EFFECTS OF PACKAGING SYSTEM, FAT CONCENTRATION AND CARBON MONOXIDE ON MICROBIOLOGY, SENSORY AND PHYSICAL PROPERTIES OF GROUND BEEF STORED AT 4 PLUS OR MINUS 1 DEGREE CELSIUS FOR 25 DAYS

By

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TABLE OF CONTENTS

page

LIST OF TABLES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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The overall appearance of a fresh meat product at the time of purchase is important to consumers as it may affect their purchasing decisions. The objective of this study was to evaluate the effects of three packaging treatments (modified atmosphere [MAP] containing 0.4% carbon monoxide [CO] and 30% carbon dioxide [CO_2], vacuum packaging [VP] and polyvinyl chloride [PVC] overwrap), three fat treatments (10, 20 and 30% fat) and storage time on microbiology, sensory and physical properties of ground beef stored at 4 plus or minus 1 degree Celsius for 25 days. The three packaging and three fat treatments were analyzed for objective color, pH, microbiology (total aerobes, total psychrotrophs, Gram negatives, *E.coli* O157:H7, total coliforms and generic *E. coli*) and thiobarbituric acid reactive substances (TBARS). Lactic acid bacteria analysis was conducted only in the MAP and VP treatments. Headspace and residual CO analyses were conducted only in the MAP treatments. Consumer panels were conducted to evaluate color and off-odors.

The CO concentrations of the package and the meat, respectively, were deemed harmless at the levels detected in this study. There were no significant increases ($P > 0.05$) in TBARS for the MAP and VP treatments throughout the storage time. Modified atmosphere and vacuum

packaging treatments showed a significant decrease ($P < 0.05$) in pH values by Day 14, whereas PVC treatments had significantly higher ($P < 0.05$) pH values by Day 7. Modified atmosphere and vacuum packaging had a bacteriostatic effect on Gram negative microorganisms. The growth of aerobic, psychrotrophic and lactic acid bacteria was not inhibited by MAP or VP. Within the MAP and VP groups, the 10% and 30% fat treatments, respectively, reached aerobic plate counts and total psychrotroph counts above spoilage levels (> 6 log CFU/g). Furthermore, consumer color and off-odor results revealed that discoloration and off-odors were detected in the 10% and 30% fat treatments within the MAP and VP groups, respectively. Objective color analysis revealed that significant decreases ($P < 0.05$) in a* values of MAP treatments had occurred by Day 21. However, consumer color scores were similar ($P > 0.05$) for all MAP treatments when compared to Day 0. Consumers should rely on "use by" dates on the package and not on the color of the meat as indicators of freshness.

CHAPTER 1
INTRODUCTION

The overall appearance of a meat product at the time of purchase is important to consumers. Several characteristics of the product are evaluated before the purchase is made. Some of these characteristics are: color, smell, texture and flavor (if applicable). Of these characteristics, the color of the product may be the one most heavily relied upon when making the purchase, given that the packaging of most meat products in their raw state prevents consumers from smelling, touching, or tasting them. Thus, methods for the enhancement of the color of meat products and its stability during storage have been studied and developed by the meat industry and retailers.

Some of the methods for color enhancement used in today's meat industry make use of a modified atmosphere system that contains either low carbon monoxide (CO) levels or high oxygen (O_2) levels. This practice is commonly referred to as modified atmosphere packaging (MAP). Furthermore, the gas mixture of MAP systems does not generally contain CO and/or O_2 alone. Other gases such as carbon dioxide (CO_2) and nitrogen (N_2) are generally included in the mixture for a series of particular reasons that will be discussed later.

Definitions: Modified atmosphere packaging has been defined as a process whereby a perishable product is placed in a package, regular air is removed by vacuum or flushing, and the package is filled with a pre-determined gas or mixture of gases with a composition different than air (regular air is composed of about 0.03% CO_2, 78% N_2 and 21% O_2 *[66]*), followed by sealing of the package *(34)*. To paraphrase, MAP consists of getting rid of an original atmosphere that would eventually affect the shelf-life of the product negatively and replacing it with another atmosphere pre-formulated so as to optimize, maximize, and preserve the shelf-life of the product. Modified atmospheres for meat and poultry are dynamic and will change with time.

Product and microbial metabolism, absorption of gases by the product, and diffusion of the gas or gas mixture through the barrier film will cause the atmosphere inside the package to vary over time *(66)*. Controlled atmosphere packaging (CAP), by contrast, is a similar packaging method wherein the gas atmosphere is kept relatively constant during the life of the package *(66)*.

Vacuum Packaging (VP), a commonly used packaging technique for fresh and cured meats, consists of the removal of all air followed by sealing of the product in a barrier film that will not allow for diffusion of gases in or out of the package. Vacuum packaging is a form of modified atmosphere packaging because, in the case of fresh meats, microbial and muscle metabolism will utilize residual O_2 to produce CO_2 and, as a result of this, a modified atmosphere that achieves significant shelf life extension is created. Modified atmosphere packaging can be differentiated from vacuum packaging by the presence of a headspace into which a larger volume of gases can be introduced into the package and also by the lack of the physical pressure that occurs when a product is vacuum packaged *(66)*.

CHAPTER 2
REVIEW OF LITERATURE

Today's meat industry has to deal with several issues. One of the most relevant issues has to do with the timely and efficient distribution over long distances of fresh meat products that are fabricated at or near centralized locations. Thus, the shelf life of the fresh product must be protected and enhanced in order to ensure optimal customer and, eventually, consumer satisfaction. Vacuum and modified atmosphere packaging are just two of the techniques developed by meat scientists and used by meat processors and retailers to achieve the ultimate goal of enhancing the shelf-life of meat products. Both VP and MAP systems provide a greater enhancement of shelf-life when compared to polyvinyl chloride (PVC) overwrap packaging *(66)*.

The predominant concern in terms of shelf life is bacterial growth, which is most often the limiting factor. In addition to microbial growth, the shelf life of a meat product is affected by other quality attributes such as color, odor, flavor and texture. Thus, an important objective of MAP systems is to minimize the changes that those attributes experience throughout the storage of the product *(66)*.

Carbon Dioxide in Modified Atmosphere Packaging

Effects of Carbon Dioxide on the Microflora of Fresh Meat

Carbon dioxide, a colorless gas with a slightly pungent odor *(46)*, is the centerpiece of MAP systems due to its ability to inhibit a wide range of microorganisms *(66)*. Furthermore, Dixon and Kell *(9)* determined that CO_2 has a greater inhibitory effect on Gram negative bacteria, which grow rapidly on fresh meat, than it does on Gram positives. However, the mechanism by which CO_2 exerts its inhibitory effect on bacteria is not yet fully understood *(66)*. It is important to mention that carbon dioxide gas has been utilized as a preservative for fresh

meat and poultry for over 100 years and, consequently, its use in MAP systems has been studied extensively *(66)*.

Although the pathway through which CO_2 exerts its inhibitory effect on bacteria is not yet fully understood, it is known that the gas dissolves readily in water and will produce carbonic acid (H_2CO_3) in solution *(23)*. Carbonic acid will then cause a drop in meat pH and, in turn, negatively affect microbial growth. All of this occurs despite the buffering ability of fresh meats *(66)*.

The antimicrobial effect of CO_2 stems from its ability to affect the chemical quality of the meat *(23)*. Dixon and Kell (9) determined that the lowering of meat pH is a result of CO_2 absorption and production of carbonic acid that dissociates to bicarbonate and hydrogen ions. A decrease in meat pH has been shown to negatively affect the rate of oxidation processes such as pigment and lipid oxidation as well as water holding capacity (WHC) of meat *(29)*.

Microflora in VP and MAP meat systems with high levels of CO_2 will change from an aerobic to an anaerobic microflora, which is the main factor responsible for extending the storage life of the products *(81)*. Mixtures of 20-100% CO_2 balanced with N_2 are normally used *(23)*. The inhibitory effect of CO_2 increases with increasing CO_2 concentration in cuts packaged in O_2-depleted atmospheres *(19)*. Primal cuts in CO_2 atmospheres containing very low residual O_2 levels and stored at -1.5 ± 0.5°C have a shelf life of 10-15 weeks or more *(27, 81)*. Carbon dioxide concentrations greater than 20-30% in MAP systems of fresh meats have been shown to exert little additional inhibitory effect on the predominant spoilage flora in aerobic environments *(21)*.

Jakobsen and Nertelsen *(23)* determined that anaerobic high (> 20-30%) CO_2 packaging of retail cuts can also be an advantageous practice. With low levels of residual O_2 and a strict

temperature control of -1.5°C, the storage period was 2-3 times longer than for aerobically packaged retail cuts. Upon exposure to O_2 the cuts will bloom and will be able to withstand a few days of aerobic display without further handling. In addition, when packaging in atmospheres with concentrations greater than 20-30% CO_2, long storage life can be achieved for both primal and retail cuts as long as certain precautions are taken. A high initial hygiene is necessary, but other critical factors are temperature and O_2 control.

