, 1982).
Development of oxidative off-flavors has long been recognized as a problem during storage of meat products. Wide fluctuations in temperature and inadequate protection from oxygen can accelerate the development of oxidative reactions in meats (Romans et al., 1994). Under proper storage without exposure to oxidizing conditions, such as light, oxygen, or high temperatures, lean raw meat is quite stable for periods of several months to a year, depending on the species from which it originated. Oxidation promotion in meat products may be attributed to the high fat content and exposure to high oxygen, prolonged storage, and/or processing operations, ingredient addition, mechanical manipulation, freeze-thawing, or temperature abuse in handling and distribution (Gray and Crackel, 1992; Rhee, 1988). The most extensively studied catalysts of lipid oxidation in red and poultry meats are the nonenzymic catalysts involving heme pigments, nonheme iron, and microsomal enzymic lipid peroxidation systems (Lin and Hultin, 1976; Player and Hultin, 1977; Harel and Kanner, 1985; Rhee et al., 1985, 1986).

The color of fresh meat is largely dictated by the concentration and chemical nature of hemoprotein and the temperature/pH history of the muscle post-slaughter. With fresh meat, oxygen is often included in the gas mixture to maintain a red bloomed color by keeping myoglobin in its oxygenated form (Bartkowski., 1982). However, studies have
shown that, for the longest shelf-life, oxygen should be excluded from the package headspace (Christopher et al., 1979), but the color of deoxymyoglobin pigment is purple.

MAP systems usually contain gaseous atmospheres whose concentrations differ from the ambient atmosphere (79% nitrogen, 20.9% oxygen, and 0.03% carbon dioxide). A dynamic gas exchange system (Mitchell, 1990) that combines the advantages of inert gas MAP for package distribution and a high concentration of oxygen for retail display has been successful in extending the shelf-life and improving the color appearance of fresh meats (McMillin et al., 1992). This system provides extended distribution time in the deoxymyoglobin state and extended display life in a bloomed color state by exchanging an inert distribution gas for high oxygen immediately before display of fresh meat (McMillin, 1994).

MAP foods have become increasingly common in North America, as food manufactures have attempted to meet consumer demands for fresh, refrigerated foods with extended shelf-life (Farber, 1991). More research will be needed to ensure the quality and safety of a wide variety of new MAP products. However, limited data are available on microbiological and biochemical properties of fresh meats in the gas exchange MAP systems. Therefore, the objectives of this research were to (1) investigate the optimal distribution and display gas mixtures for ground beef in MAP
with dynamic gas exchange; (2) determine the microbial populations and microflora changes of ground beef at normal and abuse temperatures with different MAP gas exchanges; (3) determine the lipid peroxidation potential and microsomal enzymic lipid peroxidation activity of ground beef with dynamic gas exchange MAP during refrigerated storage; and (4) investigate the relationships between lipid peroxidation and discoloration of ground beef in dynamic gas exchange MAP.
CHAPTER II
REVIEW OF LITERATURE
Properties of Fresh Meat

Hedrick et al. (1994) defined the term "fresh meat" as a meat product that has undergone the chemical and physical changes which follow slaughter, but has not been further processed by freezing, curing, smoking, or other means.

Water holding capacity

When exposed to the atmosphere, meat loses weight by evaporation and the surfaces become darker with drying out. Evaporation can be prevented by packaging, and moisture accumulates as visible free water exudate in the pack (Seman et al., 1989). Meat contains about 75% water in the lean tissue and the ability of the protein structure to retain this moisture within the tissue is of major importance to maintaining quality in packaged fresh meat. Free water exudate is undesirable, because even present in small amounts, it gives meat an unsightly appearance, causes economic loss by weight loss, and can lead to rejection by consumers (Offer and Knight, 1988). However, the occurrence of drip depends on intrinsic properties of the meat proteins, and it is difficult to overcome the problem by package design alone. Most of the water in meat is held within the myofibrils in the narrow channels between the basic muscle protein filaments. Losses of water from meat, such as occur in drip, is caused by myofibril shrinkage and then forces the fluid along the gaps between the fibers and
their surrounding connective tissue sheaths toward the cut end of the meat (Offer et al., 1984).

The greater the degree of myofibrillar shrinkage the greater the drip. Most of the shrinkage occurs during the conversion of muscle to meat as ATP became exhausted and cross-linkages were formed between the myofilaments (Hedrick et al., 1994). In addition, the pH decline from about 7.0 in living muscle to about 5.5 in rigor would cause a reduction in negative charges on the filaments and a reduction in the electrostatic repulsion between the proteins (Offer et al., 1984). The tendency to produce drip is therefore inherent in fresh meat and is difficult to eliminate during meat processing.

Postmortem differences in temperature and pH profiles cause appreciable differences in water-holding capacity and color in beef meat, which contribute to a variation in the level of drip. This source of variability, particularly in the larger bovine muscles, has received increasing research attention. One objective of hot deboning, which is processing of meat immediately after slaughter before chilling (Romans et al., 1994), often accompanied by electrical stimulation of the pre-rigor muscle, has been to reduce the degree of variability in muscle quality which may be achieved by increased temperature control during chilling (Renerre and Bonhomme, 1991). Lawrie (1991) pointed out
that the absence of marked exudation in electrically stimulated beef remains to be explained.

**Meat color**

The red color of meat is due to the presence of the heme protein myoglobin (Hedrick et al., 1994). Some residual blood may also be present in meat, but it is generally minimal and is of little practical concern in considerations of meat color. The degree of meat pigmentation is directly related to myoglobin content (Ahn and Maurer, 1989). In general, myoglobin concentration within a given muscle will be different in species, increasing with animal age, and dependent on muscle fiber distributions (Lawrie, 1991). Red muscle fibers generally tend to have a higher concentration of myoglobin than white muscle fibers because red fibers have predominantly aerobic metabolism and require more $O_2$, which is stored by myoglobin. While white fibers are predominately anaerobic (i.e., turkey thigh muscle versus breast muscles) (Seideman et al., 1984).

The heme group within myoglobin is a planar chemical structure that contains a centrally located iron atom. The iron atom has six coordination sites available for chemical bonds (Livingston and Brown, 1981). Four of these bonds anchor the iron atom within the heme structure. A fifth bond connects the iron atom to the amino acid chain of the globin protein. The sixth coordination site is available
for binding a variety of chemical groups. The chemical
group bound at the sixth site and the oxidation state of
heme iron are two major determinants of meat color
(Giddings, 1977). The oxidation state determines which
molecule may be bound at the sixth site of heme iron. Iron
is a transition metal capable of existing in an oxidized
ferric (+3) form or reduced ferrous (+2) form. In fresh
meat color, there are three oxidation-state or ligand
combinations of note, each of which has its own distinctive
color (Livingston and Brown, 1981). The principal pigment
of fresh meat is myoglobin, which can exist in three forms
or derivatives depending on the oxidation state of the heme
iron and the oxygen status of the environment surrounding
the meat (Seideman et al., 1984). These are reduced
myoglobin (Mb) oxymyoglobin (MbO₂) and metmyoglobin (MetMb).
Reduced myoglobin is purple and is responsible for the color
of meat immediately after it is cut, or for the color of
meat held in the absence of air, e.g. in a vacuum package.
Ferrous myoglobin that is exposed to O₂ will bind O₂ at the
sixth coordination site and form oxymyoglobin. Oxymyoglobin
is bright red, the typical attractive color of fully
oxygenated meat in retail cases. Metmyoglobin is brown and
is formed by oxidation of the pigment to the ferric form.
The actual color of fresh meat depends on the relative
amounts of these three derivatives present at the surface.
When heme iron is in the ferrous form and lacks a ligand at
the sixth position, it is referred to as deoxymyoglobin. The color of deoxymyoglobin is purplish-red and is characteristic of fresh meat in anoxic conditions (Giddings, 1977). The process by which deoxymyoglobin binds $O_2$ and is converted to oxymyoglobin is called oxygenation. It is important that this not be confused with oxidation. Oxidation means the loss of an electron by any chemical species (McQuarrie and Rock, 1991). The process of oxidation occurs in myoglobin when ferrous (+2) iron (deoxymyoglobin or oxymyoglobin) is converted to ferric (+3) iron and leads to the third form of myoglobin found in fresh meat, metmyoglobin. Metmyoglobin is brownish-red in color and is characterized by ferric iron with a water molecule bound at the sixth position (Livingston and Brown, 1981). The oxidation of deoxymyoglobin or oxymyoglobin leads to the formation of metmyoglobin; this process occurs gradually on the surface of meat cuts during storage and display (Ledward, 1985).

The myoglobin form which predominates in the surface of meat determines the perceived color. A typical scenario for color expression of the various myoglobin forms can be observed during the cutting of meat. The deep portion of a fresh piece of meat is anoxic, and when sliced will reveal an interior that is purplish-red in color. Following exposure to air for 20 to 30 min, the deoxymyoglobin will oxygenate to form cherry-red oxymyoglobin (McMillin et al.,
1994a). As display time increases, oxymyoglobin will oxidize to metmyoglobin and the portion of meat displaying undesirable brownish discoloration will increase. The rate of metmyoglobin formation is dependent on several factors including the specific muscle, display temperature, type and intensity of lighting, and bacterial load (Faustman and Cassens, 1990; Renerre and Labas, 1987).

Meat color is an extremely important sensory characteristic by which consumers make judgments of meat quality. Color that deviates from the accepted cherry red color for beef is discriminated by meat purchasers. This has several interesting consequences. One of these is that meat color is a primary determinant for the shelf-life of meat. Cuts of meat with too much metmyoglobin was viewed as old and undesirable for consumption (Renerre, 1990; Hood and Riordan, 1973; Greene et al., 1971).

The process of vacuum-packaging of fresh meat has several benefits, including significant shelf-life extension over that of its aerobically packaged counterpart. The lack of O₂ in the packaging atmosphere results in a purplish-red appearance. Although this is readily explained in terms of myoglobin chemistry, the nontraditional color is held suspect by a majority of consumers (Lynch et al., 1986). This has been a primary stumbling block to the meat industry’s attempts to market retail-ready, vacuum-packaged fresh meat cuts. Until the meat industry can effectively
educate consumers about meat color and its relationship to meat quality and safety, maintenance of myoglobin in the oxymyoglobin form remains an industry priority. There are two basic strategies for accomplishing this goal, the quenching of oxidative processes and continuation of the biochemical reduction of myoglobin (Ledward, 1992).

**Oxygenation of meat pigments**

The depth of $O_2$ penetration ($d$) into meat depends on the partial pressure ($c_0$) of $O_2$ at the surface, the rate of $O_2$ consumption ($A_0$) by the muscle tissue and the diffusion constant ($D$) according to the following equation (Brooks, 1938):

$$d = \sqrt{\frac{2coD}{A_0}}$$

Pre-rigor meat has a very high rate of $O_2$ consumption resulting in a minimum penetration into the surface of the meat for several hours postmortem. After a couple of days, however, meat exposed for several hours to the air becomes red and the penetration depth of $O_2$ may be 6-7 mm (Taylor, 1985). Particularly in a plentiful supply of $O_2$, myoglobin is oxygenated to oxymyoglobin, the bright-red ferrous form of the pigment. A low partial pressure of $O_2$ on the other hand favors oxidation of the pigment and formation of the brown metmyoglobin derivative. The optimum partial pressure $O_2$ for oxidation is 4 mm Hg (Brooks, 1938). Both of these reactions, oxygenation and oxidation, take place at the...
surface of a freshly cut meat surface. Where \( O_2 \) is freely available, the red oxymyoglobin is formed, but as \( O_2 \) penetration extends inward, its partial pressure is decreased due to \( O_2 \) consumption. Close to the limit of \( O_2 \) penetration, optimum conditions exist for metmyoglobin formation to occur (i.e. a partial pressure of approximately 4 mm Hg) and the brown form of the pigment predominates (Brooks, 1938). Beyond the limit of \( O_2 \) penetration where conditions are anaerobic, the purple reduced form of the pigment, myoglobin, remains at the original status without oxidation or oxygenation. Under practical conditions, all three pigments may exist together at the surface of cut meat. Oxygenation occurs rapidly so that the meat turns red within half an hour at 5°C (Hood and Mead, 1993). Oxidation to metmyoglobin, however, occurs much more slowly, first appearing close to the limit of \( O_2 \) penetration, as a fine brown layer, and gradually becoming thicker and extending outward towards the surface. The meat becomes gradually darker over the next several days by diffusion and gradual accumulation of the metmyoglobin pigment throughout the translucent surface layer.

Several factors affect the actual depth of oxymyoglobin, including duration of exposure to the atmosphere, temperature, \( O_2 \) tension, diffusion through and consumption by the tissue. Age of meat after slaughter also affected the depth of penetration of oxymyoglobin (Lawrie,
1991). Different muscles had different surviving respiratory activities, so that depth of $O_2$ penetration ($d$) varied under a given set of conditions. Lawrie (1991) indicated that after exposure of cut surfaces to the air for one hour at 0°C, the depth of oxymyoglobin was 0.94 mm in horse psoas, in which respiratory activity is relatively high, and 2.48 mm in L. dorsi, in which respiratory activity is relatively low. Similarly, O'Keeffe and Hood (1982) found that the depths of penetration were 4.3 and 5.0 mm of $O_2$ for psoas at 0°C after 48 h exposure to air in samples taken 3 and 20 days post mortem respectively. Corresponding values for the less respiratory active L. dorsi were 4.9 mm and 7.1 mm. McMillin et al. (1994a) demonstrated that depth of oxygenation in ground beef and loin steaks increased with increased air exposure time and with increased display time.

Autoxidation to metmyoglobin is also highly temperature dependent. The reaction was accelerated at lower pH values which showed to decrease the stability of the heme-globin linkage (Fronticelli and Bucci, 1963). Metal ions also stimulate the rate of oxidation of oxymyoglobin. Snyder and Skrdland (1966) found copper to be most active in this respect, while iron, aluminum and zinc were less active. The coefficient of diffusion decreases less than respiratory activity for the given fall in temperature so that the depth of the bright-red layer of oxymyoglobin will be greater at
0°C than 20°C, hence the tendency for meat surface color to be brighter with decreased temperature.

Optimum conditions required to avoid autoxidation of myoglobin pigments may be summarized. First, low O$_2$ partial pressure must be avoided, either by placing the meat under completely anaerobic conditions or by exposure to a high level of O$_2$. Storage temperatures should be kept as close to 0°C as possible and contact with metal ions, especially copper should be avoided. Bacterial contamination, with a tendency to restrict the O$_2$ available to muscle tissue pigments, should also be avoided. The high pH of dark cutting beef might be an advantage in this particular respect, but other more important factors are also at work, especially the greatly increased growth of spoilage bacteria.

**Meat pigment oxidation**

Delaying myoglobin oxidation is accomplished in a variety of ways. These include storage and display of meat under refrigerated conditions, hygienic preparation of meat cuts, and selective use of lighting. In addition, the application of antioxidants such as ascorbic acid (vitamin C), citric acid, or α-tocopherol (vitamin E) may extend color shelf-life (Mitsumoto et al., 1993).

Biochemical reduction is a process in which a chemical compound gains an electron and it works in opposition to oxidation. Metmyoglobin reduction refers to the conversion...
of ferric (+3) heme iron to ferrous (+2) heme iron. When this occurs, metmyoglobin decreases and deoxymyoglobin or oxymyoglobin increases. A source of reducing equivalents (electron donors) must be present (Brown and Snyder, 1969; Livingston et al., 1985). Metmyoglobin cannot bind $O_2$ and is physiologically inactive.

The reduction of metmyoglobin in the living state is an extremely important process for maintaining an adequate pool of ferrous myoglobin for oxygen binding. The process of reduction in living tissue is catalyzed by an enzyme called metmyoglobin reductase. Metmyoglobin reductase has been isolated from muscles of different species (Giddings, 1974; Al-Shaibani et al., 1977; Levy et al., 1985), including bovine cardiac muscle (Hagler et al., 1979) and skeletal muscles (Arihara et al., 1989). Metmyoglobin reductase is NADH-dependent and requires ferrocyanide for in vitro activity. An in vitro assay system for determination of metmyoglobin reductase activity in bovine muscle extracts was reported by Reddy and Carpenter (1991). There was a significant difference in the 0 day post-mortem enzyme activity in muscles of varying color stability, but metmyoglobin reductase maintained significant activity at pH 5.8 and 4°C, the pH and temperature typical of retail displayed meats. Madhavi and Carpenter (1993) evaluated the effects of aging and processing on color, metmyoglobin reductase activity, and $O_2$ consumption of beef muscles and
indicated that psoas steaks had higher metmyoglobin accumulation, lower metmyoglobin reductase activity, and greater O₂ consumption than longissimus steaks, and color was most stable in steaks fabricated at 4 or 7 days post-mortem. The color stability of different muscles was similar after the process of grinding, which increased O₂ consumption.

Lipid Oxidation

The lipids in meats contribute greatly to flavor. The three major classes of lipids in meat are triacylglycerols, phospholipids, and cholesterol (Hedrick et al., 1994). Phospholipids are considered as the major contributors to oxidative off-flavor in poultry, pork, lamb, and beef products (Wilson et al., 1976) and triacylglycerols enhanced the development of warmed-over flavor only when combined with the phospholipids as total lipids (Igene and Pearson, 1979). Although cholesterol has been an issue of nutritional concern, it has less impact on raw meat flavor. DeVore (1988) observed the oxidation of cholesterol in the raw and cooked ground beef patties and concluded that cholesterol oxidation in the tissue membrane was initiated by the free radicals from phospholipid oxidation because polyunsaturated fatty acids and cholesterol were integral components of the cell membrane structure.

Phospholipids are located in membranes of cells and subcellular organelles, whereas triacylglycerols predominate
in lipid droplets and fat depots (Hedrick et al., 1994). The fat that is evident on the external portion of meat cuts or as intramuscular marbling is triacylglycerol. However, phospholipids appear to be the more important lipid class in considerations of off-flavor development (Lea, 1957; Gokalp, 1981). Glycerol serves as a backbone for each of these two lipid classes. The first and second positions of these lipid molecules may be occupied by any of a variety of fatty acids (Stryer, 1988). Fatty acids are chains of carbon atoms with a carboxylic acid group at one end. The length varies according to the number of carbon atoms which comprise their backbone (Graham Solomans, 1985). Saturated fatty acids are more solid at room temperature and contain no double bonds between carbon atoms. Unsaturated fatty acids may contain one (monounsaturated) or several double bonds (polyunsaturated) between the carbon atoms and are generally liquid at room temperature (Fennema, 1985). The third position of triacylglycerol is occupied by another fatty acid, whereas that in phospholipid contains phosphate and usually a nitrogen-based chemical group (Stryer, 1988). The phosphate portion of phospholipids is important for the function of the lipid within membranes. It should be noted that the proportions of saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) found in animal tissues were species dependent (Igene et al., 1979), and were influenced by diet in monogastric species (St. John et al.,
1987; Rhee et al., 1988; Miller et al., 1990). In general, fish muscle contained the greatest concentration of polyunsaturated fatty acids followed by poultry and pork, and then beef (Igene et al., 1980; Melton, 1983).

The double bonds located within PUFAs are sites of chemical reactivity (Igene et al., 1979). Oxygen is a necessary ingredient for lipid oxidation and may react with these sites to form peroxides (Hsieh and Kinsella, 1989), which lead to rancidity. Polyunsaturated fatty acids are especially susceptible to oxidative rancidity because of their high number of reactive double bonds. The formation of lipid breakdown products leads to development of undesirable flavors and odors (Lillard, 1987). Those muscle foods with high concentrations of PUFAs, such as fish, typically develop rancid flavors and odors faster than foods with less PUFA. The oxidative rancidity process occurred when meat products were exposed to environment containing $O_2$ (Dugan, 1987; Gray and Pearson, 1987). Thus, vacuum-packaging of meat products provides longer shelf-life by excluding $O_2$ from the packaging environment. The interaction of $O_2$ with PUFA to cause rancidity is a nonenzymatic process. Lipid oxidation and rancidity may also be caused by enzymic processes occurring within the muscle food.
Measurements of lipid oxidation

Lipid oxidation in food products is usually associated with oxidative rancidity and rancidity is used to describe development of objectionable flavors and odors (Pomeranz and Meloan, 1987). The odors and flavors associated with typical oxidative rancidity were mostly due to the carbonyl type compounds from lipid degradation (Lapin and Clark, 1951; Hendick et al., 1954). Thiobarbituric acid reactive substance (TBARS) test is a frequently used method for the determination of lipid oxidation in meat products and was originally demonstrated by Kohn and Liversedge (1944) in measuring the aerobic oxidation products of animal tissue. Bernheim et al. (1947) indicated that the TBARS test measured the oxidative products of unsaturated fatty acids, mainly linolenic. Yu and Sinnhuber (1967) indicated the red pigment produced in the sensitive color reaction was a condensation product of two molecules of TBA and one molecule of malonic dialdehyde. Several modifications of the method have been recommended to improve the accuracy, such as the utilization of steam distillation to purify the TBA reactive compounds (Tarladgis et al., 1960) and the addition of an antioxidant to prevent the oxidation during operation (Rhee et al., 1986). Other methods are also available for the determination of lipid oxidation in food products, such as peroxide value for assaying the oxidation of fats and oils (Barnard and Hargrave, 1951), fat acidity...
for indicating oxidative deterioration of grain and milled products (AOAC, 1980), and active oxygen method (AOM) for determining the onset of rancidity in vegetable oils (Dugan, 1955).

Enzymic lipid oxidation

Enzymic-based lipid oxidation occurs in muscle foods and has also been termed as microsomal lipid oxidation. Microsomes are many small (~100 nm in diameter) closed vesicles which are resealed from fragments of endoplasmic reticulum (ER) when tissues or cells are disrupted by homogenization (Alberts et al., 1994). Microsomes derived from rough ER were relatively easier to purify than microsomes from smooth ER. Rough microsomes usually included the ribosomes which were attached on the rough ER and represented small versions of the rough ER, which were still capable of protein synthesis, protein glycosylation, and lipid synthesis (Alberts et al., 1994). Microsomes do not constitute a specific cellular organelle but are referred to as membrane fractions of intracellular components (e.g., sarcoplasmic reticulum.)

Microsomes isolated from liver have been shown to catalyze an NADPH-dependent peroxidation of endogenous unsaturated fatty acids (Ernster and Nordenbrand, 1978). This process required certain biochemical cofactors for activity, including reduced forms of nicotine adenine dinucleotide phosphate (NADPH), nicotine adenine
dinucleotide (NADH), adenosine diphosphate (ADP), and iron ions. Iron, either in an inorganic form or as a heme protein, played an important role of the catalytic system in tissue homogenates (Wills, 1969). Lipid peroxidation in the mitochondrial fraction was also stimulated by addition of iron (Hunter et al., 1964). Hochstein and Ernster (1963) indicated that the microsomal peroxidation was stimulated by ascorbate. Wills (1969) reported that microsomal peroxidation was accompanied by the disappearance of some polyunsaturated fatty acids from phospholipids.

The enzymatic nature of the process implies involvement of membrane-bound proteins. The process of cooking meat provides sufficient heat to denature enzymes, and thus enzymic microsomal lipid oxidation is less likely to occur in cooked meats (Rhee et al., 1984). During normal physiological functioning, enzymes found in these subcellular organelle membranes produce chemically reactive substances known as radicals. Living cells have a variety of mechanisms for protecting themselves against the undesirable actions of radicals. In postmortem muscle tissue, many of these protections are lost and radicals may hasten lipid oxidation and cause rancidity. Rhee et al. (1984) indicated the presence of an enzymic lipid peroxidation system associated with beef muscle microsomes. Rhee and Ziprin (1987) investigated the lipid oxidation in retail raw and cooked beef, pork, and chicken muscles and
found that the microsomal enzymic lipid peroxidation activities were higher in raw beef muscles than raw pork muscles during a 6-day chill storage at 4°C. Raw poultry muscles had the lowest level of microsomal enzymic lipid peroxidation compared with pork and beef muscles during storage. Differences were not found among the three species in cooked meats. Unlike other microsomal membrane systems reported for poultry and red meat products that required NADPH, McDonald et al. (1979) observed that NADH was preferred in fish muscles (red hake) rather than NADPH for the enzymic peroxidation activity.

**Fresh Meat Packaging**

The preference of consumers to purchase bright red fresh meat is strong in the case of beef and lamb, both of which have a relatively high pigment content, but is less important in pork and veal with their much paler color. The color and color stability of the fresh meat may be the most important attributes by which consumers judge freshness and quality when making purchase decisions (Tuma et al., 1973; Smith, 1981; Taylor, 1982; Reynolds, 1983). Lynch et al. (1986) surveyed 175 grocery store shoppers to determine the effect of product color and educational materials on the purchase intent for vacuum packaged (VP) ground beef and found that 74% of the consumers indicated that color was important in their product purchase decision. Even after being educated on benefits and appearance of VP ground beef,
47% of the informed consumers still indicated that they might not or would not purchase ground beef products with purple-red color.

There are three types of packaging suitable for the presentation and display of consumer cuts of meat. These are (1) conventional aerobic overwrapped trays; (2) MAP, especially using higher levels of $O_2$; and (3) vacuum-packaging. All have been used in the retail marketplace to varying extent. The use of vacuum-packaging in retail marketing is limited, due to the purple color of the meat, and attempts to educate consumers to accept the color because there is a greatly extended shelf-life have been largely unsuccessful. Nevertheless, there is a limited specialized market for the product and some interest in the system remains for special marketing situations (Hood and Mead, 1993).

**Ground Beef Production and Consumption**

Ground beef is defined as comminuted beef skeletal meat (meat that was attached to bone) without added fat, seasoning, water, binders, or extenders. The finished product contains no more than 30% fat (NLSMB, 1991). The sources of raw materials used in the production of ground beef vary widely between processors. Consumption of red meat gradually decreased from 130.2 pounds in 1970-1974 to 112.4 pounds in 1990, however, the trend reversed to 116.2 pounds in 1992. Beef was the largest group of meat consumed
and occupied about 63.5% among the different meat species (Putnam, 1992). Ground beef manufacturing is the largest segment of the processing meat industry in the United States and ground beef products account for over 50% of the total U.S. beef consumption (NLSMB, 1995). According to USDA, the annual per capita consumption of hamburger per person rose from 22 pounds in 1970 to 25 pounds in 1980, and 30 pounds in 1992 (Smith, 1994).

Packaging Considerations

Meat, as well as other foods, was originally packaged to provide a convenient container, to avoid gross contamination both of the meat itself and of other materials by the meat, and to reduce evaporative weight loss. Prolonging the storage life of fresh meat has been a very important consideration for both consumers and meat packers. With the development of new packaging materials specifically designed for meat, further attributes became possible, including improved storage life, an improved means of presentation and enhanced appeal to retail customers (Lioutas, 1988; Brody, 1993).

Meat is easily contaminated with spoilage bacteria which are present on the moist surface of the meat. Meat also provides nutrients as an ideal environment for rapid proliferation of microorganisms. Hood (1984) indicated that meat usually discolors due to intrinsic biochemical reactions long before bacterial effects become important.
Chemical deterioration such as fat oxidation and pigment autoxidation may also play a role in spoilage.

