To the Graduate Council:

I am submitting herewith a thesis written by Douglas S. Mitchell entitled " Effect of Modified Atmosphere Packaging and CO_2 Chilling on the Shelf-life of Fresh Beef Loin Steaks". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

M. James Riemann

M. J. Riemann, Major Professor

We have read this thesis
and recommend its acceptance:

Frances A. Draughon

H. Dwight Loveday

John R Mount

Accepted for the Council:

Vice Provost
and Dean of the Graduate School

EFFECT OF MODIFIED ATMOSPHERE PACKAGING AND CO_2 CHILLING ON THE SHELF-LIFE OF FRESH BEEF LOIN STEAKS

A THESIS
PRESENTED FOR THE
MASTER OF SCIENCE
DEGREE
THE UNIVERSITY OF TENNESSEE, KNOXVILLE

DOUGLAS S. MITCHELL
DECEMBER 1990

ACKNOWLEDGMENTS

I would like to thank Dr. Jim Riemann for his support, encouragement and friendship during my graduate work. Without his confidence and trust in me, I would not be in the meat science field today. I would also like to thank Dr. Dwight Loveday for his help and support during my graduate program. To my commitee members Dr. John Mount and Dr. Ann Draughon, I would also like to thank them for their help.

To the Food Technology staff, I thank them for all the assistance and guidence during my program at the University of Tennesee. I would like to espesically thank Nancy Austin for her secretarial help and friendship.

Thanks also to Bobby Grindstaff for his help and friendship throughout my college career.

I would like to express my appreciation to all my friends who helped my through some trying times inabling me to get this degree. To Fred Polhman, Sam Varner and Craig Bacon, I am especially thankfull for their support and friendship.

Finally, I would like to thank my parents for their love and support throughout my life.

ABSTRACT

Twelve boneless beef strip loins were fabricated into top loin steaks for packaging. One half of the steaks were chilled in a liquid carbon dioxide spray while the other half were unchilled. Each steak was placed into a barrier bag and the package was injected with one of the following gas mixtures: 1) 80%O_2:20%CO_2, 2) 40%O_2:15%CO_2:45%N_2 and 3) 10%O_2:15%CO_2:75%N_2. Two other treatments used in the study consisted of a vacuum packaged steak and a steak wrapped with PVC film was used for a control. The steaks were placed into a cooler at 3.3°C for a period of 0, 3, 6, 10 and 13 days of display and subsequently evaluated for relative percentages of various gases in the intact packages; Hunter 'L', 'a', and 'b' color values; myoglobin percentages of myoglobin, oxymyoglobin and metmyoglobin percentages; pH; microbial condition and sensory evaluation.

Packages initially injected with modified gas atmospheres containing O_2 increased in the amount of CO_2 with increased display time. This increase in CO_2 was due to either respiration of the meat tissue or to the conversion of O_2 to CO_2 by metabolism of certain bacteria on the meat surface.

The color of the meat was affected by the chilling time, packaging treatment and display time. The use of liquid CO_2 decreased percentage of the desireable oxymyoglobin form of the myoglobin pigment. The Hunter color values were unaffected by the chilling treatment.

The packaging treatment had different affects on the color of the meat. The amount of time the steaks were displayed also affected color.

The high oxygen packaged meat samples had increased levels of the oxymyoglobin while the meat samples packaged in a low oxygen level had high levels of myoglobin. As display time increased, the percentages of oxymyoglobin in the meat sample decreased and metmyoglobin levels increased. The decrease in oxymyoglobin was related to a decrease in the Hunter 'a' color value or redness.

The microbial condition of the meat was unaffected by chilling but was affected by packaging treatment and display time. The modified atmosphere packaged meat had an increase in the lag phase of growth attributed to increased CO_2 levels. The vacuum packaged meat had the highest aerobic plate counts among the packaging treatments but did not show any indication of bacterial spoilage. The MAP meats exhibited off-odors after 10 days of display. An increase in pH among meat samples of each of the different packaging treatments was correlated with an increase in bacterial growth.

Consumer panelists indicated that the steaks in the high oxygen MAP had an acceptable color and would purchase these products based on the color after 2 hours of display. However, due to a darkening problem, the color was found to be unacceptable throughout the remainder of the study. Steaks in the low oxygen MAP did not have an acceptable color even after 2 hours of display. The vacuum packaged meat had an unacceptable color initially, but after the 10th day of display, the panelists indicated an acceptable color.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

CHAPTER 1

INTRODUCTION

Fresh red meat is one of the few major food products processed and repackaged at the retail level (Cole,1986). Due to the cost associated with this type of system, it is essential for the red meat industry to move to a more centralized processing and packaging scheme. Centralized prepackaging allows for greater utilization of resources, better quality control, less waste through utilization of fat, bone and trimmings, more efficient use of manpower and space, improved profits, use of automated equipment, more uniform products, and better inventory control (Cole, 1986). Due to the economic as well as marketing advantages, a centralized packaging system needs to be developed which allows the meat packer to provide a true case-ready product.

In order to maintain the quality of a case ready meat product, it is essential to prevent discoloration, prevent microbial contamination, and maintain an acceptable shelf-life. Consumer studies have shown that physical appearance of a retail cut in the display case is the most important factor in determining retail selection of meat products (Jeremiah et al., 1972). The color of fresh meat is governed by the reactions between the muscle pigment and oxygen; either oxymyoglobin or metmyoglobin may be formed, the relative amounts depending on the oxygen partial pressure (Taylor and MacDougall, 1973). Modified atmosphere packaging is a method of retaining a bright red (oxymyoglobin) color as well as many other attributes. It has been well

documented that a modified atmosphere package can greatly enhance the color and shelf-life of fresh red meats (Baran, 1969; Huffman, 1975; Seideman, 1980). This packaging system typically uses a gas flushing technique and a gas impermeable package. Several gases including oxygen, carbon dioxide and nitrogen may be used in the preparation of the modified atmosphere. The advantages of a modified atmosphere include an extended transit time, higher quality maintenance, active inhibition of bacteria, molds and fungus, and reduced economic loss. However, this system has disadvantages which include visible added cost, variable product requirements, possible color changes, equipment and training requirements, and atmosphere maintenance (Wolfe, 1980).

The effect of carbon dioxide on the microbial growth on meat has been studied extensively (Baran, 1969; Clark, 1973; King and Nagel, 1975). Moran et al. (1932) found that a low concentration of carbon dioxide had a markedly inhibitory effect on the growth of molds on lean meat. Enfors (1979) concluded that shelf-life of fresh meat is significantly prolonged by storage at high partial pressures of carbon dioxide due to the selection process resulting in predominance of lactic acid producing bacteria. The bacterial inhibition by carbon dioxide involves the extension of the lag phase and generation time, thus resulting in a retarded growth rate (Holland, 1980).

Preliminary observations have indicated that liquid carbon dioxide increased color shelf-life of fresh ground beef (Melton, 1990). Little, if any, research is reported on the effect of a liquid carbon dioxide chilling treatment followed by packaging in a modified atmosphere on the shelf-life of fresh whole muscle cuts, therefore, the objective of this experiment

was to determine the effects of a liquid carbon dioxide pretreatment and a modified atmosphere package on color stability, microbial spoilage, and pH of beef loin strip steaks. This packaging process, if successful, should allow for centralization in the fresh red meat industry.

CHAPTER 2

REVIEW OF LITERATURE

Modified atmosphere packaging

The package is the primary marketing tool for transferring the product from the processing plant to the consumer. The package acts as a protective mechanism as well as providing a means for maintaining the freshness of the product for extended periods of time. Packaging cannot improve the quality of a product, it can only delay the onset of spoilage by regulating the factors that contribute to it. Therefore, the product is only protected for a limited amount of time, determined by the system used. High storage temperature and high initial bacteriological counts greatly reduce the shelf-life of meat packed in any atmosphere (Hermansen, 1983; Holland, 1980). Because of this fact, any packaging system needs to be conducted under extremely good hygiene and under the best possible temperature control.

The use of modified atmosphere packaging can be a method of retaining the quality of fresh red meat. Packaging fresh meat in a modified atmosphere is mainly done in thermoformed trays where a gas-mixture is backflushed into the package after vacuumizing (Hermanson,1983).

Modified atmosphere (MA) packaging of beef steaks has only a few advantages compared to vacuum-packaging. The bright red color of steaks packaged in a modified atmosphere is synonymous with freshness while

the purple color of vacuum-packaged meat is much less appealing to the consumer. Bone-in cuts may also be packaged more readily in a MA package than a vacuum package. Although meat in a MA package has a shorter shelf-life than meat in a vacuum package, it is considerably longer than the conventional PVC wrapped grocery store product. Gases used in MA packages include oxygen, carbon dioxide, nitrogen, carbon monoxide, or a combination of gases. Each gas has a specific function in the extension of the shelf-life of refrigerated fresh meats.

Oxygen

Oxygen has two major functions in a modified atmosphere storage environment. A high oxygen concentration inhibits the growth of certain pseudomonas strains such as Moraxella and Acinetobacter (Holland, 1980). In addition, an oxygen concentration in excess of 5% is necessary to maintain the oxygenated form of myogloin (oxymyoglobin) and to prevent the conversion of the fresh meat pigment to the brown metmyoglobin (Clark et al., 1973; Ledward, 1970).

In a modified atmosphere, as the concentration of oxygen increases, the thickness of the red surface layer of the meat increases and the color shelf-life is extended as stated by Taylor and MacDougall, (1973). Robach and Costilow (1961) examined the role of bacteria in the oxidation of myoglobin. In this study, they concluded that pigment oxidation and reduction can be controlled by physically adjusting the level of oxygen in the storage atmosphere. Furthermore, the rate of oxygen uptake on the surface of the meat tissue was correlated with microbial activity and color

change. As the microbial activity increases, there is an increase in color degradation.

Carbon Dioxide

Carbon dioxide has been found to selectively inhibit the growth of gram negative bacteria, such as pseudomonads and other related psychrotrophs which grow rapidly and produce off-odors and -flavors in raw meats and poultry. The bacterial inhibition by carbon dioxide involves the extension of the lag phase and the generation time, thus resulting in a retarded growth rate (Haines, 1933; Holland, 1980; Clark and Lentz, 1972). King and Nagel (1975) stated that carbon dioxide causes an observable block in metabolism of *Psuedomonas aeruginosa* as evidenced by a decrease in isocitrate and malate dehydrogenase activity. They explained that due to a mass action of CO_2 upon enzymatic decarboxylation, CO_2 has a specific effect on a few enzymes. However, Gill and Tan (1979) disagreed with this explanation and stated that in most cases maximun inhibition is not total and occurs at comparatively low CO_2 concentrations, whereas the postulated mechansim should result in a decrease in growth rate directly proportional to CO_2 concentration, and complete inhibition of CO_2 should be possible.

Lea (1933) found that CO_2 had a marked effect as it retarded the onset of taint in beef fat at 0°C. Lea stated that for the purpose of protecting the fat, there was comparatively little advantage from increasing the CO_2 concentrations beyond 15-20%. Those concentrations

appeared to have no harmful effect on the flavor of fat, nor on its resistence to oxidation during a storage period of 50 days.

Moran et al. (1932) stated molds such as Thamnidium, Chaetocladiodes, T. elegans, Mucor mucedo, Cladosporium herbarum and Penicillum expansum are also inhibited in low concentrations of carbon dioxide.

Carbon Monoxide

The use of carbon monoxide in MA packaging of meat raises many questions with regard to safety and efficacy. Carbon monoxide has been reported to be used as a color stabilizer, but possible toxic effects have limited CO use in commercial production. Clark et al. (1976) examined low-level carbon monoxide containing atmospheres and, in addition to visual evidence of shelf-life extension, found evidence for inhibition of psychrotrophic bacterial growth. Lanier et al. (1978) showed that the presence of CO increased the proportion of myoglobin present in a reduced state in stored meats.