General Properties of Carbon Dioxide

When high CO_2 levels are introduced into the headspace of a package, the concentration will decline as CO_2 is absorbed into the meat *(23)*. It has been shown that CO_2 dissolves in both muscle and fat tissue until saturation or equilibrium is reached *(17)*. In MAP studies in which CO_2 was used, it has been shown that its effects are usually related to the concentration of CO_2 in the food and not to its concentration in the package headspace. Thus, the gas that affects the food and the microorganisms is the one that dissolves in the food and not the one in the headspace *(8, 39)*. As a result, the full preservative potential of CO_2 will only be accomplished if the amount of CO_2 added to the headspace is greater than the amount required to saturate the meat *(20)*.

How much CO_2 a meat product can absorb is related to biological factors of the meat such as pH, water content and fat content *(17)*. Furthermore, packaging and storage conditions such as temperature, CO_2 partial pressure and headspace to meat volume ratio will also affect the rate of absorption *(89)*. Gill *(17)* concluded that the solubility of CO_2 in muscle tissue of pH 5.5 at 0°C was approximately 960 mL/kg of tissue. The solubility increased with increasing tissue pH by 360 mL/kg for each pH unit. The solubility decreased with increasing temperature by 19 mL/kg for each 1°C rise. Solubilities in beef, pork and lamb muscle tissue were comparable. Gill *(17)* also concluded that the solubility of CO_2 in fat tissues initially increased as the temperature was raised above 0°C, but then declined at higher temperatures.

To achieve maximum storage life, meat has to be stored at the lowest possible temperature without freezing the product. Even relatively small increases in storage temperature result in meaningful reductions in storage life. For example, an increase in storage temperature of 1°C results in at least 10% reduction in storage life, proving temperature control to be the most critical factor *(25)*. Storage temperature has a significant effect on weight loss, color stability, lipid oxidation *(49)* and CO_2 absorption, as previously mentioned.

In order to prevent discoloration of the product, complete removal of all O_2 from the package headspace is necessary. In addition, an impermeable film that prevents O_2 from reentering the package should be used *(25)*. Oxygen has been shown to accelerate discoloration even at low partial pressures *(36)*.

Reducing spoilage caused by bacterial growth is one of the main concerns that MAP systems in fresh meats address. However, as previously mentioned, an attractive red color of the displayed product is also desired, and, therefore, MAP systems must also address the color of the product.

After studying different pre-slaughter physiological conditions in pigs and their effect on color and lipid stability during storage, Juncher et al. *(29)* concluded that ultimate meat pH was the single most important factor affecting product quality. A low ultimate pH had a negative effect on product quality in terms of color, weight loss and lipid oxidation. Several studies investigating low levels of CO_2 (20-25%) agree that there is no effect of CO_2 on meat color *(1, 2, 3, 6, 37, 38, 51)*. However, the same cannot be said for studies that investigated the use of higher CO_2 levels.

Numerous research projects have been conducted using atmospheres containing high levels of CO_2, and concluded that a decrease in pH of 0.05-0.35 units was a direct consequence

(22, 36, 64, 69, 76). Rousset and Renerre *(64)* conducted research on normal and high pH beef packaged in 100% CO_2 and found a larger decrease in the pH of high pH meat (0.35) than of normal pH meat (0.1). The researchers attributed this effect to a larger solubility of CO_2 at higher pH, which agrees with the results obtained by Gill *(17)*. Thus, the possibility that MAP systems using high CO_2 concentrations will cause discoloration in the product is a logical conclusion.

Another major concern surrounding the use of high levels of CO_2 in MAP systems is its potentially negative effect on product weight. Seideman et al. *(68)* concluded that packaging fresh meats in 100% CO_2 will result in greater weight loss as compared to packaging in 100% N_2. The researchers concluded that CO_2 may decrease the water holding capacity of meat proteins due to its ability to bind and structurally affect the proteins and their ability to retain moisture. O'Keffe and Hood *(49)* showed that packaging in 100% N_2 is superior to 100% CO_2 in retaining water due to the possible pH lowering effect of absorbed CO_2. However, other studies *(53, 54)* have found a lower weight loss for meat products packaged in CO_2 than for products packaged in N_2. The varying physical compression of the meat during the packaging process is also responsible for part of the weight loss *(55)* and therefore, complicates the comparison of studies, according to Jakobsen and Nertelsen *(23)*.

Another potential problem with high CO_2 packaging observed in some studies is the development of a porous/fissured appearance of the meat when it is cooked. These fissures may be caused by the rapid release of carbon dioxide gas from the meat as it is exposed to increased temperatures during cooking *(23)*. Bruce et al. *(4)* determined that carbon dioxide present in either an aqueous state or bound to proteins will evolve rapidly during cooking. The researchers concluded that the increase in temperature decreases CO_2 solubility and denatures proteins, causing CO_2 to evolve rapidly. Furthermore, the researchers also concluded that the

semitendinosus muscle of beef exhibited greater fissure formation than the *psoas major* when packaged in 100% CO_2 and stored at 3-4°C, indicating that there are muscle-to-muscle differences in terms of susceptibility to fissure development.

Fissure development has been reported as a problem in beef *(4, 56)*. However, Jeremiah et al. *(26)* reported no fissures in pork upon cooking. Penney *(56)* suggested that CO_2 packaging atmospheres should result in minimal fissure development and still provide sufficient microbial control. However, after conducting research on the subject, Penney *(56)* found that for all storage times between 4-16 weeks, beef developed fissures when packaged in atmospheres of 20-100% CO_2. It was also determined that fissure development increased with increasing CO_2 level.

Because CO_2 is the active antimicrobial agent in both VP and MAP, there has been significant interest in utilizing increased concentrations of this gas in MAP systems. However, although beneficial from a microbial standpoint, using high levels of this gas when packaging fresh meats has not been a common practice because of the discoloration that occurs at more than about 30% CO_2 *(66)*. Nevertheless, research that investigated the use of CO in MAP systems has shown that as much as 99.5% CO_2 will not cause discoloration if combined with 0.5% CO *(33)*.

Carbon Monoxide in Modified Atmosphere Packaging

Effects of Carbon Monoxide on the Microflora of Fresh Meat

Packaging fresh meat products in atmospheres containing only CO has been shown to have an indirect effect on microbial growth by removing all of the O_2 from the package. Viana et al. *(84)* packaged fresh pork loins using four different packaging atmospheres that included: vacuum; 99% CO_2/1% CO; 100% O_2 and; 100% CO followed by vacuum after one hour of exposure. The loins were stored for 25 d at 5 ± 0.5°C. The researchers reported that packaging fresh pork loins in 100% CO resulted in similar bacterial growth when compared to the other O_2-free atmospheres. Except for loins exposed to 100% O_2, aerobic and anaerobic psychrotrophs

were the dominant microbiota reaching 7 log CFU/g (colony forming units) after 20 d of storage for all modified atmospheres treatments. The counts for loins packaged in 100% O_2 reached numbers greater than 8 log CFU/g. *Pseudomonas* counts reached a maximum level of 5 log CFU/g in all modified atmosphere treatments except vacuum and 100% O_2 during the 25 d of storage.

Gee and Brown *(14)* investigated the effects of different concentrations of CO on pure bacterial cultures of *Pseudomonas, Achromobacter* and *E. coli* species. It was concluded that 15-30% CO had an inhibitory effect on the growth of bacteria. These levels, however, far exceed the levels legally allowed for use in the packaging of red meat and poultry products *(83).*

General Properties of Carbon Monoxide

Carbon monoxide is used in a MAP system mainly because it has the ability to form a stable bright red or cherry red color in meat, even in very low concentrations. Concentrations of 0.4 – 1.0% CO can be regarded as sufficient and suitable for color purposes in MAP of meat *(78)*. Improved but still limited color stability is achieved by packaging meat in high O_2 atmospheres, with a minimum of 60% O_2. Thus, MAP systems using CO in the gas mixture have become more widely used by processors and retailers.

Myoglobin is the most abundant pigment found in fresh meats, and it can be found in different forms depending on the O_2 status of the environment. The different forms or states are: reduced myoglobin (Mb), oxymyoglobin (MbO_2), and metmyoglobin (MMb +), and the actual color of meat will vary depending on the presence of these three derivatives on the surface of the product. Reduced myoglobin is the form of myoglobin associated with meat immediately after it has been cut, and also with fresh meat packaged in anaerobic conditions, such as VP. Oxymyoglobin, secondly, is responsible for the typical bright red color of fully oxygenated meat,

and it forms once reduced myoglobin is exposed to O_2. Thirdly, the brown metmyoglobin is formed by oxidation of the pigment to its ferric (Fe^{3+}) form *(47)*.

According to Sørheim et al. *(78)*, the main driving force for the use of CO in MAP systems of meat is the development of a stable, bright red color as a result of the strong binding of CO to reduced myoglobin and the formation of carboxymyoglobin (MbCO). This pigment and its relative stability to oxidation are responsible for the red color retention under MAP systems containing CO. Carboxymyoglobin is much more stable towards oxidation than oxymyoglobin due to the stronger affinity of CO for the iron porphyrin site on the myoglobin molecule. Thus, the addition of CO to MAP systems at low levels to counteract discoloration issues brought about by CO_2 has received significant attention from meat processors and retailers *(47)*.