Packaging systems offer various possibilities depending on the particular conditions which are chosen, but all depend on changing the environment of the meat and especially the gaseous atmosphere within the pack. The appropriate method depended on many factors, including the type of meat to be packaged (beef, lamb, pork, or veal, etc.), the expected duration of storage, the extent of market distribution, and whether the meat was intended for wholesale or retail sale (Hood and Mead, 1993). The composition of the gaseous atmosphere determines the color of the meat and influences the rate at which it discolors, as well as the likely pattern of microbiological spoilage. Within the package, the atmosphere is a dynamic equilibrium of gaseous changes, which are dependent on the external atmosphere and the degree of gas permeability of the packaging materials. Gill (1988) reported that gases became dissolved in meat fluids at different rates according to solubility constants and the partial pressure in the atmosphere. Carbon dioxide is very soluble in both muscle and fat tissue. Ingram (1962) indicated that CO$_2$ could also be produced by respiration of the muscle tissue and microorganisms within the package.

Commercial packaging systems differ principally due to differences in O$_2$ status and in CO$_2$ within the pack. The
changes which occur and the effects that these changes produce on the quality attributes of color and bacteriological storage life are the principal factors of consideration for modified gas packaging of meat (Hood, 1980). Oxygen produced a bright-red color on meat surface because of oxygenation of myoglobin, especially at higher partial pressures. Oxygen concentration in meat tended to decrease with storage time because muscle tissues are undergoing respiration and the growth of aerobic bacteria (Ledward, 1992).

**Conventional oxygen-permeable overwrap packaging**

The conventional aerobic method of packaging is widely used by supermarkets and other self-service outlets for retail presentation. It involves placing the meat in semi-rigid foam or plastic trays, which are then overwrapped with a clear, gas permeable plastic film that allows $O_2$ to contact with the meat. The film is usually a light-gauge vinyl or polyethylene derivative which combines a low permeability to water vapor and a high permeability to oxygen ($>10000 \text{ cm}^3\text{m}^{-2}\text{day}^{-1}\text{atm}^{-1} \text{O}_2$). A wide range of films is currently available commercially for overwrapping trays based on price, suitability for use on machines, optical clarity and sealability (Taylor, 1985).

Aerobic conventional overwrapping allows for rapid growth of spoilage organisms, such as *Pseudomonas*, in retail meat. Storage life is limited due to biochemical
discoloration, intrinsic enzymic degradation and oxidation. High bacterial contamination causes an accelerated deterioration in color, due, at least initially, to competition for available $O_2$. Egan (1991) reviewed the principal microbiological considerations in meat packaging and indicated that the spoilage of fresh meat stored aerobically under chill conditions was mainly due to the growth and metabolism of *Pseudomonas* spp., including *P. fluorescens*, *P. putida* and *P. fragi* (Shaw and Latty, 1984). Other bacteria that can play a role in the spoilage of meat include cold-tolerant strains of *Enterobacteriaceae* and *Brochothrix thermosphacta*, which can cause souring off-flavor in the absence of air, but are inhibited in high concentrations of $CO_2$, especially when $O_2$ is low (Campbell et al., 1979). The conditions of low $O_2$ favor the growth of lactic acid bacteria (*Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*) which produce a typical lactic souring in the meat. A reduction in the partial pressure of $O_2$ to the critical level for oxidation to metmyoglobin results in the meat turning brown. Development of browning in meat causes 'fading' and a 'tired' appearance of the packaged meat, limiting its display life to a maximum of two to three days. Hood and Riordan (1973) reported that more than two-thirds of consumers would reject purchase of meat with 20% metmyoglobin discoloration in a retail display cabinet when compared with similar packs of bright-red meat.
Under normal commercial display, MacDougall (1982) found that color stability limited effective shelf-life to two days, depending on the muscle, before pigment oxidized to the unattractive brown metmyoglobin pigment. In practice, supermarkets generally restock every few hours or at least each day to ensure that the meat on retail display has a fresh appearance. Refrigerated meat is expected to be kept at 4°C in display case, however, poor control of temperature that is often observed in retail display cabinets result in meat that has an unattractive appearance due to the accumulation of metmyoglobin (Taylor, 1982).

Taylor (1985) pointed out that overwrapped trays provide an effective thermal insulation so that the meat temperature can be higher than the surroundings. The lighting system in display cabinets can also produce a 'greenhouse' within the pack, heating the exposed surface. The meat must be adequately cooled before packing and careful control of temperature must be maintained, not only in the cabinet, but also within the package during storage and display. Temperature of storage and display is critical in obtaining maximum shelf-life. At low refrigeration temperature, an increase of 5°C can decrease the color shelf-life by one-half, depending on species and muscle (Hood, 1984). Low-temperature storage, at ca. 0°C, is essential to obtain maximum shelf-life. Even at this low temperature, the maximum shelf-life for beef fillet was only
about four days at 0°C (Hood, 1984). Temperature is the single most important factor under practical commercial conditions, and other factors should also be taken into account, such as muscle variability, UV light and postmortem time. Some cuts, and especially certain muscles, have a very short shelf-life with respect to color stability even under ideal storage conditions, while others are less sensitive in this respect. For example, beef from the fillet (psoas major) had a color shelf-life of one to five days at 5°C, while loin steak (longissimus dorsi) retained a bright-red color for more than six days at the same temperature (O'Keeffe and Hood, 1980).

**Modified atmosphere packaging (MAP)**

Modified atmosphere packaging (MAP), although relatively new in North America, is a well established technology dating back to the 1930's when fresh beef was shipped under CO₂ storage from Australia and New Zealand to England (Empey et al., 1934). However, MAP meats have become increasingly common in meat markets, as manufacturers have attempted to meet consumer demands for fresh refrigerated meats with extended shelf life (Farber, 1991).

Koski (1988) defined MAP as "the enclosure of food products in high gas-barrier materials, in which the gaseous environment has been changed to slow respiration rates, reduce microbiological growth, and retard enzymatic spoilage - with the final effect of lengthening shelf-life." MAP is
referred as the initial introduction of a specified mixture of gases into the primary food package, without subsequent control of this atmosphere. MAP should be distinguished from controlled atmosphere storage in which the atmosphere is continuously monitored and precisely controlled throughout the whole period of storage. The latter system is more suited to large scale-storage and transportation, such as the shipping of meat to remote markets in large countries (Brody, 1989).

Farber (1991) indicated that a major turning point in MAP technology came in 1981 when Marks and Spencer in the United Kingdom introduced a wide range of meat products packaged under a modified atmosphere. The impetus behind these products came mainly from an increased consumer demand for fresh and chilled products. Other factors, such as the consumer desire for preservative-free products, the growth of centralized packaging and portion control, and a decline in the growth of canned and frozen foods certainly also played a role in the increasing demand for MAP meat (Farber, 1991).

There are numerous advantages of using MAP technology in meat products, but the major benefit of MAP is the ability to extend shelf-life of refrigerated meats (Newton et al., 1977; Thomas et al., 1984; Yi et al., 1986). Product shelf-life is one of the most important aspects to the food industry. Shelf-life usually refers to the period
of deterioration for decreased nutritional value, unfavorable color changes, development of off-flavors, and/or textural changes (Wang, 1978). Without proper handling and distribution, the shelf-life of meat products could be very limited. Shelf-life can also be extended by lower storage temperatures and low initial microbiological loads for meat products. Ayres et al. (1950) and Elliot and Michener (1961) pointed out that a lower initial bacterial load extended the shelf-life of meat products. Foster and Mead (1976) reported that foods spoil 4 times faster at 10°C and 2 times faster at 5°C than at 0°C. Shelf-life of meat stored in air at chill temperatures is often terminated by the development of off-odors that are predominantly of microbial origin. Microorganisms are ubiquitous and are the principal cause of deterioration for most foods. Growth of virtually all aerobic bacteria and molds, and particularly those which grow at lower temperatures such as the psychrotrophs, can be restrained by increasing the CO₂ level.

Oxygen, nitrogen, and carbon dioxide are the three main gases used commercially in MAP. Oxygen will generally stimulate the growth of aerobic bacteria and inhibit the growth of strictly anaerobic bacteria. One of the major functions of O₂ in MAP is to maintain myoglobin in the oxygenated form, oxymyoglobin. This is the form responsible for the bright red color of meat that most consumers

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associate with the fresh red meat. Nitrogen is an inert tasteless gas which displays little antimicrobial activity on its own. Because of its low solubility in water, the presence of $N_2$ in a MAP food can prevent package collapse that can occur because of absorption of $CO_2$ into when high concentrations of $CO_2$ are used. Utilizing $N_2$, instead of $O_2$ in the package, will delay oxidative rancidity and also inhibit the growth of aerobic microorganisms. Carbon dioxide is both water and lipid soluble and is mainly responsible for the bacteriostatic effects seen in modified atmosphere packages (Farber, 1991). Absorption of $CO_2$ into meat decreased gas volumes within the packages and resulted in pack collapse (Gill, 1988; Zhao et al., 1995). Gill (1988) reported that solubility of $CO_2$ in muscle tissue (pH 5.5, 0°C) was approximately 960 ml/kg of meat tissue. The solubility of $CO_2$ increased with increased tissue pH by 360 mL/kg meat for each pH unit and increased with temperature by 19 mL/kg meat for each 1°C rise. Zhao (1993) indicated that an optimal ratio of headspace of the package to meat volume for fresh meat could be determined based on the $CO_2$ concentration of the gas mixture and the storage temperature. Zhao et al. (1995) indicated that headspace-to-meat volume ratio was the most important packaging parameter and decreased storage temperature reduced $CO_2$ concentration remaining in headspace. With an initial 100% $CO_2$ gas atmosphere at 13°C, a 1.5-kg portion of ground beef
and chopped pork absorbed about 4.5 g and 5.3 g of CO₂, respectively, in the first 12 hr storage. The pressure drop of fresh beef packaged with 20%, 80%, and 100% CO₂ balanced with N₂ at 3°C for 12 hr were -1, 9, and 33 KPa, respectively (Zhao et al., 1995).

**Vacuum packaging**

Vacuum packaging (VP) and CO₂-enriched packaging have been the most widely used MAPs in today’s market. These types of MAP conferred an adequate storage life and reduced the weight losses of products during storage; however, the purple color of anoxic red meat has not proved acceptable to consumers (Allen and Pierson, 1986). VP is the frequently used packaging method for the storage and distribution of chilled primal or wholesale cuts of beef. VP is the simplest form of MAP, which removes air from the system and maintains the meat in an anoxic environment. In the United States, the use of VP for wholesale distribution of large pieces of meat has replaced the movement of carcasses, and the concept of boxed beef is recognized as an intrinsic link in the meat marketing chain (Breidenstein, 1982). The VP system offers several advantages compared to handling carcasses or quarters of beef. There are substantial economic savings in transporting and storing beef as boneless primal cuts rather than as quarters or sides. Only about two-thirds of the carcass is useable meat and there is a huge saving in refrigerated space by retaining low-value
trimmings and bone at the processing plant. Moreover, using boxes for transportation and storage of meat products is of great economic values to the meat industry (Hood and Mead, 1993). VP also allows individual cuts to be aged without weight loss, during which meat tenderness and eating quality characteristics are improved utilizing VP meat practices (Nortje et al., 1985). Additional advantages for the retailer are providing greater flexibility in inventory stock control and reduced butcher costs in the preparation of retail packaged cuts at the retail site (Breidenstein, 1982).

Successful vacuum packaging depends on the physical properties of the film. The film should have good mechanical strength, be puncture resistant, easily scalable, and have low water vapor transmission rate and low O2 permeability (Greengrass, 1993). Reducing the presence of O2 during cutting and preparation will allow for desirable color effect and achieve the longest storage life. Protection of bone-in cuts may be achieved by covering the bone with a reinforced plastic material before VP to prevent the package breakage (Hood and Mead, 1993).

Savage et al. (1990) indicated that the drip was composed primarily of sacroplasmic proteins and Offer and Knight (1988) reported the normal drip losses in fresh meat during storage were from 2 to 10% of the lean weight. However, VP produced a drip in excess of 9% (Ho et al.,
that was unsightly and detracts from the appearance of the final products. This may be partly overcome by vacuum skin packaging using a film that fits very tightly to the meat surface, leaving little space for the accumulation of any fluid exudate. This technique uses films which softens on heating to make it pliable so that it can be draped tightly over sharp objects such as the cut surface of bone without puncturing. The technique is especially suited to frozen meat, where the main requirement is to prevent moisture loss or transfer within the package. The accumulation of frost can otherwise have a very deleterious effect on appearance. Taylor (1985) pointed out that color is the most important feature of frozen meat and if a bright red color is required, it must be produced by oxygenation of the meat before freezing.

The color of VP fresh meat is purple, but this is not regarded as a disadvantage in the wholesale market where people are aware that this temporary effect that will be reversed when the meat is re-exposed to normal atmospheric conditions. Taylor (1985) outlined the principal changes following vacuum packaging. When meat is first vacuum packaged, there is a residual O$_2$ content remaining in the package. The O$_2$ is quickly consumed by meat pigments and muscle enzymes presented in the meat. If the meat was red at the time of packaging, the color is quickly reduced to the purple form of the pigment. A parallel effect is the
production of CO₂, which occurs as a result of respiration; the concentration of this gas increases to a maximum of about 20%. The final partial pressure of O₂ at the surface of the meat falls to less than 10 mm Hg within two days of packaging. The remainder of the gaseous atmosphere is N₂. The actual gas headspace is small after vacuum packaging and its practical effect on the meat is slight within the pack. Any oxidized pigment that occurs as a result of the low partial pressure of O₂ appears as a monomolecular layer at the surface and the purple color of the underlying unoxyxygenated tissue is unaffected. The surface layer of metmyoglobin may be as thin as 1 mm with very high barrier packaging materials, but as much as 2-3 mm when a film with permeability approaching 200 mm Hg is used. Beef stored under vacuum in low-permeability films should remain purple throughout storage. The development of a brown color due to metmyoglobin formation during storage indicates the presence of O₂, which has gained access to the pack either by the use of film with inadequate impermeability to O₂ or because the package has leaked during packaging or subsequent storage (Taylor, 1985).

The principal advantage of vacuum-packaged meat is its extended shelf-life when compared with aerobic packaging for meat. VP meat may remain in an acceptable fresh condition for many weeks after packaging if stored at proper
refrigerated temperature. However, some important precautionary measures must be taken to guarantee success.

The pH is of equal and perhaps even greater importance. Meat of pH 6.0 and above must not be vacuum packaged. The combined effects of high bacterial contamination and high pH will seriously curtail the shelf-life of the meat. Temperature is also a limiting factor and to achieve the best results, the meat must be kept close to the freezing point (Hood, 1984).

VP beef with normal pH can be stored for periods of up to 14 weeks at 0°C. The storage life of beef of high ultimate pH (>6.0) will be much shorter than this at a similar temperature. Gill and Penney (1986) gave a storage limit of 8 weeks and also applied this to most lamb cuts, which usually have some muscle tissue of high ultimate pH as well as non-respiring fat cover of neutral pH. Taylor and Shaw (1977) also showed that the storage stabilities of VP pork and lamb were less than that of beef. Lamb loins, shoulders and legs remained unspoiled in vacuum packs held at 1°C, but deteriorated rapidly during subsequent retail display at 5°C. The first practical usage of MAP containing elevated carbon dioxide level was used to ship whole chilled beef carcasses from Australia and New Zealand to Great Britain in the 1930s (Taylor, 1973). Strict attention to high standards of hygiene during processing and packaging, and rigorous control of temperature at -1°C gave largest
storage stability and subsequent retail-shelf-life. Taylor (1985) reported that the useful storage life for vacuum packaged pork primal cut was little more than two weeks at a storage temperature of 1°C. Smith et al. (1983), however, claimed a storage life of three to four weeks for vacuum-packed pork loins at 2°C.

Baltzer (1969) summarized the microbiology of meat in VP in terms of replacing Pseudomonas with lactic acid bacteria, and further noted that vacuum-packaged beef had a slower increase in total counts and a souring type of spoilage rather than putrefaction and sliminess. The strains of lactic acid bacteria present in vacuum-packaged meat have proved difficult to identify. Shaw and Harding (1984) demonstrated that 90% of strains belonged to two groups of streptobacteria, while a third group consisted of Leuconostoc spp. These authors classified 20 atypical strains of lactic acid bacteria and described a new species as Lactobacillus carnis, as well as L. divergens, which appeared to be important constituents of the vacuum packaged flora. Ahn and Stiles (1990) reported specific antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meat. When stored at 4°C, these organisms produced antagonistic substances active against closely related bacteria. Studies on meat, which carried only small amounts of bacteria (<100 CFU/cm²), have shown that an off-flavor developed and become significant after 14-16 weeks at
0°C (Dainty et al., 1983). This flavor was also described as bitter and liver-like and was probably due to chemical changes in the meat caused by enzymic activity. When meat pH was about 6.0 or higher, a number of other types of bacteria reached populations high enough to cause spoilage. In particular, the growth of Alteromonas putrefaciens, Iranians spp., or some types of Enterobacteriaceae may cause spoilage due to greening discoloration. These organisms produce hydrogen sulfide, which reacts with myoglobin to form the green pigment sulphmyoglobin. The defect is more noticeable with beef, because the higher concentration of myoglobin produces a more intense green color. It can be avoided by not packaging meat of high pH (Egan et al., 1991).

The storage life of vacuum-packaged primals may be extended by improving the bacteriological quality of carcasses from which the meat is derived. The objective is to decontaminate the surface of carcasses on the slaughter line by spraying with hot water. Dilute solutions of acetic or lactic acid may also be used to extend the storage life on high pH meat. Egan et al. (1991) reported that lamb carcasses may be treated on-line prior to chilling. If such unchilled carcasses were immersed in a 1.5% solution of acetic acid at 55°C for 10 s, there was a reduction of 95-99% in the population of bacteria on the meat. The acid treatment not only reduced the number of bacteria present,
but also had a residual bacteriostatic effect, delaying the proliferation of putrefactive bacteria and resulting in an extension of the storage life to 10-12 weeks. Greer and Jones (1991) reported the results of beef carcasses treated with either a lactic acid spray or a water spray in a research abattoir. They suggested that the marginal reduction in carcass contamination produced by the lactic acid spray did not improve the bacterial quality of subprimals nor the aerobic spoilage of steaks.

**CO₂-enriched MAP**

As long ago as the early 1930s, chilled carcass meat was successfully shipped from New Zealand and Australia to Britain to satisfy consumer demand for fresh rather than frozen meat. This system, which demanded strict control of temperature and atmospheric conditions, may be accurately described as controlled atmosphere storage. Gases surrounding the meat are continuously monitored and adjusted throughout the complete storage period. In the early days CO₂ concentration was 10-20%. Higher CO₂ were found to be effective in preventing bacterial growth, but also produced browning of the surface due to the formation of metmyoglobin. Temperature was also continuously monitored and rigorously maintained at -1°C (Haines, 1933). Controlled atmosphere storage of meat was confined to large-scale shipping or warehousing operations of this nature.
The use of CO$_2$ in the atmosphere of packaged beef strip-loins has been effective in limiting the growth of spoilage bacteria, even with high-pH beef (Gill and Penney, 1986). A system was developed in New Zealand to ship whole lamb carcasses in CO$_2$ in a foil-laminate masterpack. The hind legs of the carcass are folded into the thoracic cavity, a process known as telescoping, to give a significant saving in volume and space requirement during shipping. The package atmosphere can be maintained, irrespective of the physical shape or size of the cut of meat (Gill, 1982). A reduction in partial pressure within a package may result from absorption of the gas into the meat unless a sufficient excess is included to counteract the effect. Shay and Egan (1987) used high concentrations of CO$_2$ to extend the storage life of packaged lamb and pork. The carcasses may be vacuum packaged, but it is difficult to remove all the air from the system. Flushing with CO$_2$ or N$_2$ removes residual O$_2$ from the system. Garout et al. (1989) reported an increased storage life for lamb loins and carcasses, packed in CO$_2$, compared with similar vacuum-packaged meat in consignments transported by air from New Zealand to Saudi Arabia. Given similar chilling conditions, the storage life of CO$_2$-packed lamb was about 40 days longer than that of vacuum-packed lamb. Storage life was limited by the development of putrid spoilage, principally due to psychrotrophic enterobacteria.
Case-ready packaging

There is a considerable economic advantage to be gained by distributing meat cuts in a final packaged form, prepared and packaged at a central packing location. Straightforward overwrapping does not give a long enough color shelf-life to achieve this, with the color deteriorating about two days after packaging. This has led to the development of other forms of packaging which prolong shelf-life. Shelf-life of 8-10 days was considered as sufficient for the retail sale of O₂-containing MAP meat in European Market (Lioutas, 1988). This objective could be achieved by using vacuum or an inert atmosphere in an anaerobic pack (Kropf, 1980), or alternatively an elevated concentration of O₂ in an aerobic system (Georgala and Davidson, 1970). In the former, storage life is increased at the expense of color. In the latter, although an enhanced bright-red color is achieved, aerobic spoilage bacteria continue to proliferate.

The suitability of vacuum packaging for retailing fresh red meat has been a subject of much debate for a long time. The advantages derived in the wholesale market indicate that there are considerable potential benefits which ought to be extended to retail marketing of consumer portions. First, there is an obvious advantage of extending storage life to several weeks during which aging continues to improve tenderness (Bidner et al., 1985; Nortje et al., 1985). Second, with tight fitting film, the appearance of drip can
be curtailed to some extent (Weakley et al., 1986). The purple color of vacuum-packaged beef and lamb is a disadvantage in terms of packed consumer acceptability; in fact it is the major disadvantage of the system for retail marketing. At the same time, the stability of the purple color is a significant advantage in terms of storage life compared with other retail packaging methods. Kropf (1980) reported a shelf-life of 21 days for the storage life of vacuum-packaged consumer portions at 2°C, which is sufficient to allow distribution from centralized packaging plants. Morgan et al. (1991) reported that the average time for beef to reach the retail store was 17 days postmortem, with a range of 13 to 30 days.

Previous attempts to sell vacuum-packaged retail cuts, have had only limited success, in spite of considerable commercial investment, notably in Sweden, in educating consumers to accept the color (Taylor, 1985). Dumont (1980) pointed out that the importance of color, as an indicator of freshness in retailing fresh meat, was probably overestimated, but agreed with the assertion that "the psychological attractiveness of the bright-red color makes it a fundamental criterion." Nevertheless, Taylor (1985) reported that a range of vacuum-packaged beef cuts had been marketed regionally in the UK with some success, but with little impact on marketing, and vacuum-packed lamb legs, loins and shoulders have been imported for many years from
New Zealand. In the case of lamb, the joints are generally removed from the packs before display and perhaps they should not be regarded as vacuum-packed retail meat. Color is not so important with pork since it is much paler and the difference between oxygenated and reduced myoglobin is less pronounced. Taylor (1985) reported that VP was widely used for pork retailing in the UK and Europe. A shelf-life of two weeks at 1°C was sufficient for distribution and display of centrally packed retail joints over short distances.

With vacuum-packaged retail cuts, the ratio of residual \( O_2 \) volume to meat was higher than with primals, which means that \( O_2 \) was depleted more quickly. Consequently there was a greater hazard of increased metmyoglobin formation and browning. If retail cuts were to be vacuum-packaged, it would be better done immediately after cutting and packages contained the minimum volume of air (Seideman et al., 1976). For a similar reason, the use of a very low permeability film laminate was recommended for vacuum-packaged consumer cuts.

Vacuum skin packaging is also used for frozen retail cuts. Taylor et al. (1990) compared the performance of vacuum skin packaging and MAP in prolonging the shelf-life of fresh beef and pork loin steaks at 1°C under retail display. MAP (75% \( O_2 \), 25% CO\(_2\)) developed the typical red color in beef and pink color in pork, which gradually oxidized to brownish red and brownish pink respectively.
after 12 days. Similar samples in vacuum skin packs (VSP) remained purple throughout the storage period. Off-odors developed more rapidly in MAP (8-12 days), due to more extensive growth of *Brochothrix thermosphacta*, suppressing the effects of lactic acid bacteria which predominate in both types of pack (Taylor et al., 1990).

Both the meat and the package are subjected to mechanical strain with VP. Pressure on the meat increases the incidence of drip, while the packaging film may be punctured if sharp bone surfaces are present. The effect may be alleviated by incorporating CO$_2$, or a mixture of CO$_2$ and N$_2$, after evacuation and before sealing. The anaerobic conditions ensure that color is maintained in the purple, reduced form of the myoglobin pigment and storage life can be extended similar to that achieved in vacuum packaging. It is essential to eliminate O$_2$ completely from the system, if discoloration due to metmyoglobin is to be avoided. The reaction occurs optimally when O$_2$ is about 10%. The elimination of O$_2$ can be achieved by introducing hydrogen (about 8%), sufficient to react with any O$_2$ entering the system, together with an O$_2$ scavenging catalyst (O’Keeffe and Hood, 1982). Rousset and Renerre (1990) reported that high-pH beef could be stored for six weeks in CO$_2$ at 2°C using an oxygen scavenger. The CO$_2$ inhibited *Pseudomonas* spp., *Brochothrix thermosphacta*, and *Enterobacteriaceae*. 

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The shelf-life of comparable high-pH beef stored under vacuum was considerably shorter.

Anaerobic storage followed by aerobic display may be achieved in a single composite pack. Hirsch et al. (1977) patented a process in which a meat portion was sealed in a gas-impermeable tray in an atmosphere of CO\textsubscript{2} and N\textsubscript{2}. Oxygen was excluded during storage, but immediately before display the tray was peeled off, exposing a highly O\textsubscript{2} permeable film underneath. Following oxygenation, the bright-red color was claimed to be stable for up to seven days at 2\textdegree C (Hirsch et al., 1977).

Use of carbon monoxide in MAP meat

The cherry, red color of the carbon monoxide (CO) derivative, carboxymyoglobin, is more stable than oxymyoglobin. Carbon monoxide has been suggested on this basis as a possible alternative to atmospheres with O\textsubscript{2} for prepackaged fresh meat. The possibility of increased fat oxidation at higher O\textsubscript{2} concentration has also been a reason for examining the potential use of CO. Matamoros and Rama (1973) showed that the system was successful and the meat developed a stable bright-red color. Several other workers have also obtained satisfactory results. The hazardous nature of CO gas requires obvious precautionary safeguards in handling, which would also increase processing costs, were it to be allowed. Carbon monoxide has not been approved by regulatory authorities, although it was pointed
out that the health hazard may have been exaggerated, since
the body’s exposure would be minimal at the relatively low
concentration (0.5-1.0%) required to produce the color
effect (Wolfe, 1980).

MAP with aerobic atmospheres

The gases of interest in aerobic MAP of meat are O\textsubscript{2},
CO\textsubscript{2} and N\textsubscript{2}, especially the first two. Nitrogen is inert and
provides no bactericidal or bacteriostatic function, but it
may be included to help prevent the collapse of the package
as CO\textsubscript{2} dissolves in the meat fluids. To achieve the best
effect, there must be excess gas present (approximately 1.5-
2 times the volume of the meat). A deep-draw impermeable
plastic pack is used with a dimpled base, which allows
access of the gas to the lower surface of the meat (Hood and
Mead, 1993).