Nitrogen

Nitrogen is the most abundant component in the atmosphere. Nitrogen is used as an inert filler to displace or dilute oxygen and to maintain a pillow pack to prevent package collapse from carbon dioxide absorption into the meat product (Cole, 1986). Nitrogen gas is also used to overcome extreme exudation and meat distortion caused by some vacuum

packaging techniques (Holland, 1980). However, nitrogen has been shown to increase the incidence of off-odor, increase visual microbial damage, and decrease the attractiveness of subcutaneous fat (Smith et al., 1977).

Gas mixtures

Optimum meat color and bacterial inhibition may be accomplished through mixtures of the three main gases (oxygen, carbon dioxide and nitrogen). Research has indicated that certain mixtures of these gases can prolong the shelf-life and preserve the bright red color of fresh red meat. Clark and Lentz (1973) indicated that meat packaged in a 15% CO_2 : 50-85% O_2 gas mixture had a longer odor and color shelf-life than when individual gases were used alone. Bartkowski et al. (1982) stated that meat packaged in gas mixtures containing 15% CO_2 : 75% O_2 had lower psychrotrophic bacterial counts than meat in atmospheres containing single gases.

The effect of freezing/chilling on meat

The freezing of meat has been used as a preservation method for many years. The preservation action of refrigeration is based on the prevention of multiplication of harmful bacteria, yeasts, and molds by mechanically lowering the temperature. The lack of available water, due to formation of ice, causes the inhibition of growth of most microorganisms.

Two unfavorable changes occur as a result of freezing. First, the physical state of the muscle plasm (globulin and albumen proteins) is considerably altered (Gracey, 1986). The formation of ice crystals occurs when meat is frozen below -2°C, which elevates the concentration of the muscle proteins so that they become insoluble and do not regain their solubility upon thawing (Gracey, 1986). Secondly, the speed of freezing has an important bearing on the size of the ice crystal and the future quality of the product. When meat is frozen slowly (-0.5 to -4°C), large ice crystals are formed and are located outside of the muscle fibers. If the meat is stored at this temperature, the crystals will continue to grow in size and eventually will cause rupture of the muscle cells and tissues upon thawing (Gracey, 1986). This phenomenon will cause excessive water loss or weep. On the other hand, if meat is frozen rapidly to a temperature below -4°C, the ice crystals are small and lie mainly within the muscle fibers (Gracey, 1986). With the rapid freezing method, there is little 'weep' or 'drip' in the product upon thawing.

Kraft et al. (1970) examined the use of liquid nitrogen for rapid freezing as a pretreatment for fresh beef steaks. This study compared conventional air-freezing to a liquid nitrogen freezing method. The results indicated the liquid nitrogen freezing treatment increased the lag phase of the bacteria studied. Bacterial numbers were also considerably greater on the meat frozen in air and stored for 3 or 4 days at 5°C than on the liquid nitrogen frozen samples. Percentages of metmyoglobin were also measured to determine the effect of freezing on color. Formation of metmyoglobin was slowest on meat frozen with liquid nitrogen.

Gas packaging: The effect of various gas atmospheres on the color of meat

Consumer studies have shown that physical appearance of a retail cut in the display case is the most important factor in determining retail selection of meat products (Jeremiah et al., 1972). Factors which influence the visual appearance of meat are rapidly assessed by a consumer and interpreted into a response: to buy or not to buy; to eat or not to eat (Mackinney et al., 1966). The acceptability of fresh meat is determined primarily by the color of the lean, a quality trait that is most sensitive to environmental conditions. Some factors which affect the color of packaged retail beef cuts include origin of the cut, package type and storage condition (Fellers et al., 1963).

The color of meat is related to the proportions of the three different forms of the muscle pigment myoglobin- reduced myoglobin, oxymyoglobin and metmyoglobin. Reduced myoglobin (purple) is the predominant muscle color form in the absence of oxygen. Oxymyoglobin (bright cherry red) is the oxygenated form of the muscle pigment, while metmyoglobin (brown) is the oxidized form of myoglobin or oxymyoglobin. Metmyoglobin may be formed from oxidation at low oxygen partial pressures or from the oxidation of the iron heme in the myoglobin molecule (Seideman et al., 1984).

The reaction of myoglobin at certain partial pressures is very important in the packaging of fresh red meat. Low oxygen partial pressures must be avoided to reduce chances of metmyoglobin formation. However, high partial pressures of oxygen may also cause metmyoglobin

formation (Cole, 1986). On the other hand, Watts (1954) stated oxygen tension at intermediate levels was demonstrated to be more rapid in converting myoglobin to metmyoglobin than very high or very low tensions.

Exposure to oxygen before packaging allows for oxygen molecules to adhere to the muscle surface (Cole, 1986). Because of this, the packaging of fresh red meat should be done within 15 minutes after slicing to minimize metmyoglobin formation (Cole, 1986).

Research has indicated certain gas atmospheres may effect the color of beef steaks. In a study conducted by Bartkowski et al. (1982), the effect of modified atmospheres on beef color was examined. Steaks were placed on styrofoam trays and packaged according to their randomly assigned treatments. These treatments included 40% O_2: 15% CO_2: 45% N_2; 10% O_2: 15% CO_2: 75% N_2; 10% O_2: 10% CO_2: 80% N_2; and 75% O_2: 15% CO_2: 10% N_2. The steaks were placed in open top display cases and held at 4°C. The research found, using a index of fading, steaks packaged in an atmosphere containing 40% O_2: 15% CO_2: 45% N_2 had the lowest degree of fading at 9 days of storage. They also found that an atmosphere containing 75% O_2: 15% CO_2: 10% N_2 was adequate for maintaining the color during storage. From these findings, they concluded that carbon dioxide at concentrations of 15% inhibited microbial growth but did not promote darkening of the meat and when combined with high levels of oxygen appeared to provide a gas atmosphere mixture which was capable of increasing shelf-life of beef cuts to at least 9 days of retail display.

Huffman et al. (1975) looked at the effect of various gas atmospheres on color and microbial growth of beef. These researchers

found that samples stored in O_2 had better color (higher reflectance) than samples stored in pure air, N_2, CO_2, or a mixture (5% O_2: 25% CO_2 :70% N_2) of these gases. From a subjective standpoint, the steaks stored in a pure nitrogen atmosphere, had the least desirable color and all steaks had an undesirable color after 23 days of storage. However, steaks stored in CO_2 and the mixture of gases had significantly ($P< .05$) lower aerobic bacterial counts through 27 days post-slaughter than any of the other treatments.

Partmann et al. (1970a, b) examined the behavior of beef in controlled atmospheres at 7°C and 3°C. They found that meat in mixtures with high levels of CO_2 and air had a more satisfactory color acceptance at 7 days of storage than meat in a gas mixture of 1% O_2 and 99% N_2, but the color of the meat, with high CO_2 levels, was considered unacceptable when stored at either temperature.

Carbon dioxide and nitrogen gas mixtures have received considerable attention in preserving beef steaks. The most frequently used combination of the gases is a mixture of 20% carbon dioxide and 80% nitrogen. Partmann et al. (1975) examined beef longissimus steaks stored in this mixture with a defined volume and surface area at 1°C. The steaks stored in a still atmosphere had an excellent color and visual appearance of freshness after 6 weeks of storage while the steaks stored in a flowing atmosphere had poorer color stability. It was concluded that traces of oxygen present in the package of the flowing atmosphere were responsible for the color change.

In a study conducted by O'Keefe et al. (1975), the color changes of the muscle groups Psoas major, Semimembranosus, and Gluteus medius stored in various gas atmospheres at 0°C were examined. They concluded

that pure CO_2 had a deteriorating effect on meat color, but the color of meat stored in O_2 + CO_2 enriched atmospheres remained acceptable longer than meat stored in air. The extension of the keeping time increased as the concentration of O_2 increased in the gas mixture. They also found that after a storage period of several days in pure N_2, meat re-exposed to air 'bloomed' to an acceptable red color.

Seideman et al (1979) examined the effect of packaging beef longissmus and loin steaks in gas atmospheres containing either 100% O_2, 100% CO_2, or 100% N_2. These steaks were stored for periods of 7, 14, 21 or 28 days at 1-3°C. These researchers found that an atmosphere containing 100% CO_2 could be an alternative method of packaging some fresh red meat products. After 3 days of retail display, longissimus steaks stored in an atmosphere initially containing 100% CO_2 had lower psychrotrophic counts and less surface discoloration than steaks stored in oxygen or nitrogen. Loin steaks, on the other hand, stored up to 28 days in 100% carbon dioxide were more discolored and had a lower percent of oxymyoglobin than similar steaks stored in oxygen or nitrogen. Ledward (1970), found increased formation of metmyoglobin (browning) in steaks packaged in high concentrations of carbon dioxide and low oxygen partial pressures. A problem Seideman et al (1979) found was that the steaks packaged in 100% CO_2 had considerably longer 'bloom' time than vacuum packaged steaks and concluded that CO_2 may bind to the meat proteins decreasing their ability to hold moisture and bloom rapidly. The principal disadvantages of use of high CO_2 atmospheres in fresh meat storage are color darkening due to metmyoglobin formation and continuation of fat oxidation as stated by Wolfe (1980).

Taylor and MacDougall (1973) examined the changes in gas composition since gases dissolve in meat and respiration of tissue consumes oxygen and produces carbon dioxide. They studied changes in hue (Gardner Color Difference Meter model Ac2a) and saturation (Gardner Color Difference Meter model Ac2a) in the surface of fresh beef samples stored in mixtures of CO_2 and O_2 and in air for up to 12 days at 1°C. They found samples packed in air had a gradual increase in hue as red changed to brown, accompanied by a fairly rapid loss of saturation as grayness developed. The steaks packaged in the O_2 and CO_2 mixtures remained red longer and had a slow loss of saturation. The research also found that increasing the oxygen concentration in the controlled atmopshere, increased the thickness of the oxymyoglobin surface layer. They concluded best results would be obtained using the highest possible concentrations of oxygen together with sufficient carbon dioxide to be most effective in delaying saturation.

Brooks (1933) looked at the effect of carbon dioxide on the color changes of lean beef. This researcher found with moderate concentrations (30-40%) of CO_2 there is no marked decrease in the depth of oxygen penetration which would be favorable to rapid discoloration. He also concluded that oxidation of myoglobin in muscle is not affected by concentrations of CO_2 below 20%.

Gas packaging: The effect of various gas atmospheres on the microbiology of meat

The first practical use of modified atmospheres containing elevated levels of carbon dioxide as a preservative in the handling of fresh meat was in the shipment of whole chilled beef carcasses from Australia and New Zealand to Great Britian in the 1930's (Silliker and Wolfe, 1980). Since that time, many researchers have studied the effects of various gas atmospheres on the microbiology of fresh meat.

Ogilvy and Ayres (1952) were some of the early researchers who examined the effect of carbon dioxide on microbial growth on stored frankfurters. In this study, they stored frankfurters in several different percentages of carbon dioxide at 4.4°C. The results indicated that carbon dioxide at high levels had an inhibitory effect on the growth of micrococci, molds, yeasts and to a lesser extent lactobacilli. Carbon dioxide was found not only to influence the growth of microorganisms, but also to have a selective action on the types of organisms developing on the frankfurter surface which ultimately cause spoilage.

The effect of carbon dioxide on individual meat spoilage organisms has been studied very little. Some researchers have indicated that gram-negative organisms are more susceptible to inhibition by carbon dioxide than gram-positive organisms. Gill and Tan (1979) found this to be an over-simplification of the situation, because certain species of both groups can be completely insensitive to CO_2. However, they indicated the most important gram-positive organisms are the facultative or strict anaerobes, and anaerobic growth is evidently not inhibited by CO_2, whereas the most

important gram-negative bacteria are strict aerobes and are susceptible to CO_2 inhibition.