It has been known for more than 50 years that the color spectrum of carboxymyoglobin is very similar to that of oxymyoglobin *(79)*. What is more important in terms of color stability is the fact that carboxymyoglobin is more resistant to oxidation than is oxymyoglobin *(35)*. Without CO present, atmospheres containing a gas mixture comprised of CO_2 and N_2 are vulnerable to discoloration by myoglobin oxidation due to residual O_2. Discoloration due to myoglobin oxidation occurs in beef when less than 0.1% O_2 is present *(18)*, and/or due to a decreased meat pH in the case of high CO_2 packaging, as previously discussed.

Research has shown that the use of CO at levels between 0.1 – 2.0% improve meat color and color stability. These reports include beef, pork, and poultry. The color improvement by CO seems to be valid if the other gases in the atmosphere are CO_2, N_2 and O_2 *(78)*. However, when the CO concentration was increased to 2.0%, the color was characterized as “too artificial” by a sensory panel *(61)*.

Fresh beef steaks stored in CO concentrations of 0.5% in MAP systems remained red for 8 weeks in a study conducted at Utah State University *(24)*. The study used the Hunter Lab Miniscan portable colorimeter (Reston, VA) for the objective measurement of color in ground beef stored with varying CO concentrations in MAP systems. Using this system, larger hue angle values are associated with less red color, where hue-angle 0 = red and hue-angle 90 = yellow. Fresh beef steaks stored in MAP systems that contained 0.5% CO had lower hue angle values compared with 5% CO in VP systems and steaks stored in PVC overwrap *(24)*. It was concluded that fresh ground beef stored in 0.5% CO-MAP systems remained red for the full 8-week length of the study, while the red color was lost within the first week of storage of ground beef in PVC. The ground beef in 0.5% CO-MAP systems had lower hue angle values than did the ground beef in PVC.

Researchers at the Universidad de Zaragoza *(41)* objectively evaluated the color on the surface of meat samples using a reflectance spectrophotometer (Minolta Chroma Meter CM-2002) 30 minutes after opening the package. This device yielded color measurements in the form of L*, a*, and b* values. A total of six different gas mixtures were used in the experimental units. These consisted of

- CMA (Control Modified Atmosphere): 70% O_2 + 20% CO_2 + 10% N_2
- LO-CO 0.1: 24% O_2 + 50% CO_2 + 25.9% N_2 + 0.1% CO
- LO-CO 0.25: 24% O_2 + 50% CO_2 + 25.75% N_2 + 0.25% CO
- LO-CO 0.5: 24% O_2 + 50% CO_2 + 25.5% N_2 + 0.5% CO
- LO-CO 0.75: 24% O_2 + 50% CO_2 + 25.25% N_2 + 0.75% CO
- LO-CO 1: 24% O_2 + 50% CO_2 + 25% N_2 + 1% CO

The study revealed that a* values increased as CO concentration increased, demonstrating that redness was influenced by the concentration of CO used. The values of a* for beef steaks did not differ ($P > 0.05$) within the same group, while differences were significant ($P < 0.01$) amongst groups *(41)*. The researchers concluded that the three types of atmospheres that

could be established according to their increasing ability for maintaining red color in the meat included

Type 1: Packages stored in the LO-CO 0.1 and LO-CO 0.25 experimental units that did not increase red color stability with respect to steaks stored in the CMA.

Type 2: Packages stored in the LO-CO 0.5 experimental unit that increased color stability.

Type 3: Packages stored in the LO-CO 0.75 and LO-CO 1 experimental units that greatly increased red color stability.

Oxygen in Modified Atmosphere Packaging

Effects of Oxygen on the Microflora of Fresh Meat

It is logical to conclude that if O_2 is present in the package aerobic bacteria will proliferate and cause the spoilage of the product. This is due to the fact that aerobic bacteria are commonly responsible for food spoilage due to their higher growth rates with respect to anaerobic bacteria under refrigeration conditions. This conclusion is supported by research projects that investigated the combined effects of storage temperature and O_2 concentration on the shelf life of different types of fish and fishery products *(58, 59, 60, 85)* and meat products *(84)*.

General Properties of Oxygen

Oxygen is a colorless, odorless gas that has a relatively low solubility in water, supports combustion (is explosive) and is very reactive with a wide variety of biological compounds *(46)*. Oxygen, given its reactive nature, is involved in many of the reactions that are detrimental to the quality of food products. These reactions include browning, rancidity development, fat oxidation and pigment oxidation *(66)*. The main reason for the inclusion of O_2 in MAP systems of fresh meat is the development of the bright red or cherry-red color and its maintenance. These are two factors that are considered essential to the display and acceptance of fresh meat. Even though the

red oxymyoglobin pigment is formed readily under normal atmospheric O_2 pressure, an elevated O_2 concentration (65-80%) in MAP systems helps to form a deeper layer of oxymyoglobin pigment that will extend the time the color appears attractive. However, the presence and increased level of O_2 will also promote the growth of rapidly-proliferating, aerobic microorganisms. Oxygen in MAP systems for fresh meat is usually combined with 20-25% carbon dioxide to achieve improved microbial control *(34)*.

Fresh meat is particularly susceptible to discoloration when low levels of O_2 are present in the packaging environment. A partial O_2 pressure in the range of 5-10 mm. of mercury (normal atmospheric partial pressure of O_2 is 159.2 mm Hg) will quickly cause the myoglobin pigment in meat to convert to metmyoglobin, which is brown. Low levels of residual O_2 in MAP packages of fresh meat will result in at least some metmyoglobin formation *(66)*. Therefore, it is recommended that the residual O_2 in fresh meat MAP systems be no more than 0.01% (10 ppm) immediately after packaging, and essentially 0% within 24 hours after packaging *(74)*.

Atmospheric or greater concentration of O_2 results in an attractive red color for fresh red meat. Complete elimination of O_2, as is the case in VP, prevents color deterioration. Upon exposure to O_2, however, formation of the attractive red color will occur. If a package has air leaking into it due to a poor seal or if low O_2 levels remain in it due to poor flushing, it is likely to discolor quickly. Low O_2 (> 0.01% O_2) levels can also be a problem in cooked or cured cooked meats where color fading and rancidity may occur as a result. In this case, 0.5% or less O_2 in package atmospheres is recommended *(66)*.

The problems of excess residual O_2 can sometimes be solved if O_2 scavengers or absorbers that react with any residual O_2 that may remain in the package are used. Iron powders in small packages are most often used for this purpose and can be frequently found in packages

of highly O_2-susceptible products such as dried snack sticks and jerky and other ready-to-eat products *(66)*.

Another problem created by exposing beef to high O_2 atmospheres is called "premature browning." The phenomenon develops when the beef is cooked and turns brown at lower-than-usual cooking temperatures *(31, 70, 71)*. In this case, meat that is cooked to a medium degree of doneness (71.1°C internal) or less appears to have been cooked to a well-done degree of doneness *(66)*. Research has shown that premature browning can occur in ground beef even when cooked to internal temperatures as low as 49°C *(28)*. Furthermore, ground beef patties stored in atmospheres containing 80% O_2 at 2°C were shown to develop premature browning in nearly 100% of the patties evaluated *(28)*. From a microbial standpoint, this creates a food safety concern due to the fact that many consumers use cooked color as an indicator of the temperature achieved during cooking (doneness) *(66)*.

Nitrogen Gas in Modified Atmosphere Packaging

Effects of Nitrogen on the Microflora of Fresh Meat

In addition to CO_2 and O_2, N_2 is the other gas used in significant amounts in MAP systems. Nitrogen is an inert gas that is colorless, odorless and tasteless *(46)*. Some of its characteristics include lower density than air, low solubility in water and fat and it is non-flammable.

Research demonstrates that N_2 can affect meat product shelf life indirectly because when N_2 is used to completely displace O_2, the atmosphere will not allow growth of aerobic microorganisms. Nitrogen is used to replace oxygen in packages to retard oxidative rancidity and inhibit growth of aerobic microorganisms, as an alternative to VP *(73)*. However, no direct effect of N_2 on microbial growth has been observed and, as a result, it has no impact on anaerobic bacteria *(66)*.

General Properties of Nitrogen

"Pack collapse," which can occur in MAP systems as a consequence of absorption of CO_2 into meat tissue, is usually avoided by using N_2 in combination with CO_2 as an inert filler *(5)*. Nitrogen acts as an inert filler due to its very low solubility in meat. As mentioned previously, N_2 has no antibacterial properties and does not affect meat color *(47)*.