The use of a high concentration of O\textsubscript{2} produces a deep
layer of bright-red oxymyoglobin and ensures that the
formation of metmyoglobin occurs at a maximum distance from
the surface. The brown discoloration of pigment is thus
retarded, giving an extension to color shelf-life. For a
successful centralized packaged operation, it is necessary
to maintain the red color for at least a week. This can be
achieved by modifying the packaging technique to delay
aerobic deterioration due to the proliferation of spoilage
bacteria. The incorporation of CO\textsubscript{2} and storage at low
refrigeration temperature are essential for this purpose.
MAP will not compensate for poor bacteriological quality or poor temperature control, but it will extend shelf-life provided both these prerequisites are met.

Gas mixtures containing O\textsubscript{2} and CO\textsubscript{2} have been used commercially for a considerable time (Brody, 1970). In a patent published in 1970, Georgala and Davidson specified a range of O\textsubscript{2} and CO\textsubscript{2} concentrations suitable for MAP of beef, with O\textsubscript{2} above 70\% combined with at least 10\% CO\textsubscript{2}. Results demonstrated that at least 60\% O\textsubscript{2} is required to achieve a color shelf-life of 9 days and the patent claimed that a mixture of 80\% O\textsubscript{2} plus 20\% CO\textsubscript{2} keeps meat red for up to 15 days at 4\degree C. Atmospheric mixtures of 60-80\% O\textsubscript{2} and 20-40\% CO\textsubscript{2} are commonly used. Provided a low storage temperature is also achieved, meat color can be maintained for a week using this mixture of gases. Shay and Egan (1987) reported that beef stored at 5\degree C in MAP (80\% O\textsubscript{2} and 20\% CO\textsubscript{2}) had a shelf-life more than three times that of similar beef and lamb meat stored in conventional overwrapped trays. Storage life under retail display is dependent on muscle type, species and the length of storage in vacuum pack before retail packaging. Because of the high O\textsubscript{2} content, the color remains bright red during the period of retail display. The color display life also depends on the previous storage history of the meat. Meat that has been stored in vacuum packs prior to retail display will have a reduced retail display life. For example, the color shelf-life of meat
stored at 5°C for longer than six weeks before retail
display at 0°C was less than half that of fresh meat. This
effect was due to a combination of factors, which include an
increased bacterial load on the surface of the meat and
decreased metmyoglobin reducing activity in muscle enzyme
systems with age of the meat. Patterson (1990) found that
microbiological and sensory quality of pork was improved by
the combined effects of MAP (25% CO₂, 75% N₂) and
irradiation treatment at 1.75 kGy.

Egan et al. (1991) reported that consumer portions
packaged in conventional overwrapped trays could be placed
in a master-pack, which consists of a large impermeable bag,
evacuated and filled with a gas mixture of 20% O₂ and 80%
CO₂. The master-pack is stored at a low temperature until
packs are required for retail display. Storage temperature
is again extremely important; it should preferably be as low
as possible without actually freezing, i.e. 0°C or -1°C.
The combined effects of O₂ to produce an attractive red
color and the bacteriostatic effect of CO₂ were employed in
this system. The master-packs were opened and the
individually overwrapped trays removed as required for
refrigerated retail display. Meat stored for up to nine
days in the master-pack at -1°C to 0°C had a retail display
life of 3 more days. With longer periods of storage in the
master-pack, the retail display life was shorter, i.e. less
than was obtained with fresh meat in overwrapped trays.
Master-packs of this type are suitable for centralized prepacking operations.

**MAP with dynamic gas exchange**

A dynamic gas exchange system (Mitchell, 1990) combines the advantages of inert gas MAP for package distribution and a high concentration of \( O_2 \) for retail display that has been successful in extending the shelf-life and improving and color appearance of fresh beef and pork (McMillin et al., 1992; Huang et al., 1995). In gas exchange MAP system, the meat is initially packaged in MAP barrier styrofoam or plastic tray and impermeable lidding film with \( N_2 \) and \( CO_2 \). After two or three weeks of distribution and storage, the gas atmosphere is exchanged for a mixture of high \( O_2 \) and 20% \( CO_2 \), using a patented gas exchange machine, immediately before retail display. This system provides extended distribution time in the deoxymyoglobin pigment state and extended display life in a bloomed color state. Less packaging material is used because no film or trays are removed or discarded after initial packaging. A major disadvantage is the need for gas exchange equipment (McMillin, 1994).

**Factors Affecting the Shelf-Life of Fresh Meats in MAP**

**Growth of spoilage microorganisms**

Microorganisms on intact or uncomminuted muscle tissue are usually confined to the surface. Interior muscle tissue from animals sacrificed under reasonable hygiene conditions
are usually sterile (Brown, 1982). Microorganisms cannot penetrate muscle tissues until they produce proteolytic enzymes, which does not occur until very late in exponential microbiological growth (Dainty et al., 1985). By that time, the muscle is usually covered by slime and is obviously spoiled (Lee et al., 1985). Microorganisms exposed to a rich source of nutrients preferentially utilize some substrate like simple sugars. Simple sugars such as glucose are present in very low concentrations in meat (Price and Schweigert, 1987). When the surface concentration of the preferred substrate is reduced to undetectable levels, then the microorganisms attack the secondary substrates such as protein degradation products, e.g., amino acids (Price and Schweigert, 1987). Initial microbiological attacks on fatty triglycerides play almost no part in the onset of spoilage (Brown, 1982), and so a fat cover on the carcass assists in maintaining a low microbiological count. Fatty tissue usually does not enter into most microbial degradation. Spoilage in comminuted ground meat develops similar to that in solid muscle, but the substrate is available to microorganisms, which are distributed throughout the mass. Higher counts of microorganisms are reached before spoilage is evident because of the enhanced substrate availability (Brody, 1989). A comparative study of vacuum packaging alone or vacuum followed by N₂ back-flushing of fresh pork loins stored at -4, 0, 3, and 7°C for 49 days showed that
pork stored at -4°C exhibited small changes in microbial numbers, pH, exudate, appearance and off-odor (Lee et al., 1985). However, there were no significant differences observed in microbiological, physicochemical and sensory changes between the two atmosphere packages.

Typically, *Pseudomonas* constitute a numerically important, if not dominant, element of the spoilage flora and are therefore assumed to be an important source of the off-odors (Dainty et al., 1983). The most numerous types of volatiles produced by pure culture inoculation of *Pseudomonas* in aerobic sterile beef at 5°C were esters and sulphur-containing compounds (Dainty et al., 1984). Jackson et al. (1992) indicated that 20% CO₂:80% O₂ was not as efficient as other atmospheric conditions (vacuum, 40% CO₂:60% N₂, and 100% CO₂) at extending the shelf-life of strip loin steaks. While the flora of samples packaged in 20% CO₂:80% O₂ became dominated by *Pseudomonas putida*, loins stored in the other atmospheric conditions became dominated by lactic acid bacteria (*Lactobacillus plantarum* in vacuum and 40% CO₂:60% N₂, *Leuconostoc mesenteroides subsp. mesenteroides* in 100% CO₂). Edwards et al. (1987) examined the microflora of normal and high pH pork stored in vacuum-package at 5°C. They reported that the microflora of the normal pH pork became dominated by lactic acid bacteria and gram-negative organisms dominated in high pH pork.

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Robach and Costilow (1961) studied the oxidation of myoglobin in beef and found that the addition to steaks ofcell suspensions of a number of aerobic bacteria, such as Saccharomyces cerevisiae, greatly increased the rate of discoloration, while no such effect was observed with Lactobacillus plantarum. They concluded that the primary effect of bacteria on meat discoloration was the reduction of O$_2$ tension in the surface tissues. Grant (1955) demonstrated that the blocking of succinic dehydrogenase with malonate protected the interior of frozen ground meat from discoloration. This treatment eliminated practically all O$_2$ uptake, since the pigment remained in the oxygenated state. However, malonate did not prevent the oxidation of myoglobin in surface tissue of steaks held at refrigerator temperature, although it did greatly reduce O$_2$ uptake of contaminating bacteria. Iodoacetate, inhibitor of glyceraldehyde phosphate dehydrogenase (a glycolytic enzyme) and other sulfur-containing enzymes, was more effective than malonate on steak surface, but was without any measurable effect when the O$_2$ tension in the atmosphere was reduced to 10 mm Hg. It is believed that the reduction of O$_2$ tension in meat tissue, either by microbial growth or by physical means, resulted in a great increase in the reduced myoglobin, which, in turn, was oxidized by metabolic H$_2$O$_2$ produced either by the meat tissue or by the bacteria. If the O$_2$ tension is reduced enough, little or no H$_2$O$_2$ can be
formed and no appreciable oxidation occurs. Any oxidized myoglobin present was rapidly reduced under such conditions (Reddy and Carpenter, 1991; Arihara et al., 1993).

Stutz et al. (1991) compared volatile compounds in ground beef maintained at 10°C in atmospheres of 2 or 18% O₂ spoiled by indigenous microbial flora to those spoiled by inoculating isolates from spoiled ground beef. Similar putrid spoilage occurred at both concentrations of O₂, although the microbial flora differed. Samples stored at 2% O₂ spoiled in 10 days and those stored at 18% O₂ in 5 days. Fluorescent *Pseudomonas* was at a higher percentage (30%) in ground beef spoiled with 2% O₂ than in that under 18% O₂ (4%). The percentages of nonfluorescent *Pseudomonas* and *Moraxella* were higher in samples stored under 18% O₂ than in 2% O₂. Shaw and Latty (1982) stated that the nonfluorescent *Pseudomonas* strains predominated on meats. Sulzbacher (1952) noted generation times of 4.5 hours in ground beef, and 4.81 and 5.99 hours in ground pork for *Pseudomonas* type organisms at 44.6°F (7°C).

Hood (1975) indicated that the rate of discoloration in meat primarily depends on bacteriological and biochemical considerations, the latter becoming increasingly significant as bacterial effects are reduced. Jensen (1945) stated that microorganisms, both living and dead, and their enzymes on the surface of meat oxidized both fresh and cured meat pigments to metmyoglobin.
Safety Aspects of MAP Meats

There has been an increasing consideration of food safety issues regarding MAP meats. The great vulnerability of MAP meats from a safety standpoint is that a particular modified atmospheric condition may inhibit organisms that might warn consumers of spoilage while either allowing or promoting the growth of pathogens (Farber, 1991). In the past, the major concerns have been with anaerobic pathogens, especially the psychrotrophic, nonproteolytic clostridia. However, because of the incorporation of $O_2$ in MAP gases, the growth of psychrotrophic pathogens such as *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on refrigerated and ready-to-eat meat has been raised as the new safety issue of the MAP meats. With extended shelf-life, pathogens may be allowed to grow for an extended period of time and reach a dangerous level in meat products. Farber (1991) indicated that there are some MAP safety issues that have not been properly addressed, including product safety under conditions of temperature abuse, gas-flush system failure, and loss of packaging integrity.

The microorganisms that are pathogenic to humans (*Salmonella* spp. and *Campylobacter* spp.) were present on meats at the time of slaughter and at the retail level (National Research Council, 1987). Suppressed growth by spoilage organisms on pathogens in normal conditions works
as a safeguard for consumers. While MAP changes the atmospheric environment in the package, another concern for food safety is the possibility that the changed environment may encourage the growth of certain pathogens.

Investigations by Silliker and Wolfe (1980) showed that the growth of *Salmonella* in inoculated ground beef was affected by CO$_2$-enriched packaging. There were a thousand-fold higher counts of beef stored under air controls than those samples stored in CO$_2$-enriched packages after 10 days at 10°C. Sander and Soo (1978) used ice packed, vacuum, and vacuum back-flushed with two addition rates of CO$_2$ at $3.61 \times 10^{-4}$ and $7.22 \times 10^{-4}$ m$^3$/kg body weight to pack whole chickens and stored them at 1.1°C. Every 4 days, *Clostridium perfringens*, *Salmonella* spp., *Staphylococcus aureus*, and coliform were enumerated. Aerobic and anaerobic organisms were counted at 2 day intervals. Both aerobic and anaerobic microbial growth were limited by CO$_2$ back-flushed packaging, but there were no differences in the presence of potential pathogens. Gray et al. (1984) inoculated fresh chicken thighs with *Salmonella enteritidis* and *Staphylococcus aureus* to study the inhibitory effects of a combination of potassium sorbate/CO$_2$ treatment on these organisms during storage at 10°C. They reported the growth rate and maximum cell number of both organisms exposed to a 100% CO$_2$ atmosphere were reduced, even in the absence of the sorbate treatment. Marshall et al. (1991) showed that the
growth of *Pseudomonas fluorescens* was inhibited by MAP to a greater extent than the growth of *Listeria monocytogenes* in chicken nuggets. *Listeria monocytogenes* was still capable of growing at 3°, 7°, and 11°C, even though it was inhibited by MAP. The effectiveness of MAP on the growth of microorganism decreased as temperature increased.

MAP conditions may favor the growth of *Clostridium botulinum*, an obligate anaerobe, and its non-proteolytic strains known to produce toxin at temperatures as low as 3.3°C (Schmidt et al., 1961). Furthermore, it might be assumed that any abuse temperature would tend to increase the risk, while germination of *Clostridium botulinum* spores would be stimulated by CO$_2$ in the pack (Wynne and Foster, 1948). The main question, however, is whether CO$_2$ inhibition of the normal aerobic spoilage organisms and development of a slower-growing microflora could leave the consumer without any reliable indication that the meat had become unsafe to eat.

In comparison with pre-cooked products that require only minimum reheating, e.g. breaded and fried chicken, the use of MAP for raw meats is seen as less hazardous (Hotchkiss, 1988) because of the need for proper cooking before consumption, and hence destruction of any *Clostridium botulinum* toxin, which is heat-sensitive and can be inactivated by heating at 80°C for 10 min or boiling temperature for 3-5 min (Licciardello et al., 1967). The
possibility of improving safety by incorporating low levels of O\textsubscript{2} (range of 5-10\%, Hintlian and Hotchkiss, 1987) in the pack to prevent growth of anaerobes does not appear to be feasible for raw meats. Small amounts of O\textsubscript{2} would soon be taken up by residual respiration in the meat and would not necessarily prevent toxin formation by \textit{C. botulinum} (Genigeorgis, 1985).

The effects of modified atmosphere conditions on growth of spoilage bacteria diminishes outside the temperature range used for proper chill storage (0-4°C) and, with meats held at ambient temperature, toxin formation by \textit{C. botulinum} is little affected by gaseous environment. For example, Silliker and Wolfe (1980) showed that high CO\textsubscript{2} atmospheres had no significant effect on growth of \textit{C. botulinum} at 27°C on pork. More important is the relationship between the product becoming toxic and the onset of spoilage. Vacuum-packed raw beef, inoculated with spores of type A and B strains and stored at 25°C, was toxic after six days, but by this time, the meat had already spoiled (Hauschild et al., 1985). At lower temperatures, 100\% CO\textsubscript{2} has been found to delay growth of both \textit{C. botulinum} and \textit{C. perfringens} (Doyle, 1983). Also, CO\textsubscript{2} concentrations of 45-75\% delayed toxin production by \textit{C. botulinum} in pork stored at 15°C (Lambert et al., 1991). Thomas et al. (1984) packaged both whole and cut-up chickens separately in conventional stretch wrap in air, vacuum, and vacuum-CO\textsubscript{2} back-flushed environment (14-15
inch Hg), and held at 5°C. Mesophiles, psychrophiles, lactobacilli, Clostridium perfringens, salmonellae, and staphylococci were enumerated. They reported an atmosphere containing 75% CO$_2$, 15% N$_2$, and 10% O$_2$ effectively retarded the growth of Pseudomonas fragi, Salmonella typhimurium, Staphylococcus aureus, and Clostridium perfringens, but the difference in microbiological quality with different packaging was negligible.

Hintlian and Hotchkiss (1987) studied the effects of 75% CO$_2$ with 0, 2, 5, 10, and 25% O$_2$ (balance N$_2$) on mixed cultures of Pseudomonas fragi, Clostridium perfringens, Salmonella typhimurium, and Staphylococcus aureus. An atmosphere with 75% CO$_2$, 15% N$_2$, and 10% O$_2$ effectively retarded the growth of Pseudomonas fragi, Salmonella typhimurium, Staphylococcus aureus, and Clostridium perfringens. However, MAP was less effective in inhibiting Salmonella typhimurium. Baker et al. (1986) reported that 80% CO$_2$ (balance air) had significantly better inhibitory effects on the growth of Salmonella typhimurium, Pseudomonas fragi, and Staphylococcus aureus inoculated into ground chicken meat and broth when compared to air at 2, 7, and 13°C. No effect was observed for Clostridium sporogenes.

As with Clostridium, experience involving other food-borne pathogen suggests that any hazard is unlikely to be increased by modified atmosphere storage of meats. Most strains of Campylobacter spp. are unable to multiply at
temperatures below 30°C, but survival of this microaerophilic organism under modified atmosphere conditions could be favored by the virtual absence of O₂. However, Wesley and Stadelman (1985) studied the behavior of C. jejuni at 4°C on broilers in O₂-permeable packs and in packs containing 100% CO₂. The CO₂-enriched atmosphere had no detectable effect on the organism.

In ground beef inoculated with six strains of Salmonella and held at 10°C for 7 days, Silliker and Wolfe (1980) observed a 1000-fold decline in viability with 60% CO₂:25% O₂:15% N₂, but not in air after 10 days. Another food-borne pathogen that is sensitive to high concentrations of CO₂ is Staphylococcus aureus. In this case, there appeared to be a synergism between CO₂ concentration and temperature in controlling growth of the organism in MAP beef and chicken products (Hintlian and Hotchkiss, 1986). Salmonella showed evidence of inhibition in MAP cooked beef containing 75% CO₂, 15% N₂, and 10% O₂ (Hintlian and Hotchkiss, 1986).

Recently, attention has centered on the more psychrotrophic pathogens, particularly Listeria monocytogenes and Yersinia enterocolitica, both of which are capable of growth at 0-3°C. While Gill and Reichel (1989) showed that L. monocytogenes could grow between 0 and 10°C in high-pH beef under CO₂, or in vacuum packs, other studies have shown that some conditions are inhibitory. The growth
potential of *L. monocytogenes* on meats appears to be influenced both by the type of meat and by its pH value, so that growth would occur more readily and possibly at a lower temperature on high pH meat. Thus, Bolder et al. (1991) observed higher growth of *L. monocytogenes* at 0-4°C on chicken thigh portions with skin than breast portions without skin. In both cases, however, growth was markedly inhibited by 100% CO₂, while 20% CO₂ or vacuum packaging had much less effect. With skinless breast meat (pH 5.8), Hart et al. (1991) found that *L. monocytogenes* failed to grow at 1°C, even without MAP. At 6°C, some growth occurred under aerobic conditions before spoilage was evident, but not with 100% CO₂.

Concern over possible growth of *Y. enterocolitica* in MAP meats appeared to have originated from the study of Hanna et al. (1976), which showed the organisms resembling this species reached high levels during chill storage vacuum-packed beef and lamb. *Y. enterocolitica* is relatively common on meats of all kinds, including poultry, but the pig is regarded as the most important source of serotypes that are pathogenic to human. Strains isolated from meats other than pork are usually of the 'environmental', i.e. non-pathogenic type. Although *Yersinia* grow readily in vacuum packs and in packs containing 20% CO₂, higher CO₂ concentrations inhibit their growth on chill-stored pork (Enfors et al., 1979).
In conclusion, use of MAP for raw meats does not appear to increase the hazard from food-borne pathogens, especially when packs are held under chill conditions and used before the expiration date. On the contrary, evidence suggested that CO$_2$ alone, or at high concentration in combination with other gases, retarded growth of food-borne pathogens that would otherwise multiply during chill storage. However, Genigeorgis (1985) suggested that packaging of muscle foods under low partial pressure of O$_2$ should not extend to meat for retail sale. This is because of the potential hazard from *C. botulinum* and the risk of temperature abuse by consumers. Nevertheless, the real risk of botulism from such MAP products remains largely unresolved.
CHAPTER III

EFFECTS OF DISTRIBUTION AND DISPLAY GAS MIXTURES ON SHELF-LIFE PROPERTIES OF GROUND BEEF IN GAS EXCHANGE MODIFIED ATMOSPHERE PACKAGING
Introduction

Modified atmosphere packaging (MAP) can increase the shelf-life of fresh meat products, reduce economic loss, decrease distribution costs and improve product quality (Wolfe, 1980; Savell et al., 1981; Farber, 1991; Zhao et al., 1993; McMillin, 1994). Vacuum packaging (VP) and carbon dioxide-enriched (CO\textsubscript{2}) packaging have been the most widely used MAP in retail markets to extend the shelf-life of many meat products by 50-400% (Hotchkiss, 1988). However, meat products in VP or CO\textsubscript{2} packages have deoxymyoglobin pigments that possess a purple color, which is not acceptable to consumers (Allen and Pierson, 1986). A dynamic gas exchange system (McMillin, 1994) provides extended distribution and a prolonged display shelf-life with a bloomed (oxymyoglobin) red color by exchanging an inert distribution gas for high O\textsubscript{2} gas immediately before retail display (Mitchell, 1990). Ground beef is the major fresh item of beef in the United States, accounting for more than 50 percent of the total U.S. beef consumption (NLSMB, 1995).

The processes of mixing and grinding subject ground beef to increased quality degradation by increasing microbial contamination and oxidative deterioration (Madden and Moss et al., 1987; Ho et al., 1995). Lopez-Lorenzo et al. (1980) indicated that three major factors limiting the shelf life of chilled meat were spoilage by slime-forming
psychrotrophic bacteria, lipid oxidation, and metmyoglobin (MetMb) formation. It is of great interest and importance to meat processors to improve the storage stability of refrigerated ground beef because of economic and food safety considerations. For a successful centralized packaged operation, it is necessary to maintain the quality of meat for at least two weeks during distribution (Morgan et al., 1991). Previous reports showed that CO$_2$ in meat packages effectively decreased the growth of aerobic spoilage microorganisms (Clark and Lentz, 1969; Taylor, 1972), but produced brown discoloration (Hall et al., 1980; Seideman et al., 1980; Fu et al., 1992), and package collapse (Zhao et al., 1995).

High O$_2$ gas mixtures produced a bright-red color on the meat surface, however, lipid oxidation and myoglobin autoxidation occurred rapidly and were the shelf-life limiting factors in ground meat products because of the increased ratio of volume to surface area (Lopez-Lorenzo et al., 1980). High O$_2$ produced a bright-red color on meat surfaces, but accelerated the lipid instability in ground beef at 4.4°C (Huang et al., 1993a). Cornforth et al. (1985) found that normally dark, intact, pre-rigor muscles turned bright red when held at both low temperatures (3°C) and high O$_2$ for 30 min. This effect was reversible and the muscles turned dark when returned to air at room temperature (20°C); however, actual O$_2$ concentration in the package was
not given in that study. Price and Schweigert (1987) indicated that muscle would not normally bloom until the pH of the muscles fell below about 6.0. Rizvi (1981) indicated that myoglobin of beef muscles packaged in polyvinylchloride (PVC) overwrapping packaging was easily oxygenated and expressed as a bloomed red color with greater than 40 torr partial pressure of \(O_2\). However, Pierson et al. (1970) indicated that color fading and oxidation of the pigments to brown metmyoglobin form occurred in meat with PVC within 5 days of storage at 38°C because the \(O_2\) in air depleted the natural meat oxidation-reduction systems that maintained the bloomed meat color.

Huang et al. (1993a) determined that effects of different distribution temperatures on MAP ground beef packaged in 80% \(N_2:20\% CO_2\) distribution gas and gas exchanged for 80% \(O_2:20\% CO_2\) display gas on day 14 and reported that ground beef distributed at 4.4°C had higher HunterLab "a" values and psychrotrophic plate counts than patties stored at -12.2 and -3.8°C during retail display. Ho et al. (1995) indicated that MAP ground beef patties packaged with 80% \(N_2:20\% CO_2\) at 4.4°C distribution storage and gas exchanged on day 13 for 80% \(O_2:20\% CO_2\) display gas had higher HunterLab "L" values and microbial growth than patties stored at -4.4 and 0°C, while storage temperature did not influence the oxidative stability of the ground beef. Microbial growth increased with the increased gas
exchange time for MAP beef loin steaks (McMillin et al., 1994b).

McMillin et al. (1994c) packaged beef steaks in gas exchange MAP with 80% N\textsubscript{2}:20% CO\textsubscript{2} or VP and then gas exchanged with 80% O\textsubscript{2}:20% CO\textsubscript{2} or 60% O\textsubscript{2}:40% CO\textsubscript{2} for MAP samples or changed to PVC overwrapping for VP samples on day 16 post-packaging. There were no differences in HunterLab "L", "a", "b" values, pH, microbial growth rates, lipid stability, and weight loss due to different display gas mixtures. However, VP-PVC samples had lower HunterLab "L" values than MAP samples gas exchanged with 80% O\textsubscript{2}:20% CO\textsubscript{2} display gas, but had higher "a" values than MAP samples gas exchanged with 60% O\textsubscript{2}:40% CO\textsubscript{2}. Steaks with VP-PVC had higher microbial growth rate and lower weight retention when compared with other MAP treatments. Ho et al. (1995) reported that no differences were observed for gas exchange MAP beef loin steak (80% N\textsubscript{2}:20% CO\textsubscript{2} or 50% N\textsubscript{2}:50% CO\textsubscript{2} for distribution and 80% O\textsubscript{2}:20% CO\textsubscript{2} or 60% O\textsubscript{2}:40% CO\textsubscript{2} for display) among different gas treatments, but lipid oxidation increased with the increased storage time. The objective of this study was to identify the levels of CO\textsubscript{2}:O\textsubscript{2}:N\textsubscript{2} gas mixtures for optimal shelf-life during distribution and retail display of case-ready ground beef in a dynamic gas exchange MAP system.
Materials and Methods

Two beef steers (Angus breed, 118 days on feed) of choice or high select quality were slaughtered and the carcasses were chilled to 4.4°C in the Louisiana State University Agricultural Center Meat Laboratory. The ground beef manufactured from chuck rolls (infraspinatus and supraspinatus) was ground through a 1.27 cm plate and then through a 0.32 cm plate using a Butcher Boy Grinder (Model TCA 32, LASAR MFG. Comp., Los Angeles, CA). Square patties (10.16 cm x 10.16 cm) were formed by Hollymatic® Super Food Portioning Machine (Model 54, Hollymatic Co., Countryside, IL) with an average weight of 113.4 g. Patties were randomly assigned to MAP or VP treatments. Patties for MAP were packed (InPack tray sealer, Model 580, Ross Industries, Midland, VA) with an absorbent pad (Dri-Loc® 50 pad, Sealed Air Co., Food Packaging Div., Patterson, NC) in barrier laminated foam trays (SealFresh™, 3P White, Amoco Foam, Atlanta, GA; oxygen permeability of 1.55 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 3.10 g/m²/24 hr at 38°C, 90% R.H.) using lidding film (Type Curlam, Curwood, New London, WI; oxygen permeability of 0.1 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 1.5 cc/m²/24 hr at 22.8°C, 0% R.H.) in distribution gas mixtures of 20% N₂:80% CO₂, 50% N₂:50% CO₂, or 80% N₂:20% CO₂. Patties for VP were placed on absorbent pads and packaged in vacuum pouches (3 mil nylon/polyethylene,
20.32 cm x 25.4 cm, Koch Supplies Inc., Kansas City, MO; oxygen permeability of 9.3 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 9.3 cc/m²/24 hr at 23°C, 90% R.H.) using a vacuum machine (Model VM200H, Westglen Co., Los Angeles, CA). All packages contained one patty and were stored at -1°C in cardboard boxes during distribution storage. After 15 days of post-packaging storage, gaseous contents of MAP were exchanged (Windjammer, Pakor, Inc., Livingston, TX) for 80% O₂:20% CO₂:0% N₂, 50% O₂:50% CO₂:30% N₂, or 20% O₂:20% CO₂:60% N₂ before display under simulated retail conditions of 7°C and 1345 lux cool white fluorescent light. VP patties were removed from pouches, transferred to foam trays for polyvinyl chloride film (PVC, Borden Resinite, O₂ transmission rate of 325 cc/cm²/24 hr at 23°C, 0% R.H.; CO₂ transmission rate, 2,500 cc/cm²/24 hr at 23°C, 90% R.H.) overwrapping, and then displayed under the same conditions as patties in MAP. Duplicate packages of each treatment combination were randomly sampled on day 0, 8, 15 (day 0 after gas exchange) and at 2 day intervals after gas exchange until day 21.