The growth of psychrotrophic slime-producing bacteria are a major source of spoilage in refrigerated meats. Since carbon dioxide inhibits growth of slime-producing bacteria, its use in the atmosphere of transport vehicles was demonstrated to be a method of solving the slime problem. Clark and lentz (1969) looked at this effect of carbon dioxide on the growth of slime-producing bacteria on fresh beef. They found 20% CO_2 markedly inhibited the growth of bacteria that cause the formation of slime on fresh beef stored at a high humidity, provided the gas was applied before the organisms have become adjusted to the environmental conditions. In a similar study, Clark and Lentz (1972) looked at the use of carbon dioxide for extending the shelf-life of prepackaged beef. Steaks from the rump (round) muscle were inoculated with strains of *Psuedomonas* and *Achromobacter* and placed into paperboard trays, overwrapped with a polyvinylchloride (PVC) film, and incubated at various temperatures in saturated atmospheres of different carbon dioxide concentrations (0, 5, 10, 15 and 20%). Results indicated CO_2 continuously present would extend the shelf-life to more than 10 days, depending on the CO_2 concentration, initial number of bacterial cells on the meat surface and storage temperature. Fifteen percent carbon dioxide was the most practical level of treatment since it was nearly as effective as 20% and was almost twice as effective as 10% CO_2.

Wolfe (1980) stated that the use of carbon dioxide enriched atmospheres for meat products can retard growth of spoilage organisms, accompanied by rapid attainment of low temperatures. Christopher et al.

(1979) looked at the microbiology of beef packaged in various gas atmospheres. In this study, psychrotrophic and lactobacilli counts of conventional vacuum-packaged beef and comparable roasts stored in six gas atmospheres were compared. Unlike data found by many other researchers, the differences in microbial counts were not statistically significant. However, they concluded there was inhibition of Pseudomonas in atmospheres containing 20 to 50% CO_2 indicating a direct action of CO_2 on gram negative bacteria.

Silliker et al. (1977) tried to determine whether long term exposure of meat to atmospheres enriched in carbon dioxide would result in a post-treatment extension of shelf-life. Treatments used included 1) air atmosphere, 2) 20% O_2: 60% CO_2: 20% N_2, and 3) 25% O_2: 20% CO_2: 55% N_2. The samples were stored for 7 and 14 days at 1°C. After 14 days, samples were placed air desiccators for further study. Results showed after 7 days of storage, both samples stored in carbon dioxide had 10^2 lower counts than the air control samples. After 14 days of storage, the samples stored in CO_2 had 10^5 lower counts, and still exhibited satisfactory odor. In contrast, the samples stored in air had extensive microbial growth on the surface and the meat exhibited an off-odor. The most important finding in this study was not only that there was clear evidence of an anti-microbial effect of CO_2, but a residual effect as evidenced by continued inhibition of microbial growth after exposure to CO_2.

Silliker and Wolfe (1980) examined the microbiological safety considerations of ground beef stored in various gas atmospheres. They looked at samples inoculated with salmonella, staphylococcus,

enterococcus and putrefactive anaerobes (Clostridium botulinum). On the basis of their findings, there was no evidence that elevated levels of carbon dioxide would increase the salmonella hazard. Furthermore, storage in CO_2- enriched atmospheres should not increase the hazard of staphylococcus food poisoning. The data also clearly indicated that environments enriched with carbon dioxide did not selectively favor the growth of Enterococcus. Finally, with respect to botulism, there was no indication that controlled atmospheres do not increase the hazard of this type of food poisoning.

Gas packaging: The effect of various gas atmospheres on pH of meat.

The pH of normal meat varies from approximately 5.2 to 6.6, owing largely to differences in amount of glycogen available for transformation into lactic acid following slaughter. Fresh meat should be in the mid-range (5.6-6.0) of the pH scale, since low pH values were found to be directly related to accelarated oxidation of fresh meat which resulted in rapid formation of metmyoglobin at low pH values (Watts, 1954).

Muscle pH can drastically affect microflora growth and fresh muscle must be controlled or selected for pH in retail packaging systems. Microorganisms have a pH range for optimum growth that is generally near neutrality (pH 7.0). Tomkins (1932) stated carbon dioxide lowers the pH of aqueous solutions and thus reduces the growth rate of organisms. Haines (1933) studied the influence of carbon dioxide on the rate of

multiplication of certain bacteria and found that the change in pH caused by CO_2 did not account for the retardation of growth. It was suggested that an explanation may be found in the action of the gases on the dehydrogenating enzymes of the cell. King and Nagel (1967) concluded that the influence of low pH caused by CO_2 in unbuffered solutions was a major factor in slowing microbial growth rate. On the other hand, Huffman et al. (1975) indicated the decrease in microbial growth caused by CO_2 was not the result of lowered pH.

Clark and Lentz (1973) looked at mixtures of carbon dioxide and oxygen and its effect on surface pH of fresh beef steaks. They found that 70-80% oxygen and 15% carbon dioxide had no effect on pH of the meat or weight loss due to weep.

Regarding the use of high carbon dioxide levels, Ledward (1970) reported an increased CO_2 adsorption on the meat surface at high CO_2 partial pressures resulting in a reduced muscle surface pH. Ledward (1970) concluded the decrease in pH of steaks stored in CO_2 enriched atmospheres was attributed to activity of Lactobacilli bacteria and/or dissolving of CO_2 in meat fluids. Seideman et al. (1979) stated that mean values for surface pH of steaks stored in atmospheres containing 100% oxygen were significantly higher than those of vacuum-packaged steaks or steaks packaged in 100% CO_2. They concluded the increase in pH was attributed to the result of bacterial proteolysis.

Consumer retail Acceptance

From the viewpoint of the meat processor, the consumer is the final determinant in whether the product is acceptable or not. Steps must be taken to assure the product will pass the consumers acceptance test.

Quality grading standards of the USDA are very subjective and are subject to wide variations in interpretation (Jeremiah et al., 1972). The ideal color of beef is considered "cherry red" but since people differ in their individual response to visual colors, they will also differ in their interpretations of subjectively defined terms for color (Jeremiah et al., 1972). Therefore, an objective method, as well as a subjective method of color determination, is necessary before accurate color characteristics can be made between laboratories.

As stated earlier, color is a very important factor involved in consumer acceptance of fresh beef. Data presented in a study done by Jeremiah et al. (1972) indicated that pigment concentration alone does not serve as a reliable guide for monitoring visual color of beef cuts. A variety of instruments are needed for accurately estimating meat color.

Elliott (1969) examined the relationship between the subjective and objective measurement of pork color. In this study, a spectophotometer, a simple color reflectometer, and ten untrained graders were used to determine color characteristics. There was considerable variation between panelists score, while the results obtained from the color meter and the spectophotometer were quite similar and reproducible. Elliott concluded that both subjective and objective measurements of color are required for the determination of color quality.

CHAPTER 3

MATERIALS AND METHODS

Experimental design

Twelve boneless strip loins were purchased from IBP, Inc. through Lay's Market in Knoxville, Tennessee. The strip loins were stored for 21 days in a vacuum package at the outset of the study. The strip loins were fabricated with minimal exposure to the air, into top loin steaks (2.54 cm thick) at the University of Tennessee meats laboratory. Each steak was individually marked so that randomization would occur in assigning steaks to treatments. Before the steaks were able to "bloom", one half of the steaks were placed into a Kryospray freezer (Model BF300SD, Cryo-Chem Inc., Gardena, CA) for chilling in liquid carbon dioxide spray (8 to 10 minutes at -59.4°C) before packaging. No prechilling treatment was used on the other half of the steaks. The steaks were individually packaged in barrier bags (bag #B650, Cryovac Division, W.R. Grace & Co., Simpsonville, S.C.). Packaging treatments for the steaks included: modified atmospheres of 80%O_2:20%CO_2 (treatment 1), 40%O_2:15%CO_2:45%N_2 (treatment 2), and 10%O_2:15%CO_2:75%N_2 (treatment 3); vacuum packaging (treatment 4), and a control (air) that consisted of wrapping a steak placed in a styrofoam tray with polyvinyl chloride (PVC) film (treatment 5). All steaks, except the PVC wrapped steaks, were packaged using a Koch Multivac packaging machine (Model AG4, Koch Industries, Kansas City, MO) with gas flush capability.

The steaks were then placed into a simulated retail counter and stored for periods of 0, 3, 6, 10, and 13 days at approximately 5°C. One steak represented each storage period within each packaging and chilling treatment. There were three replications of this study.

Analysis of samples

Package headspace

The relative weight percentages of oxygen, nitrogen and carbon dioxide in the headspace of the packaged meat were determined according to Seideman et al. (1980). The analysis was repeated at the end of each of the storage periods. A rubber septum was applied to the exterior of the packaging film to facilitate sampling of the gas atmosphere. A 4 ml sample of headspace gas was drawn through an 18-gauge needle with a gas-tight syringe and immediately analyzed by gas chromatography.

The gas analysis was conducted using a Hewlett Packard (Model 5890) gas chromatagraph (GC) with a thermal conductivity detector and a Hewlett Packard (Model 3393A) electronic integrator. Separation of the gases was accomplished using a 4.8 M x 0.3 cm i.d. stainless steel column packed with carbosieve B (Supelco Inc., Belefonte, PA). The GC oven was programmed at 70°C and calibration of the integrator was achieved using standard gas mixtures (Supelco Inc., Belefonte, PA). Carrier gas (Helium) flow rate was kept at 37.5 ml/min.

Color

Surface color values for each steak under different atmospheric treatments were measured using the Hunterlab Color/Difference Meter (Model D25-2). The Hunter colorimeter was standardized with a pure pink color standard (L-69.0; a- 22.0; b-11.9). Measurements taken included the 'L' value denoting lightness, 'a' value denoting redness, and 'b' value denoting yellowness. The Hunter L, a, and b values of each were compared.

The percentages of myoglobin (Mb), oxymyoglobin (OMb), and metmyoglobin (MMb) on the surface of each steak were estimated by a modified procedure of Snyder and Aryes (1961). Spectra for Mb, OMb and MMb were determined using a procedure of Warriss (1979). Forty mg of crystallized horse skeletal myoglobin (Sigma Chemical Co., St. louis, MO) was dissolved in 50 ml of phosphate buffer (0.04 ionic strength, pH 6.8) to form MMb and the percent transmission (%T) was determined using an IDL Color-eye (Model D-1, Instrument Development Laboratories, Attleboro, MA) at 20 nm intervals from 400 to 700 nm wavelengths. A small quantity (0.02 g) of sodium hydrosulfite was then added to reduce MMb to Mb and the %T of Mb at the same wavelengths was determined. Care was taken to keep the solution under nitrogen at all times to reduce exposure to oxygen. Reduced Mb was then shaken in air until it became oxygenated to form OMb and the %T from 400 to 700 nm was determined.

From the spectra obtained for the pure myoglobin forms, 520 nm (isobestic point for all forms of Mb), 540 nm (wavelength of maximum absorbance for OMb), 560 nm (wavelength of maximum absorbance for Mb), and 640 nm (wavelength of maximum absorbance for MMb), were

chosen as analytical wavelengths for measurement of different forms of myoglobin. The absorptivity of each form of myoglobin at each of these wavelengths was determined from the spectra. Solving simultaneous equations, the following equations were derived to determine the concentration (mg/100ml) of Mb, OMb, and MMb (Melton, 1990);

OMb- 13.924*COMb - 4.922*A at 540 - 1.489*A at 520 - 3.561*A at 560,

MB- 12.969*CMb + 8.901*COMb - 3.561*A at 560 - 3.063*A at 520,

MMb- 6.25*CMb + 3.75*COMb + 10.0*CMMb - A at 640,

where, A at 520 - Log Ro/%R at 520 nm,

A at 540 - Log Ro/%R at 540 nm,

A at 560 - Log Ro/%R at 560 nm,

A at 640 - Log Ro/%R at 640 nm,

Ro - Percent reflectance of white standard

%R - Percent reflectance of meat sample

C - Concentration of the pigment.