High concentrations (usually 100%) of N_2 are often used in packages of cooked, cured meats, particularly sliced items where slice adhesion is to be prevented *(65)*. In these packages, given the cooked and cured nature of the products, it is recommended that O_2 be reduced to 0.5% or less to ensure cured color stability *(45)*. When dealing with uncured, cooked products, the elimination of O_2 in order to retard rancidity development and diminish flavor losses is of utmost importance. Due to the fact that cooked products are expected to have low microbial loads, prevention of flavor changes during storage is often more critical to shelf life of these products than microbial inhibition. In this case, the inclusion of a gas with bacteriocidal or bacteriostatic properties is not necessary. Instead, the use of 100% N_2 can extend shelf life of the products by preventing the chemical changes and flavor losses brought about by exposure to O_2 *(66)*.

Regulatory Status for the Use of Carbon Monoxide in Modified Atmosphere Packaging Systems

The approved use of CO in the packaging of fresh meat, poultry and fish products is a relatively new practice in the US. However, requests from processors to regulatory agencies began to appear as early as 1985 *(88)* and in 1999 the most significant patent for fish applications was issued *(32)*. A patent for the use of CO in fresh red meats was issued in 2001 *(72)*.

The United States Food and Drug Administration (FDA) approved master-bag packaging with 0.4% CO in 2002 *(82)*. In 2004 this approval was extended to retail, case-ready packaging *(83)*. In other parts of the world, however, government acceptance of the use of CO is not as

high. In fact, the use of CO in MAP systems in other countries is very limited. Norway, for example, used CO in MAP systems from 1985 *(77)* until trade agreements between it and the European Union caused the use of CO in MAP systems to cease in 2004 *(66)*.

Current Applications of Carbon Monoxide in the Food Industry

Today CO is being applied to a variety of seafood as a single gas with variable concentrations, as a component in filtered or "tasteless" smoke (TS), and as so-called artificial-filtered smoke based on gas blends to exemplify "tasteless smoke." Commercial use is expanding primarily with fish from either traditional harvests or culture operations in most seafood producing nations around the world. The primary market driving this trend is based in the US due to particular market acceptance, regulatory allowances, and the necessity for frozen products due to extensive transportation distances *(52)*.

The relatively recent approval for the use of CO in the packaging of fresh meats in the US is expected to increase the percentage of low-O_2 packaging formats and also to increase retail acceptance of case ready at retail stores throughout the country *(10)*. Modified atmosphere packaging of fresh meats would qualify under the "case ready" category. A study conducted in 2004 to audit and report trends in fresh meat packaging at retail level concluded that 60% of the packages audited were case ready (up from 49% in 2002). Modified atmosphere packages increased by 4% from 2002 to 2004 from 9% to 13%, respectively. Conversely, the traditional Styrofoam tray with PVC overwrap decreased from 51% in 2002 to 47% in 2004 *(44)*.

Disadvantages of Modified Atmosphere Packaging Systems

The major hurdle that MAP systems, especially those that make use of CO in the gas mixture, will have to clear before they become widely used by processors in the US is consumer acceptance. Several companies throughout the country have already asked the FDA to prohibit the use of CO in the packaging of fresh meats. In February of 2006, Kalsec, Inc. (Kalamazoo,

MI) officially petitioned that the FDA ban the use of CO in fresh meats because “the use of carbon monoxide deceives consumers and creates an unnecessary risk of food poisoning by enabling meat and ground beef to remain fresh-looking beyond the point at which typical color changes would indicate ageing or bacterial spoilage. If not banned, consumers should at least be notified through labeling if a meat product has been treated with carbon monoxide *(87)*.”

Another major disadvantage of the use of MAP systems, including those that make use of CO, is the costs associated with this practice. Specialty packaging films with specific gas permeability rates are required, and state-of-the-art equipment is also required when packaging a fresh meat product in a modified atmosphere of any kind.

Table 2-1. Advantages and disadvantages of modified atmosphere packaging systems as they apply to fresh meat products

Advantages	Disadvantages
Increase in shelf life	Controversy surrounding the use of CO (consumer perception)
Reduced economic losses due to increased shelf life	Added costs of production (special equipment and trained personnel)
Centralized packaging and greater portion control	Gas mixture is product-dependent (one gas mixture for each product category)
Improved presentation and limited or no need for chemical preservatives	Increased volume of the package will increase transportation costs
Packaging films will prevent cross-contamination of the product	Loss of benefits once the package has been opened
Convenient packaging	Spoilage of the product may be masked

CHAPTER 3
MATERIALS AND METHODS

The purpose of this study was to evaluate the effects of three packaging treatments (MAP containing 0.4% CO, VP and PVC overwrap), three fat treatments (10, 20 and 30% fat) and storage time on shelf life attributes of ground beef stored at 4 ± 1°C. The shelf life attributes evaluated were: microbial counts, objective color, sensory color and odor, 2-thiobarbituric acid reactive substances (TBARS), pH, headspace CO and residual CO. Two trials were conducted, each of which consisted of two phases. Phase 1 consisted of formulating, blending, grinding, packaging and storage of the meat. Phase 2 involved the evaluation of the different parameters associated with the shelf life of ground beef and also with CO levels in both the meat and the headspace of the packages.

Phase 1: Formulation, Packaging and Storage of Ground Beef

Formulation

Beef trimmings were obtained from the University of Florida Animal Science USDA inspected meat processing facility (EST. 6537), located in Gainesville, FL. Beef trimmings of different fat compositions (50/50 and 90/10) were blended in the appropriate proportions using a Pearson's square to obtain 10, 20 and 30% fat. After blending, the meat was ground through a 1/8" (0.3175 cm) grinding plate, packaged in the appropriate systems and stored at 4 ± 1°C.

Sample Treatment

A total of nine treatments were evaluated throughout the study (Table 3-1). Following mixing and grinding of the beef trimmings, approximately 454 g portions were packaged under the three packaging atmospheres.

Packaging, Gas Injection and Storage of Ground Beef

After grinding, the ground beef was divided into three equal aliquots and packaged in either Genpak 2 Styrofoam trays and over wrapped with one layer of PVC film (Companions, product#: 12073), vacuum packaged in 9 x 18" Cryovac B4770 barrier bags (0.5-0.6 g/100 in^2/24 hr @ 100°F, 100% relative humidity [RH] for water vapor transmission rate, and 1 cm^3/m^2/24 hr atm @ 40°F at 0% RH for O_2 transmission rate, Simpsonville, SC), or vacuum packaged in Cryovac B4770 barrier bags containing a manually, aseptically installed septum valve (Cole-Parmer, Vernon Hills, IL 60061-1844, catalog#: 00095XR) to allow for the injection of the gas blend into them. After sealing of these bags, they were transported to the University of Florida's Food Science and Human Nutrition (FSHN) Department in Styrofoam coolers with ice, where each package was injected with the gas blend. Three (3.0) L of a custom gas blend containing 0.4% CO, 30% CO_2 and 69.6% N_2 (Airgas Specialty Gases, Gainesville, FL 32609) were measured using a mass flow meter (Alicat Scientific, Inc., Tucson, AZ 85745, model M-50SLPM-D) and injected into each bag.

After the injection of the gas blend was completed, all bags were transported to the University of Florida's Department of Animal Sciences (ANS) for storage in a walk-in cooler for 25 d at 4 ± 1°C. The temperature was monitored daily and continuously using a circular-chart thermometer installed in the cooler (Partlow, Elizabethtown, NC 28337, model RTF) thermometer throughout the duration of the study. The lights inside the cooler were kept on at all times. The type of light bulbs inside the cooler emitted fluorescent light.

Phase 2: Evaluation of Shelf Life Parameters

Different parameters associated with ground beef shelf life were evaluated throughout the study. Those parameters were: objective color, microbial counts, sensory color and odor, rancidity, pH, headspace CO and residual CO.

Objective Color

Except for the PVC overwrap treatments, all treatments were evaluated on Days 0, 1, 3, 5, 7, 14, 21 and 25 for color. The PVC overwrap treatments were analyzed on Days 0, 1, 3, 5 and 7 due to spoilage of the meat. To objectively evaluate the effects of the different treatments used on the color of the ground beef, a Minolta colorimeter (Minolta Corp., Ramsey, NJ, model CR-310 with 50 mm aperture) was employed.

On each sampling day, two bags of ground beef per treatment were removed from the storage cooler and evaluated for objective color. Instrumental data of the ground beef was measured using the L* a* b* color spectrum. This spectrum includes L* (lightness), which is a measure of total light reflected on a scale ranging from 0 = black to 100 = white. The a* (red/green) value is a measure of the red (positive values) and green (negative values) colors of the sample. As the value of a* increases, the sample has an increase in red tones. As the value of a* decreases, the sample has an increase in green tones. The b* (blue/yellow) value is a measure of the yellow (positive values) and blue (negative values) colors of a sample. As the value of b* increases, the sample takes on a more yellow coloration. As the value of b* decreases, the sample takes on more of a blue coloration.

The colorimeter was calibrated as described in the user's manual on each sampling day. Furthermore, since two different packaging films were used (PVC and Cryovac B4770 film), the colorimeter was calibrated with each film separately before the color measurements were conducted. After calibration, two measurements per treatment were taken and averaged. Each treatment was evaluated in duplicates.