**HunterLab "L", "a", and "b" values**

Objective color as HunterLab "L", "a", and "b" values was measured using a Hunter Associates LabScan reflectance spectrophotometer, (Model LABSCAN-2 0/45, Hunter Associate Laboratory, Inc., Reston, VA). Samples were removed and analyzed within 30 sec after opening the packages. "L"
(lightness), "a" (degree of red/green), and "b" (degree of yellow/blue) were averaged on each patty by rotating 90° between sample readings. A white plate ("L" = 92.4, "a" = -0.7, and "b" = -0.9) and a black plate were used for instrument standardization described by the manufacturer.

**Metmyoglobin formation**

Reflectance at wavelengths that were isobestic (equal reflectance for two or more of the three myoglobin forms, Hunt et al., 1991) for different forms of myoglobin was measured and converted to K/S ratios. Metmyoglobin formation was estimated using K/S ratio of spectral reflectance (K/S 572 nm)/(K/S 525 nm) and calculations as described by Stewart et al. (1965).

**Headspace O₂ and CO₂ concentrations**

Headspace O₂ and CO₂ were measured with a Food Package Analyzer (1450, Servomex, Sussex, England), allowing 20 sec for equilibration of readings per sample.

**Weight loss**

For determination of exudation and weight retention during storage, ground beef and absorbent pads in each tray were weighed separately at the time of initial packaging and at the time of sampling. Weight loss was calculated as the difference of final sample weight and initial sample weight divided by the initial weight for ground beef patties.
Thiobarbituric acid reactive substances (TBARS) test

Lipid stability analyses were performed by determining TBARS values using a distillation method as outlined by Tarladgis et al. (1960). The distillate optical absorbances were determined by using a U-2000 spectrophotometer (Hitachi, Conroy, TX) at 535 nm.

Psychrotrophic plate counts (PPC)

Microbial sampling was conducted on day 1, 8, 15, 17, 19 and 21 post-packaging. Psychrotrophic plate counts (PPC) were determined by standard plate count procedures (APHA, 1976). Ten grams of sample were excised and placed in a sterile Stomacher bag with 90 mL sterile 0.1% peptone solution and pummeled for 1 min (Stomacher Lab-Blender 400, Model No. STO-400, Tekmar Co., Cincinnati, OH). Using a "spreading" method (Vanderzant and Splittstoesser, 1992), suitable serial dilutions of the beef-peptone homogenate were plated with Standard Plate Count Agar (Bacto®-Peptone, DIFCO Laboratories Inc., Detroit, MI) in duplicate and plates were incubated at 6°C for 10 days. The average number of colonies from the duplicate plates were reported as log colony forming units (CFU)/g meat sample.

Statistical analyses

The statistical model of the present study was a split-plot design with main plot represented by 3 x 3 factorial arrangement of distribution gas mixture and display gas mixture treatment combinations and VP-PVC as the control and
the sub-plot was represented by storage time. Animal sources were used as the replication and data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS, 1985). Treatment means were separated by least square means procedures if a significant difference was detected at $P<0.05$ level.

Results and Discussion

The initial composition of ground beef patties was 66.5% water, 18.9% protein and 14.6% fat. Each steer was used as replication in the data analyses and results are shown in Table A.3.1. ANOVA (Table A.3.2) indicated that CO$_2$ and O$_2$ in the atmospheres of different MAP treatments were affected ($P<0.05$) by animal source, packaging treatment, storage time, and interactions of packaging and storage time. However, after package atmosphere changes, O$_2$ decreased ($P<0.05$) and CO$_2$ increased ($P<0.05$) in the packages as the display time increased (Figure 3.1). The interaction of gas treatment and display time was not significant ($P>0.05$) in the gas concentrations of MAP packages. Table 3.1 presents the actual concentrations of CO$_2$ and O$_2$ in each treatment combination before and after the gas exchange operation. The gas exchange system could not completely change the gas atmospheres in the packages to equal the O$_2$ and CO$_2$ in the gas mixtures. In MAP samples containing 80%, 50%, and 20% CO$_2$ in the distribution gas,
Figure 3.1 - Average gas concentrations of $O_2$ (SEM = 1.54) and $CO_2$ (SEM = 0.62) after gas exchange in MAP with ground beef during refrigerated storage. Bars for same gas with same letter were not different ($P > 0.05$).
Table 3.1 - Concentrations of O$_2$ and CO$_2$ in packages of ground beef patties on day 15 before and after gas exchange.

<table>
<thead>
<tr>
<th>Dist. 1</th>
<th>Disp. 2</th>
<th>CO$_2$ %</th>
<th>O$_2$ %</th>
<th>BF$^3$</th>
<th>AF$^4$</th>
<th>BF$^3$</th>
<th>AF$^4$</th>
<th>Eff. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$%:O$_2$%</td>
<td>O$_2$%:CO$_2$%:N$_2$%</td>
<td>BF$^3$</td>
<td>AF$^4$</td>
<td>BF$^3$</td>
<td>AF$^4$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80:20</td>
<td>80:20:0</td>
<td>79.9</td>
<td>32.7</td>
<td>0</td>
<td>54.0</td>
<td>67.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50:20:30</td>
<td>79.9</td>
<td>33.5</td>
<td>0</td>
<td>30.2</td>
<td>60.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:20:60</td>
<td>79.9</td>
<td>31.1</td>
<td>0</td>
<td>15.9</td>
<td>79.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50:50</td>
<td>80:20:0</td>
<td>39.7</td>
<td>26.3</td>
<td>0</td>
<td>58.8</td>
<td>73.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50:20:30</td>
<td>39.7</td>
<td>26.2</td>
<td>0</td>
<td>38.0</td>
<td>77.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:20:60</td>
<td>39.7</td>
<td>27.0</td>
<td>0</td>
<td>14.5</td>
<td>72.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:80</td>
<td>80:20:0</td>
<td>18.1</td>
<td>18.6</td>
<td>0</td>
<td>63.3</td>
<td>79.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50:20:30</td>
<td>18.1</td>
<td>19.8</td>
<td>0</td>
<td>39.7</td>
<td>79.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:20:60</td>
<td>18.1</td>
<td>19.9</td>
<td>0</td>
<td>14.5</td>
<td>72.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>73.6</strong></td>
</tr>
</tbody>
</table>

3. BF (Before): gas concentration before gas exchange. The value was the average of two packages from each distribution gas treatment.
4. AF (After): gas concentration after gas exchange.
5. Efficiency of gas exchange process = O$_2$ in package / O$_2$ in the gas mixture x 100%.
6. The O$_2$ concentrations in packages were below the limit that the gas analyzer could detect.

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the percentages of CO$_2$ for display were 32.4%, 26.5%, and 19.4%, respectively. Oxygen was lower than the level of detection for the gas analyzer in the initial distribution gas mixtures. After gas exchange, O$_2$ was 58.7%, 36.0%, and 15.0% in MAP packages. The equipment used in the present study had an average relative efficiency of gas exchange of 73.6% based upon the O$_2$ after gas exchange.

Results of analyses of variance indicated that weight loss of the ground beef patties was affected (P<0.05) by animal source, packaging treatments and storage time (Table A.3.3). Weight loss increased (P<0.05) with extended storage time (Table 3.2). VP-PVC samples had higher (P<0.05) percentages of weight loss than MAP samples (Table 3.3). Different gas mixtures in MAP packages had no (P>0.05) effect on the weight loss of ground beef patties. Lee et al. (1985) indicated that the weight losses of pork in MAP were identical to VP samples, while Gill (1988) observed that high CO$_2$ gave a more viscous exudate. Seman et al. (1989) reported deer loins in 100% CO$_2$ were higher in drip loss than VP samples. The data indicates that MAP packages reduced the weight loss of ground beef patties during refrigerated storage to approximately one tenth that
Table 3.2 - Weight loss and pH values of ground beef patties during refrigerated storage.

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>Trait</th>
<th>Weight Loss</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0.26&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>1.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.83&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>1.64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.83&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>2.45&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.86&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>0.26</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Weight Loss: (initial weight-final weight)/initial weight x 100%

2 SEM: standard error of the mean.

<sup>abcde</sup> means in column with same letter were not different (P>0.05).
Table 3.3 - Effects of gas treatments on weight loss and pH of ground beef patties during retail display.

<table>
<thead>
<tr>
<th>Distribution CO₂%:N₂%</th>
<th>Display O₂%:CO₂%:N₂%</th>
<th>Weight Loss¹</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>80:20</td>
<td>80:20:0</td>
<td>0.75ᵃ</td>
<td>5.85ᵃ</td>
</tr>
<tr>
<td></td>
<td>50:20:30</td>
<td>0.97ᵃ</td>
<td>5.85ᵃ</td>
</tr>
<tr>
<td></td>
<td>20:20:60</td>
<td>0.81ᵃ</td>
<td>5.85ᵃ</td>
</tr>
<tr>
<td>50:50</td>
<td>80:20:0</td>
<td>1.04ᵃ</td>
<td>5.89ᵃᵇ</td>
</tr>
<tr>
<td></td>
<td>50:20:30</td>
<td>0.79ᵃ</td>
<td>5.84ᵃ</td>
</tr>
<tr>
<td></td>
<td>20:20:60</td>
<td>0.87ᵃ</td>
<td>5.88ᵃᵇ</td>
</tr>
<tr>
<td>20:80</td>
<td>80:20:0</td>
<td>1.22ᵃ</td>
<td>5.91ᵇ</td>
</tr>
<tr>
<td></td>
<td>50:20:30</td>
<td>1.17ᵃ</td>
<td>5.91ᵇ</td>
</tr>
<tr>
<td></td>
<td>50:20:60</td>
<td>0.94ᵃ</td>
<td>5.90ᵇ</td>
</tr>
<tr>
<td>VP</td>
<td>PVC²</td>
<td>8.95ᵇ</td>
<td>5.91ᵇ</td>
</tr>
<tr>
<td>SEM³</td>
<td></td>
<td>0.47</td>
<td>0.025</td>
</tr>
</tbody>
</table>

¹ Weight Loss: (initial weight-final weight)/initial weight x 100%

² PVC: polyvinylchloride.

³ SEM: standard error of the mean.

abc means in column with same letter were not different (P>0.05).
of VP (Table 3.3). The results confirmed other studies with gas exchange MAP (Huang et al., 1993a, b; Ho et al., 1995).

ANOVA (Table A.3.3) indicated that the pH values were influenced (P<0.05) by animal source, packaging treatment, and storage time. The initial pH value of the raw ground beef was 5.2. Patties in 80% and 50% CO$_2$ distribution gas had lower (P<0.05) pH values than in 20% CO$_2$ (Table 3.3). Bendall (1972) indicated that dissolution of CO$_2$ in muscle tissue produced a fall in pH regardless of the buffering capacity of the tissue. pH values (Table 3.2) show a decreasing (P<0.05) trend with the increasing storage time. This decline in pH values during storage might be due to the accumulations of lactic acid because of muscle anaerobic respiration and/or the degradation products of carbohydrates because of microorganism growth.

Analyses of variance indicated that psychrotrophic plate counts were affected (P<0.05) by animal source, packaging treatment, storage time, and the interactions of packaging and storage time (Table A.3.4). Psychrotrophic plate counts were higher (P<0.05) in VP-PVC samples than in MAP samples (Table 3.4). Patties in 80% CO$_2$:20% N$_2$ distribution gas had lower microbial counts than other treatments, except for 50% N$_2$:50% CO$_2$ exchanged for 50%
Table 3.4 - Effects of gas treatments on ground beef patties during retail display.

<table>
<thead>
<tr>
<th>Distribution CO₂%:N₂%</th>
<th>Display O₂%:CO₂%:N₂%</th>
<th>PPC¹</th>
<th>K/S Ratio²</th>
</tr>
</thead>
<tbody>
<tr>
<td>80:20</td>
<td>80:20:0</td>
<td>4.75ab</td>
<td>1.17abc</td>
</tr>
<tr>
<td></td>
<td>50:20:30</td>
<td>4.77ab</td>
<td>1.66bc</td>
</tr>
<tr>
<td></td>
<td>20:20:60</td>
<td>4.69a</td>
<td>1.70bc</td>
</tr>
<tr>
<td>50:50</td>
<td>80:20:0</td>
<td>5.15c</td>
<td>1.87ab</td>
</tr>
<tr>
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<td>1.75abc</td>
</tr>
<tr>
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<td>5.19cd</td>
<td>1.61c</td>
</tr>
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<td>80:20:0</td>
<td>5.59e</td>
<td>1.93a</td>
</tr>
<tr>
<td></td>
<td>50:20:30</td>
<td>5.52de</td>
<td>1.80abc</td>
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<tr>
<td></td>
<td>20:20:60</td>
<td>5.71e</td>
<td>1.65bc</td>
</tr>
<tr>
<td>Vacuum</td>
<td>PVC³</td>
<td>6.73f</td>
<td>1.69bc</td>
</tr>
</tbody>
</table>

SEM⁴ | 0.36 | 0.23 |

¹ PPC: psychrotrophic plate counts, log CFU/g meat.
² K/S Ratio: higher K/S ratio indicates less metmyoglobin formation.
³ PVC: polyvinylchloride.
⁴ SEM: standard error of the mean.

abcdef means in column with same letter were not different (P>0.05).
O₂:20% CO₂:30% N₂. Increased CO₂ in distribution gases decreased (P<0.05) the microbial growth rates (Figure 3.2). Hence, the bacteriostatic activity of CO₂ on ground beef patties was more effective (P<0.05) than VP treatment. MAP samples with 20% CO₂ during distribution had the highest (P<0.05) microbial growth among MAP treatments. McMillin et al. (1994c) packaged beef steaks in 80% N₂:20% CO₂ and VP then gas exchanged with 80% O₂:20% CO₂ or 60% O₂:40% CO₂ for MAP samples or changed to PVC overwrapping for VP samples on day 16 post-packaging. They found no differences in psychrotrophic plate counts due to different display gas mixtures, but indicated that psychrotrophic plate counts were higher in VP-PVC samples than MAP treatments.

HunterLab "L" values indicate the lightness of the tested samples (Hunter and Harold, 1987). HunterLab "L" values were affected (P<0.05) by animal source, packaging treatment, storage time, and the interactions of packaging and storage time (Table A.3.4). VP-PVC patties had lower HunterLab "L" values than MAP patties (P<0.05), except for patties in 20% CO₂:80% N₂ distribution gas and gas exchanged with 50% O₂:20% CO₂:30% N₂ and 20% O₂:20% CO₂:60% N₂ (Table 3.5). McMillin et al. (1994b) indicated that gas exchange MAP ground beef patties with 80% O₂:20% CO₂ display gas increased in HunterLab "L" values than VP samples. Georgala
Figure 3.2 - Psychrotrophic plate counts of ground beef patties in different distribution gas mixtures during refrigerated storage (SEM = 0.10).
Table 3.5 - Effects of gas treatments on ground beef patties during retail display.

| Distribution | Display | HunterLab | | |
|--------------|---------|-----------|---|---|---|
| CO₂%:N₂% | O₂%:CO₂%:N₂% | "L" | "a" | "b" |
| 80:20 | 80:20:0 | 37.66^a | 14.54^abc | 9.71^ab |
| | 50:20:30 | 37.56^ab | 13.68^c | 9.42^abcd |
| | 20:20:60 | 36.17^ab | 13.54^c | 9.23^cd |
| 50:50 | 80:20:0 | 37.03^ab | 15.97^ab | 9.78^a |
| | 50:20:30 | 36.71^ab | 14.38^bc | 9.32^bcd |
| | 20:20:60 | 36.99^ab | 12.92^c | 8.98^de |
| 20:80 | 80:20:0 | 36.20^ab | 16.48^a | 9.58^abc |
| | 50:20:30 | 35.76^bc | 14.85^abc | 9.00^de |
| | 20:20:60 | 35.64^bc | 12.96^c | 8.77^e |
| Vacuum | PVC | 34.34^c | 13.92^c | 9.22^cde |

SEM^2 | 1.51 | 2.03 | 0.45 |

1 PVC: polyvinylchloride.
2 SEM: standard error of the mean.

Means in column with same letter were not different (P>0.05)
and Davidson (1970) reported that color purity increased with increased levels of $O_2$ in MAP.

Major ($P<0.05$) effects on ground beef patties were observed for HunterLab "a" values in ANOVA from animal source, packaging treatment, storage time, and the interactions of packaging and storage time (Table A.3.5). MAP patties that were gas exchanged with 80% $O_2$:20% $CO_2$ had higher ($P<0.05$) HunterLab "a" values than other treatments, except samples that were in 80% $N_2$:20% $CO_2$ distribution gas. HunterLab "b" values of ground beef patties were affected by animal source, packaging treatment, storage time, and the interactions of packaging and storage time (Table A.3.5). The VP-PVC patties had lower ($P<0.05$) HunterLab "b" values (less yellowness) than patties in 80% $CO_2$:20% $N_2$ and 50% $CO_2$:50% $N_2$ distribution and gas exchanged for 80% $O_2$:20% $CO_2$ display gas mixture (Table 3.5). However, the meaning of HunterLab "b" values related to bloomed color of meat samples is more difficult to interpret, while HunterLab "a" values, which refer to redness, are more meaningful in the subjective color measurement of meat samples. More results and discussion for HunterLab "L", "a", and "b" values regarding different distribution gases, display gases, storage time and interactions are presented in later sections.

Wavelength of 610 nm is isobestic for deoxymyoglobin and metmyoglobin, 572 nm for deoxymyoglobin and
oxymyoglobin, and 474 nm for oxymyoglobin and metmyoglobin. Isobestic wavelengths are where compounds have the same reflectance. Therefore, \((K/S\ 572\ \text{nm})/(K/S\ 525\ \text{nm})\) was used as the indicator for metmyoglobin formation. Higher values of \((K/S\ 572\ \text{nm})/(K/S\ 525\ \text{nm})\) indicate decreased metmyoglobin formation on the surface. \(K/S\) ratios were influenced by animal source, packaging treatment, storage time, and the interactions of packaging and storage time (Table A.3.6). Patties in 80% CO\(_2\):20% N\(_2\) and gas exchanged with 80% O\(_2\):20% CO\(_2\) had lower (\(P<0.05\)) \(K/S\) ratios (higher levels of metmyoglobin) than samples in 50% CO\(_2\):50% N\(_2\) and 20% CO\(_2\):80% O\(_2\) distribution gas and gas exchanged with 80% O\(_2\):20% CO\(_2\) (Table 3.4). Therefore, patties packaged in 80% CO\(_2\):20% N\(_2\) distribution gas needed a higher level of O\(_2\) in the display gas to oxygenate the myoglobin to oxymyoglobin (bloom the red color) and reduce the formation of metmyoglobin on the surface. Lynch et al. (1986) reported that the purple color of deoxymyoglobin pigment resulting from MAP with inert gases or vacuum packaging decreased customer acceptance of the meat products. Metmyoglobin formation on the meat surface is a limiting factor for the marketing of MAP meats (Seideman et al., 1980; Asensio et al., 1988; Przybylski et al., 1989).

To further clarify the influences of different distribution and display gases on the MAP ground beef patties, the data (excluding VP-PVC) was analyzed as a
split plot design of a 3 (distribution gases) x 3 (display gases) factorial arrangement as the main plot and storage time (Day 1, 8, 15, 17, 19, and 21) as the sub-plot. ANOVA (Table A.3.7) indicated that weight loss of ground beef patties increased (P<0.05) with increased storage time (Table 3.2), but was not influenced (P>0.05) by either distribution or display gas mixtures.

pH values of the ground beef patties were affected (P<0.05) by distribution gas and storage time (Table A.3.7), but no interactions among treatments were observed (P>0.05). Samples with higher CO₂ in the distribution gas mixture resulted in higher pH values. The carbonic acid formed in the ground beef patties because of the dissolution of CO₂ in the headspace gas mixtures (Enfors et al., 1979) might have resulted in the lower pH values in samples with higher CO₂ distribution gas mixtures. pH values of ground beef patties were higher (P<0.05) on day 0 and day 15 than other storage periods, while pH values increased (P<0.05) immediately after gas exchange on day 15 and then decreased (P<0.05) as storage time increased.

Psychrotrophic microorganism growth was mainly affected (P<0.05) by distribution gases, storage time and distribution gases and time interaction (Table A.3.8). Growth of psychrotrophs was inhibited by distribution gas mixtures with higher CO₂ (P<0.05) and increased (P<0.05) during storage with different rates in each gas treatment.
(Figure 3.2). However, there were no differences (P>0.05) in the psychrotrophic growth rates among different display gas mixtures. Psychrotrophic microorganism growth rates in MAP packaged ground beef patties were determined primarily by the distribution gas mixtures rather than display gas mixtures (P<0.05), probably because of the CO$_2$ concentrations.

HunterLab "L" values were also influenced (P<0.05) by distribution gases, storage time and distribution gases and time interaction (Table A.3.8). Figure 3.3 presents the changes in HunterLab "L" values of ground beef patties in various distribution gas mixtures during the refrigerated storage. Patties with 20% CO$_2$:80% N$_2$ had lower (P<0.05) HunterLab "L" values and expressed a darker color than patties with 80% CO$_2$:20% N$_2$ and 50% CO$_2$:50% N$_2$. Distribution gas mixtures with higher CO$_2$ resulted in a lighter (P<0.05) surface color of ground beef patties during storage. Ledward (1970) indicated that meat stored in high concentrations of CO$_2$ often developed a grayish-tinge and believed it was due to the lowering of the pH and subsequent precipitation of some of the sarcoplasmic protein.

HunterLab "a" values of the ground beef patties were affected (P<0.05) by display gas mixtures (Table A.3.9) instead of distribution gas mixtures (P>0.05). Ground beef patties that had higher (P<0.05) HunterLab "a" values (redder) were gas exchanged for 80% O$_2$:20% N$_2$ than for 50%
Figure 3.3 - HunterLab "L" values of ground beef patties in different distribution gas mixtures during refrigerated storage (SEM = 0.43).
O₂:20% CO₂:30% N₂ and 20% O₂:20% CO₂:60% N₂ gas mixtures
(Figure 3.4). Rikert et al. (1958) indicated that fresh
meat stored under various partial pressures of O₂ still lost
redness, but high O₂ temporarily delayed the discoloration.
It was indicated that no increased benefit was derived from
the use of O₂ at partial pressures higher than those of air
(Rikert et al., 1958). However, in the present study, high
CO₂ (80%) resulted in beef patties having a darker surface
color than patties packaged in 50% and 20% CO₂ during
distribution, but did not affect the bloomed color (redness)
of ground beef patties during display storage after gas
exchange with different O₂ levels of display gas mixtures.

The amount of yellowness was shown by the HunterLab "b"
values (Hunter and Harold, 1987). HunterLab "b" values were
influenced by the interaction of display gas and storage
time (Table A.3.9). Patties packaged in 80% O₂:20% N₂ had
higher (P<0.05) HunterLab "b" values than patties that were
gas exchanged with other display gases during storage
(Figure 3.6). HunterLab "b" values increased (P<0.05) after
gas exchange on day 15 and reached a higher level on day 17,
but HunterLab "b" values dropped (P<0.05) with display time
after day 17. Huang et al. (1993a) found that HunterLab "b"
values were higher in MAP ground beef packaged with 80%
O₂:20% CO₂ stored at 4.4°C than gas exchange MAP samples
with 80% N₂:20% CO₂ distribution gas and 80% O₂:20% CO₂ for
Figure 3.4 - HunterLab "a" values of ground beef patties in different display gas mixtures during refrigerated storage (SEM = 0.48).
Figure 3.5 - HunterLab “b” values of ground beef patties in different display gas mixtures during refrigerated storage (SEM = 0.13).
display at -12.2, -3.8, and 4.4°C distribution temperature during storage.

However, ANOVA (Table A.3.10) of K/S ratios in this study indicated that metmyoglobin formation of the ground beef patties were affected (P<0.05) by display gas mixtures and storage time, and interactions of display gas and storage time. Higher K/S ratios indicated less metmyoglobin formation on the surface of meat samples, and the K/S ratios decreased (P<0.05) at the extended display time (Figure 3.5). Greene et al. (1971) claimed that consumers rejected beef at metmyoglobin concentration greater than 40%. Meat color is usually considered as the most critical appearance factor, because consumers associate the bright red retail meat with good quality (Taylor, 1982). Hood and Riordan (1973) indicated that there was a linear increase in discrimination by consumers against discolored meat as the percentage of metmyoglobin increased in the fresh beef.