The initial readings were taken approximately two hours after the steaks were packaged. The other readings were taken immediately after the consumer panel evaluated each steak.

pH

The pH of each steak was determined by blending a 10 gram meat sample in 200 ml of distilled water for 2 minutes as described by Ockerman (1972). The pH of this solution was then measured using a calibrated Fisher Accumet pH meter (Model 600). The electrode used to measure the pH was carefully washed with distilled water and wiped between samples.

Microbial analysis

The effect of the chilling and packaging treatments on microbial growth was determined by aseptically removing a random portion of each steak after the end of each storage period. The samples were prepared by placing 25 grams of meat into a sterile stomacher bag containing 225 ml of sterile 0.1% peptone diluent and blending (Stomacher 400 Trekmar, Inc., Cincinati, OH) for 2 minutes. Aerobic plate counts were determined using Standard Methods Agar pour plates incubated at 32°C for 48 hours and the pyschrotrophic bacterial counts were determined using SMA pour plates incubated at 7°C for 72 hours.

Sensory analysis

At the termination of each storage period, the steaks were examined by a consumer panel under simulated retail display conditions. A retail display case was to be used in the experiment, but due to a temperature malfunction in the display case, the steaks were placed on a table in a cooler at 3.3°C. The steaks were constantly exposed to light, except the light had fewer foot candles than the light in the the display case. Twenty five consumer panelists visually evaluated each steak and indicated whether the color was acceptable or unacceptable and then, based solely on that color, indicated whether or not they would be willing to purchase the product. Since there was no basis for expecting a yes or no response, it was considered a two-tailed test. Using binomial distribution, 18 of 25 concurring responses were needed for the steak to be considered accptable (Stone and Sedel. 1982).

Statistical analysis

The General Linear Models procedure of SAS (1985) was used to analyze the data with the independent variables of gas treatments, chilling treatment, display time and replication. Mean separation was accomplished using the Student-Newman-Keuls test. Sensory data was analyzed using binomial distribution for number of responses for color acceptability of each steak for each display period and packaging treatment.

CHAPTER 4

RESULTS AND DISCUSSION

Package headspace

A modified atmosphere was created inside the package, but the composition of the package headspace changed throughout the display period. Changes in headspace are very important and affect the color as well as the microbial condition of meat products.

The package headspace was sampled for each display period and the relative mean percentages of carbon dioxide, oxygen and nitrogen are shown in table 1. Due to a problem with the gas chromatograph, the data for 3 and 6 days of display were unavailable. Seideman et al. (1979) reported steaks packaged initially in 100% O_2 or 100% N_2 had an increase in the percentage of CO_2 as storage period increased, whereas steaks packaged in 100% CO_2 showed a gradual decrease in the percentage of CO_2. In this study, the same trend was assumed to have occurred. The percentages of CO_2 increased as display time increased with a concurrent decrease in the percentage of O_2. Taylor and MacDougall (1973) reported that both O_2 and CO_2 dissolve in meat tissue, while respiration consumes O_2 and produces more CO_2. In another study done by Seideman et al. (1980), the report indicated little changes of the atmospheres in packages injected with modified atmospheres. The percentage of CO_2 usually increased with prolonged storage, concurrent with a decrease in the percentage of O_2. Daun et al. (1971) concluded that the result of the

Table 1. Relative mean weight percentages of carbon dioxide, oxygen and nitrogen of beef loin steaks displayed in different packaging treatments.

		Packaging treatment		
Display days	Type of gas	80%O_2 20%CO_2	40%O_2 15%CO_2 45%N_2	10%O_2 15%CO_2 75%N_2
0*	O_2	80.40	39.80	10.10
	CO_2	19.60	14.30	15.30
	N_2	0.00	45.90	74.60
3	O_2	-	-	-
	CO_2	-	-	-
	N_2	-	-	-
6	O_2	-	-	-
	CO_2	-	-	-
	N_2	-	-	-
10	O_2	68.34	26.54	6.28
	CO_2	31.66	21.89	27.09
	N_2	0.00	51.57	66.63
13	O2	66.50	13.20	5.70
	CO2	33.51	29.56	32.15
	N2	0.00	57.24	62.15

*bottle lable values.

interaction between gas solubility, temperature and headspace volume caused the increase in CO_2 in modified gas atmosphere packages. On the other hand, Enfors and Molin (1984) stated that not only the meat tissue, but also the bacteria, respire and convert O_2 to CO_2.

Taylor and MacDougall (1973) indicated that it was important that the package does not come in contact with the meat surface. Should this happen, the oxygen would no longer have access to the meat surface which is necessary in this type of packaging. This problem occurred in our study since the packaging film came in contact with the meat surface of some steaks causing darkened areas which affected other color attributes.

Color

Hunter color/difference meter values

The Hunter color/difference meter determines the differences in lightness ('L'), redness ('a'), and yellowness ('b'). This instrument determines differences in color which, in many cases, the human eye can not (Mount, 1990).

The results of the mean Hunter 'L' values for each packaging treatment and display period are presented in table 2. The Hunter 'L' values for the chilling treatments were pooled and appear as packaging treatment means in table 2. The 'L' value indicates the degree of lightness/darkness, with 100 representing white and 0 representing black. Hunter 'L' values for the steaks were not affected ($P > .05$) by

Table 2. Mean and standard deviation of Hunter 'L' values[a] for beef loin strip steaks displayed in different packaging treatments.

	Display days					Treatment
Packaging treatment	0	3	6	10	13	means
1. 80%O_2 : 20%CO_2	28.9[b] ±0.92	30.8[b] ±4.46	28.7[b] ±2.28	29.3[b] ±3.59	30.4[b] ±2.04	29.7[x]
2. 40%O_2 : 15%CO_2 : 45%N_2	25.7[b] ±2.96	31.3[b] ±1.92	29.5[b] ±2.91	29.1[b] ±4.76	30.8[b] ±2.51	29.8[x]
3. 10%O_2 : 15%CO_2 : 75%N_2	26.9[b] ±1.34	28.3[b] ±2.20	30.0[b] ±4.62	28.1[b] ±2.64	31.0[b] ±3.40	29.2[x]
4. Vacuum	32.7[b] ±0.70	28.2[b] ±2.23	29.7[b] ±2.84	28.5[b] ±1.91	31.5[b] ±3.32	30.2[x]
5. PVC (control)	33.6[b] ±3.25	28.6[b] ±1.83	31.2[b] ±2.54	30.4[b] ±1.89	30.2[b] ±2.60	29.7[x]

[a] Data for chilling treatments were pooled.

[b] Row means for display days with different superscripts are different ($P<0.05$).

[x] Treatment means (column) with different superscripts are different ($P<0.05$).

packaging, chill treatment or display time (Appendix A). The chilling treatment had no significant effect ($P < .05$) on the degree of lightness of any of the packaging treatments.

Significant differences (Appendix A) were found among treatments over display days (Table 3) for Hunter 'a' values. For treatment 1 ($80\%O_2:20\%CO_2$), 2 ($40\%O_2:15\%CO_2:45\%N_2$) and 5 (PVC), the Hunter 'a' values decreased ($P < .05$) over the 13 day display period while treatment 3 ($10\%O_2:15\%CO_2:75\%N_2$) had no decrease ($P > .05$) in redness over the storage period. The high oxygen level affected the conversion of oxymyoglobin to metmyoglobin as evidenced by the decrease in redness. Treatment 1 had a higher ($P < .05$) degree of redness, or overall treatment mean, than treatment 2 ($40\%O_2:15\%CO_2:45\%N_2$) which had the lowest overall degree of redness. This was due to the high initial percentages of oxymyoglobin. It was interesting that the Hunter 'a' value for the vacuum treatment actually increased ($P < .05$) over the storage period while the others decreased.

The analysis of Hunter 'a' values showed a significant ($P < .05$) packaging treatment by day interaction (Appendix A). At day 0, there were differences in Hunter 'a' values among all treatments. This was related to the amount of oxygen in the headspace of each of the packaging treatments. As oxygen levels decreased over the display period, the differences in Hunter 'a' color values decreased.

The mean Hunter 'b' values are listed in table 4. The chilling treatment compared to the unchilled treatment caused no difference in the yellowness (Hunter 'b' values) of the product (Appendix A). The modified gas atmosphere packages (treatments 1, 2, and 3) and the

Table 3. Mean and standard deviation of Hunter 'a' values[a] for beef loin strip steaks displayed in different packaging treatments.

Packaging treatment	Display days				
	0	3	6	10	13
1. 80%O_2 : 20%CO_2	$16.1^{b,xy}$ ±2.90	$11.8^{cd,x}$ ±3.20	$14.1^{bc,x}$ ±1.50	$9.4^{de,xy}$ ±3.32	$6.4^{e,y}$ ±1.87
2. 40%O_2 : 15%CO_2 : 45%N_2	$12.0^{b,z}$ ±3.64	$12.3^{b,x}$ ±1.63	$8.9^{bc,y}$ ±2.00	$7.2^{c,y}$ ±3.53	$7.0^{c,y}$ ±1.67
3. 10%O_2 : 15%CO_2 : 75%N_2	$12.4^{b,yz}$ ±2.89	$10.9^{b,x}$ ±2.57	$9.8^{c,y}$ ±2.57	$11.6^{b,x}$ ±2.30	$9.5^{b,x}$ ±2.39
4. Vacuum	$7.4^{c,ym}$ ±0.70	$11.1^{b,x}$ ±1.01	$11.6^{b,xy}$ ±0.83	$11.4^{b,x}$ ±0.85	$9.8^{b,x}$ ±2.57
5. PVC (control)	$17.5^{b,x}$ ±1.76	$12.9^{c,x}$ ±2.10	$11.7^{c,xy}$ ±3.08	$6.9^{d,y}$ ±0.56	$7.8^{d,xy}$ ±1.43

[a] Data for chilling treatments were pooled

^{b,c,d,e} Row means for display days with different superscripts are different ($P<0.05$).

^{x,y,z,m} Treatment means (column) with different superscripts are different ($P<0.05$).

Table 4. Mean and standard deviation of Hunter 'b' values[a] for beef loin strip steaks displayed in different packaging treatments.

	Display days					Treatment
Packaing treatment	0	3	6	10	13	means
1. 80%O_2 : 20%CO_2	8.2^{b} ±0.92	7.5^{b} ±2.25	7.7^{b} ±0.83	7.1^{b} ±1.54	6.8^{b} ±0.53	7.3^{x}
2. 40%O_2 : 15%CO_2 : 45%N_2	6.1^{b} ±2.12	8.0^{b} ±0.87	6.6^{b} ±0.70	6.6^{b} ±1.51	6.6^{b} ±0.76	6.9^{xy}
3. 10%O_2 : 15%CO_2 : 75%N_2	6.8^{b} ±1.20	6.4^{b} ±1.05	6.7^{b} ±1.56	6.2^{b} ±1.17	6.5^{b} ±1.23	6.5^{yz}
4. Vacuum	6.3^{b} ±0.21	6.1^{b} ±0.52	6.1^{b} ±0.89	5.4^{b} ±0.72	5.8^{b} ±0.87	5.8^{z}
5. PVC (control)	9.0^{b} ±1.27	7.3^{c} ±0.89	7.5^{c} ±1.12	6.2^{c} ±0.71	6.1^{c} ±0.96	6.9^{x}

[a] Data for chilling treatments were pooled.