Microbial Analyses

Except for the PVC overwrap treatments, all treatments were evaluated on Days 0, 1, 7, 14, 21 and 25 for microbial counts. The PVC overwrap treatments were analyzed on Days 0, 1,

3, 5 and 7 due to spoilage of the meat. All treatments were analyzed for the following microorganisms: total aerobes, total psychrotrophs, Gram negatives, lactic acid bacteria, total coliforms, generic *E. coli* and *E. coli* O157:H7. All media (Difco Laboratories, Detroit, MI 48232) and materials used for the cultivation and maintenance of the bacteria were purchased from Fisher Scientific (Pittsburgh, PA 15238). Twenty-five grams of ground beef from each treatment were placed in sterile 18 x 30 cm Fisherbrand stomacher bags (400 mL, Fischer Scientific, Pittsburgh, PA 15238) along with 225 mL of sterile 0.1% peptone water (Cat. No. DF1807-17-4). Each treatment was done in duplicate. The stomacher bags were massaged by hand for two minutes to loosen up any surface bacteria. One mL of the sample mixture was transferred to a test tube containing 9 mL of sterile 0.1% peptone water, from which 10^{-1} to 10^{-6} (or more if needed) serial dilutions were prepared for each treatment.

One µL of the dilutions was pipetted and spread (using a glass hockey stick previously flame-sterilized) BBL Trypticase Soy Agar (TSA, Cat. No. B11043) for total aerobe and total psychrotrophs counts, Sorbitol MacConkey agar (SMAC, Cat. No. OXCM0813B) supplemented with a Cefixime-Tellurite supplement (CT, Cat. No. OXSR0172E) for *E. coli* O157:H7 counts, Lactobacilli MRS agar (MRS, Cat. No. DF0882170) for lactic acid bacteria counts and GN Broth (GN, Cat. No. DF0486-17-4) with added granulated agar (Cat. No. DF0145-17-0) for Gram negative bacteria counts. One mL of each dilution was added onto 3M Petrifilm E. coli/Coliform Count Plates (3M Company, St. Paul, MN 55144, Cat. No. 6414) for the evaluation of generic *E. coli* and total coliforms.

The TSA plates for total aerobic counts, GN and MRS plates were incubated at 35 ± 1°C for 48 hr, CT-SMAC plates were incubated at 37 ± 1°C for 24 hr, 3M Petrifilm E. coli/Coliform Count Plates were incubated at 35 ± 1°C for 24 hr, and TSA plates for total psychrotrophs counts

were incubated at 7 ± 1°C for 10 d. After incubation, suspected colony forming units (CFU) were counted, recorded and averaged.

Analysis of pH

Except for the PVC overwrap treatments, all treatments were evaluated on Days 0, 1, 7, 14, 21 and 25 for pH. The PVC overwrap treatments were analyzed on Days 0, 1, 3, 5 and 7 due to spoilage of the meat. Analysis of pH was conducted immediately after the microbiological analyses were completed. An Accumet Basic pH meter (Fisher Scientific, Pittsburgh, PA 15238, model AB15) was used and pH measurements were recorded for all treatments. The probe was standardized using standard buffer solutions of pH 4.0 and pH 7.0. After standardization, the probe was placed inside the sample homogenate and allowed to reach equilibrium for 1 minute before the reading was taken. The probe was rinsed with distilled water and dried with a Kimwipe (Kimberly-Clark Corporation, Roswell, GA, Cat. No. 06-666) between each sample measurement.

Headspace Carbon Monoxide

On Days 1, 7, 14, 21 and 25 of the experiment, the CO concentration in the headspace of the MAP treatments was analyzed. Two bags of ground beef per treatment were removed from the storage cooler, placed in Styrofoam coolers containing ice, and transported to the University of Florida's FSHN Department for analysis.

The CO concentration in the headspace and CO residuals in the meat was determined using an Agilent Technologies 6890N Network Gas Chromatograph (GC) System. The GC was equipped with a flame ionization detector (FID), a Supelco 80/100 Porapak Q column (1.82 m long) and a hydrogen aided nickel catalyst (to convert carbon monoxide and carbon dioxide into methane and make it detectable for the FID). The GC settings used for CO evaluation are listed in Table 3-2.

Two 100 µL samples from each bag were injected into the GC using a 100 µL Hamilton syringe (Cat. No. 14-815-80) and analyzed. One peak was obtained for each 100 µL sample that was injected into the GC. The two peaks per bag of ground beef were then averaged and plugged into the following formulas to determine CO concentration in the headspace of the package:

% CO = 0.000196 X – 0.001458 (where X = Average peak area)

ppm CO = % CO * 1,000,000

Residual Carbon Monoxide

On Days 0, 1, 3, 5, 7, 9, 11, 14, 21 and 25 of the experiment, the CO concentration per gram of beef in the MAP treatments was measured. A 6 g sample from each treatment was collected in duplicate on each of the sampling days, placed in 60 mL I-Chem Economy 100-series tubes (Cat. No. 05-719-398) and frozen at -20 ± 1°C for no more than 30 d before sampling. Samples were thawed overnight at 4 ± 1°C and transported to the University of Florida's FSHN department for CO analysis. The following protocol was followed to determine ppm CO/g meat:

1. Transfer a 6 g core sample into a 60 mL I-Chem bottle.
2. Add 3 drops of octanol (antifoaming) (Cat. No. S80109).
3. Add 12 mL of 10% sulfuric acid (H_2SO_4) (Cat. No. 815032).
4. Shake mixture for 10 sec by hand.
5. Incubate for 5 min at 40°C.
6. Shake at room temperature for 15 min in a shaker.
7. Inject 100 µL into GC and analyze.

Two 100 µL samples from each tube were injected into the GC using a 100 µL Hamilton syringe (Cat. No. 14-815-80) and analyzed. One peak was obtained for each 100 µL sample that was injected into the GC. The two peaks per tube were then averaged and plugged into the following formulas to determine ppm CO/g meat:

% CO = 0.000196 X – 0.001458 (where X = Average peak area)

g CO/g Meat = (0.000001145 * % CO * 430)/6

Adjusted g CO/g Meat = (6 * g CO/g meat)/Weight of Sample

ppm CO/g Meat = Adjusted g CO/g meat * 1,000,000

Thiobarbituric Acid Reactive Substances

Except for the PVC overwrap treatments, all treatments were evaluated on Days 0, 1, 3, 5, 7, 14, 21 and 25 for rancidity in the form of TBARS. The PVC overwrap treatments were analyzed on Days 0, 1, 3, 5, 7 and 9 due to spoilage of the meat. Samples were frozen at -20 ± 1°C for no more than 30 d in sterile 18 x 30 cm Fisherbrand stomacher bags (400 mL, Fischer Scientific, Pittsburgh, PA 15238) and allowed to thaw overnight in a cold room at 4 ± 1°C before they were analyzed. The TBARS distillation procedure for meat and poultry was adapted using procedures from Tarladgis et al. *(80)*, Rhee *(62)* and Ke et al. *(30)*. In the adapted procedure, the sample was read against the blank at the optical wavelength of 535 nm. In addition, 66% recovery was obtained, compared to 69% in Tarladgis et al. *(80)*, resulting in a variation in K (distillation value). Two bags per treatment were analyzed on each of the days mentioned above. Two absorbance readings per sample were taken and then averaged.

Consumer Sensory Panel Analysis

Except for the PVC overwrap treatments, all treatments were evaluated on Days 0, 1, 3, 7, 9, 14, 21 and 25 for consumer sensory panel color and off-odors. The PVC overwrap treatments were analyzed on Days 0, 1, 3, 7 and 9 due to spoilage of the meat. The sensory

facilities at the University of Florida's Animal Science Meat Research laboratory were used. Samples of ground beef were removed from their initial packaging atmospheres, placed in transparent Zip-lock bags and placed on white countertops for panelist evaluation.

The panelists were instructed to evaluate all samples for color and off-odors. The color scale used was an 8 - point hedonic scale where 1 = extremely dark brown, 2 = very dark brown, 3 = dark brown, 4 = dark red, 5 = slightly dark red, 6 = cherry red, = 7 moderately light cherry-red and 8 = light cherry-red. The scale used to evaluate off-odors was a 7 - point hedonic scale where 1 = none, 2 = barely perceptible, 3 = perceptible, 4 = slightly strong, 5 = moderately strong, 6 = very strong and 7 = very strong. A hedonic scale is a rating scale that measures the level of liking or disliking of food products *(50)*.

Statistical Analysis

The statistical analysis for this project was performed using SAS Windows *(65)*. A split-plot design with three packaging treatments and three fat treatments was used for evaluating all the parameters associated with the shelf life of ground beef described above. The analysis of variance of the Mixed Procedures (PROC MIXED) of SAS® software and the LSMEANS procedure for generating standard errors of the mean (SEM) were used to analyze trial, day, fat, packaging, day by fat, day by packaging, fat by packaging and day by fat by packaging interaction. Variations in data were accounted for by eight treatment effects: trial, day, fat, packaging, day*fat, day*packaging, fat*packaging and day*fat*packaging. Any significant differences were analyzed by the multiple comparison procedure of Duncan's Multiple range test, using a level of significance of alpha = 0.05.