Concentrations of CO₂ in the distribution gas mixtures had no effects (P>0.05) on HunterLab "a" values and metmyoglobin formation. Metmyoglobin formation was lower (P<0.05) in 80% O₂:20% CO₂ samples than samples with 50% O₂:20% CO₂:30% N₂% and 20% O₂:20% CO₂:60% N₂. Ledward (1970) found that no metmyoglobin formation would occur in atmospheres containing high CO₂ as long as the O₂ partial pressure was above a limiting concentration of 5%. However, results in the present study do not agree with this. Higher O₂ levels of
Figure 3.6 - Metmyoglobin formation \((K/S \ 572 \ \text{nm})/(K/S \ 525 \ \text{nm})\) of ground beef patties in different display gas mixtures during refrigerated storage \((SEM = 0.053)\).
the display gases bloomed the red color of beef patties more effectively and reduced the formation of metmyoglobin on the beef patties more than patties in display gases with lower \( \text{O}_2 \). K/S ratio was highly correlated (\( P<0.05 \)) to HunterLab "a" values of MAP ground beef patties (Table 3.6). Ordonez and Ledward (1977) indicated that the formation of metmyoglobin at the surface of the fresh pork muscles was independent of \( \text{CO}_2 \) concentration, while data in the present study indicates the metmyoglobin formation on the ground beef patties more likely depended on the \( \text{O}_2 \) in the display gas mixtures, regardless of the \( \text{CO}_2 \) in distribution gas mixtures for gas exchange MAP system. Bartkowski et al. (1982) reported that \( \text{CO}_2 \) at 15% inhibited the microbial growth, but did not promote darkening of the beef steaks. However, we found that 80% \( \text{O}_2:20\% \text{CO}_2 \) display gas mixture was capable of blooming the ground beef patties packaged with 80, 50, or 20% \( \text{CO}_2 \) after 15 days of distribution storage.

ANOVA (Table A.3.10) indicated that the values of thiobarbituric acid reactive substances (TBARS) were affected by animal source, display gas mixtures, and storage time. Distribution gas mixtures did not (\( P>0.05 \)) influence the oxidative stability of ground beef samples. No interaction (\( P>0.05 \)) among the treatments was observed in the study. TBARS values were similar (\( P>0.05 \)) among different display gas treatments, but were higher (\( P<0.05 \))
Table 3.6 - Correlation coefficients\(^1\) of HunterLab "L", "a", and "b" values, K/S ratio, and TBARS values.

<table>
<thead>
<tr>
<th></th>
<th>L(^1)</th>
<th>a(^2)</th>
<th>b(^3)</th>
<th>K/S(^4)</th>
<th>TBARS(^5)</th>
</tr>
</thead>
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<tr>
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<td>.</td>
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</tr>
<tr>
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<td>.</td>
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<td>0.35</td>
</tr>
<tr>
<td>K/S</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>1.00</td>
<td>-0.52</td>
</tr>
<tr>
<td>TBA</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>1.00</td>
</tr>
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</table>

\(^1\) Correlation coefficients with associated probability values.

\(^2\) L = HunterLab L values.

\(^3\) a = HunterLab a values.

\(^4\) b = HunterLab b values.

\(^5\) K/S = K/S ratio.

\(^6\) TBARS = thiobarbituric acid reactive substance values (mg malondialdehyde/kg meat).
in samples that were gas exchanged with 80% O₂:20% CO₂ display gas than other display gases and increased (P<0.05) with extended display time (Figure 3.7). Ground beef patties displayed in a gas mixture containing 80% O₂ had more oxidation than displayed in gas mixtures containing lower O₂. Oxygen atmospheres are often used in MAP for the retention of color acceptable to consumers. Taylor et al. (1990) indicated the use of O₂ limited the shelf life of most meats to approximately one week because of the growth of microorganisms and lipid oxidation; however, with the gas exchange MAP technology using CO₂ and O₂, the shelf-life of ground beef could be extended to two weeks for distribution storage and one more week for retail display with a bloomed red color. Lopez-Lorenzo et al. (1980) evaluated the effect of O₂- and CO₂-enriched atmospheres on refrigerated ground pork and found O₂-enriched (80% O₂) packages with 20% CO₂ greatly depressed the lipid oxidation rate and extended the storage time from 5 days to 12 days when compared with packages containing 100% O₂, but without CO₂. Our data agreed with their results regarding higher O₂ resulting in higher lipid oxidation, but the shelf-life for the ground beef patties in the present study after 2 weeks of distribution storage was about 7 days when considering the microbial growth and discoloration. Lopez-Lorenzo et al. (1980) also indicated that the time needed for ground pork to reach 40% discoloration was extended from 4 days to 13
Figure 3.7 - TBARS (thiobarbituric acid reactive substances) values of ground beef patties in different display gas mixtures during refrigerated storage (SEM = 0.35).
days by O₂ enrichment packaging (80-100%). However, when considering the microbial growth, the psychrotrophic plate counts of the MAP ground beef samples in the present study reached log 7 CFU/g meat in 7 days after 14 days of distribution storage under refrigeration temperature. Huang et al. (1993a) indicated that oxidative stability of ground beef in gas exchange MAP increased with storage time regardless distribution temperatures (4.4, -3.8, -12.2°C), while samples packaged with 80% O₂:20% CO₂ had higher TBARS values than other gas exchange MAP samples (80% N₂:20% CO₂ for distribution and 80% O₂:20% CO₂ for display). Ho et al. (1995) reported that no differences were observed for gas exchange MAP beef loin steak (80% N₂:20% CO₂ or 50% N₂:50% CO₂ for distribution and 80% O₂:20% CO₂ or 60% O₂:40% CO₂ for display) among different gas treatments, but lipid oxidation increased with the increased storage time. Therefore, storage time tended to have profound effects on the lipid oxidative stability of gas exchange MAP meat more than the distribution and display gas mixtures.

Conclusions

Higher CO₂ in distribution gas inhibited the growth of psychrotrophic microorganisms on ground beef patties. Patties had more red color if displayed with higher levels of O₂, but lower oxidative stability. Oxidative stability decreased with extended display in VP-PVC and MAP samples. Display gas mixtures containing 80% O₂ in the ground beef
patties packages resulted in the highest color values and least metmyoglobin formation. Therefore, the combination of 50% CO₂:50% N₂ distribution gas mixture exchanged with 80% O₂:20% CO₂ for display was concluded to be an optimal gas mixtures for highest degree of color retention with moderate microbial growth.
CHAPTER IV

EFFECTS OF ABUSE TEMPERATURE ON THE MICROFLORAL CHANGES AND QUALITY OF GROUND BEEF IN GAS EXCHANGE MODIFIED ATMOSPHERE PACKAGING
Introduction

The growth of microorganisms on meat products is highly influenced by interactions among competing species and environmental conditions (Gill, 1982). Storage of meat products in modified atmosphere packaging (MAP) using different combinations of carbon dioxide (CO₂), nitrogen (N₂) and/or oxygen (O₂) has been suggested as an effective alternative to conventional vacuum packaging (VP) and polyvinylchloride (PVC) overwrapping (Lee et al., 1985) because of the extension of shelf life and improved economics (Lee et al., 1985).

There is a possibility that microorganisms with pathogenicity to humans are present on meats at the time of slaughter and at the retail level (National Research Council, 1987). Hintlian and Hotchkiss (1986) suggested that suppressed growth of pathogens by spoilage microorganisms in normal conditions could be considered as a safeguard for consumers. The noxious warning odors produced by spoilage organisms in refrigerated meat products could alert the consumer of temperature abuse to prevent the consumption of a product which might contain pathogens and cause illness. While MAP changes the atmospheric environment in the package, there are concerns for food safety because of the possibility that the changed environment may encourage the growth of certain pathogens (Farber, 1991). Hotchkiss (1988) indicated that the
distinguishing traits of MAP meats from other refrigerated meat products was not the ability to support pathogen growth, but rather the inability of spoilage microorganisms to co-develop with the pathogens.

Studies have been conducted to determine the patterns of microflora for fresh meats in various MAP. Combinations of elevated CO$\textsubscript{2}$ concentrations and restricted quantities of O$_2$, a growth-limiting factor for aerobes, produced a synergistic inhibitory effect on the growth of refrigerated meat spoilage microorganism *Pseudomonas* spp. (Ahmad and Marchello, 1989; Enfors and Molin, 1980). The CO$_2$-enriched packaging discouraged the growth of Gram-negative, rod-shaped microorganisms, typified as *Pseudomonas* spp., by effectively extending the lag growth period (King and Nagel, 1967) and enhanced the growth of Gram-positive, lactic acid-producing organisms such as *Lactobacillus* spp. (Bailey et al., 1979; Banks et al., 1980). Dainty et al. (1979) reported that the extended shelf-life of vacuum packaged beef was due to the replacement of normal aerobic spoilage flora by one comprised mainly of lactic acid bacteria. Lactic acid bacteria are considered of less significance in spoilage than other microorganisms (Gill and Newton, 1978; Newton and Rigg, 1979). Nitrogen serves as a filler gas in MAP to prevent the packages from collapsing as CO$_2$ dissolves into the meat product (Botchkiss, 1988).
Inhibitory effects of CO\(_2\) on common psychrotrophic spoilage microorganisms, such as *Pseudomonas*, have been demonstrated (Clark and Lentz, 1969; King and Nagel, 1967; Hintlian and Hotchkiss, 1987). King and Nagel (1967) indicated that the inhibitory effects of CO\(_2\) on bacteria growth was because CO\(_2\) blocked the metabolism of Gram-negative bacteria on enzymatic decarboxylation. Sears and Eisenberg (1961) found that CO\(_2\) affected the permeability of cell membranes and caused cell damage. Packaging with elevated levels of CO\(_2\) can extend the shelf life of refrigerated meats and retard the growth of aerobic spoilage microorganisms, permitting lactic acid bacteria and other CO\(_2\)-resistant organisms to dominate the microbial population (Blickstad and Molin, 1983; Enfors et al., 1979). Under humid conditions, aerobic floras are usually dominated by *Pseudomonas* and anaerobic floras are dominated by *Lactobacillus* (Gill and Newton, 1978).

Furthermore, the normal retail display temperature of the retail display case should be at the refrigeration temperatures of 0.5-3.3°C; however, in some instances the display temperature may reach a higher level, such as 15°C, than the expected normal refrigeration temperature (Lioutas, 1988). Temperature of storage and display is critical in obtaining maximum shelf-life. At low refrigeration temperature, an increase of 5°C can decrease the color shelf-life by one-half, depending on species and muscle
Gas exchange MAP involves use of atmospheres without O₂ for initial packaging and distribution with replacement of the gaseous package environment for one with high amounts of O₂ for retail display. However, the gas environments in the MAP with gas exchange will have two types of conditions that change from CO₂-enriched anoxic atmospheres to high O₂-containing environments. The influences of gas exchange MAP technology on the microflora in ground beef is unknown and the possibilities of abuse temperatures increase the uncertainty of microorganism interactions in the packaging system. Therefore, the first objective of the study was to determine the microfloral patterns in ground beef patties with gas exchange MAP CO₂-enriched packaging to an environment with CO₂ and high concentration of O₂. The second objective of the study was to evaluate how abuse temperature (15°C) influences microflora changes in ground beef with gas exchange MAP.

**Materials and Methods**

**Sample preparation**

Two beef steers (Angus, 120 days on feed) of choice or high select quality were slaughtered and the carcasses were chilled to 4.4°C in the LSU Agricultural Center Meat Laboratory. The ground beef manufactured from chuck rolls (infraspinatus and supraspinatus) was ground through a 1.27 cm plate and then through a 0.32 cm plate using Butcher Boy Grinder (Model TCA 32, LASAR MFG. Comp., Los Angeles, CA).
Square patties (10.16 cm x 10.16 cm) were formed by Hollymatic® Super Food Portioning Machine (Model 54, Hollymatic Co., Countryside, IL) with an average weight of 113.4 g. Patties were randomly assigned to MAP or vacuum packaging (VP) treatments. Patties for MAP were packed (InPack tray sealer, Model 580, Ross Industries, Midland, VA) with an absorbent pad (Dri-Loc® 50 pad, Sealed Air Co., Food Packaging Div., Patterson, NC) in barrier laminated foam trays (3P White, Amoco Foam, Atlanta, GA; oxygen permeability of 1.55 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 3.10 g/m²/24 hr at 38°C, 90% R.H.) using lidding film (Type Curlam, Curwood, New London, WI; oxygen permeability of 0.1 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 1.5 cc/m²/24 hr at 22.8°C, 0% R.H.) with distribution gas mixture of 50% N₂:50% CO₂. Patties for VP were placed on absorbent pads and packaged in vacuum pouches (SealFresh™, 3 mil nylon/polyethylene, 20.32 cm x 25.4 cm, Koch Supplies Inc., Kansas City, MO; oxygen permeability of 9.3 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 9.3 cc/m²/24 hr at 23°C, 90% R.H.) using vacuum machine (Model VM200H, Westglen Co., Los Angeles, CA). All packages contained one patty and were stored at -1°C in cardboard boxes during distribution storage. After 15 days post-packaging storage, gaseous contents of MAP were exchanged (Windjammer, Pakor, Inc., Livingston, TX) for 80%
O₂:20% CO₂ before display under simulated retail conditions of 7 or 15°C and 1345 lux cool white fluorescent light. VP patties were removed from pouches, transferred to foam trays for overwrapping with polyvinyl chloride film (PVC, Borden Resinite, O₂ transmission rate of 325 cc/cm²/24 hr at 23°C, 0% R.H.; CO₂ transmission rate, 2,500 cc/cm²/24 hr at 23°C, 90% R.H.), and then displayed under the same conditions as patties in MAP. Duplicate packages of each treatment combination were randomly sampled on day 0, 8, 15 (day 0 after gas exchange) and at one day intervals after gas exchange until day 18.

HunterLab "L", "a", and "b" values

Objective color changes were evaluated on Day 1 and 8, immediately before gas exchange (day 15), 30 minute after gas exchange (day 15.1), and every 12 hours after day 15 until day 18 (15.5, 16, 16.5, 17, 17.5, 18 and 18.5) using a Hunter Associates LabScan reflectance colorimeter (Model LABSCAN-2 0/45, Hunter Associate Laboratory, Inc., Reston, VA). Samples were removed from the packages and analyzed within 30 seconds to avoid moisture loss on the meat surface. HunterLab "L" (lightness) "a" (degree of red/green) and "b" (degree of yellow/blue) readings were averaged on each sample by rotating the patties 90° between three readings. A standard white plate (number 6274) with "L" = 92.4, "a" = -0.7, and "b" = -0.9 and black plate were used for instrument standardization.
Microbial sampling and identification

Microbial sampling was conducted on day 1, 8, 15, 16, 17 and 18 post-packaging. Psychrotrophic plate counts (PPC) were determined by standard plate count procedures (APHA, 1976). Ten grams of sample was excised and placed in a sterile Stomacher bag with 90 mL sterile 0.1% peptone solution and pummeled for 1 min (Stomacher Lab-Blender 400, Model No. STO-400, Tekmar Co., Cincinnati, OH). Using a "spreading" method (Vanderzant and Splittstoesser, 1992), suitable serial dilutions of the beef-peptone homogenate were plated with Standard Plate Count Agar (Bacto®-Peptone, DIFCO Laboratories Inc., Detroit, MI) and plates were incubated at 6°C for 10 days. The average number of colonies from the duplicate plates were reported as log colony forming units (CFU)/g meat sample.

Physiological and biochemical studies of microorganisms

The psychrotrophic plate counts (PPC) plates of initial day, day 8, day 15 (before gas exchange), and at 1 day intervals until day 18 were used for the identification of microflora species. Plates with approximately 30 colonies were selected for colony isolation (Vanderzant et al., 1985). If available plates had more than 30, but less than 250 colonies, each colony was assigned a number and 30 colonies were chosen at random for identification. A random number table was used to determine which colonies would be selected for isolation. Those selected colonies were
streaked with a sterile needle on plate count agar and incubated for 24 hr at 35°C before initiation of identification procedures. The isolates were purified and transferred to slants. Identification was accomplished by separating the microbes into two groups according to their reaction to Gram's stain. The Gram-positive and the Gram-negative organisms were identified at the genus level according to Vanderzant and Nickelson (1969) (Figure 4.1, 4.2, 4.3). Confirmation of Gram's stain was also conducted by streaking colonies on MacConkey's agar (Bacto®-MacConkey Agar, DIFCO Laboratories Inc., Detroit, MI). Further identification of the Gram-negative microorganisms were accomplished using API 20E system (bioMérieux Vitek, Inc., Hazelwood, MO) for the identification of Enterobacteriaceae and non-fermentative Gram-negative rods.

Percentage distributions of microbial types were calculated based on the occurrence among the total number of isolates. The following tests as described by Collins and Lyne (1970) were used to characterize the isolates, including (1) Gram's stain using Hucker's modification as recommended by the Society of American Bacteriologists (1957); (2) motility by (a) agar method using tubes with motility agar (Bacto®-Motility Test Medium, DIFCO Laboratories Inc., Detroit, MI) inoculated by stabbing inoculum through the center of the tubes and incubation at
Figure 4.1 - Scheme for identifying Gram-positive bacteria isolated from various beef samples (adapted from Vanderzant and Nickelson, 1969).
Figure 4.2 - Scheme for identifying Gram-negative oxidase-positive bacteria isolated from various beef samples (adapted from Vanderzant and Nickelson, 1969).

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Figure 4.3 -- Scheme for identifying Gram-negative oxidase-negative bacteria isolated from various beef samples (adapted from Vanderzant and Nickelson, 1969).
25°C for 12 days with growth through the agar considered to be a positive result, (b) hanging drop method where a small drop of fresh liquid culture (24 hr) in nutrient broth was examined for motility under a microscope; (3) Hugh-Leifson test having each culture inoculated into duplicate tubes of Hugh-Leision medium (Bacto®-OF Basal Medium, DIFCO Laboratories Inc., Detroit, MI) with one tube capped for aerobic reaction and the other sealed with wax and covered with mineral oil for anaerobic reaction during incubation at 35°C for 48 hr or longer; (4) catalase test with hydrogen peroxide solution (3%) added to culture slants and the production of gas recorded as a positive reaction; (5) cytochrome oxidase test by placing bacteria colonies on filter paper previously moistened with a 1% tetramethylphenylenediamine dihydrochloride solution (SpotTest™ Oxidase Reagent, DIFCO Laboratories Inc., Detroit, MI) and a formation of deep blue color regarded as a positive reaction; and (6) API 20E used to biochemically characterize the properties of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrogen sulfide production, urease, tryptophane deaminase, metabolism of tryptophan, VP (Voges-Proskauer) test, catalase, nitrate utilization, carbohydrate utilization, and gelatin liquefaction (bioMérieux Vitek, Inc., Hazelwood, MO).
Statistical Analyses

A split-plot design with a 2x2 factorial arrangement (2 package types and 2 temperatures) as the main-plot and storage time as the sub-plot was used in the study. Animal sources were used as the replication and data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS, 1985). Treatment means were separated by least square means procedures if a difference was detected at $P<0.05$ level.

Results and Discussion

Growth of psychrotrophic microorganisms

Microorganisms that grow in foods at refrigeration temperatures are called psychrotrophic bacteria (Gilliland et al., 1992). Cousin et al. (1992) defined the psychrotrophs as the microorganisms that could have a visible growth at $7 \pm 1^\circ C$ within 7 to 10 days, regardless of their optimum growth temperatures. The analysis of variance (Table A.4.1) indicated that the growth of psychrotrophic microorganisms during storage was influenced ($P<0.05$) by packaging, temperature, storage time, interactions of storage time and packaging, storage time and temperature, packaging and temperature. The initial psychrotrophic microorganism count was 3.88 log CFU/g meat. There were no differences ($P>0.05$) in microbial counts between MAP and VP samples from days 0 to 8 post-packaging during distribution storage at $-1^\circ C$ (Figure 4.4). The initial psychrotrophic
Figure 4.4 - Growth of psychrotrophic microorganisms as affected by packaging and temperature during storage.
counts of log number of 3.88 were enumerated for the ground beef in the present study.

The total psychrotrophic counts of VP-PVC and MAP ground beef samples increased \((P<0.05)\) when storage temperatures were changed from \(-1^\circ\) to \(7^\circ\) or \(15^\circ\)C after overwrapping of patties in VP with PVC \((6.53 \text{ log CFU/g meat to } 6.86 \text{ log CFU/g meat at } 7^\circ\text{C and } 8.25 \text{ log CFU/g meat at } 15^\circ\text{C})\) or gas exchange of MAP for \(80\% \text{ O}_2:20\% \text{ CO}_2\) \((4.16 \text{ log CFU/g meat to } 4.50 \text{ log CFU/g meat at } 7^\circ\text{C and } 4.56 \text{ CFU/g meat at } 15^\circ\text{C})\) on day 16. The rate of increase in psychrotrophic count was faster at \(15^\circ\text{C} (1.72 \text{ log CFU/g meat for VP-PVC and } 0.4 \text{ log CFU/g meat for gas exchange MAP})\) than at \(7^\circ\text{C} (0.33 \text{ log CFU/g meat for VP-PVC and } 0.34 \text{ log CFU/g meat for gas exchange MAP})\). The increase of psychrotrophic plate counts in VP-PVC samples for \(15^\circ\text{C} \) display on day 16 was 3.3 times higher than that in gas exchange MAP samples at the same display temperature.

The psychrotrophic plate counts \((3.88-4.20 \text{ log CFU/g})\) of beef patties under MAP condition did not increase \((P>0.05)\) until day 16 \((4.5 \text{ log CFU/g})\). With retail display in two different display temperatures \((7 \text{ and } 15^\circ\text{C})\), microbial counts of VP-PVC samples displayed at \(15^\circ\text{C} \) reached a high level of \(8.25 \text{ log CFU/g ground beef in one day (day 16) while the microbial counts of samples displayed at } 7^\circ\text{C remained in the } 6.86 \text{ log CFU/g meat range and did not exhibit major growth (P>0.05) until day 18 (8.01 log CFU/g).} \)
Fagerman et al. (1984) reported that the rate of spoilage of fresh poultry at 10°C was about twice of the rate at 5°C and that at 15°C was three times of the rate at 5°C. The large increase of the psychrotrophic plate counts in VP-PVC samples displayed at 15°C might be contributed by contamination during the overwrapping operation or from packaging materials and was enhanced by the higher display temperature. The lower degree of human handling involved in gas exchange MAP compared with overwrapping might also be an advantage for reducing the contamination in the meat products during processing.

MAP samples at the two display temperatures had microbial counts at the same level (P>0.05) of 4.50-4.56 log CFU/g meat throughout day 16. After day 16, MAP samples displayed at 15°C increased rapidly to 6.64 log CFU/g meat in one day (day 17) and 8.45 log CFU/g on day 18, while the microbial growth was at 4.16-5.17 log CFU/g meat levels for gas exchange MAP samples at 7°C display temperature. Growth of psychrotrophic microbial counts of MAP samples with gas exchange displayed at 15°C did not increase (P<0.05) until day 17. Microbial counts of MAP samples with gas exchange displayed at 7°C had lower levels (P<0.05) of microbial counts (4.16-5.17 log CFU/g) throughout the 18 day storage period. CO₂ in the display gas mixture of gas exchange MAP ground might play a role in retarding the microbial growth, however, the ease and efficiency of gas exchange MAP
operations would decrease the chances of contamination due to product mishandling.

Huang et al. (1993a) reported that psychrotrophic plate counts of beef patties and steaks stored at 4.4°C in 80% O₂:20% CO₂ on day 0 were higher (P<0.05) than in samples packaged in 80% N₂:20% CO₂ followed by gas exchange for 80% O₂:20% CO₂ after 14 days distribution storage at -3.9° or -12.2°C. Ho et al. (1995) also reported that psychrotrophic plate counts increased with increased distribution temperatures from -4.4 and 0 to 4.4°C for samples packaged in 80% N₂:20% CO₂ and gas exchanged for 80% O₂:20% CO₂ after 13 days of distribution storage. Psychrotrophic microorganism growth was higher in VP-PVC than in MAP with gas exchange.

The psychrotrophic bacteria in VP samples increased after a relatively short lag phase of 1 day at 15°C and 2 days at 7°C for VP-PVC beef patties compared with 2 days at 15°C and more than 4 days at 7°C for MAP samples after gas exchange. MAP treatment with gas exchange was more capable of delaying the psychrotrophic microbial growth in ground beef patties than VP-PVC packaging. However, increased display temperature removed the benefit of MAP in retarding the growth of microorganisms. Ogrydziak and Brown (1982) also indicated that MAP should not be used as a replacement or backup for proper storage temperature. The effectiveness of MAP might have been decreased because increased display
temperature, decreased solubility of CO$_2$ in meat, and increased respiration rate of meat tissue and microorganisms result in a decreased shelf-life (Hotchkiss, 1988).

**Microflora changes of ground beef as affected by packaging types and temperatures**

The microflora distributions (%) of ground beef patties in different packaging systems and display at 7°C are presented in Table 4.1. *Pseudomonas* and *Flavobacterium* were the dominant bacteria in initial samples, which constituted about 50% of the total microflora. Ingham et al. (1990) indicated that *Pseudomonas* spp. was a prevalent group of spoilage bacteria found on raw and cooked meat products. Numerous species of bacteria have been isolated from fresh and spoiled ground beef; however, the most common spoilage microorganisms of refrigerated red meat and chicken products were *Pseudomonas*, *Acinetobacter* and *Moraxella* (Elliott et al., 1985). Bacteria of *Pseudomonas-Acinetobacter-Moraxella* group constituted 43% of the initial microflora in the present study. Collins and Lyne (1970) indicated that the genus of *Flavorbacterium* contained many species that were difficult to identify, however, the Gram-negative bacilli forming yellow colonies were frequently isolated from food and water samples (Collins and Lyne, 1970).

*Pseudomonas* are Gram-negative rods and are the most important spoilage microorganisms for refrigerated meat items (Jay, 1992). *Pseudomonas* spp. became the predominate
Table 4.1 – Microfloral distributions of ground beef patties in different packaging and displayed at 7°C during storage.

<table>
<thead>
<tr>
<th>Microflora (% of total)</th>
<th>MAP</th>
<th>VP-PVC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Packaging</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Storage Time (day)</strong></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td><strong>Storage Temp. (°C)</strong></td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td><strong>Pseudomonas</strong></td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td><strong>Acinetobacter</strong></td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td><strong>Moraxella</strong></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td><strong>Achromobacter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brochothrix</strong></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td><strong>Lactobacillus</strong></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td><strong>Yersinia</strong></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Aeromonas</strong></td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td><strong>Enterobacter</strong></td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><strong>Serratia</strong></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td><strong>Salmonella spp.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococci</strong></td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td><strong>Coryneform</strong></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Flavobacterium</strong></td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td><strong>Flavobacterium cytophaga</strong></td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Blank = not detected.
organisms on the surface of spoilage poultry flesh during storage, but were inhibited by storage in vacuum or CO₂ atmospheres, allowing *Lactobacillus* spp. to predominate as a spoilage organisms (Bailey et al., 1979). Many microorganisms, such as *Pseudomonas*, favor the utilization of carbohydrates as the source of energy for growth and grow aerobically on the surface of meat (Price and Schweigert, 1987). Barnes (1976) indicated that the predominate microflora of freshly slaughtered carcasses was mesophilic in nature and there were shifts in microflora to psychrotrophs of the *Pseudomonas-Acinetobacter-Moraxella* group because of the chilling and refrigerated holding of the carcasses. Vanderzant and Nickelson (1969) identified the isolates from beef *Biceps femoris* muscle on blood agar plates (BAP) incubated in 37°C and reported that the microorganisms on beef carcasses immediately after slaughter included *Staphylococcus* (55.5%), *Micrococcus* (0.5%), *Coryneform* (43.5%), *Bacillus* (0.5%) and *Flavobacterium* (0.5%). After three days at a chill temperature of 1°C, microflora became *Staphylococcus* (52.7%), *Micrococcus* (13.8%), *Coryneform* (32.4%) and unidentified (0.8%). The contamination either during sampling or handling from sample tissues, media, diluents, or equipment for sterility resulted in the high levels of *Staphylococcus* and *Coryneform* in the fresh beef samples, however, the sources of
*Staphylococcus* and *Coryneform* were not identified in their study.