[b,c] Row means for display days with different superscripts are different ($P<0.05$).

[x,y,z] Treatment means (column) with different superscripts are different ($P<0.05$).

vacuum package (treatment 4) had no changes ($P > .05$) in Hunter 'b' values over the display times, while the control (treatment 5) exhibited a decrease ($P < .05$) in Hunter 'b' values (Appendix A). The vacuum treatment had the lowest mean Hunter 'b' value which was lower ($P < .05$) than that of treatments 1, 2, 3 and 5. Treatment 1 ($80\%O_2$:$20\%CO_2$) had the highest overall mean Hunter 'b' value and was different ($P < .05$) than treatments 3 and 4.

Another indicator of color is the relative percentage of the different forms of the myoglobin pigment. The reduced form of the myoglobin pigment is purplish red in color and is predominant in the absence of oxygen. When exposed to oxygen, the pigment becomes oxygenated to produce oxymyoglobin, a highly desirable bright red color. However, when the surface is continuously exposed to oxygen, the pigment becomes oxidized, turning to an undesirable brownish red color (Cole, 1986).

Percent myoglobin

The mean percentages of myoglobin, the reduced form of the pigment, for the different packaging treatments and display times, are presented in table 5. For the high oxygen containing atmospheres (treatments 1 and 2), there was a high initial percent of myoglobin followed by a steadily decreasing amount of the pigment. Treatment 3 had a stable amount of myoglobin over time due to the low initial amount of oxygen. As would be expected, the vacuum treatment exhibited the highest amount of myoglobin over the storage period as well as the overall treatment mean due to the lack of oxygen. Pierson et al. (1970)

Table 5. Mean[a] percentages and standard deviations of myoglobin in beef loin strip steaks displayed in different packaging treatments.

	Display days					Treatment
Packaging treatment	0	3	6	10	13	means
1. 80%O_2 : 20%CO_2	51.1^{b} ±5.65	32.4^{b} ±9.51	32.8^{b} ±13.57	13.8^{c} ±8.40	9.2^{c} ±9.11	24.3^{z}
2. 40%O_2 : 15%CO_2 : 45%N_2	55.1^{b} ±3.18	35.3^{b} ±8.45	33.7^{b} ±17.96	24.2^{b} ±22.98	18.3^{b} ±25.00	29.6^{yz}
3. 10%O_2 : 15%CO_2 : 75%N_2	43.5^{b} ±1.13	41.2^{b} ±22.00	35.8^{b} ±22.45	57.4^{b} ±14.92	35.2^{b} ±30.70	42.4^{y}
4. Vacuum	56.8^{b} ±33.94	63.1^{b} ±3.74	65.8^{b} ±6.40	59.7^{b} ±17.31	63.6^{b} ±27.11	62.5^{x}
5. PVC (control)	42.7^{b} ±4.17	31.1^{b} ±10.37	24.6^{b} ±10.65	12.9^{b} ±8.61	27.9^{b} ±29.80	25.6^{z}

[a] Data for chilling treatments were pooled and appear as packaging treament means.

[b,c] Row means for display days with different superscripts are different ($P<0.05$).

[x,y,z] Treatment means (column) with different superscripts are different ($P<0.05$).

studied the effect of vacuum packaging on myoglobin formation. They found that there was a rapid decrease in oxymyoglobin after packaging while myoglobin levels increased to 100% within 8 hours. At the same time, there was an increase in metmyoglobin formation to a certain point then a decrease in metmyoglobin levels.

The high oxygen containing treatment (1) had the lowest overall treatment mean probably due to the myoglobin pigment being oxidized. There were no differences ($P > .05$) between chilling treatments on the effect of percent myoglobin.

Percent oxymyoglobin

The mean percentages of oxymyoglobin for beef loin steaks displayed in different packaging treatments are listed in table 6. Since the initial readings were taken two hours after packaging, there was indication of oxygenation of the myoglobin pigment at zero days of storage. The high oxygen treatment (80%O_2:20%CO_2) had a relatively high amount of oxymyoglobin initially and throughout the display period with a significant decrease ($P < .05$) from day 10 to day 13 (Appendix A). This indicates that the product should have a color shelf-life around 10 days. The high percentage of oxygen in the headspace, allowed for the conversion of the oxygenated form of myoglobin to the oxidized form of myoglobin. With treatment 2 (40%O_2:15%CO_2:45%N_2), there was a significant decrease ($P < .05$) in percentage of oxymyoglobin between the third and sixth days of display but additional decreases were not significant ($P < .05$). Treatment 3 had no decrease ($P > .05$) in oxymyoglobin over the display period, but there was a decreasing trend in

Table 6. Mean[a] percentages and standard deviations of oxymyoglobin in beef loin strip steaks displayed in different packaging treatments.

	Display days				
Packaging treatment	0	3	6	10	13
1. 80%O_2 : 20%CO_2	$39.4^{b,xy}$ ±0.42	$36.6^{b,x}$ ±8.58	$37.9^{b,x}$ ±3.07	$32.9^{b,x}$ ±13.92	$14.3^{c,x}$ ±13.45
2. 40%O_2 : 15%CO_2 : 45%N_2	$34.1^{b,y}$ ±1.69	$32.9^{b,x}$ ±7.64	$19.9^{c,y}$ ±10.08	$21.4^{c,x}$ ±8.61	$14.1^{c,x}$ ±8.22
3. 10%O_2 : 15%CO_2 : 75%N_2	$32.1^{b,y}$ ±1.06	$26.5^{b,xy}$ ±7.71	$24.7^{b,xy}$ ±10.41	$20.5^{b,x}$ ±5.74	$17.6^{b,x}$ ±10.04
4. Vacuum	$13.7^{b,z}$ ±1.97	$20.0^{bc,y}$ ±2.91	$21.1^{bc,y}$ ±3.62	$22.3^{b,x}$ ±5.11	14.17^{x} ±4.48
5. PVC (control)	$46.9^{b,x}$ ±14.42	$36.0^{bc,x}$ ±7.37	$32.1^{bc,xy}$ ±11.17	$20.2^{c,x}$ ±10.92	27.4^{xy} ±5.52

[a] Data for chilling treatments were pooled and appear as packaging treatment means.

[b,c,d] Row means for display days with different superscripts are different ($P<0.05$).

[x,y,z] Treatment means (column) with different superscripts are different ($P<0.05$).

oxymyoglobin. The low relative weight percentages of oxygen in the headspace of treatment 2 and 3 could explain why the oxymyoglobin levels decrease. Pierson et al. (1970) stated that in an aerobic environment oxymyoglobin levels will decrease over 7 days to levels under 10 percent. The vacuum treatment had a significant increase in oxymyoglobin, possibly due to excess exposure of the samples to air during the testing procedure.

The difference in percentages of oxymyoglobin among treatments over display time was related to the amount of oxygen in the headspace. Initially, there were significant differences between oxymyoglobin percentages, but as time increased, the level of oxygen decreased followed by a decrease in percentage of oxymyoglobin. At the end of the 13th day of display, there was no difference ($P < .05$) among the treatments for percentage of oxymyoglobin at the surface of the meat.

The chilling treatment had a detrimental effect ($P < .05$) on the percentage of oxymyglobin in the steaks (Appendix A). The unchilled steaks had higher ($P < .05$) mean oxymyoglobin percentages indicating a brighter, more desirable color than the chilled steaks. The CO_2 may have been absorbed into the meat surface causing a decrease in the oxygen levels at the meat surface.

Satterlee and Hansmeyer (1974) reported that fluorescent light increases the rate of conversion of oxymyoglobin to metmyoglobin of meat products. This might explain some of the reason why there was a short color shelf-life among all packaging treatments.

Percent metmyoglobin

The mean percentages of metmyoglobin for the beef loin steaks in the different packaging treatments and display times are in table 7. The liquid carbon dioxide treatment had no effect ($P > .05$) on the percent of metmyoglobin (Appendix A).

As would be expected, the packaging treatments with high oxygen levels (treatments 1, 2, and 5) had the lowest initial mean percentages of metmyoglobin (Table 7). However, as display time increased, the percent of metmyoglobin also increased. The package headspace is a possible indicator of why the metmyoglobin levels increased. As the display time increased, the percentages of oxygen decreased among all treatments. With the low oxygen concentrations, as in treatment 3 and the vacuum package (treatment 4), there was no increase ($P > .05$) in metmyoglobin due to low oxygen percentages in the package headspace. As far as color is concerned, the vacuum treatment had a lower ($P < .05$) percentage of metmyoglobin than all other treatments which should indicate a more desirable color. On the other hand, the high oxygen treatments had relatively high percentages of metmyoglobin in the early days of the display, which suggests an undesirable color in these products. Seideman et al. (1979) suggested that conversion of muscle pigments to their oxidized form was mostly associated with color deterioration. Robach and Costilow (1961) stated that aerobic bacteria reduced the O_2 availability to the meat surface causing an increase in metmyoglobin formation.

The percentage of metmyoglobin was affected ($P < .01$) by the treatment and display day interaction (Appendix A). As the display time

Table 7. Mean[a] percentages and standard deviations of metmyoglobin in beef loin strip steaks displayed in different packaging treatments.

	Display days				
Packaging treatment	0	3	6	10	13
1. 80%O_2 : 20%CO_2	$9.5^{d,y}$ ±5.23	$30.9^{cd,x}$ ±14.97	$29.3^{cd,xy}$ ±16.11	$53.2^{bc,x}$ ±22.18	$76.4^{b,x}$ ±22.06
2. 40%O_2 : 15%CO_2 : 45%N_2	$10.8^{c,y}$ ±4.94	$31.8^{c,x}$ ±11.09	$48.5^{c,x}$ ±22.71	$54.4^{b,x}$ ±26.24	$67.6^{b,x}$ ±28.34
3. 10%O_2 : 15%CO_2 : 75%N_2	$38.6^{b,x}$ ±20.15	$32.3^{b,x}$ ±16.78	$39.6^{b,xy}$ ±16.71	$22.2^{b,y}$ ±18.28	$47.2^{b,xy}$ ±26.85
4. Vacuum	$29.4^{b,x}$ ±31.96	$16.8^{b,x}$ ±5.07	$13.3^{b,y}$ ±5.43	$18.6^{b,y}$ ±13.01	$21.8^{b,y}$ ±27.70
5. PVC (control)	$10.5^{c,y}$ ±10.25	$32.9^{bc,x}$ ±14.86	$43.2^{bc,xy}$ ±20.44	$66.8^{b,x}$ ±17.20	$57.1^{b,xy}$ ±30.22

[a] Data for chilling treatments were pooled and appear as packaging treatment means.

[b,c,d] Row means for display days with different superscripts are different ($P<0.05$).

[x,y] Treatment means (column) with different superscripts are different ($P<0.05$).

increased, each treatment was affected differently. Treatments1, 2 and 5 followed similar patterns for metmyoglobin formation. Initially, there was a low percentage of metmyoglobin at the surface. The levels of metmyoglobin increased as display time increased. For treatments 3 and 4, they had higher percentages of metmyoglobin initially than the other 3 treatments. These differences among treatments was related to the amount of oxygen in the package headspace.

pH

Huffman et al. (1972) indicated that the typical pattern for pH changes of fresh meat stored in gas atmospheres increases gradually from pH 5.5 to approximately pH 5.8 at 1.1°C. In this study, due to the length of time the whole muscles were vacuum packaged prior to gas packaging, the mean pH of the steaks was initially very low as indicated in table 8. King and Nagel (1967) postulated that CO_2 inhibited growth of bacteria by lowering the pH of the medium. The CO_2 was dissolved into the moisture on the meat surface causing an increase in carbonic acid which lowers the pH. The PVC treatment (control) showed significant increases ($P < .05$) in pH among all of the display periods but the treatment mean was not different ($P > .05$) from the means of the other packaging treatments. The increase in pH during display for treatments 1, 2, 3 and 4 was not significant ($P > .05$) between 0 and 3 days or 3 and 6 days or 6 and 10 days but was significant ($P < .05$) between 10 and 13 days. The low oxygen treatment ($10\%O_2$:$15\%CO_2$:$75\%N_2$) had the greatest increase in pH over time, but there were no significant differences ($P > .05$) between overall

Table 8. Mean[a] and standard deviation of pH values for beef loin strip steaks displayed in different packaging treatments.