Table 3-1. Treatments evaluated during a 25-d storage period to determine the effects of packaging system, fat concentration and carbon monoxide on the storage stability of ground beef

1 - MAP[1] – 10% fat	4 - VP[2] – 10% fat	7 - PVC[3] – 10% fat
2 - MAP – 20% fat	5 - VP – 20% fat	8 - PVC – 20% fat
3 - MAP – 30% fat	6 - VP – 30% fat	9 - PVC – 30% fat

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride

Table 3-2. Gas Chromatograph settings used for analyzing packaged ground beef for carbon monoxide gas headspace and residuals in the meat

Parameters	Settings
Injection temperature	100°C
Carrier gas and flow rate	Helium at 26.9 mL/min (splitless)
Nickel catalyst temperature	375°C
Oven temperature	30°C isotherm
Runtime	3 min
Detector temperature	250°C
Column	80/100 Porapak Q packed column (1.82 m)

CHAPTER 4
RESULTS AND DISCUSSION

The objectives of this study were to evaluate the effects of three packaging atmospheres (MAP containing 0.4% CO, VP and PVC overwrap) and three fat percentages (10, 20 and 30%) on pH, color, TBARS, microbiological and sensory characteristics of ground beef, and to determine the concentration of CO in the headspace of the package and in the meat during 25-d storage at 4 ± 1ºC.

Evaluation of Ground Beef Stored in Polyvinyl Chloride Overwrap for 7 d

Microbiological Analyses

Aerobic plate counts

Total aerobic counts in PVC treatments significantly increased ($P < 0.09$) on Day 5 compared to Day 0 regardless of fat level (Table 4-1). However, a slight but not significant decrease ($P > 0.05$) in log CFU/g was detected on Day 7 compared to Day 5. These results indicate that, once maximum levels were attained, aerobic bacteria entered the death phase of their growth curve. An alternative conclusion would be that, as shown by other microbiological analyses, the slight decrease in Gram negative counts was caused by competitive inhibition. No fat effect was detected ($P > 0.05$).

Gram negative counts

Gram negative microorganisms in all PVC treatments significantly increased ($P < 0.05$) on Day 5 compared to Day 0 regardless of fat level (Table 4-2). Furthermore, a decrease of almost 1 log CFU/g was detected in all treatments between Day 5 and Day 7, suggesting that Gram negative microorganisms had entered the death phase of their growth curve. Gram negative counts for the 30% fat treatment were significantly higher ($P < 0.05$) than those in the 20% fat treatment on Day 1. However, no overall fat effect was detected ($P > 0.05$).

Psychrotroph counts

Psychrotroph counts in all PVC treatments significantly increased ($P < 0.05$) by Day 5 compared to Day 0 regardless of fat level (Table 4-3). In the 30% fat treatment, however, a significant increase ($P < 0.05$) in psychrotroph counts was detected as early as Day 1 compared to Day 0. In both the 20 and 30% fat treatments, psychrotroph counts slightly decreased ($P > 0.05$) by Day 7 compared 5, indicating that psychrotrophic microorganisms had entered the death phase of their growth curve by Day 7. Psychrotroph counts for the 30% fat treatment were significantly higher ($P < 0.05$) than all other fat levels on Day 1. However, no overall fat effect was detected ($P > 0.05$).

Analysis of pH

A significant increase ($P < 0.05$) in pH values for all PVC treatments was detected by Day 7 compared to Day 0 regardless of fat level (Table 4-4). Although no overall fat effect was detected ($P > 0.05$), the pH values for the 30% fat treatment were significantly higher ($P < 0.05$) than all other fat treatments on Day 5.

Evaluation of Ground Beef Stored in Modified Atmosphere, Vacuum Packaging and Polyvinyl Chloride Overwrap for 25 d

Microbiological Analyses

Aerobic plate counts

Aerobic microorganisms (APCs) were less than 7 log CFU/g for all treatments through 25 d storage (Table 4-5). In general, APCs increased for all treatments as storage time increased. In the MAP group, significant ($P < 0.05$) increases in APCs were detected by Day 14 when compared to Days 0 and 1 for all fat treatments. However, for the 20 and 30% fat treatments, significant increases ($P < 0.05$) in APCs were detected by Day 7. Counts were similar ($P > 0.05$) for all MAP treatments from Days 14-25 and remained less than 6 log CFU/g. This suggested

that aerobic microorganisms in all MAP treatments had entered the stationary phase of their growth curve by Day 14.

Similar results were observed in the VP group. Significant ($P < 0.05$) increases in APCs were detected by Day 14 when compared to Days 0 and 1 regardless of fat level. Counts were similar ($P > 0.05$) from Days 14-25. This suggested that aerobic microorganisms in all VP treatments had entered the stationary phase of their growth curve by Day 14.

The APCs for the PVC group significantly ($P < 0.09$) increased by Day 7 when compared to Day 0 for the 10 and 20% fat levels. However, APCs were similar ($P > 0.05$) for the 30% fat treatment through 7-d storage.

Counts within each day were similar ($P > 0.05$) throughout the storage time for all treatments. However, the 20% fat treatment in the PVC group on Day 1 had significantly lower ($P < 0.05$) APCs than all other treatments.

Gram negative counts

Gram negative microorganisms were less than 4 log CFU/g for all treatments through 25-d storage (Table 4-6). No significant ($P > 0.05$) increase in Gram negative counts was detected in MAP and VP treatments during storage. In addition, Gram negative counts were similar ($P > 0.05$) within and between all MAP and VP treatments. These results suggested that both packaging methods had a bacteriostatic effect on Gram negative microorganisms.

Gram negative counts for the PVC group increased significantly ($P < 0.05$) by Day 7 when compared to Day 0 regardless of fat level. These results suggested that exposure to O_2 promoted the growth of Gram negative microorganisms. Gram negative counts for the 10 and 30% fat treatments in the PVC group were higher ($P < 0.05$) than all MAP and all VP treatments on Day 7.

Lactic acid bacteria counts

Lactic acid bacteria counts were less than 6 log CFU/g for all treatments throughout 25-d storage (Table 4-7). Similar results were observed for all MAP and VP treatments. Lactic acid bacteria counts significantly increased ($P < 0.05$) in all treatments by Day 7 when compared to Day 0 regardless of fat level and packaging method. In addition, lactic acid bacteria counts significantly increased ($P < 0.09$) in all treatments by Day 1 when compared to Day 0 regardless of fat level and packaging method. Lactic acid bacteria counts remained similar ($P > 0.05$) from Days 7 through 25. No significant ($P > 0.05$) differences were detected between treatments on each individual day throughout the storage time.

Psychrotroph counts

Psychrotroph counts were less than 7 log CFU/g for all treatments through 25-d storage (Table 4-8). In general, psychrotroph counts increased for all treatments as storage time increased. All treatments in the MAP group had significantly higher ($P < 0.05$) counts on Day 14 when compared to Day 0. Counts on Days 21 and 25 were significantly higher ($P < 0.05$) when compared to Day 0 for the 10 and 20% fat treatments, but similar ($P > 0.05$) for the 30% treatment.

The 10 and 20% fat treatments in the VP group showed significantly higher ($P < 0.05$) levels of bacteria by Day 21 compared to Day 0. In the 30% fat treatment, however, no significant changes ($P > 0.05$) were detected throughout the storage time.

The 10 and 20% fat treatments in the PVC group showed significantly higher ($P < 0.05$) levels of bacteria by Day 7, when compared to Day 0. In the 30% fat treatment, however, no significant changes ($P > 0.05$) were detected throughout the storage time. On Day 7, counts in the PVC group were all above 5 log CFU/g regardless of fat level, whereas they did not exceed 4.61 log CFU/g for all MAP and VP treatments. Furthermore, the 10% fat treatment in the PVC

group had significantly higher ($P < 0.05$) counts compared to all VP treatments and to the 20 and 30% fat treatments within the MAP group.

E. coli O157:H7 counts

No *E. coli* O157:H7 was detected throughout the duration of the study.

Total coliforms and _E. coli_ counts

No *E. coli* was detected throughout the duration of the study. Total coliforms were detected only in the first trial conducted. Thus, the statistical analysis could not be carried out.

Anaerobic conditions have been shown to inhibit the growth of aerobic bacteria and increase microbiological shelf life of meat products *(16, 57, 63, 67)*. Increased bacterial inhibition occurs with CO_2 levels of 20% or more in MAP systems *(11, 41)*. Vacuum packaging is a form of MAP because, in the case of fresh meats, microbial and muscle metabolism will utilize residual O_2 to produce CO_2 *(66)*. Low levels (0.1 – 1.0%) of CO, on the other hand, have little effect on meat microflora *(40)*.