*Lactobacillus* were not identified until day 15 for MAP samples and day 8 for VP samples. An increase of 0 to 24% for VP-PVC samples on day 8 and 0 to 7% for gas exchange MAP samples on day 15 in *Lactobacillus* were observed (Table 4.1). *Aeromonas* (13%) were found in MAP, but not in VP samples on day 8 at -1°C distribution storage, while the increase in *Lactobacillus* (24%) and *Staphylococci* (7%) were observed in VP packaged beef patties. *Aeromonas* were small Gram-negative rods with flagella with some strains being psychrotrophic and suspected of being pathogenic in foods (Collins and Lyne, 1970).

The increases in *Lactobacillus* and *Enterobacter* coincided with the disappearance of Gram-positive *Staphylococcus*, *Coryneform* and *Brochothrix* in VP samples after 15 days in the present study. Gardner (1981) indicated that the wrapping of meats in oxygen-impermeable films retarded surface growth and selected for microaerophilic bacteria, such as *Lactobacillaceae* and *Brochothrix thermosphacta*, at the expense of the *Pseudomonas-Acinetobacter-Moraxella* group. *Pseudomonas*, *Acinetobacter*, *Serratia* and *Aeromonas* were the prevalent microorganisms of the microflora in MAP beef patties, while *Pseudomonas*, *Enterobacter*, and *Lactobacillus* comprised the microflora in VP samples on day 15. *Yersina*, *Aeromonas*,

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Enterobacter, Serratia, Salmonella spp., Staphylococci are commonly considered as foodborne bacteria, which cause health hazards if consumed by humans (Jay, 1992). Dainty et al. (1985) indicated that Pseudomonas and Brochothrix thermosphacta were the major sources resulting in the spoilage flavor. Silliker and Wolfe (1980) demonstrated that an atmosphere of 60% CO₂ inhibited the growth of Salmonella and Staphylococci at 10°C in ground beef. Sanders and Soo (1978) also indicated that Salmonella and Staphylococci did not proliferate on fresh chicken packaged in low levels of CO₂ during refrigerated storage. However, the levels of Salmonella and Staphylococci isolated from ground beef were very low or none in the present study. Gas exchange MAP and VP-PVC might not support the growth of these two pathogens.

Percentages of Pseudomonas increased and became the prevalent microorganisms in MAP patties after gas changing to 80% O₂:20% CO₂ atmosphere. The same trend was also observed for Pseudomonas in VP-PVC samples. Percentages of isolated Moraxella were increased to 27% on day 16 and then were not observed in MAP samples because of the increases in Aeromonas (44%). On day 18, 81% of the microorganisms in gas exchange MAP ground beef were Pseudomonas, while the percentage was 57% in VP-PVC patties.

Table 4.2 represented the microflora changes of ground beef patties in different packaging and displayed at 15°C.
Table 4.2 - Microfloral distribution of ground beef patties in different packaging and displayed at 15°C during storage.

<table>
<thead>
<tr>
<th>Packaging</th>
<th>Microflora (% of total)</th>
<th>MAP</th>
<th>VP-PVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Time (day)</td>
<td>0</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Storage Temp. (°C)</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
</tbody>
</table>

- **Pseudomonas**: 20 37 37 25 17 20 28 23 40 63 10
- **Acinetobacter**: 10 3 13 4 3 3
- **Moraxella**: 13 13 3 14 3 7 7 20
- **Achromobacter**: 37
- **Brochothrix**: 7 30 7 7
- **Lactobacillus**: 7 39 7 3 24 37
- **Yersinia**: 3 3
- **Aeromonas**: 7 13 13 11 13 10 3 10 4 10
- **Enterobacter**: 7 3 3 20 27 14 30 10 20 10
- **Serratia**: 17 7 23 13 27
- **Salmonella spp.**: 3 7 3
- **Staphylococci**: 3 10 3
- **Coryneform**: 3 3
- **Flavobacterium cytophaga**: 30 23 3 7
- **Flavobacterium**: 10 7 3

* Blank = not detected.
during storage. *Pseudomonas* (37%), *Aeromonas* (13%), *Moraxella* (13%), and *Flavobacterium* (23%) constituted a total of 86% of the microorganisms in the MAP samples on day 8 and *Pseudomonas* (28%), *Enterobacter* (14%), and *Lactobacillus* (24%) were the prevalent microorganisms in the VP samples. On day 15, *Lactobacillus* increased to 37% in the VP samples and was the dominant microorganism species. After changing to PVC overwrapping, percentages of *Pseudomonas* increased rapidly during display and reached 63% on day 17 in VP-PVC beef patties at 15°C, while MAP samples displayed at 15°C had 17-25% *Pseudomonas*, but more *Serratia*, *Salmonella* spp., *Aeromonas*, and *Enterobacter* during storage. *Brochothrix*, *Serratia*, *Aeromonas*, and *Enterobacter* were dominant in beef patties in MAP at 15°C during display, while these microorganisms did not become prevalent in VP-PVC samples at 15°C until day 18.

Percentages of *Lactobacillus* in MAP samples increased from 7% on day 15 to 39% on day 16 at 15°C (Table 4.2) and were 13% at 7°C (Table 4.1) at the same time. Thirty percent *Brochothrix* and 20% *Enterobacter* were identified in MAP samples displayed at 15°C on day 17. *Brochothrix* has been implicated in spoilage off-flavor (Dainty et al., 1985; Gill and Harrison, 1989; McMullen and Stiles, 1990) and *Enterobacter* was a pathogen to human health (Vanderzant et al., 1992; Brown, 1982). *Aeromonas* was 44% of microorganisms isolated from MAP samples displayed at 7°C on
day 17. 81% of microorganisms in gas exchange MAP ground beef patties were *Pseudomonas* on day 18 at 7°C. 20% *Pseudomonas*, 23% *Serratia*, 27% *Enterobacter*, and 10% *Aeromonas* comprised the major microflora compositions for MAP patties at the 15°C display temperature. Percentage of *Pseudomonas* decreased from 63% on day 17 to 10% on day 18 for VP-PVC samples displayed at 15°C. However, *Achromobacter* (37%), *Serratia* (27%), *Aeromonas* (10%) and *Enterobacter* (10%) were predominant on the ground beef patties displayed at 15°C on day 18 (Table 4.2). In contrast, *Pseudomonas* was the dominant microorganisms in VP-PVC samples displayed at 7°C on day 16 (50%), day 17 (65%), and day 18 (57%) after PVC overwrapping packaging (Table 4.1).

In an early report, Coyne (1933) reported that CO$_2$ increased the lag phase of the growth curves of several spoilage organisms; combinations of higher CO$_2$ and lower temperature gave the greatest inhibition of growth. Ahmad and Marchello (1989) studied the psychrotrophic microflora of MAP packaged steak stored at 4°C in O$_2$, CO$_2$, and N$_2$ combination atmospheres. Several microorganisms survived and became the predominant genera, including *Serratia liquefaciens*, *Enterobacter aerogenes*, *Moraxella* spp., *Yersinia Enterocolitica*, and *Pseudomonas* spp. Packaging type and film with different O$_2$ and CO$_2$ concentrations directly influenced the changes of microflora. An
atmosphere with high CO$_2$ successfully inhibited the growth of *Pseudomonas*, which are the dominant spoilage bacteria. McMullen and Stiles (1991) packaged pork loin cuts in 40% CO$_2$:60% N$_2$ atmosphere using films with three different O$_2$ transmission rate (OTR). They reported *Brochothrix thermosphacta* dominated in the higher OTR film packed samples, and lactic acid bacteria was the major organism in the lower OTR film samples. This was partially observed in the present study where *Brochotrix* grew in MAP while *Lactobacillus* decreased with exposure to O$_2$ in display atmospheres for both package types. Gill and Penney (1985) stated that for packaging of species with CO$_2$ flushing, *Enterobacteriaceae* were dominant (100%) at week 3, B. *thermophacta* (83.3%) and *Enterobacteriaceae* (16.7%) at week 6 and 100% B. *thermophacta* at week 12.

Several investigators have discussed the microflora changes for vacuum packaged meat (Lee et al., 1985, Dainty et al. 1985; Dainty et al., 1979). Dainty et al. (1979) demonstrated that vacuum packaged refrigerated beef developed a low spoilage potential microflora of *Lactobacillus*. Initial flora of vacuum packaged lamb chops were composed predominantly of mesophilic *Enterococci* with chill temperature spoilage microorganisms of *Pseudomonas*, *Enterobacteria* or *B. thermosphacta* forming no more than 2% of each genera (Gill and Penney, 1985). After three weeks at -0.5°C, flora composition had changed to 100%
Lactobacillus (Gill and Penney, 1985). Shift in the microflora continued and became dominantly B. thermophacta (16.7%) and Enterobacteriaceae (83.3%) after six weeks and were mostly Lactobacillus (62.5%) and Enterobacteriaceae (37.5%) after nine weeks. After refrigerated storage periods of 7 to 21 days and depending on temperature and O₂ permeability of the packaging film, Gram-positive, facultatively anaerobic, lactic acid producing bacteria such as Lactobacillus spp., Micrococcus spp. and Streptococcus spp. became dominant (Egan and Shay, 1982; Hitchener et al., 1982). Other bacterial genera have also been shown to survive in vacuum-packaged meat, including Brochothrix thermodracta (Grau et al., 1985) and, most importantly, members of the Enterobacteriaceae because of their indication of fecal contamination and enteric pathogenicity (Mossel, 1985). At 4.4°C, lactic acid bacteria were predominant, but at 10°C the members of Enterobacteriaceae spp. predominated, regardless of packaging film (McMullen and Stiles, 1991). The initial microflora on cubed pork stored at 0-3°C was reported to be primarily of Pseudomonas spp., however, these organisms diminished as Lactobacillus spp. multiplied (Lee et al., 1985). The results of the present study were similar to those of Egan and Shay (1982), Grau et al. (1985), and McMullen and Stiles (1991), but different from findings of Gill and Penney (1985) and Dainty et al. (1979).
Microflora populations for ground beef patties of VP-PVC or gas exchange MAP displayed at 7°C during storage are in Table 4.3. Populations of *Pseudomonas* in gas exchange MAP displayed at 7°C increased from 0.78 log CFU/g meat on day 0 to 3.98 log CFU/g meat on day 18. Silliker and Wolfe (1980) indicated that *Pseudomonas* was the most sensitive and lactic acid bacteria and anaerobes were the most resistant microorganisms to the inhibitory effects of CO₂. However, the VP environment was more likely to encourage the growth of *Lactobacillus* than MAP. The populations of *Lactobacillus* increased from 0 log CFU/g meat on day 0, to 0.94 log CFU/g meat on day 8, and to 2.39 log CFU/g meat on day 15 in VP samples (Table 4.3), while the population of *Lactobacillus* was 0.28 log CFU/g meat in MAP samples on day 15. King and Nagel (1967) indicated that MAP affected the microbial growth by extending the lag growth period. The populations of microorganisms in MAP ground beef did not decrease during distribution storage, but remained at the same levels (Table 4.3), which could be considered as an extended lag phase.

Gram-negative bacteria were more sensitive to environment changes, such as pH, temperature, osmotic pressure, than Gram-positive bacteria (Brown, 1982). Microorganisms growth produced some metabolic products, such as CO₂, acids, fatty acids, and ammonia (Price and Schweigert, 1987) because of the degradation of carbohydrates, fats, and proteins. Our data indicates Gram-
Table 4.3 - Microfloral populations of ground beef patties in different packaging and displayed at 7°C during storage.

<table>
<thead>
<tr>
<th>Storage Time (day)</th>
<th>Microflora (log CFU/g meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.78</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0.39</td>
</tr>
<tr>
<td>Moraxella</td>
<td>0.52</td>
</tr>
<tr>
<td>Achromobacter</td>
<td></td>
</tr>
<tr>
<td>Brochothrix</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.28</td>
</tr>
<tr>
<td>Yersinia</td>
<td>0.14</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>0.26</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0.26</td>
</tr>
<tr>
<td>Serratia</td>
<td>0.69</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0.13</td>
</tr>
<tr>
<td>Staphylococci</td>
<td></td>
</tr>
<tr>
<td>Coryneform</td>
<td></td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>1.16</td>
</tr>
<tr>
<td>Flavobacterium cytophaga</td>
<td>0.39</td>
</tr>
<tr>
<td>Total</td>
<td>3.88</td>
</tr>
</tbody>
</table>

* Blank = not detected.
negative bacteria were more sensitive to bacterial metabolic processes or products than Gram-positive bacteria. For example, *Moraxella*, a Gram-negative bacteria, appeared to be more sensitive to either the CO₂ environment of MAP or the presence of bacterial metabolic products (Table 4.3) because the populations decreased from 0.56 log CFU/g meat on day 8 to 0.14 log CFU/g meat on day 15. In contrast, Gram-positive bacteria such as *Lactobacillus* increased from not being detected to 0.28 log CFU/g meat on day 8.

Hotchkiss (1988) indicated that the distinguishing characteristics of meat in MAP from other refrigerated meat products was not their ability to support the pathogen growth, but rather the inability of spoilage bacteria to co-develop with the pathogens. The similar results were also observed in the present gas exchange MAP study for beef displayed at 15°C (Table 4.4). The populations of spoilage microorganisms, such as *Pseudomonas*, did not decrease on day 18 in gas exchange MAP displayed at 15°C, while the populations of pathogenic microorganisms increased to a high level (2.25 log CFU/g meat for *Enterobacter* and 1.97 log CFU/g meat for *Serratia*) at the same time. However, the spoilage microorganisms of the ground beef were less competitive than some of the pathogenic microorganisms at 15°C display. The populations of *Pseudomonas* still had a small increase from 1.11 to 1.69 log CFU/g meat.
Table 4.4 - Microfloral populations of ground beef patties in different packaging and displayed at 15°C during storage.

<table>
<thead>
<tr>
<th>Packaging</th>
<th>Microflora (log CFU/g meat)</th>
<th>MAP</th>
<th>VP-PVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Time (day)</td>
<td>0</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Storage Temp. (°C)</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.78</td>
<td>1.54</td>
<td>1.53</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0.39</td>
<td>0.14</td>
<td>0.55</td>
</tr>
<tr>
<td>Moraxella</td>
<td>0.52</td>
<td>0.56</td>
<td>0.14</td>
</tr>
<tr>
<td>Achromobacter</td>
<td>3.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brochothrix</td>
<td>0.33</td>
<td>1.99</td>
<td>0.27</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.28</td>
<td>1.79</td>
<td>0.44</td>
</tr>
<tr>
<td>Yersinia</td>
<td>0.14</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Aeromonas</td>
<td>0.26</td>
<td>0.56</td>
<td>0.55</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0.26</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Serratia</td>
<td>0.69</td>
<td>0.44</td>
<td>1.97</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0.22</td>
<td>0.56</td>
<td>0.29</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>0.13</td>
<td>0.14</td>
<td>0.50</td>
</tr>
<tr>
<td>Coryneform</td>
<td>0.22</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>1.16</td>
<td>0.98</td>
<td>0.28</td>
</tr>
<tr>
<td>Flavobacterium cytophaga</td>
<td>0.39</td>
<td>0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>Total</td>
<td>3.88</td>
<td>4.20</td>
<td>4.16</td>
</tr>
</tbody>
</table>

* Blank = not detected.
In contrast, the populations of *Pseudomonas* decreased from 5.27 on day 17 to 0.86 log CFU/g meat on day 18 at 15°C display, while a significant growth of *Achromobacter* and *Serratia* occurred (Table 4.4). *Achromobacter* is a acid-producing bacteria (Collins and Lyne, 1970) and *Serratia* is capable of degrading proteins and amino acids by its proteolytic enzymes (Jay, 1992). The metabolic products of the predominate microorganisms in VP-PVC ground at 15°C ground might inhibit the growth of *Pseudomonas*. However, the microflora of the VP-PVC ground beef displayed at 15°C was more dominated by spoilage microorganisms, such as *Achromobacter, Pseudomonas, and Acinetobacter*, than pathogenic microorganisms during storage (Table 4.4).

**Objective color evaluation**

The analysis of variance indicated that HunterLab "L", "a" and "b" values were affected by packaging, temperature, interactions of packaging and temperature, storage time and packaging, temperature and storage time, and packaging, temperature and storage time (P<0.05), with the exception that "b" values were not affected by the interaction of packaging and temperature (Table A.4.1, Table A.4.2).

Ho et al. (1995) evaluated the shelf-life of ground beef in different distribution temperatures and found that beef packaged in a distribution gas mixture of 80% N₂:20% O₂ and gas exchanged with 80% O₂:20% CO₂ had higher HunterLab "L" and "a" values than VP-PVC patties during 48 hr.
Figure 4.5 - HunterLab "L" values affected by packaging and temperature during storage.
simulated retail display. This result was consistent with the findings in the present study. Bentley et al. (1989) packaged ground beef patties in N₂, CO₂, and VP and stored at 0, 4, and 8°C. They found that patties in N₂ had highest browning discoloration scores, followed by CO₂ and VP. Display temperature at 0°C resulted in higher discoloration scores than samples displayed at 4 and 8°C. Lopez-Lorenzo et al. (1980) packaged ground pork in 100% O₂, 20% O₂:80% O₂, 20% CO₂:80% air, and air. They reported that elevated O₂ (80-100%) depressed percentage of metmyoglobin formation which increased the time to reach 50% metmyoglobin formation from 4 to 13 days.

Results of HunterLab "L" values are in Figure 4.5. The higher the HunterLab "L" values, the lighter the surface color. HunterLab "L" values in the samples of different treatments fluctuated, but stayed in the range of 38-42. All samples had about the same levels of HunterLab "L" values until day 16 (P>0.05). After day 16, only VP-PVC samples displayed at 15°C had lower HunterLab "L" values (P<0.05) than other treatments.

Results of HunterLab "a" values are in Figure 4.6. Increased HunterLab "a" values indicate more red and less green in the surface color. After initial packaging, both VP and MAP samples decreased (P<0.05) in HunterLab "a" values because of the formation of metmyoglobin on the sample surface in the anaerobic environment. MAP samples
Figure 4.6 - HunterLab "a" values affected by packaging and temperature during storage.
increased (P<0.05) in HunterLab "a" values after exchange of the initial gas for 80% O₂:20% CO₂ display gas, which oxygenated myoglobin in the samples. MAP samples reached the highest redness in the first 30 min after gas exchange. However, HunterLab "a" color decreased at a faster rate (P<0.05) in MAP samples displayed at 15°C than MAP samples displayed at 7°C after gas exchange. VP samples increased in HunterLab "a" values after transferring to PVC overwrap packaging, but the magnitude of the increase was much smaller in VP-PVC samples than for the MAP samples after gas exchange. HunterLab "a" values of MAP samples were bloomed to a higher level (P<0.05) after gas exchange than the ground beef patties on day 0; however, VP samples after changing to the O₂ permeable PVC film still had lower (P<0.05) HunterLab "a" values than the ground beef patties on day 0. The gas exchange of 80% O₂ was much more efficient (P<0.05) in blooming the red color in ground beef patties of MAP samples than VP-PVC packaging. There were no differences (P>0.05) in HunterLab "a" values of VP-PVC samples which were displayed at 15°C or 7°C.

In Figure 4.7, which presents the results of HunterLab "b" values in ground beef patties during storage, it was indicated that MAP samples had higher (P<0.05) HunterLab "b" values than VP-PVC samples after gas exchange, which indicated more yellowness in the surface color.
Figure 4.7 - HunterLab "b" values affected by packaging and temperature during storage.
HunterLab "L" values were influenced by packaging type and temperatures as reported by Ho et al. (1995). It was concluded that steaks stored at 0°C during distribution had higher HunterLab "L" and "a" and "b" values than samples stored at -4.4 and 4.4°C. MAP packaged samples were also brighter and redder than VP-PVC samples when all samples were displayed at 4.4°C. Huang et al. (1993a) reported that HunterLab "L" and "b" values of beef patties and steaks were higher (p<0.10) with initial packaging in 80% O₂:20% CO₂ compared with other treatments where 80% N₂:20% CO₂ was followed by gas exchange at 14 days with 80% O₂:20% CO₂. The "a" values (redness) for patties during retail display were lower (P<0.05) with distribution at -12.2°C for steaks and with 4.4°C and 80% O₂:20% CO₂ for ground beef compared with other temperature treatments.

Conclusions

MAP was more effective in retarding the rate of psychrotrophic growth on ground beef patties during storage compared with VP-PVC. Gas exchange with display gas containing 80% O₂ increased the red color of the ground beef patties more than VP patties changed to PVC overwrapping. Abusive display temperatures increased (P<0.05) the microbial growth rate and decreased the shelf-life of ground beef during retail display. Predominant microorganism species were Pseudomonas, Yersinia, Aeromonas, Enterobacter for MAP samples and Pseudomonas, Brochothrix, Aeromonas,
Enterobacter, Moraxella, Lactobacillus for VP-PVC samples. Lower levels of pathogenic bacteria were isolated in MAP ground beef patties with a lower display temperature (7°C) when compared with MAP samples at 15°C and VP-PVC samples, indicating that MAP samples with abusive high display temperature might be potential hazards to consumers. Therefore, good manufacturing procedures should be followed with MAP meat to reduce the risk of health hazards from pathogenic microbial growth.
CHAPTER V
MICROSOMAL LIPID OXIDATION AND COLOR STABILITY
OF GROUND BEEF IN DYNAMIC GAS EXCHANGE MAP

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Introduction

Lipid oxidation and discoloration are the major factors responsible for the quality deterioration of muscle food products. Oxidative changes of the meat products become a serious problem when subjecting meat products to size reduction (grinding, chunking), freeze-thawing, and/or prolonged storage (Rhee et al., 1986). The disruption of the cell membrane integrity promoted lipid oxidation (Buege and Aust, 1978). Early studies (Igene et al., 1979; Kwoh, 1971; Love and Pearson, 1971; Younathan and Watts, 1959) indicated that the relative roles of heme iron versus non-heme iron as catalysts were most responsible for the lipid oxidation of red meat products. However, later studies showed that the oxidation could also be initiated by the microsomal enzymes lipoxygenase and peroxidase (Buege and Aust, 1978; Player and Hultin, 1977; Rhee, 1984; Rhee and Ziprin, 1987).

Lipid peroxides, as measured by the development of thiobarbituric acid reactive substances (TBARS), are formed in homogenates of many different tissues after incubation (Tarladgis et al., 1960). Wills (1969) indicated that separated nuclei, mitochondria, lysosomes and microsomes of muscle tissue were all capable of forming peroxides during incubation, but the quantity and the rate of lipid peroxides formed in the microsomal fraction were much greater than in other fractions. Microsome peroxidation was stimulated by
ascorbate NADPH (Hochstein and Ernster, 1963) and was accompanied by the disappearance of polyunsaturated fatty acid from the phospholipids (Wills, 1969).

The enzymic systems in microsomal fractions of skeletal muscles from chicken and fish catalyzed the oxidation of microsomal lipids in the presence of cofactors (Lin and Hultin, 1977; Player and Hultin, 1977; McDonald et al., 1979; Slabyj and Hultin, 1982). A similar lipid oxidation system associated with beef and pork muscle microsomes has also been observed (Rhee et al., 1984; Rhee and Ziprin, 1987).

MAP has been suggested as an alternative for fresh meat packaging with an extended shelf-life (Farber, 1991). MAP systems usually contain gaseous atmospheres whose concentrations differ from the ambient atmosphere (79% nitrogen (N\textsubscript{2}), 20.9% oxygen (O\textsubscript{2}), and 0.03% carbon dioxide (CO\textsubscript{2})). A dynamic gas exchange system (Mitchell, 1990) that combines the advantages of inert gas MAP for package distribution and a high concentration of O\textsubscript{2} for retail display has been successful in extending the shelf-life and improving the color appearance of fresh meats (McMillin et al., 1992).

McMillin et al. (1994c) packaged beef steaks in 80% N\textsubscript{2}:20% CO\textsubscript{2} or vacuum packaging (VP) and then gas exchanged with 80% O\textsubscript{2}:20% CO\textsubscript{2} or 60% O\textsubscript{2}:40% CO\textsubscript{2} for MAP samples or PVC overwrapping for VP samples on day 16 post-packaging. There

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were no differences in lipid oxidation in MAP treatments with different gas mixtures and VP-PVC samples. Huang et al. (1993b) packaged pork chops with ascorbic acid and citric acid dip in VP or MAP with 80% N₂:20% CO₂ during distribution storage. After seven days, samples in VP were transferred into PVC overwrapping and MAP was gas exchanged with 80% O₂:20% CO₂ for retail display. Samples in VP-PVC generally had lower lipid oxidation when compared with MAP samples. Huang et al. (1995) commercially packaged ground beef with 80% N₂:20% CO₂ distribution gas mixture before gas exchange for 80% O₂:20% CO₂ on days 15, 18, 20, 22, 25, 27 and 29 post-packaging. Lipid oxidation increased with extended gas exchange and display times and the rates of increase during display storage were greater for samples from later exchange times. Huang et al. (1993a) packaged steaks and ground beef in 80% O₂:20% CO₂ at 4.4°C as a control and in 80% N₂:20% CO₂ with distribution temperatures of 4.4, -3.8 and -12.2°C before gas exchange with 80% O₂:20% CO₂ after 14 days of storage. Ground beef and steaks in control packages of 80% O₂:20% CO₂ had higher lipid oxidation during display storage compared with other treatments. Ho et al. (1995) packaged steaks and ground beef with 80% N₂:20% CO₂ or VP during distribution at temperatures of -4.4, 0 and 4.4°C and changed packaging from VP to PVC or 80% N₂:20% CO₂ to 80% O₂:20% CO₂ on Day 13 for ground beef and Day 17 for steaks. Lipid oxidation was not
affected by different distribution temperatures, but 80% O₂:20% CO₂ increased lipid oxidation when compared with VP-PVC. Frozen storage (-12.2°C) caused discoloration of steaks and ground beef while -3.8 or 4.4°C provided increased redness and increased oxidative stability with gas exchange MAP compared to MAP containing 80% O₂:20% CO₂ at initial packaging (Huang et al., 1993a).

Carbon dioxide inhibited the microbial growth and O₂ promoted the bloomed meat color after gas exchange (McMillin et al. 1994b; Huang et al., 1995). It is generally believed that lipid peroxidation in muscle food is non-enzymic in nature, but there are evidences for the presence of enzymic lipid peroxidation systems associated with muscle microsomes. However, the interactions among lipid oxidation, microsomal lipid peroxidation activity, discoloration, and gas atmospheres in the gas exchange MAP ground beef have not been well defined. The objective of this study was to investigate relationships between lipid peroxidation and discoloration of MAP ground beef in dynamic gas exchange system with focus on lipid peroxidation potential and microsomal enzymic lipid peroxidation activity.