Packaging treatment	Display days 0	3	6	10	13	Treatment means
1. 80%O_2 : 20%CO_2	5.18^{d} ±0.02	5.19^{cd} ±0.05	5.32^{c} ±0.05	5.32^{c} ±0.07	5.79^{b} ±0.10	5.39^{x}
2. 40%O_2 : 15%CO_2 : 45%N_2	5.16^{d} ±0.03	5.19^{cd} ±0.01	5.28^{cd} ±0.03	5.31^{c} ±0.08	5.85^{b} ±0.15	5.39^{x}
3. 10%O_2 : 15%CO_2 : 75%N_2	5.15^{c} ±0.01	5.19^{c} ±0.12	5.32^{c} ±0.04	5.33^{c} ±0.06	5.92^{b} ±0.03	5.42^{x}
4. Vacuum	5.16^{d} ±0.04	5.24^{cd} ±0.10	5.28^{cd} ±0.12	5.38^{c} ±0.06	5.79^{b} ±0.03	5.40^{x}
5. PVC (control)	5.14^{f} ±0.04	5.23^{e} ±0.04	5.31^{d} ±0.02	5.40^{c} ±0.04	5.77^{b} ±0.02	5.41^{x}

[a] Data for chilling treatments were pooled.

b,c,d,e,f Row means for display days with different superscripts are different ($P<0.05$).

[x] Treatment means (column) with different superscripts are different ($P<0.05$).

treatment means. Clark and Lentz (1972) found no significant change ($P > .05$) in pH in steaks treated with carbon dioxide but indicated that bacterial growth caused a sharp increase in pH of samples at a high population of cells. On the other hand, Ledward (1970) reported steaks packaged in CO_2 enriched atmospheres had a decrease in pH. Again, the decrease in pH was attributed to activity of lactobacilli bacteria and/or dissolving of CO_2 in meat fluids.

Microbial growth

The mean mesophile or aerobic plate counts (APC) for each treatment over display days are shown in Figure 1 and the psychrotrophic counts are presented in Figure 2. There was a low initial count on all treatments with a gradual increase in the counts as storage time increased. Beginning with day 6 and continuing through day 10, APC (Figure 1) and psychrotrophic counts (Figure 2) were lower ($P < .05$) for the gas treated steaks (treatments 1, 2, and 3). Huffman et al. (1975) reported that steaks stored in CO_2 enriched atmospheres had lower aerobic bacterial counts than steaks stored in air. In our study, the lag phase of growth was prolonged due to the effect of the CO_2 present while the control steaks continued to have logarithmic bacterial growth throughout display. Clark and Lentz (1972) found similar results since inhibition of bacterial growth was associated to an increase in the lag phase of growth. The effect of CO_2 was also indicated by the slower bacterial growth

Figure 1. Mean total aerobic plate count (APC) for beef strip loin steaks packaged in different packaging treatments

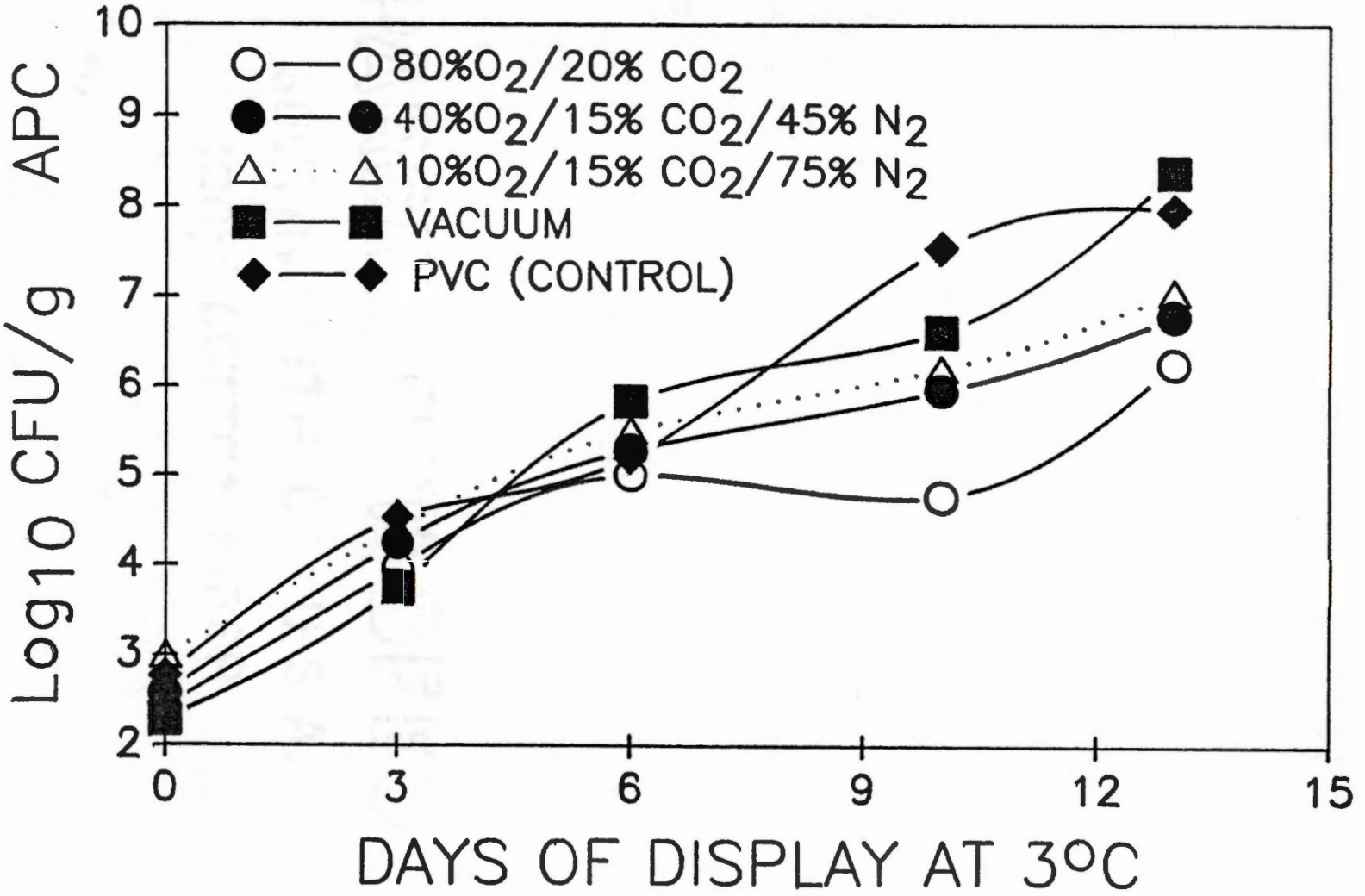
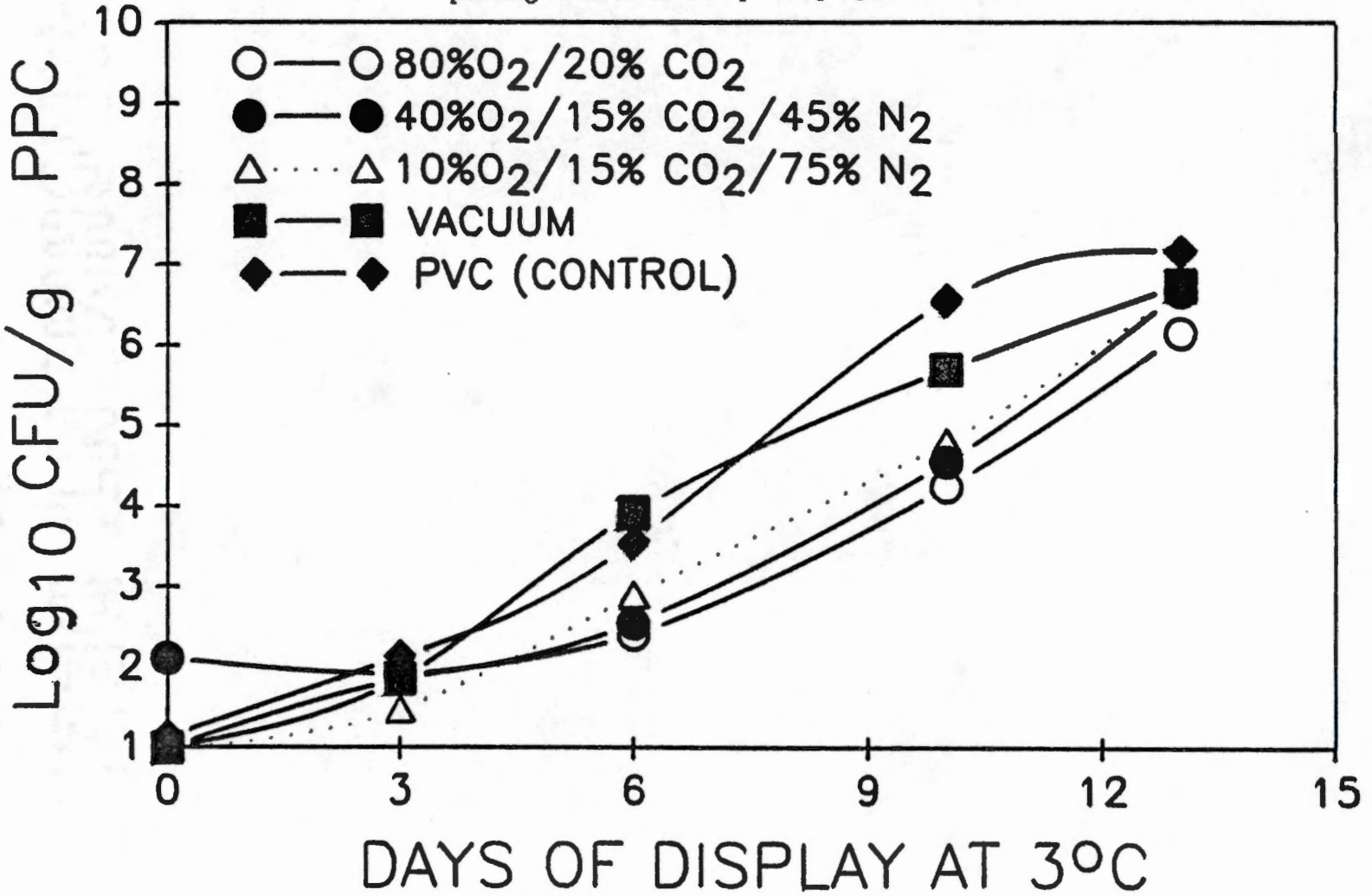


Figure 2. Mean psychrotrophic bacterial counts for beef strip loin steaks packaged in different packaging treatments



among the three gas treatments. The higher CO_2 concentration (treatment 1) had the lowest mean APC and psychrotrophic counts over the entire study compared to the other two gas atmospheres. It was shown by Haines (1933) that carbon dioxide is effective against the spoilage bacteria of fresh meats. Ogilvy and Ayres (1952) showed the effect of carbon dioxide on growth curves of microorganisms on frankfurters stored at 4.4°C. A 50% CO_2 level had the lowest log count over the entire storage period (30 days).