Results from this study indicated that MAP and VP had a bacteriostatic effect on Gram negative microorganisms (Table 4-6). Dixon and Kell *(9)* determined that CO_2 has a greater inhibitory effect on Gram negative bacteria, which grow rapidly on fresh meat, than on Gram positives. The growth of aerobic (Table 4-5), psychrotrophic (Table 4-8) and lactic acid bacteria (Table 4-7), on the other hand, was not inhibited by MAP or VP. Significant increases ($P < 0.05$), compared to Day 0, in aerobic (Day 14), psychrotrophic (Day 14) and lactic acid bacteria (Day 7) in all MAP treatments were observed. Similarly, significant increases ($P < 0.05$), compared to Day 0, in aerobic (Day 14), psychrotrophic (Day 21 [$P < 0.09$]) and lactic acid bacteria (Day 1) in all VP treatments were also observed. Mean log CFU/g of total aerobes and lactic acid bacteria generally did not significantly increase ($P > 0.05$) after Day 7 of storage, while total psychrotroph counts did ($P < 0.05$). It is important to mention that, within the MAP

(10% fat, Day 21) and VP (30% fat, Day 25) groups, two of the six treatments reached aerobic plate counts and total psychrotrophs counts above spoilage levels (> 6 log CFU/g). Consumer panel sensory color (Table 4-14) and off-odors (Table 4-15) results suggested that discoloration and off-odors were detected in those treatments on those days.

Packaging of ground beef in PVC overwrap favors the growth of aerobic microorganisms due to its O_2 permeability. Bacterial growth is favored in PVC-wrapped meats compared with vacuum packaged meats *(67)*. Vacuum packaging favors the growth of lactic acid bacteria, whereas *Pseudomonads* spp. are generally the dominant spoilage microflora of PVC-wrapped meats *(16, 57, 63)*. These conclusions are in agreement with results obtained during this study. Packaging ground beef in PVC overwrap allowed for significant increases ($P < 0.05$) in Gram negative (Table 4-2), total aerobes (Table 4-1) and psychrotrophic (Table 4-3) bacteria by Day 5 of storage. In addition, total psychrotroph counts for the 20 and 30% fat treatments in the PVC group reached spoilage levels (>6 log CFU/g) by Day 5 of storage. Consumer panel sensory color (Table 4-14) and off-odors (Table 4-15) results suggested that discoloration and off-odors were detected in those treatments as early as Day 3.

Product Analyses

Analysis of pH

The pH values varied between 5.00 and 6.17 (Table 4-9). The pH values for all MAP and VP treatments decreased significantly ($P < 0.05$) by Day 14 when compared to Day 0 regardless of fat level. These results agree with those observed in the microbiological analysis of lactic acid bacteria, whose levels rose significantly ($P < 0.05$) by Day 7 compared to Day 0 regardless of fat level and packaging method (Table 4-7).

The pH values for the PVC group increased significantly ($P < 0.05$) by Day 7 when compared to Day 0. Furthermore, pH values for the PVC group on Day 7 were significantly

higher ($P < 0.05$) than those for all MAP and all VP treatments regardless of fat level. No significant differences ($P > 0.05$) were detected between fat levels throughout the storage time regardless of packaging method.

The modified atmosphere used in this study contained 30% CO_2, the active antimicrobial agent in both anaerobic MAP and VP *(66)*. When CO_2 is introduced into the headspace of a package, its concentration will decline as CO_2 is absorbed into the meat *(23)*. It has been shown that CO_2 dissolves in both muscle and fat tissue until saturation or equilibrium is reached, and the rate at which it is absorbed by tissues is affected by initial meat pH, temperature and water activity *(17)*. It is also known that the gas dissolves readily in water and will produce H_2CO_3 in solution *(23)*. Carbonic acid will then cause a drop in meat pH and, in turn, negatively affect microbial growth. Numerous research projects have been conducted using atmospheres containing high levels of CO_2, and concluded that a decrease in pH of 0.05-0.35 units was a direct consequence *(22, 36, 64, 69, 75)*.

Results from this study showed that a significant decrease ($P < 0.05$) in pH occurred after Day 14 of storage for all MAP and VP treatments (Table 4-9). Thus, the absorption of CO_2 into the meat could have been responsible for this significant drop in pH values. However, as discussed previously, log CFU/g of lactic acid bacteria significantly increased ($P < 0.05$) by Day 7 of storage regardless of packaging method and fat level (Table 4-7). The absence of O_2 from the packaging system, as is the case in anaerobic MAP and VP, has been shown to favor the growth of lactic acid-producing bacteria *(16, 57, 63)*. This production of lactic acid may have led to the significant decrease in pH values observed in all MAP and VP treatments by Day 14.

Results observed in the PVC group contrasted from those observed in the MAP and VP groups in that a significant increase ($P < 0.05$) in pH was observed by Day 7 of storage (Table 4-

9). Analyses of aerobic (Table 4-1), Gram negative (Table 4-2) and psychrotrophic (20 and 30% fat treatments only [Table 4-3]) bacteria showed a slight decrease ($P > 0.05$) in log CFU/g counts on Day 7 compared to Day 5, suggesting that the organisms in question had entered the death phase of their growth curves. Thus, the increase in pH observed in all PVC treatments may have been caused by the by-products of bacterial cell death.

Thiobarbituric acid reactive substances

There were no significant increases ($P > 0.05$) in TBARS for the MAP and VP treatments throughout the storage time when compared to Day 0 (Table 4-10). These results suggested that the MAP and VP systems have an antioxidant effect on ground beef regardless of fat level.

Significant increases ($P < 0.05$) in TBARS for the PVC group were observed for both the 10 and 20% fat treatments by Day 7 when compared to Day 0. Thiobarbituric acid reactive substances values for the 30% fat treatment were similar ($P > 0.05$) over time. Furthermore, on Days 3, 5 and 7, the 20% fat treatment within the PVC group yielded significantly higher ($P < 0.05$) TBARS values than all VP treatments. These levels were also higher ($P < 0.05$) when compared to the values observed in the 10 and 30% fat treatments within the MAP group on those days, suggesting that exposure to O_2 accelerates lipid oxidation.

It is also important to mention that, within the MAP group, the 30% fat treatment yielded higher ($P < 0.05$) TBARS values than the 10% fat treatment on Days 1, 7, 9 and 25, results which suggest that fat level has an effect on lipid oxidation. These results, however, were not significant ($P > 0.05$) when they were compared to those from the 20% fat treatment.

A low ultimate pH has been shown to have a negative effect on product quality in terms of color, weight loss and lipid oxidation *(29)*. However, TBARS values did not significantly increase ($P > 0.05$) for all MAP and VP treatments throughout the duration of the study (Table 4-

10) even though the pH values for those treatments had significantly decreased by Day 14 of storage (Table 4-9). Since rancidity is an oxidative process, CO can be viewed as an antioxidant.

Oxygen, given its reactive nature, is involved in many of the reactions that are detrimental to the quality of food products. These reactions include browning, rancidity development, fat oxidation and pigment oxidation *(66)*. Results from this study showed significant increases ($P < 0.05$) in TBARS in the PVC group for both the 10 and 20% fat treatments by Day 7 when compared to Day 0. Thiobarbituric acid reactive substances values for the 20% fat treatment in the PVC group were significantly higher ($P < 0.05$) than all VP treatments and the 10 and 30% fat treatments within the MAP group on Days 3, 5 and 7. These results emphasize the shorter shelf life achieved by packaging fresh meats in PVC overwrap compared to both anaerobic MAP and VP.

Objective Color Evaluation

L* values

L* values, which measure total light reflected on a scale ranging from 0 = black to 100 = white, did not change ($P > 0.05$) over time in all MAP and PVC treatments (Table 4-11). These results suggest that both of these packaging methods had similar effects on L* values. Conversely, L* values for all VP treatments significantly decreased ($P < 0.05$) by Day 1 when compared to Day 0 regardless of fat level. L* values for all VP treatments remained constant ($P > 0.05$) after Day 1.

Fat treatment within each packaging treatment had an effect ($P < 0.05$) on L* values. On Days 0, 1, 3, 5 and 7, L* values for the 30% fat treatment within each packaging treatment were significantly higher ($P < 0.05$) than those observed for the 10% fat treatment. Similar results were observed on Days 14 and 21, where L* values for the 30% fat treatment within both the MAP and the VP groups were significantly higher ($P < 0.05$) than those observed in the 10% fat

treatment. These results suggest that fat level influences the brightness scores of ground beef under the conditions used in this study.

L* values for the 10% fat treatment within the VP group were significantly lower ($P < 0.05$) from all MAP and PVC treatments on Days 1, 3, 5, 7 and were also lower ($P < 0.05$) than all MAP treatments on Days 14, 21 and 25. These results suggest that VP had the opposite effect on L* values than fat level did under the conditions used in this study.

b* values

Table 4-12 summarizes the b* values obtained during this study. Within the MAP and the VP treatments, all fat levels showed significant decreases ($P < 0.05$) in b* values by Day 1 compared to Day 0. No significant changes ($P > 0.05$) were detected after Day 1 for any of these treatments.