**Materials and Methods**

Chuck rolls from three steers (*infraspinatus* and *supraspinatus*) of choice or high select quality were obtained from Hyde's Slaughter House (Robert, LA) at 72 hr
postmortem, ground through a 1.27 cm plate and then through a 0.32 cm plate using a Butcher Boy Grinder (Model TCA 32, Laser Mfg. Comp., Los Angeles, CA). Square patties (10.16 cm x 10.16 cm) were formed by Hollymatic® Super Food Portioning Machine (Model 54, Hollymatic Co., Countryside, IL) with an average weight of 113.4 g. Patties were randomly assigned to MAP or vacuum packaging (VP) treatments. Patties for MAP were packed (InPack tray sealer, Model 580, Ross Industries, Midland, VA) with an absorbent pad (Dri-Loc® 50 pad, Sealed Air Co., Food Packaging Div., Patterson, NC) in barrier laminated foam trays (3P White, Amoco Foam, Atlanta, GA; oxygen permeability of 1.55 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 3.10 g/m²/24 hr at 38°C, 90% R.H.) using lidding film (Type Curlam, Curwood, New London, WI; oxygen permeability of 0.1 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 1.5 cc/m²/24 hr at 22.8°C, 0% R.H.) in a distribution gas mixture of 50% N₂:50% CO₂. Patties for VP were placed on absorbent pads and packaged in vacuum pouches (SealFresh™, 3 mil nylon/polyethylene, 20.32 cm x 25.4 cm, Koch Supplies Inc., Kansas City, MO; oxygen permeability of 9.3 cc/m²/24 hr at 23°C, 0% R.H.; moisture vapor transmission rate of 9.3 cc/m²/24 hr at 23°C, 90% R.H.) using vacuum machine (Model VM200H, Westglen Co., Los Angeles, CA). All packages contained one patty and were stored at -1°C in cardboard.
boxes during distribution storage. After 15 days post-packaging storage, gaseous contents of MAP were exchanged (Windjammer, Pakor, Inc., Livingston, TX) for 80% O₂:20% CO₂ before display under simulated retail conditions of 7° and 1345 lux cool white fluorescent light. VP patties were removed from pouches, transferred to foam trays for polyvinyl chloride film (PVC, Borden Resinite, O₂ transmission rate of 325 cc/cm²/24 hr at 23°C, 0% R.H.; CO₂ transmission rate, 2,500 cc/cm²/24 hr at 23°C, 90% R.H.) overwrapping, and then displayed under the same conditions as patties in MAP. Duplicate packages of each treatment combination were randomly sampled on day 0, 8, 15 (day 0 after gas exchange) and at 2-day intervals after gas exchange until day 19.

Headspace O₂ and CO₂ concentrations

Headspace O₂ and CO₂ were measured with a Food Package Analyzer (Series 1400, Servomex, Sussex, England), allowing 20 sec for equilibration of readings per sample.

Weight losses

For determination of exudation and weight retention during storage, ground beef and absorbent pads in each tray were weighed separately at the time of initial packaging and at the time of sampling. Weight loss was calculated as the difference of final sample weight and initial sample weight divided by the initial weight for ground beef patties.

HunterLab "L","a", and "b" values
Objective color analysis as HunterLab "L" (lightness), "a" (redness), and "b" (yellowness) values were measured using a Hunter Associates LabScan reflectance spectrophotometer, (Model LABSCAN-2 0/45, Hunter Associate Laboratory, Inc., Reston, VA). Samples were removed and analyzed within 30 sec. "L" (lightness), "a" (degree of red/green), and "b" (degree of yellow/blue) were averaged on each patty by rotating 90° between sample readings. A white plate ("L" = 92.4, "a" = -0.7, and "b" = -0.9) and a black plate were used for instrument standardization.

Metmyoglobin formation

Reflectance at wavelengths that are isobestic for different forms of myoglobin was measured and converted to K/S ratios. Metmyoglobin formation was estimated using K/S ratio of spectral (K/S 572 nm)/(K/S 525 nm) and calculations as described by Stewart et al. (1965).

Determination of overall lipid peroxidation (OTBA)

Oxidative stability analyses were performed by determining TBARS values using a distillation method as outlined by Tarladgis et al. (1960). The distillate optical absorbance was ascertained by using U-2000 spectrophotometer (Hitachi, Conroy, TX) at 535 nm.

Preparation of microsomes

Microsomes were prepared from ground beef patties of each treatment on each sampling day followed the procedures described by Rhee et al. (1984) with slight modifications.
Thirty grams of ground meat were homogenized for 90 sec (Waring blender, in cycle of 30 sec-on/20 sec-off) with 100 mL 0.12 M KCl and 5 mM histidine buffer pH 5.4. The homogenates were centrifuged at 4°C at 17,000 x g (10,840 rpm, Model RC-5C, Sorvall Instruments) for 30 min. The supernatant fractions were then filtered through 4 layers of cheesecloth and centrifuged at 4°C at 105,000 x g (357,000 RPM, VTi 50 rotor, XL 70 Centrifuge, Beckman, CA) for 60 min to recover the crude microsomal fraction. The sediment was suspended in 25 mL of 0.6 M KCl and 5 mM histidine buffer (pH 5.4) to solubilize myofibrillar protein contaminants and centrifuged again at 105,000 x g for 60 min. The final sediment was resuspended in 10 mL of 0.12 M KCl and 5 mM histidine buffer (pH 5.4). Protein content of the microsomal preparation was determined by the modified Lowry procedure of Markwell et al. (1978). Microsomal fractions were used immediately after preparation on each sampling day.

Microsomal enzymic lipid peroxidation activity

The reaction mixtures contained 0.20 mM NADPH, 0.2 mM ADP and 0.015 mM FeCl₃ in 0.12 M KCl and 5 mM histidine buffer (pH 5.4) and were incubated at 36°C for 30 min. The reaction was initiated by the addition of 0.25 mg/mL microsomal protein.
Determination of thiobarbituric acid reactive substances of microsomal lipid peroxidation activity (MTBA)

Thiobarbituric acid reactive substance values of the microsomal enzymic lipid peroxidation activity (MTBA values), expressed as malondialdehyde that was produced during incubation, were determined by the procedure of Buege and Aust (1978). A 1.0 mL aliquot of the incubation mixture was added to 2.0 mL of TCA-TBA-HCl solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid) and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1000 x g for 10 min. The absorbances of the samples were determined at 535 nm against a blank containing all reagents except the microsomal mixture. The results were reported as nmole malondialdehyde (MDA) per g microsomal protein using a molar extinction coefficient of $E_{532\text{nm}} = 1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Statistical analyses

The statistical model of the present study was a split-plot design with main plot as two packaging types (MAP and VP-PVC) and the sub-plot as storage time. Animal sources (3 steers) were used as the replication and data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS, 1985). Treatment means were separated by least square means procedures if a difference was detected at $P<0.05$ level.
Correlation coefficients of shelf-life traits were obtained using correlation procedure (CORR) (SAS, 1985).

**Results and Discussion**

Chuck meat from each steer was used for replications in the data analyses and results of variances for steers are presented in Table A.5.1. The analysis of variance indicated that O$_2$ and CO$_2$ in the MAP samples were affected (P<0.05) by storage time (Table A.5.2). Headspace O$_2$ in MAP packages after gas exchange decreased as the display time increased, and were 58.0, 53.3 and 51.5% (SEM = 0.50) on days 15, 17 and 19 post-packaging. Carbon dioxide after gas exchange increased (P<0.05) from 26.4% on day 15 to 30.3% on day 17, and then decreased (P<0.05) to 28.7% on day 19 (SEM = 0.3).

Packaging type, storage time and interactions of packaging type and storage time affected (P<0.05) weight loss (Table A.5.3). Weight losses were much higher (P<0.05) in VP-PVC samples than in MAP samples (Table 5.1). Weight loss of 8.98% for VP-PVC samples was observed on day 8 and increased to 12.18% on day 19 during the display. However, MAP samples had a relatively lower (P<0.05) weight loss (0.3% of the initial weight) than VP samples during 15 days distribution storage period and had a final weight loss of 2.59% on day 19 after the 5 days retail display. Bentley et al. (1989) evaluated the effects of gas atmospheres (100% N$_2$, 100% CO$_2$, and vacuum packaging) on the shelf-life and
Table 5.1 - Weight loss, pH values, HunterLab "a", and HunterLab "b" values of ground beef patties in different packaging during refrigerated storage.

<table>
<thead>
<tr>
<th>Tr</th>
<th>Pk</th>
<th>Storage Time (Days)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>8</td>
<td>15</td>
<td>17</td>
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<tr>
<td>WL</td>
<td>MAP</td>
<td>0d</td>
<td></td>
<td>0.39d</td>
<td>0.30d</td>
<td>1.67c</td>
<td>2.59c</td>
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</tr>
<tr>
<td></td>
<td>VP</td>
<td>8.98b</td>
<td></td>
<td>9.84b</td>
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<td>12.18a</td>
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<td>pH</td>
<td>MAP</td>
<td>5.99ab</td>
<td></td>
<td>5.86cd</td>
<td>5.91abc</td>
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<td>5.80d</td>
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<tr>
<td></td>
<td>VP</td>
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<td></td>
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<td>5.82cd</td>
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<td>a</td>
<td>MAP</td>
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<td>16.17c</td>
<td>13.48de</td>
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</tr>
<tr>
<td>b</td>
<td>MAP</td>
<td>8.36c</td>
<td></td>
<td>8.22c</td>
<td>11.18a</td>
<td>11.20a</td>
<td>10.13b</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>VP</td>
<td>8.61c</td>
<td></td>
<td>8.92c</td>
<td>6.89d</td>
<td>10.24ab</td>
<td>10.14b</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>MAP</td>
<td>1.81de</td>
<td></td>
<td>1.86cd</td>
<td>2.67a</td>
<td>2.27b</td>
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<td>1.98c</td>
<td>1.72e</td>
<td>1.90cd</td>
<td>1.50f</td>
<td></td>
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</tbody>
</table>

1 Tr: Traits.
2 Pk: packaging type, MAP = ground beef patties packaged in 50%CO₂:50%N₂ distribution gas and then gas exchanged with 80%O₂:20%CO₂ on day 15. PVC = ground beef patties packaged in vacuum pouch and transferred to PVC overwrapping packages on day 15.
3 SEM: standard error of the means.
4 WL: weight loss = (initial weight - final weight) / initial weight x 100%.
5 a: HunterLab "a" values.
6 b: HunterLab "b" values.
7 KS: K/S ratio = (K/S 572 nm)/(K/S 525 nm), higher K/S ratio indicates less metmyoglobin formation.

abcdef means in the same trait with same letter were not different (P>0.05)
sensory attributes of ground beef patties and reported that VP ground beef resulted in higher (P<0.05) purge production and lower taste panel scores than CO₂ packaged patties. The results that VP-PVC samples had higher weight loss during storage than meat in gas exchange MAP agreed with previous studies (Huang et al., 1993a, b; McMillin et al., 1994b; Ho et al., 1995). Lee et al. (1983) investigated the effects of high N₂ on MAP ground beef and found that use of N₂ minimized both weight loss and protein extraction and increased the water holding capacity of ground beef, possibly by permitting a more swollen structure of the meat to develop and persist.

pH values were affected by animal source, storage time, and the interactions of packaging and storage time (P<0.05). MAP samples had a pH of 5.99 on day 0 and pH values decreased (P<0.05) to 5.80 on day 19 with extended storage (Table 5.1). Ground beef patties in VP had a pH of 5.87 on day 0, and pH increased (P<0.05) to 5.99 on day 15 after changing to PVC overwrapped packages. However, the pH of the ground beef patties used in the present study were slightly higher than the normal pH (<5.8) (Gill and Penney, 1988) of postmortem beef. The chuck meat was obtained from commercial slaughter and the background information regarding the meat or the animals was not available. The accumulation of lactic acid produced by anaerobic fermentation of glucose and glycogen in muscle tissue after
slaughter usually resulted in a pH decrease from approximately pH 7.4 in living muscle to a pH about 5.6-5.7 within 6-8 hr postmortem (Hedrick et al., 1994). The higher pH might be due to the stress before slaughter, age, breed, or chilling condition regarding the animals or meat.

HunterLab "L" color (lightness) values were not influenced (P>0.05) by packaging type and storage time or the interactions of packaging and time (Table A.5.4). There were no differences (P>0.05) in HunterLab "L" values between VP-PVC and MAP samples during the refrigerated storage (Table A.5.8). The previous study in Chapter III indicated that VP samples displayed lower HunterLab "L" values than gas exchange MAP samples with 50% N₂:50% CO₂ distribution gas and 80% O₂:20% CO₂ display gas. The lower HunterLab "L" values of gas exchange MAP ground beef in this study might be because of the higher initial pH values observed. McMillin et al. (1994c) also indicated that gas exchange MAP ground beef patties with 80% O₂:20% CO₂ display gas increased in HunterLab "L" values compared with VP-PVC samples during retail display.

HunterLab "a" (redness) values were influenced by packaging type and storage time and the interactions of packaging and time (Table A.5.4). HunterLab "a" values were at the same level of 13.17-14.06 for MAP and VP samples before gas exchange or change to PVC overwrapping. HunterLab "a" values of MAP samples increased (P<0.05) from
13.24 to 24.60 immediately after gas exchange for the 80% O₂:20% CO₂ display gas mixture, but HunterLab "a" values for VP-PVC samples decreased (P<0.05) from 14.06 to 12.63 after changing to overwrapping on day 15 and then increased (P<0.05) to 16.17 on day 17. MAP samples had higher HunterLab "a" values throughout the retail display period than VP-PVC samples (Table 5.1). VP samples changed from O₂ impermeable vacuum pouch to O₂ permeable PVC film had less (P<0.05) and slower (P<0.05) effects on the red color blooming of ground beef patties than MAP samples that were gas exchanged with high O₂ gas mixtures in the simulated retail display period. This agreed with results of previous studies (Huang et al., 1993a; McMillin et al., 1994b; Ho et al., 1995).

HunterLab "b" (yellowness) values were influenced by packaging, storage time and the interactions of packaging and storage time (Table A.5.5). VP and MAP samples had the same levels (P>0.05) of HunterLab "b" values before changing the packaging (Table 5.1). However, HunterLab "b" values of MAP samples increased (P<0.05) from 8.22 to 11.18 after gas exchange and decreased (P<0.05) to 10.13 on day 19 during display storage period. HunterLab "b" values of VP-PVC samples decreased (P<0.05) from 8.93 to 6.87 after changing to PVC overwrapping packaging and increased (P<0.05) to 10.24 on day 17 (Table 5.1). Huang et al. (1993b) found that HunterLab "b" values were higher in MAP ground beef.
packaged with 80% O₂:20% CO₂ stored at 4.4°C than gas exchange MAP samples with 80% N₂:20% CO₂ distribution gas and 80% O₂:20% CO₂ for display at -12.2, -3.8, and 4.4°C distribution temperature during storage.

Packaging type, storage time and the interactions of packaging type and storage and time affected (P<0.05) K/S ratios as indicators of metmyoglobin formation (Table A.5.5). Higher metmyoglobin ratios indicated lower metmyoglobin formation. A decreasing trend (P<0.05) was observed for samples in MAP and VP-PVC after gas exchange or changing of packaging for display (Table 5.1). K/S ratios in MAP samples reached the highest level (least metmyoglobin formation) immediately after gas exchange, however, the highest value for K/S ratio in VP-PVC samples were not reached until day 17. Greene et al. (1971) claimed that consumers would reject beef at metmyoglobin concentration greater than 40%. Meat color is usually considered as the most critical appearance factor, because consumers associate the bright red retail meat with good quality (Taylor, 1982). Hood and Riordan (1973) indicated that there was a linear increase in discrimination by consumers against discolored meat as the percentage of metmyoglobin increased in the fresh beef. However, K/S ratios reported in the present study were relative values of metmyoglobin formation in different samples instead of percentages of metmyoglobin formation.
The analysis of variance table (Table A.5.6) indicated that the overall lipid oxidation activity was affected (P<0.05) by storage time, but not (P>0.05) packaging type (Table A.5.8). TBARS values of the overall lipid oxidation activities increased from 0.277 on day 1 to 2.152 on day 8, and reached 4.6 mg malondialdehyde/kg meat on day 15 after changing the packaging of MAP or VP-PVC samples (Figure 5.1). The TBARS value of the ground beef patties increased (P<0.05) as the storage time increased. The high O₂ environment of the MAP packages after gas exchange did not increase the overall lipid oxidation of the ground beef patties during refrigerated storage.

McMillin et al. (1994c) packaged beef steaks in 80% N₂:20% CO₂ or VP before gas exchange with 80% O₂:20% CO₂ or 60% O₂:40% CO₂ for MAP samples or PVC overwrapping for VP samples on day 16 post-packaging. They indicated that there were no differences in lipid oxidation in MAP treatments with different gas mixtures and VP-PVC samples. Huang et al. (1993b) packaged pork chops with ascorbic acid and citric acid dip in VP or MAP with 80% N₂:20% CO₂ during distribution storage. After seven days, samples in VP were transferred into PVC overwrapping and MAP was gas exchanged with 80% O₂:20% CO₂ for retail display. Samples in VP-PVC generally had lower lipid oxidation when compared with MAP samples. Huang et al. (1995) gas exchanged commercially packaged ground beef with 80% N₂:20% CO₂ distribution gas.
Figure 5.1 - Microsomal lipid peroxidation activity (MTBA) and overall lipid oxidation activity (OTBA) of ground beef patties during refrigerated storage (Data in Table A.5.7).
mixture and gas exchanged for 80% O₂:20% CO₂ on Day 15, 18, 20, 22, 25, 27 and 29 post-packaging. Lipid oxidation increased with extended gas exchange and display times and the rates of increase during display storage were greater for samples from later exchange times. Huang et al. (1993a) packaged steaks and ground beef in 80% N₂:20% CO₂ with distribution temperatures of 4.4, -3.8 and -12.2°C and 80% O₂:20% CO₂ at 4.4°C as a control. Samples were gas exchanged with 80% O₂:20% CO₂ after 14 days of storage. Ground beef and steaks in control of 80% O₂:20% CO₂ had higher lipid oxidation during display storage compared with other treatments. Ho et al. (1995) packaged steaks and ground beef with 80% N₂:20% CO₂ or VP during distribution at temperatures of -4.4, 0 and 4.4°C and changed packaging from VP to PVC or 80% N₂:20% CO₂ to 80% O₂:20% CO₂ on Day 13 for ground beef and Day 17 for steaks. Lipid oxidation was not affected by different distribution temperatures, but 80% O₂:20% CO₂ increased lipid oxidation when compared with VP-PVC. Frozen storage (-12.2°C) caused discoloration of steaks and ground beef while -3.8 or 4.4°C provided increased redness and increased oxidative stability with gas exchange MAP compared to MAP containing 80% O₂:20% CO₂ at initial packaging (Huang et al., 1993a). Therefore, MAP meat with 80% N₂:20% CO₂ and gas exchange for 80% O₂:20% CO₂ had higher TBARS values than VP-PVC samples during retail
display in previous studies. No differences between gas exchange MAP and VP-PVC were detected in the present study.

The analysis of variance (Table A.5.6) indicated that microsomal enzymic lipid peroxidation activity was affected (P<0.05) by storage time, but not packaging type (Table A.5.8). There were no differences (P>0.05) between MAP and VP-PVC ground beef patties in the enzymic lipid peroxidation activity of microsomal protein during refrigerated distribution and retail display storage. High O₂ display gas mixtures also did not cause higher (P>0.05) levels of microsomal enzymic lipid peroxidation activity in the MAP samples than in VP-PVC samples. TBARS values indicated the microsomal enzymic production activity in ground beef patties increased with the increased storage time (Figure 5.1). Microsomal enzymic lipid peroxidation activities (MTBA) in ground beef patties after package environment changes increased (P<0.05) in each sampling day, while the TBARS values of the overall lipid oxidation determined by distillation method remained at the same level (P>0.05) during the first two days of display after gas exchange.

Rhee et al. (1986) investigated the nonenzymic factors affecting lipid peroxidation in ground beef at 4°C storage for 7 days and found that microsomal lipid peroxidation activity was positively correlated (P<0.05) with the overall lipid oxidation. Pearson correlation coefficients indicated that microsomal enzymic lipid peroxidation activity was
positively correlated (P<0.05) with O₂ concentration, weight loss, pH values, HunterLab "a", HunterLab "b" values, and overall lipid oxidation activity (Table 5.2). Oxygen concentrations were positively correlated (P<0.05) to microsomal enzymic lipid peroxidation activity, overall lipid oxidation, and HunterLab "a" values, but negatively correlated (P<0.05) to metmyoglobin formation of the ground beef patties. Weight losses were positively correlated (P<0.05) with enzymic lipid peroxidation and overall lipid oxidation, but negatively correlated to metmyoglobin formation. Ground beef patties with a higher percentage of weight loss would result in economic loss due to decreased product weight and were accompanied by quality deterioration problems such as higher metmyoglobin formation and more rapid lipid oxidation. pH values were negatively correlated to the overall lipid oxidation (P<0.05), but positively correlated to the microsomal enzymic lipid peroxidation (P<0.05) of the ground beef patties. Cornforth and Egbert (1985) reported that pre-rigor ground beef yielded higher HunterLab "a" values with decreasing pH values below 6.0 because of inhibited mitochondrial respiration rate. They concluded that pH influences on meat color were due to pH effects on mitochondrial activity, rather than direct influences of myoglobin affinity to O₂. However, overall lipid peroxidation was positively (P<0.05) correlated to enzymic lipid peroxidation activity and metmyoglobin
Table 5.2 - Correlation coefficients\(^1\) of O\(_2\), CO\(_2\), weight loss, pH, HunterLab "a", and "b" values, K/S ratio, overall lipid oxidation activity, and microsomal lipid peroxidation activity.

<table>
<thead>
<tr>
<th></th>
<th>O(_2)</th>
<th>CO(_2)</th>
<th>WL</th>
<th>pH</th>
<th>a</th>
<th>b</th>
<th>K/S</th>
<th>OTBA</th>
<th>MTBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(_2)</td>
<td>1.0000</td>
<td>0.4423</td>
<td>-0.4371</td>
<td>-0.3299</td>
<td>0.8460</td>
<td>0.6831</td>
<td>0.6619</td>
<td>0.4697</td>
<td>0.5094</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>0.0</td>
<td>0.0144</td>
<td>0.0157</td>
<td>0.0751</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0088</td>
<td>0.0040</td>
</tr>
<tr>
<td>WL</td>
<td>1.0000</td>
<td>-0.7533</td>
<td>-0.1693</td>
<td>0.2801</td>
<td>0.1387</td>
<td>0.3056</td>
<td>-0.1621</td>
<td>-0.1465</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.0</td>
<td>0.0001</td>
<td>0.3711</td>
<td>0.1338</td>
<td>0.4649</td>
<td>0.1006</td>
<td>0.3920</td>
<td>0.4400</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.0000</td>
<td>-0.1828</td>
<td>0.3029</td>
<td>0.0590</td>
<td>0.6315</td>
<td>0.0089</td>
<td>0.0190</td>
<td>0.0156</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>0.0</td>
<td>0.3337</td>
<td>0.2650</td>
<td>0.7083</td>
<td>0.0304</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K/S</td>
<td>1.0000</td>
<td>-0.0095</td>
<td>0.0939</td>
<td>0.0001</td>
<td>0.0939</td>
<td>0.0389</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTBA</td>
<td>0.0</td>
<td>0.9603</td>
<td>0.9280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTBA</td>
<td>1.0000</td>
<td>0.8980</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Correlation coefficients with associated probability values.
formation of the ground beef samples during storage. Some studies indicated that heme iron played an important role in the raw meat lipid oxidation (Govindarajan, et al., 1977; Greene, 1969; Hutchins et al., 1967). Kwoh (1971) indicated that oxidized myoglobin (metmyoglobin) in the raw meat product catalyzed the lipid peroxidation of the unsaturated fatty acids. Rhee et al. (1985) reported that the lipid peroxidation was highly correlated to the discoloration in raw meat products. However, the influences of microsomal enzymic lipid peroxidation activity in the ground beef patties were not exactly the same as observed in the overall lipid oxidation, suggesting that other lipid oxidation systems, such non-enzymic lipid oxidation or mitochondria lipid oxidation, also played roles in the lipid oxidation of ground beef patties during refrigerated distribution and display storage. The lipid oxidation in gas exchange ground beef might be due to the synergic effects of the enzymic or non-enzymic lipid oxidation which developed at different rate during refrigerated storage.

Conclusions

High O₂ (80%) display gas mixtures in the MAP samples resulted in higher (P<0.05) HunterLab "a" values and less (P<0.05) metmyoglobin formation on the surfaces of ground beef patties than in VP-PVC samples, without elevating (P>0.05) lipid oxidation during display storage. Lipid oxidation and microsomal preparation of ground beef
increased with the storage time regardless of MAP or VP-PVC packaging. Advantages of MAP ground beef patties with gas exchange technology over VP-PVC packaging observed in the present study included lower percentages of weight loss (P<0.05), higher HunterLab "a" values (P<0.05), and less metmyoglobin formation (P<0.05) during refrigerated storage. Enzymic lipid peroxidation activity was increased (P<0.05) in ground beef patties during display, whereas lipid oxidation measured in the ground beef patties was relatively small (P>0.05) after changing packaging types for retail display. Further studies regarding enzymic and non-enzymic lipid oxidative stability for gas exchange MAP meat should be conducted.
CHAPTER VI

SUMMARY AND CONCLUSIONS
Modified atmosphere packaging has become commonly used as an alternative for refrigerated food products in extending the shelf-life. However, MAP has not been widely accepted by the meat industry and retail meat stores because of the unfamiliar purple meat color due to formation of deoxymyoglobin in the anoxic MAP environment. Gas exchange MAP changes the initial CO\textsubscript{2}-containing gas mixture to a high O\textsubscript{2} containing gas mixture before retail display, which provides a bloomed meat product with extended shelf-life.

Effects of distribution (80% CO\textsubscript{2}:20% N\textsubscript{2}, 50% CO\textsubscript{2}:50% N\textsubscript{2}, or 20% CO\textsubscript{2}:80% N\textsubscript{2}) and display (20% CO\textsubscript{2}:80% O\textsubscript{2}:0% N\textsubscript{2}, 20% CO\textsubscript{2}:50% O\textsubscript{2}:30% N\textsubscript{2}, or 20% CO\textsubscript{2}:20% O\textsubscript{2}:60% N\textsubscript{2}) gas mixtures on shelf-life properties of ground beef patties in gas exchange MAP were compared with samples in VP-PVC. Carbon dioxide in the distribution gas mixtures retarded the growth of microorganisms, while O\textsubscript{2} in the display gas mixtures improved the color values of ground beef patties during simulated retail display. Ground beef patties in VP-PVC had higher weight loss, higher microbial growth rates, and lower color quality when compared with samples in gas exchange MAP. The combination of 50% N\textsubscript{2}:50% CO\textsubscript{2} in distribution and 80% O\textsubscript{2}:20% CO\textsubscript{2} for display was considered to be optimal for highest color scores and moderate microbial growth for gas exchange MAP.