The steaks packaged under vacuum had higher log counts of aerobic bacteria after day 6 than the other modified atmospheres. However, Baran et al. (1970) indicated that aerobic bacterial growth reaches a stationary phase after 6 days of vacuum packaging storage. Pierson et al. (1970) on the other hand, stated that aerobic bacterial counts increased up to 15 days for anaerobically packaged beef. Hodges et al. (1974) found that aerobic microbial growth of beef increased up to 28 days of storage under anaerobic conditions.

The overall mean APC was significantly lower ($P<.05$) for treatment 1 ($80\%O_2:20\%CO_2$) than the other treatments (Appendix A). This is evidence that the higher initial level of CO_2 reduced bacterial growth. Treatments 2 ($40\%O_2:15\%CO_2:45\%N_2$) and 3 ($10\%O_2:15\%CO_2:75\%N_2$) also had significantly lower ($P< .05$) APC from the control (PVC). Again, the effect of the CO_2 on reducing bacterial growth was evident. In analyzing the psychrotrophic bacterial counts, the modified gas atmospheres had lower counts ($P< .05$) than the PVC control. There was indication of an increased lag phase in the growth of psychrotrophic bacteria counts. Bartkowski et al. (1982) found similar results when a modified

atmosphere packaging treatment (75%O_2:15%CO_2:10%N_2) had lower ($P<.05$) mesophilic and psychrotrophic counts than a control stored in air.

The interaction between treatment and display days was significant ($P<.0002$) for both APC and psychrotrophic bacterial counts. As the storage time increased, there was a significant effect on the APC and psychrotrophic bacteria among the different treatments. Off-odors are an indication of bacterial spoilage. Off-odors were evident in all treatments, except for the vacuum packaged meat, at the end of the 13th day of display . Even though the vacuum packaged meat had the highest total aerobic plate count on day thirteen among all treatments, there was no indication of bacterial spoilage. Clark and Lentz (1972) reported that off-odors in meat stored in 20% CO^2 at 0°C, could not be detected over the 20 day storage period used.

As stated earlier, the rise in APC and psychrotrophic counts was highly correlated with rise in pH. However, the percent oxymyoglobin was negatively correlated ($r = -.51$) with APC. This would indicate the aerobic microorganisms utilized enough of the atmospheric oxygen inside the package to reduce the oxygen level below the level needed to maintain the oxymyoglobin which resulted in conversion of the pigment to metmyoglobin. Seideman and Durland (1982) noted that certain species of aerobic bacteria were shown to discolor meat by reducing the oxygen tension to the meat surface while the facultative anaerobes did not cause metmyoglobin formation because it doe not consume oxygen in any appreciable extent. Furthermore, there was a negative correlation with APC and Hunter 'a' and 'b' values, -0.46 and -0.36, respectively. From this data, it could be said that color is a fair indicator of bacterial condition.

The rate of bacterial growth was also found to be related to the rise or fall of the pH as stated by Huffman et al. (1975). As the pH increased, the rate of bacterial growth also increased. In this study, changes in pH followed aerobic and psychrotrophic plate counts very closely as indicated by the significant Pearsons correlation coefficents ($P<.0001$) of .70 and .80, respectively.

Sensory evaluation

A twenty five member consumer panel evaluated one steak from each packaging and chill treatment at the end of each storage period for color acceptability and purchasing preferrence The panelists were asked to give a yes or no response to each of the given questions (Appendix B) and the analysis of the discrimination test responses was based on binomial distribution (Stone and Sedel, 1982). In the evaluation, the panelists compared each steak to their own mental standard for color. Since there was no basis for expecting a yes or no response, it was considered a two-tailed test. Therefore, each steak had to have 18 of 25 concurring responses for the product to be considered acceptable or not acceptable ($P< .05$) as indicated by Stone and Sedel (1982). The overall consumer response to each treatment is presented in Table 9.

Since the initial evaluation was conducted two hours after packaging, there was time for reactions to occur inside each package. Initially, the high oxygen treatments were considered acceptable and

Table 9. Mean number of responses for acceptable[a] color of beef loin steaks display in different packaging treatments.

	Packaging treatment				
Display days	80%O_2 20%CO_2	40%O_2 15%CO_2 45%N_2	10%O_2 15%CO_2 75%N_2	Vacuum packaged	PVC
0	21	20	11	14	21
3	13	11	9	10	25
6	11	3	5	15	19
10	12	3	5	19	8
13	2	3	7	18	3

[a] Color was acceptable if there were 18 or more acceptable responses from 25 panelists ($P < .05$).

would be bought by the panelists. Treatment 3 (10%O_2:15%CO_2:75%N_2), which had a low concentration of oxygen that resulted in a high degree of myoglobin or purplish color, was considered unacceptable throughout the study by the consumer panelists. The vacuum packaged steak was considered unacceptable at the beginning of the study. This statistic backs up the hypothesis that the color of vacuum packaged meat is unacceptable to the consumer. However, at display day 10, the vacuum package was considered acceptable while no other treatments were acceptable. A possible reason for this unusual response is that the panelists compared the purplish color of the vacuum packaged steaks to the brownish color of the other steaks. Supporting this response, Seideman et al. (1980) found that retail cuts stored in a modified atmosphere (20%CO_2:80%N_2) after a 21 day storage period in a vacuum package had significantly greater ($P < .05$) surface discoloration than vacuum packaged retail cuts.

A problem occurred in this study with the gas treated steaks which might have altered the panelists decisions on color acceptability. The meat surface had darkened areas where the packaging material touched the meat surface. This problem was probably due to a low oxygen partial pressure. Taylor and MacDougall (1973) indicated that high oxygen partial pressures during storage produced a thick bright red surface layer of oxymyoglobin which masked the development of metmyoglobin in the underlying tissue. With low oxygen partial pressures, the development of the metmyoglobin is more pronounced. In this study, a microenvironment was created where the package touched the meat surface, possibly causing a decrease in oxygen partial pressure. This, in turn, allowed an increase in the formation of metmyoglobin.

CHAPTER 5

CONCLUSION

The objective of this study was to determine if a modified atmosphere and chilling treatment affected the color stability, microbial condition and pH of fresh beef loin steaks. With the growing interest in alternatives to vacuum packaging, the use of modified atmosphere packaging of beef steaks appears to have potential advantages over vacuum packaging.

Although little difference occurred in the color of vacuum packaged steaks and the modified atmosphere packaged steaks, as far as, the Hunter color values were concerned, the percentages of myoglobin, oxymyoglobin and metmyoglobin were different. The treatments with the high initial oxygen concentrations, had a higher percentage of the more bright red desirable oxymyoglobin through 10 days of display compared to the vacuum packaged steaks. This gives the indication of an approximate 10 day shelf-life for the treatment with 80%O_2:20%CO_2. However, due to a problem with low partial pressure of oxygen in the modified packages, the steaks had darkened areas where there was an increase in metmyoglobin formation. Without the darkening problem, the panelists' might have indicated a greater willingness to purchase the product based on a more acceptable color.

Although the panelists indicated that the steaks had an unacceptable color after the initial day of display, the microbial condition

of the steaks was acceptable through 10 days for the steaks packaged in 80%O_2:20%CO_2. After the 10th day of display, the condition of the meat deteriorated rapidly. The decline in microbial activity was correlated with the increase in pH, as well as, an increase in metmyoglobin formation. The low pH of the meat at the time of initial packaging was probably due to lactic acid producing bacteria. There was an increase in CO_2 which was attributed to an increase in bacterial growth and to the conversion of O_2 to CO_2 by respiration of the meat tissue.

In this study, different concentrations of carbon dioxide were used to determine what levels of CO_2 are needed to control bacterial growth. Even though the lower levels were effective in controlling bacterial growth, the higher level (20%) of carbon dioxide was most effective for reduction of growth. The combination of a high oxygen concentration and a high level of carbon dioxide was found to be the most effective in controlling the rate of microbial degradation and the color stability of the beef loin steaks.

A somewhat unexpected result occurred with the chilling treatment. There were no differences found between chilled and nonchilled steaks for pH and microbial condition. However, the chilled steaks had a lower percentage of oxymyoglobin which was detrimental to the color of the steaks.

Modified atmosphere packaging is a method of increasing the shelf-life of beef loin steaks. However, there needs to be more research done on the actual volume of gas in the package and meat color. If the packaging material could be kept away from the meat surface, the problem with darkened areas on the meat surface could be eliminated. Once this

problem is eliminated, the use of modified atmosphere packaging could be justified due to the increased shelf-life.

LITERATURE CITED

LITERATURE CITED

Baran, W.L., A.A. Kraft, and H.W. Walker. 1969. Effects of carbon dioxide and vacuum packaging on color and bacterial count of meat. J. Milk Food Tech. 33:77-82.

Bartkowski, L., F.D. Dryden, and J.A. Marchello. 1982. Quality changes of beef steaks stored in controlled gas atmospheres containing high or low levels of oxygen. J. Food Prot. 45:41-45.

Brooks, J. 1933. The effects of carbon dioxide on the colour changes or bloom of lean meat. J. Soc. Chem. Ind. 52:17t-19t.

Christopher, F.M., S.C. Seideman, Z.L. Carpenter, G.C. Smith, C.W. Dill, and C. Vanderzant. 1979. Microbiology of beef packaged in various gas atmospheres. J. Food Prot. 42:240-244.

Clark, D.S. and C.P. Lentz. 1969. The effect of carbon dioxide on the growth of slime producing bacteria on fresh beef. J. Can. Inst. Food Tech. 2:72-75.

Clark, D.S. and C.P. Lentz. 1972. Use of carbon dioxide for extending shelf-life of prepackaged beef. J. Can. Inst. Food Tech. 5:175-178.

Clark, D.S. and C.P. Lentz. 1973. Use of mixtures of carbon dioxide and oxygen for extending shelf-life of prepackaged fresh beef. J. Can. Inst. Food Tech. 6:194-196.

Clark, D.S., C.P. Lentz and L.A. Roth. 1976. Use of carbon monoxide for extending shelf-life of prepackaged fresh beef. J. Can. Inst. Food Tech. 9:114-117.

Cole, A.B. 1986. Retail packaging systems for fresh red meat cuts. Proc. Recip. Meat Conf. 39:106-111.

Daun, H., M. Solberg, W. Franke and S. Gilbert. 1971. Effect of oxygen-enriched atmospheres on storage quality of packaged fresh meat. J. Food Sci. 36:1011-1014.

Elliott, R.J. 1969. The relationship between the subjective and objective measurement of pork colour. J. Food Technol. 4:147-156.

Enfors, S.O. 1979. Effects of packaging under carbon dioxide, nitrogen, or air on the microbial flora of pork stored at 4°C. J. Appl. Bacteriol. 47:197-208.

Enfors, S.O. and G. Molin. 1984. Carbon dioxide evolution of refrigerated meat. Meat Sci. 10:197-206.

Fellers, D.A., I.J. Wahba, J.C. Caldano, and C. Olin Ball. 1963. Factors affecting the color of packaged retail beef cuts- origin of cuts, package type, and storage conditions. Food Technol. 17:95.

Gracey, J. F. 1986. Meat Hygiene. 8th ed. Bailliere Tindall. London. pp 248-278.

Gill, C.O. and K.H. Tan. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. Appl. Environ. Microbiol. 39:317-319.

Haines, R.B. 1933. The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. J. Soc. Chem. Ind. 52:13t-17t.

Hermansen, P. 1983. Comparison of modified atmosphere versus vacuum packaging to extend the shelf-life of retail fresh meat cuts. Proc. Recip. Meat Conf. 35:60-65.

Hodges, J.H., V.R. Cahill, and H.W. Ockerman. 1974. Effect of vacuum packaging on weight loss, microbial growth and palatability of fresh beef wholesale cuts. J. Food Sci. 39:143-146.