Modified atmosphere packaging was more effective in retarding the growth of psychrotrophic microorganism growth.
on ground beef patties compared with VP-PVC samples. *Pseudomonas, Acinetobacter, and Moraxella* are considered as the general spoilage microorganisms in refrigerated ground beef products, while *Enterobacter, Serratia, Yersinia, Staphalococci*, and *Aeromonas* may cause health hazards if consumed by humans. *Pseudomonas* was the predominate microorganism on ground beef in gas exchange MAP and in VP-PVC samples displayed at 7°C. However, the magnitude of the microbial populations were higher in VP-PVC sample than gas exchange MAP samples displayed at 7°C. Increased display temperature reduced the effects of gas exchange MAP on the extension of shelf-life. The incidences of pathogenic microorganisms identified in the gas exchange MAP at 15°C display temperature were higher than other treatments. Abusive display temperatures of ground beef patties reduced shelf-life and eliminated the benefits for gas exchange MAP, but also indicated potential pathogenic microorganism hazards to consumers not observed in VP-PVC samples.

Lipid oxidation of ground beef and lipid peroxidation of microsomes extracted from the ground beef increased with storage time after packaging regardless of the packaging type. Immediate color blooming was observed in MAP samples gas exchanged with 80% O₂, while VP-PVC samples contained higher levels of metmyoglobin formation even after changing to O₂-permeable packaging. Microsomal enzymic lipid peroxidation activity was elevated in MAP ground beef that
was gas exchanged with high $O_2$ display gases for the retail display, but color values were improved more than in VP-PVC samples.

Ground beef patties with 14 days of distribution storage and at least four days of display shelf-life were observed in the present research. Gas exchange MAP increased the shelf-life of ground beef to more than 2 weeks in distribution storage, and provided a bloomed red color during simulated retail display. This research demonstrated the usefulness of this type of case-ready meat packaging technology for implementation of commercial practice for meat plant packaging, retail store merchandising of red meat. The case-ready consumer package ground beef can be manufactured in the central plant packaged with 50% $N_2$:50% $CO_2$ distribution gas mixture and delivered to the retail store within the 14 days of distribution storage period. The case-ready packaged ground beef will then be gas exchanged with 80% $O_2$:20% $CO_2$ display gas mixture immediately before put on the retail shelf. Within 20 min, the case-ready ground beef will have a bloomed meat color and be suitable for customer purchase for 4 days when displayed at a temperature lower than 7°C. However, future investigations are needed regarding the flavor and sensory properties of the fresh meat as well as organoleptic study of the cooked products. The information can provide further understanding to the meat processor in regards to the gas
exchange MAP meat products and the customer acceptance of the gas exchange MAP meat products. Off-flavor or odor that may develop in the package headspace during refrigerated storage may influence the consumer acceptance to the MAP meat products, although the microbial populations and rancidity of the meat products might meet the requirement of safety for consumption.
REFERENCES


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Hsieh, R.J. and Kinsella, J.E. 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and


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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
lamb stored in vacuum or modified gas atmospheres. J. Food Prot. 43:252-258.


Wynne, E.S. and Foster, J.W. 1948. Physiological studies on spore germination, with special reference to Clostridium botulinum. III. Carbon dioxide and

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APPENDIX

Table A.3.1 - Shelf-life traits for replications of two steers.

<table>
<thead>
<tr>
<th></th>
<th>Steer&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>SEM&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight Loss (%)</td>
<td>1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>pH</td>
<td>5.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>PPC&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>HunterLab &quot;L&quot;</td>
<td>37.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34</td>
</tr>
<tr>
<td>HunterLab &quot;a&quot;</td>
<td>13.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46</td>
</tr>
<tr>
<td>HunterLab &quot;b&quot;</td>
<td>9.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>K/S Ratio</td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>TBA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<sup>1</sup> Two steers were used in this study.

<sup>2</sup> SEM = standard error of the mean.

<sup>3</sup> PPC = psychrotrophic plate count (log CFU/g sample).

<sup>4</sup> TBA = thiobarbituric acid reactive substance values (mg malondialdehyde/kg meat).

<sup>ab</sup> means in row with same letter were not different (P>0.05).
Table A.3.2 - Analysis of variance for headspace $O_2$ and $CO_2$% concentrations.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>6.13</td>
<td>0.4228</td>
<td>114.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>8</td>
<td>41506.62</td>
<td>0.0001</td>
<td>7307.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>8</td>
<td>241.91</td>
<td>0.0029</td>
<td>263.30</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day</td>
<td>3</td>
<td>402.20</td>
<td>0.0001</td>
<td>316.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>24</td>
<td>258.46</td>
<td>0.3190</td>
<td>245.08</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P | 1  | 41506.62    | 0.0001 | 7307.80     | 0.0001|

Table A.3.3 - Analysis of variance for weight loss and pH values.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Weight loss</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>0.19</td>
<td>2.2724</td>
</tr>
<tr>
<td>Trt (P)</td>
<td>9</td>
<td>1364.30</td>
<td>0.1784</td>
</tr>
<tr>
<td>Anim*P</td>
<td>9</td>
<td>4.69</td>
<td>0.0544</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>310.11</td>
<td>0.3903</td>
</tr>
<tr>
<td>Day*P</td>
<td>45</td>
<td>289.37</td>
<td>0.3047</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*P as an error term

| P | 9  | 1364.30     | 0.1784 | 0.0458|
Table A.3.4 - Analysis of variance for psychrotrophic plate counts (PPC) and HunterLab "L" values.

<table>
<thead>
<tr>
<th>Source</th>
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<th>PPC Type III SS</th>
<th>P&gt;F</th>
<th>HunterLab &quot;L&quot; Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>3.21</td>
<td>0.0001</td>
<td>70.37</td>
<td>0.0001</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>9</td>
<td>70.55</td>
<td>0.0001</td>
<td>190.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>9</td>
<td>2.69</td>
<td>0.0129</td>
<td>47.72</td>
<td>0.0207</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>642.74</td>
<td>0.0001</td>
<td>176.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>45</td>
<td>33.25</td>
<td>0.0001</td>
<td>205.05</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P | 9  | 70.55 | 0.0001 | 190.14 | 0.0258 |

Table A.3.5 - Analysis of variance for HunterLab "a" values and HunterLab "b" values.

<table>
<thead>
<tr>
<th>Source</th>
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<th>HunterLab &quot;a&quot; Type III SS</th>
<th>P&gt;F</th>
<th>HunterLab &quot;b&quot; Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>116.13</td>
<td>0.0001</td>
<td>14.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>9</td>
<td>305.28</td>
<td>0.0001</td>
<td>25.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>9</td>
<td>86.62</td>
<td>0.0002</td>
<td>4.26</td>
<td>0.0344</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>3077.00</td>
<td>0.0001</td>
<td>179.86</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>45</td>
<td>817.82</td>
<td>0.0001</td>
<td>71.16</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P | 9  | 305.28 | 0.0372 | 25.56 | 0.0068 |
Table A.3.6 - Analysis of variance for thiobarbituric acid reactive substances (TBARS) values and K/S ratios.

<table>
<thead>
<tr>
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<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>92.23</td>
<td>0.0001</td>
<td>1.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>41.93</td>
<td>0.0008</td>
<td>2.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>32.35</td>
<td>0.0077</td>
<td>1.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>1344.40</td>
<td>0.0001</td>
<td>38.83</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>58.67</td>
<td>0.5764</td>
<td>6.81</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P            | 9  | 41.93 | 0.3528 | 2.22    | 0.1694 |

Table A.3.7 - Analysis of variance for weight loss and pH values.

<table>
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<th>Source</th>
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<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal(Rep)</td>
<td>1</td>
<td>0.00</td>
<td>0.9585</td>
<td>2.11</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1</td>
<td>2</td>
<td>2.23</td>
<td>0.0316</td>
<td>0.10</td>
<td>0.0005</td>
</tr>
<tr>
<td>PK2</td>
<td>2</td>
<td>0.97</td>
<td>0.2191</td>
<td>0.01</td>
<td>0.4385</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>1.61</td>
<td>0.2739</td>
<td>0.02</td>
<td>0.5801</td>
</tr>
<tr>
<td>Anim<em>PK1</em>PK2</td>
<td>8</td>
<td>2.97</td>
<td>0.3171</td>
<td>0.05</td>
<td>0.3835</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>162.85</td>
<td>0.0001</td>
<td>0.40</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*Day</td>
<td>10</td>
<td>5.69</td>
<td>0.0648</td>
<td>0.12</td>
<td>0.0469</td>
</tr>
<tr>
<td>PK2*Day</td>
<td>10</td>
<td>1.56</td>
<td>0.8920</td>
<td>0.05</td>
<td>0.5502</td>
</tr>
<tr>
<td>PK1<em>PK2</em>Day</td>
<td>20</td>
<td>4.29</td>
<td>0.8421</td>
<td>0.10</td>
<td>0.7371</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

| PK1           | 2  | 2.23 | 0.1064 | 0.0969 | 0.0154 |
| PK2           | 2  | 0.97 | 0.3235 | 0.0101 | 0.4941 |
| PK1*PK2       | 4  | 1.64 | 0.4181 | 0.0176 | 0.6315 |
Table A.3.8 - Analysis of variance for psychrotrophic plate counts (PPC) and HunterLab "L" values.

<table>
<thead>
<tr>
<th>Source</th>
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<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>4.46</td>
<td>0.0001</td>
<td>38.57</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1</td>
<td>2</td>
<td>20.53</td>
<td>0.0001</td>
<td>81.85</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK2</td>
<td>2</td>
<td>0.02</td>
<td>0.9076</td>
<td>7.25</td>
<td>0.1992</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>0.22</td>
<td>0.7940</td>
<td>6.87</td>
<td>0.5447</td>
</tr>
<tr>
<td>Anim<em>PK1</em>PK2</td>
<td>8</td>
<td>1.28</td>
<td>0.2737</td>
<td>17.45</td>
<td>0.4528</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>549.13</td>
<td>0.0001</td>
<td>183.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*Day</td>
<td>10</td>
<td>10.46</td>
<td>0.0001</td>
<td>101.21</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK2*Day</td>
<td>10</td>
<td>1.22</td>
<td>0.4916</td>
<td>41.61</td>
<td>0.0531</td>
</tr>
<tr>
<td>PK1<em>PK2</em>Day</td>
<td>20</td>
<td>5.46</td>
<td>0.0054</td>
<td>47.95</td>
<td>0.3775</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

<table>
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<tr>
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<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK1</td>
<td>2</td>
<td>20.53</td>
<td>0.0001</td>
<td>81.85</td>
<td>0.0010</td>
</tr>
<tr>
<td>PK2</td>
<td>2</td>
<td>0.02</td>
<td>0.9261</td>
<td>7.25</td>
<td>0.2491</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>0.22</td>
<td>0.8465</td>
<td>6.87</td>
<td>0.5647</td>
</tr>
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</table>

Table A.3.9 - Analysis of variance for HunterLab "a" values and HunterLab "b" values.

<table>
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<tr>
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<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
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<td>127.88</td>
<td>0.0001</td>
<td>11.20</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1</td>
<td>2</td>
<td>29.38</td>
<td>0.0054</td>
<td>5.41</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK2</td>
<td>2</td>
<td>254.28</td>
<td>0.0001</td>
<td>21.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>24.07</td>
<td>0.0698</td>
<td>0.55</td>
<td>0.6187</td>
</tr>
<tr>
<td>Anim<em>PK1</em>PK2</td>
<td>8</td>
<td>70.50</td>
<td>0.0019</td>
<td>2.65</td>
<td>0.1296</td>
</tr>
<tr>
<td>Day</td>
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<td>3391.59</td>
<td>0.0001</td>
<td>167.99</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*Day</td>
<td>10</td>
<td>79.20</td>
<td>0.0023</td>
<td>3.49</td>
<td>0.0892</td>
</tr>
<tr>
<td>PK2*Day</td>
<td>10</td>
<td>359.11</td>
<td>0.0001</td>
<td>32.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1<em>PK2</em>Day</td>
<td>20</td>
<td>47.10</td>
<td>0.6282</td>
<td>4.22</td>
<td>0.4444</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

<table>
<thead>
<tr>
<th>Source</th>
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<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK1</td>
<td>2</td>
<td>29.38</td>
<td>0.2483</td>
<td>5.41</td>
<td>0.0116</td>
</tr>
<tr>
<td>PK2</td>
<td>2</td>
<td>254.28</td>
<td>0.0022</td>
<td>21.15</td>
<td>0.0002</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>24.07</td>
<td>0.6233</td>
<td>0.55</td>
<td>0.7938</td>
</tr>
</tbody>
</table>
Table A.3.10 - Analysis of variance for thiobarbituric acid reactive substances (TBARS) values and K/S ratios.

<table>
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<th>Type III SS TBA</th>
<th>P&gt;F</th>
<th>Type III SS Metmyoglobin</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
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<td>97.67</td>
<td>0.0001</td>
<td>1.87</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1</td>
<td>2</td>
<td>13.42</td>
<td>0.0120</td>
<td>0.47</td>
<td>0.0015</td>
</tr>
<tr>
<td>PK2</td>
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<td>15.33</td>
<td>0.0065</td>
<td>1.33</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*PK2</td>
<td>4</td>
<td>10.88</td>
<td>0.1233</td>
<td>0.34</td>
<td>0.0478</td>
</tr>
<tr>
<td>Anim<em>PK1</em>PK2</td>
<td>8</td>
<td>28.37</td>
<td>0.0179</td>
<td>0.97</td>
<td>0.0009</td>
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<tr>
<td>Day</td>
<td>5</td>
<td>1236.81</td>
<td>0.0001</td>
<td>37.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1*Day</td>
<td>10</td>
<td>8.74</td>
<td>0.8184</td>
<td>0.97</td>
<td>0.0030</td>
</tr>
<tr>
<td>PK2*Day</td>
<td>10</td>
<td>27.93</td>
<td>0.0499</td>
<td>3.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK1<em>PK2</em>Day</td>
<td>20</td>
<td>16.00</td>
<td>0.9439</td>
<td>0.60</td>
<td>0.6248</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS TBA</th>
<th>P&gt;F</th>
<th>Type III SS Metmyoglobin</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK1</td>
<td>2</td>
<td>13.42</td>
<td>0.2125</td>
<td>0.47</td>
<td>0.2064</td>
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<td>PK2</td>
<td>2</td>
<td>15.33</td>
<td>0.1776</td>
<td>1.33</td>
<td>0.0312</td>
</tr>
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<td>PK1*PK2</td>
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<td>10.88</td>
<td>0.5758</td>
<td>0.34</td>
<td>0.6145</td>
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Table A.4.1 - Analysis of variance for psychrotrophic plate counts (PPC) and HunterLab "L" values.

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<th>P&gt;F</th>
<th>Type III SS HunterLab &quot;L&quot;</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.8178</td>
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</tr>
<tr>
<td>PK</td>
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<td>16.83</td>
<td>0.0001</td>
<td>75.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temp</td>
<td>1</td>
<td>6.31</td>
<td>0.0001</td>
<td>37.20</td>
<td>0.0001</td>
</tr>
<tr>
<td>PK*Temp</td>
<td>1</td>
<td>0.05</td>
<td>0.6568</td>
<td>22.48</td>
<td>0.0006</td>
</tr>
<tr>
<td>Anim<em>PK</em>Temp</td>
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<td>0.77</td>
<td>0.4090</td>
<td>2.64</td>
<td>0.6564</td>
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<tr>
<td>Day</td>
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<td>100.42</td>
<td>0.0001</td>
<td>26.28</td>
<td>0.1791</td>
</tr>
<tr>
<td>PK*Day</td>
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<td>21.74</td>
<td>0.0001</td>
<td>134.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temp*Day</td>
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<td>10.59</td>
<td>0.0002</td>
<td>46.74</td>
<td>0.0123</td>
</tr>
<tr>
<td>PK<em>Temp</em>Day</td>
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<td>6.00</td>
<td>0.0071</td>
<td>42.05</td>
<td>0.0153</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS PPC</th>
<th>P&gt;F</th>
<th>Type III SS HunterLab &quot;L&quot;</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>1</td>
<td>16.83</td>
<td>0.0039</td>
<td>75.38</td>
<td>0.0027</td>
</tr>
<tr>
<td>Temp</td>
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<td>6.31</td>
<td>0.0156</td>
<td>37.20</td>
<td>0.0074</td>
</tr>
<tr>
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<td>1</td>
<td>0.05</td>
<td>0.6835</td>
<td>22.47</td>
<td>0.0149</td>
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</table>
Table A.4.2 - Analysis of variance for HunterLab "a" values and HunterLab "b" values.

<table>
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<th>HunterLab &quot;a&quot; Type III SS</th>
<th>P&gt;F</th>
<th>HunterLab &quot;b&quot; Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>1</td>
<td>0.23</td>
<td>0.5646</td>
<td>0.71</td>
<td>0.0095</td>
</tr>
<tr>
<td>PK</td>
<td>1</td>
<td>182.62</td>
<td>0.0001</td>
<td>4.73</td>
<td>0.0001</td>
</tr>
<tr>
<td>Temp</td>
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<td>113.73</td>
<td>0.0001</td>
<td>3.74</td>
<td>0.0001</td>
</tr>
<tr>
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<td>0.0001</td>
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<td>0.5098</td>
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<tr>
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<td>0.9208</td>
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<td>0.4069</td>
</tr>
<tr>
<td>Day</td>
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<td>0.0001</td>
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<tr>
<td>PK*Day</td>
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<td>0.0001</td>
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<td>0.0001</td>
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<td>Temp*Day</td>
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<td>125.99</td>
<td>0.0001</td>
<td>4.09</td>
<td>0.0001</td>
</tr>
<tr>
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<td>6</td>
<td>115.42</td>
<td>0.0001</td>
<td>3.51</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for Anim*PK1*PK2 as an error term

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>1</td>
<td>182.62</td>
<td>0.0001</td>
<td>4.73</td>
<td>0.0059</td>
</tr>
<tr>
<td>Temp</td>
<td>1</td>
<td>113.73</td>
<td>0.0001</td>
<td>3.74</td>
<td>0.0082</td>
</tr>
<tr>
<td>PK*Temp</td>
<td>1</td>
<td>148.23</td>
<td>0.0001</td>
<td>0.04</td>
<td>0.5518</td>
</tr>
</tbody>
</table>
Table A.5.1 - Shelf-life traits for replications of three steers.

<table>
<thead>
<tr>
<th></th>
<th>Steer&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
<th></th>
<th>SEM&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Weight Loss (%)</td>
<td>4.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25</td>
</tr>
<tr>
<td>pH</td>
<td>5.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>HunterLab &quot;L&quot;</td>
<td>34.86</td>
<td>35.29</td>
<td>36.30</td>
<td>3.06</td>
</tr>
<tr>
<td>HunterLab &quot;a&quot;</td>
<td>14.57</td>
<td>15.91</td>
<td>16.18</td>
<td>1.28</td>
</tr>
<tr>
<td>HunterLab &quot;b&quot;</td>
<td>8.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25</td>
</tr>
<tr>
<td>K/S Ratio</td>
<td>1.83</td>
<td>1.96</td>
<td>1.98</td>
<td>0.09</td>
</tr>
<tr>
<td>OTBA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.71</td>
<td>3.57</td>
<td>3.04</td>
<td>1.27</td>
</tr>
<tr>
<td>MTBA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19.49</td>
<td>21.66</td>
<td>22.20</td>
<td>1.38</td>
</tr>
</tbody>
</table>

<sup>1</sup> Three steers were used in this study.

<sup>2</sup> SEM = standard error of the mean.

<sup>3</sup> OTBA: TBARS (thiobarbituric acid reactive substances, mg malondialdehyde/kg meat) of the ground beef patties.

<sup>4</sup> MTBA: TBARS (thiobarbituric acid reactive substances, nmole malondialdehyde/mg microsomal protein) in the microsomal fraction.

<sup>ab</sup> means in row with different letters were different (P>0.05).
Table A.5.2 - Analysis of variance for headspace $O_2$ and $CO_2\%$.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>0.87</td>
<td>0.5717</td>
<td>0.06</td>
<td>0.8667</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>7064.44</td>
<td>0.0001</td>
<td>7375.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>0.87</td>
<td>0.5717</td>
<td>0.06</td>
<td>0.8667</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>5064.81</td>
<td>0.0001</td>
<td>66.70</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>5064.81</td>
<td>0.0001</td>
<td>66.70</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P | 1  | 7375.22 | 0.0001 | 4906.88 | 0.0001 |

Table A.5.3 - Analysis of variance for weight loss and pH values.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>P&gt;F</th>
<th>Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>1.66</td>
<td>0.2429</td>
<td>0.16</td>
<td>0.0001</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>349.33</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.0650</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>0.12</td>
<td>0.8906</td>
<td>0.01</td>
<td>0.3390</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>180.97</td>
<td>0.0001</td>
<td>0.06</td>
<td>0.0221</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>98.91</td>
<td>0.0001</td>
<td>0.05</td>
<td>0.0305</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P | 1  | 349.33 | 0.0002 | 0.01     | 0.2053 |

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Table A.5.4 - Analysis of variance for HunterLab "L" and HunterLab "a" values.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>HunterLab &quot;L&quot; Type III SS</th>
<th>P&gt;F</th>
<th>HunterLab &quot;a&quot; Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>11.52</td>
<td>0.2101</td>
<td>4.48</td>
<td>0.0301</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>27.64</td>
<td>0.0117</td>
<td>79.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>18.75</td>
<td>0.0920</td>
<td>3.26</td>
<td>0.0668</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>2.60</td>
<td>0.9359</td>
<td>130.84</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>3.95</td>
<td>0.8735</td>
<td>161.33</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

P 1 27.64 0.2281 79.50 0.0199

Table A.5.5 - Analysis of variance for HunterLab "b" values and K/S ratios.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>HunterLab &quot;b&quot; Type III SS</th>
<th>P&gt;F</th>
<th>K/S Ratio Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>1.80</td>
<td>0.0343</td>
<td>0.06</td>
<td>0.0468</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>4.87</td>
<td>0.0003</td>
<td>0.53</td>
<td>0.0001</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>0.12</td>
<td>0.7448</td>
<td>0.02</td>
<td>0.3406</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>19.12</td>
<td>0.0001</td>
<td>1.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>24.44</td>
<td>0.0001</td>
<td>1.11</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

P 1 4.87 0.0126 59.38 0.0167
Table A.5.6 - Analysis of variance for MTBA (microsomal lipid peroxidation activity) and OTBA (overall lipid oxidation activity).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MTBA Type III SS</th>
<th>P&gt;F</th>
<th>OTBA Type III SS</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (Rep)</td>
<td>2</td>
<td>1.89</td>
<td>0.7386</td>
<td>4.34</td>
<td>0.1341</td>
</tr>
<tr>
<td>Packaging (P)</td>
<td>1</td>
<td>0.07</td>
<td>0.8834</td>
<td>0.04</td>
<td>0.8349</td>
</tr>
<tr>
<td>Rep*P</td>
<td>2</td>
<td>3.83</td>
<td>0.5488</td>
<td>3.22</td>
<td>0.2139</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>4121.00</td>
<td>0.0001</td>
<td>118.68</td>
<td>0.0001</td>
</tr>
<tr>
<td>Day*P</td>
<td>4</td>
<td>30.09</td>
<td>0.0933</td>
<td>3.54</td>
<td>0.4648</td>
</tr>
</tbody>
</table>

Test of hypotheses using Type III MS for packaging*animal as an error term

| P                | 1  | 0.07 | 0.8667 | 4906.88 | 0.8865 |

Table A.5.7 - Microsomal lipid peroxidation activity (MTBA) and overall lipid oxidation activity (OTBA) of ground beef patties during refrigerated storage.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Storage Time (Days)</th>
<th>SEM(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>OTBA(^2)</td>
<td>0.28^a</td>
<td>2.15^b</td>
</tr>
<tr>
<td>MTBA(^3)</td>
<td>3.72^a</td>
<td>12.37^b</td>
</tr>
</tbody>
</table>

1 SEM: standard error of the mean.

2 OTBA: TBARS (thiobarbituric acid reactive substances, mg malondialdehyde/kg meat) of the ground beef patties.

3 MTBA: TBARS (thiobarbituric acid reactive substances, nmole malondialdehyde/mg microsomal protein) in the microsomal fraction.

abcde means in the same row with same letter were not different (P>0.05)
Table A.5.8 - HunterLab "L" values, microsomal lipid peroxidation (MTBA), and overall lipid oxidation (OTBA) of ground beef patties during refrigerated storage.

<table>
<thead>
<tr>
<th>Pk.</th>
<th>HunterLab &quot;L&quot;</th>
<th>MTBA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>OtBA&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>VP-PVC</td>
<td>MAP</td>
</tr>
<tr>
<td>1</td>
<td>36.83</td>
<td>34.31</td>
<td>3.91</td>
</tr>
<tr>
<td>8</td>
<td>36.69</td>
<td>31.12</td>
<td>11.68</td>
</tr>
<tr>
<td>15</td>
<td>36.64</td>
<td>34.75</td>
<td>24.60</td>
</tr>
<tr>
<td>17</td>
<td>36.03</td>
<td>33.48</td>
<td>33.37</td>
</tr>
<tr>
<td>19</td>
<td>36.03</td>
<td>35.39</td>
<td>36.48</td>
</tr>
<tr>
<td></td>
<td>SEM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.34</td>
<td>1.29</td>
</tr>
</tbody>
</table>

<sup>1</sup> MTBA: TBARS (thiobarbituric acid reactive substances, nmole malondialdehyde/mg microsomal protein) in the microsomal fraction.

<sup>2</sup> OTBA: TBARS (thiobarbituric acid reactive substances, mg malondialdehyde/kg meat) of the ground beef patties.

<sup>3</sup> Pk: packaging type, MAP = ground beef patties packaged in 50%CO₂:50%N₂ distribution gas and then gas exchanged with 80% O₂:20% CO₂ on day 15. VP-PVC = ground beef patties packaged in vacuum pouch and transferred to PVC overwrapping packages on day 15.

<sup>4</sup> SEM: standard error of the means.
Chung-Ping Ho, son of Yee-Ming Ho and Hsiu-Ying Chou Ho, was born January 3, 1964, in Taiwan, Republic of China. He received his Bachelor of Science (Animal Science) degree from National Taiwan University in 1987. After two years of military service, he worked as a research assistant in Meat Research Laboratory of National Taiwan University for one year. The Master of Science was received in Animal Science at Auburn University in 1992. In August, 1992, he entered Graduate School at Louisiana State University to pursue the Doctor of Philosophy degree in Animal Science. The Ph.D. degree will be awarded in December of 1995. He married Nai- Yun Huang on July 25, 1992.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Chung-Ping Ho
Major Field: Animal Science
Title of Dissertation: Biochemical and Microbiological Characteristics of Ground Beef in Modified Atmosphere Packaging With Gas Exchange

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: November 3, 1995