Holland, G.C. 1980. Modified atmospheres for fresh meat distribution. Proc. 16th Meat Ind. Res. Con. pp. 21-39.

Huffman, D.L., K.A. Davis, D.N. Marple, and J.A. McGuire. 1975. Effect of gas atmospheres on microbial growth, color and pH of beef. J. Food Sci. 40:1229-1231.

Jeremiah, L.E., Z.C. Carpenter, and G.C. Smith. 1972. Beef color as related to comsumer acceptance and palatability. J. Food Sci. 37:476-479.

King, A.D. and C.W. Nagel. 1967. Growth inhibition of a Pseudomonas by carbon dioxide. J. Food Sci. 32:575-579.

King, A.D. and C.W. Nagel. 1975. Influence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa* J. Food Sci. 40:362-366.

Kraft, A.A., C.R. Rey, W.L. Baran, R.E. Rust and H.W. Walker. 1970. Use of carbon dioxide and liquid nitrogen for preserving meats. Proc. Meat Ind. Res. Conf. pp. 39-53.

Lanier, T.C., J.A. Carpenter, R.T. Toledo and J.O. Reagan. 1978. Metmyoglobin reduction in beef systems as affected by aerobic, anaerobic and carbon monoxide-containing environments. J. Food Sci. 43:1788-1793.

Lea, C.H. 1933. Chemical changes in the fat of frozen and chilled meat. PartIV. The protective influence of carbon dioxide on the fat of beef stored at 0°C. J. Soc. Chem. Ind. 52:9t-12t.

Ledward, D.A. 1970. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. J. Food Sci. 35:33-36.

Mackinney, G., A.C. Little, and L. Brinner. 1966. Visual appearance of foods. Food Technol. 43:1300-1306.

Melton, C. 1990. Personal communication.

Melton, S.L. 1990. Personal communication.

Moran, T., E.C. Smith and R.G. Tomkins. 1932. The inhibition of mould growth on meat by carbon dioxide. J. Soc. Chem. Ind. 51:114t-116t.

Mount, J.R. 1990. Personal communication.

Ogilvy, W.S. and J.C. Ayres. 1951. Post-mortem changes in stored meats. III. The effect of atmospheres containing carbon dioxide in prolonging the storage life of frankfurters. Food Technol. 5:300-303.

Ogilvy, W.S. and J.C. Ayres. 1952. Post-mortem changes in stored meats. V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. Food Technol. 5:121-130.

O'keefe, M., D.E. Hood and M.G. Harrington. 1975. Gas packaging: The effect of various gas atmospheres on color of fresh beef. Proc. European Meeting of Meat Research Workers. 20:150-152.

Partmann, W., H.K. Frank, and J. Gutschmidt. 1970a. The behavior of meat in controlled atmospheres at 7°C. Die Fleischwirtschaft 8:1067.

Partmann, W., H.K. Frank, and J. Gutschmidt. 1970b. Investigations concerning the storage of meat in controlled atmospheres at 3°C. Die Fleischwirtschaft 9:1205.

Partmann, W., M. Bomar, A. Halek, H. Bohling, and A.H. Schlaszus. 1975. Application of controlled atmospheres containing 20% CO_2 to the storage of beef. Die Fleischwirtschaft 8:1441.

Pierson, M.D., D.L. Collins-Thompson, and Z. John Ordal. 1970. Microbiological, sensory and pigment changes of aerobically and anaerobically packaged beef. Food Technol. 24:129-133.

Rea, R.H., G.C. Smith, and Z.L. Carpenter. 1972. Protective packaging materials for fresh beef shipments. J. Food Sci. 37:739-742.

Robach, D.L. and R.N. Costilow. 1961. Role of bacteria in the oxidation of myoglobin. Appl. Microbiol. 9:529-533.

SAS. 1985. Users Guide: Statistics. Cary, NC.

Satterlee, L.D. and W. Hansmeyer. 1974. The role of light and surface bacteria in color stability of prepackaged beef. J. Food Sci. 39:305-306.

Seideman, S.C. and P.R. Durland. 1982. Vacuum packaging of fresh beef: A review. Meat Sci. 1:29-45.

Seideman, S.C., C. Vanderzant, G.C. Smith, C.W. Dill and Z.L. Carpenter. 1984. Appearance of beef, pork, and lamb stored in vacuum or modified gas atmospheres. J. Food Prot. 43:252-258.

Seideman, S.C., G.C. Smith, Z.L. Carpenter, T. R. Dutson and C.W. Dill. 1980. Modified gas atmospheres and changes in beef during storage. J. Food Sci. 44:1036-1040.

Seideman, S.C., Z.L. Carpenter, G.C. Smith, C.W. Dill and C. Vanderzant. 1979. Physical and sensory characteristics of beef packaged in modified gas atmospheres. J. Food Prot. 42:233-239.

Silliker, J. H., R.E. Woodruff, J.R. Lugg, S.K. Wolfe and W.D. Brown. 1977. Preservation of refrigerated meats with controlled atmospheres: Treatment and post treatment effects of carbon dioxide on pork and beef. Meat Sci. 1:195-204.

Silliker, J.H. and S.K. Wolfe. 1980. Microbiological safety considerations in controlled atmosphere storage of meats. Food Technol. pp. 59-63.

Smith, G.C., Z.L. Carpenter and K.E. Hoke. 1976. Shipment of fresh beef. Proc. Meat Ind. Res. Conf. pp. 121-135.

Snyder, H. E. and J.C. Ayres. 1961. The autoxidation of crystallized beef myoglobin. J. Food Sci. 26:469-472.

Stone, H. and J.L. Sidel. 1985. Sensory Evaluation Practices. Academic Press Inc., New York, NY. pp. 132-139.

Tan, K.H. and C.O. Gill. 1981. Physiological basis of CO_2 inhibition of a meat spoilage bacterium, Pseudomonas fluorescens. Meat Sci. 7:9-17.

Taylor, A.A. and D.B. MacDougall. 1973. Fresh beef packed in mixtures of oxygen and carbon dioxide. J. Food Tech. 8:453-461.

Warriss, P. D. 1979. The extraction of heme pigments from fresh meat. J. Food Technol. 14:75-79.

Watts, B. M. 1954. Oxidative rancidity and discoloration in meat. Advances in Food Research. 5:1.

Wolfe, K. 1980. Use of CO^- and CO_2 enriched atmospheres for meats, fish and produce. Food Technol. 3:55-58.

APPENDIXES

Appendix A

Analysis of Variance

Dependent variable: Hunter color value 'L'

Source	DF	Sum of squares	F value	PR>F
Treatment	4	40.16	1.21	0.3098
Days	4	49.25	1.49	0.2115
Chilling	1	1.34	0.16	0.6877
Treat*Days	16	173.05	1.31	0.2083
Treat*Chill	4	53.37	1.61	0.1771
Days*Chill	4	56.43	1.71	0.1549
Error	96	793.82		

Analysis of Variance

Dependent variable: Hunter color value 'a'

Source	DF	Sum of squares	F value	PR>F
Treatment	4	60.25	3.25	0.0152
Days	4	343.31	18.51	0.0001
Chilling	1	8.76	1.89	0.1724
Treat*Days	16	357.16	4.81	0.0001
Treat*Chill	4	43.32	2.34	0.0609
Days*Chill	4	17.46	0.94	0.4434
Error	96	445.27		

Analysis of Variance

Dependent variable: Hunter color value 'b'

Source	DF	Sum of squares	F value	PR>F
Treatment	4	30.82	6.32	0.0001
Days	4	16.62	3.41	0.0119
Chilling	1	1.18	0.97	0.3262
Treat*Days	16	19.05	0.98	0.4881
Treat*Chill	4	4.50	0.92	0.4540
Days*Chill	4	10.14	2.08	0.0896
Error	96	117.06		

Analysis of Variance

Dependent variable: Percent myoglobin

Source	DF	Sum of squares	F value	PR>F
Treatment	4	17378.52	13.84	0.0001
Days	4	3594.95	2.86	0.0274
Chilling	1	302.40	0.96	0.3288
Treat*Days	16	7080.83	1.41	0.1536
Treat*Chill	4	2321.61	1.85	0.1258
Days*Chill	4	542.08	0.43	0.7854
Error	96	30139.24		

Analysis of Variance

Dependent variable: Percent oxymyoglobin

Source	DF	Sum of squares	F value	PR>F
Treatment	4	2546.31	9.06	0.0001
Days	4	4672.23	16.62	0.0001
Chilling	1	320.42	4.56	0.0353
Treat*Days	16	2336.14	2.08	0.0154
Treat*Chill	4	318.60	1.13	0.3455
Days*Chill	4	67.94	0.24	0.9140
Error	96	6746.52		

Analysis of Variance

Dependent variable: Percent metmyoglobin

Source	DF	Sum of squares	F value	PR>F
Treatment	4	7555.18	4.79	0.0014
Days	4	14524.14	9.21	0.0001
Chilling	1	832.02	2.11	0.1496
Treat*Days	16	13348.39	2.12	0.0133
Treat*Chill	4	3201.45	2.03	0.0963
Days*Chill	4	3201.45	0.18	0.9463
Error	96	37852.95		

Analysis of Variance

Dependent variable: pH

Source	DF	Sum of squares	F value	PR>F
Treatment	4	0.01	0.39	0.8189
Days	4	7.52	308.46	0.0001
Chilling	1	0.00	0.07	0.7983
Treat*Days	16	0.14	1.46	0.1328
Treat*Chill	4	0.04	1.57	0.1883
Days*Chill	4	0.01	0.55	0.6979
Error	96	0.58		

Analysis of Variance

Dependent variable: Total aerobic plate count (Log_{10})

Source	DF	Sum of squares	F value	PR>F
Treatment	4	15.57	8.51	0.0001
Days	4	244.17	133.49	0.0001
Chilling	1	0.23	0.51	0.4761
Treat*Days	16	24.41	3.34	0.0001
Treat*Chill	4	1.99	1.09	0.3661
Days*Chill	4	6.48	3.54	0.0097
Error	96	43.90		

Analysis of Variance

Dependent variable: Psychrotrophic plate count (Log_{10})

Source	DF	Sum of suares	F value	PR>F
Treatment	4	14.13	5.38	0.0006
Days	4	496.72	189.10	0.0001
Chilling	1	0.41	0.63	0.4289
Treat*Days	16	17.99	1.71	0.0568
Treat*Chill	4	6.19	2.36	0.0592
Days*Chill	4	3.02	1.15	0.3377
Error	96	63.04		

Appendix B

Name_____ Date _____

Number_____
Check the statement that reflects your evaluation of the color.
Color is acceptable _____
Color is unacceptable _____
If you were basing your decision solely on color, would you buy this product?
yes ____
no ____

Number_____
Check the statement that reflects your evaluation of the color.
Color is acceptable _____
Color is unacceptable _____
If you were basing your decision solely on color, would you buy this product?
yes ____
no ____

Number_____
Check the statement that reflects your evaluation of the color.
Color is acceptable _____
Color is unacceptable _____
If you were basing your decision solely on color, would you buy this product?
yes ____
no ____

VITA

Douglas S. Mitchell was born July 4, 1962 in Pittsburgh, Pennsylvania, the son of Mr. David S. Mitchell and Mrs. Lota E. Mitchell. He graduated June, 1980 from Mount Lebanon High School and enrolled that fall at the University of Tennessee, Knoxville. He received a bachelor of science degree in Animal Science in December, 1985. In september of 1986, he accepted a position at Monfort of Colorado, Inc. in Greeley, Colorado. He worked three years as a Quality Assurance Technician before returning to the University of Tennessee to continue his education. In the summer of 1989, he accepted a research assistantship in the Food Technology and Science department. He earned a Master of Science degree in December, 1990.

The author is a member of the American Meat Science Association, American Society of Animal Scientists and the Institute of Food Technologists.