In the PVC group, similar results were observed. The b* values significantly decreased ($P < 0.05$) by Day 1 in the 10 and 20% fat treatments and by Day 3 in all treatments when compared to Day 0. All PVC treatments showed significantly higher ($P < 0.05$) b* values on Day 1 when compared to all MAP and all VP treatments. However, on Days 3, 5 and 7, b* values for all PVC treatments did not differ significantly ($P > 0.05$) from all MAP treatments while they remained higher ($P < 0.05$) than those observed in all VP treatments.

All MAP treatments showed significantly higher ($P < 0.05$) b* values than those observed in all VP treatments regardless of fat level on Days 3, 5, 7, 14, 21 and 25. These results indicated that the MAP system used in this study had a similar effect on b* values of ground beef as did conventional PVC overwrap regardless of fat level and under the conditions of this study.

a* values

A significant decrease ($P < 0.05$) in a* values was observed in the 10 and 20% fat treatments within the MAP group by Day 21 when compared to Day 0 (Table 4-13). All fat

treatments within the MAP group, however, showed significant ($P < 0.05$) decreases in a* values on Day 21 when compared to Day 1. In addition, a* values within the MAP group reached their highest levels on Day 5 regardless of fat level.

Vacuum packaging caused a significant decrease ($P < 0.05$) in a* values by Day 1 compared to Day 0 regardless of fat level. After Day 1, however, no significant ($P > 0.05$) changes were observed. The a* values for all VP treatments were significantly lower ($P < 0.05$) than all MAP treatments on Days 1, 5 and 7.

All treatments within PVC overwrap group, on the other hand, showed significant decreases ($P < 0.05$) in a* values by Day 3. Furthermore, a* values for all treatments within the PVC group were significantly lower ($P < 0.05$) than all MAP and all VP treatments on Days 3, 5 and 7.

Red color can be maintained in low-CO treated meats that have spoiled, emphasizing the need for adherence to label instructions for product shelf life and the use of odor and overall appearance as spoilage indicators *(7).* As results from this study indicate, a significant decrease ($P < 0.05$) in a* values was observed in the 10 and 20% fat treatments within the MAP group by Day 21 when compared to Day 0. In addition, all fat levels within the MAP group showed significant ($P < 0.05$) decreases in a* values on Day 21 when compared to Day 1, suggesting that the appealing red color associated with fresh ground beef was lost after 21 days of storage.

It is general consensus that consumer acceptance of vacuum packaged retail beef has been low because of its dark reddish-purple color *(43).* Reduced myoglobin is the form of myoglobin associated with fresh meat packaged in anaerobic conditions, such as VP. Results from this study indicated that ground beef that was vacuum packaged was darker in appearance

as shown by the initial decrease in a* values and lower L* and b* values when compared to that ground beef in the MAP system.

Fresh meat is particularly susceptible to discoloration when low levels of O_2 are present in the packaging environment. A partial O_2 pressure in the range of 5-10 mm of mercury (normal atmospheric partial pressure of O_2 is 159.2 mm Hg) will quickly cause the myoglobin pigment in meat to convert to metmyoglobin, which is brown. Low levels of residual O_2 in MAP packages of fresh meat will result in at least some metmyoglobin formation *(15, 66, 75)*.

Results from this study suggested that exposure to O_2 had a negative effect on redness values (Table 4-13). These results agree with previous research projects that concluded that packaging fresh meats in PVC overwrap allows rapid surface pigment oxygenation and red color development, but brown color discoloration occurs within 1-7 days *(42)*.

Consumer Sensory Evaluation

Color

The average consumer panel color scores for the 10% fat treatment within the MAP group were similar ($P > 0.05$) on any day compared to Day 0 (Table 4-14). However, a clear drop in the consumer panelist scores was noticed on Day 21. This drop in color scores was significant ($P < 0.05$) when compared to Day 14. Similar results were obtained with the 20% fat treatment within this group ($P < 0.05$). The 30% fat treatment, however, did not show a clear drop in average consumer panel scores until Day 25. These results allow us to conclude that the modified atmosphere used in this study was able to enhance the color of the meat until Day 14 of storage under the conditions used in this study.

Panelists rated the color of all ground beef in the VP group significantly higher ($P < 0.05$) on Day 3 when compared to Day 0 regardless of fat level. The color of all samples was rated similar ($P > 0.05$) for Days 9-25.

Color scores significantly decreased ($P < 0.05$) for the PVC group on Day 3 when compared to Days 0 and 1 regardless of fat level. In addition, color scores for all PVC treatments were significantly lower than all VP treatments and the 20 and 30% fat treatments within the MAP group on Days 3 and 7 ($P < 0.05$). Color scores for all PVC treatments were significantly lower ($P < 0.05$) than all VP and all MAP treatments on Day 7.

Off-odors

Off-odor consumer panel scores for MAP treatments were significantly higher ($P < 0.05$) on Days 21 and 25 when compared to Days 0 and 1 regardless of fat level (Table 4-15). These results indicate that the modified atmosphere used in this study prevented the development of off-odors up until Day 14.

Within the VP group, all treatments showed higher ($P < 0.05$) off-odor scores on Days 7, 14, 21 and 25 compared to Day 0 regardless of fat level. Prior to Day 7, however, no significant increases ($P > 0.05$) in off-odor scores were detected.

All PVC treatments showed significant increases ($P < 0.05$) in off-odor scores after Day 3 of storage regardless of fat level. Further increases ($P < 0.05$) in off-odors were shown on Day 9, indicating that PVC overwrap prevented the development of off-odors for only 1 d after packaging under the conditions used in this study. In addition, all PVC treatments showed significantly higher ($P < 0.05$) off-odor scores than all MAP treatments on Days 7 and 9 regardless of fat level.

The 10% fat treatment within the MAP group reached spoilage levels of psychrophilic bacteria by Day 21 of storage (Table 4-4). Although by this day consumer panel color scores were significantly lower ($P < 0.05$) than those for Day 14, they did not significantly differ ($P > 0.05$) from Day 0. These results indicate packaging fresh ground beef in an atmosphere

containing 0.4% CO may mask spoilage from a visual standpoint. Cornforth and Hunt *(7)*, after reviewing literature on the use of carbon monoxide in fresh meats, reached the same conclusion.

After comparing a* values obtained during this study (Table 4-13) to consumer panel color scores (Table 4-14), a clear discrepancy can be seen. The a* values for the 10 and 20% fat treatments within the MAP group significantly decreased ($P < 0.05$) by Day 21 compared to Day 0. Panelists, conversely, were not able to detect this decrease ($P > 0.05$).

Off-odor consumer panel scores, conversely, showed that all MAP treatments had developed off-odors by Day 21 ($P < 0.05$). Thus, it can be concluded that, even though consumer panel color scores did not significantly decrease ($P > 0.05$) throughout the storage time, panelists were still able to detect the onset of off-odors.

Panelist color scores for all VP treatments did not significantly decrease ($P > 0.05$) over time. Off-odor scores, conversely, significantly increased ($P < 0.05$) after 7-d storage, which coincided with a significant increase ($P < 0.05$) in lactic acid bacteria levels (Table 4-7). These results suggested that the growth of lactic acid-producing bacteria within the packaging environment may lead to the development of off-odors.

The rapid onset of discoloration and off-odors in all PVC treatments further supported the conclusion that exposure to O_2 has detrimental effects on shelf life attributes such as color and odor.

Carbon Monoxide Analyses

Headspace carbon monoxide

A significant decrease ($P < 0.09$) in CO concentration was detected on Day 14 compared to Day 1 in both the 20 and 30% fat treatments (Table 4-16). The 10% fat treatment, conversely, did not show significant changes ($P > 0.05$) in CO levels throughout the duration of the study.

Although fat level did not appear to have an effect on the CO concentration in the headspace of the package, the 20 and 30% fat treatments showed significantly higher ($P < 0.05$) concentrations of CO than the 10% fat treatment on Day 1. These results suggested that fat level has an effect on how rapidly CO is absorbed into the ground beef.

Residual carbon monoxide

Significantly higher ($P < 0.05$) residual levels of CO were detected on Days 5, 7, 9, 11, 14, 21 and 25 compared to Day 0 regardless of fat level (Table 4-17). Although no overall fat effect was detected ($P > 0.05$), the residual levels of CO in the 20 and 30% fat treatments were significantly lower ($P < 0.05$) than those in the 10% fat treatment on Days 11 and 14.

Residual levels of CO peaked on Days 11, 21 and 25 for the 10, 20 and 30% fat treatments, respectively. These results suggested that fat level had an effect on how rapidly maximum levels of residual CO were reached under the conditions of this study.

Although the concentration of CO in the headspace of the bags (Table 4-12) appears to be high enough to present a health risk to humans, the quantity of CO in one or several packages would still be too low to cause health concerns when packages are opened at home *(7)*. It has been concluded that, for MAP packages containing 0.4% CO with a headspace of 1.5 L, opened in a typical room with a volume of 150 m^3, opening of 216 packages would be required to exceed the Environmental Protection Agency (EPA) National Ambient Air Quality Standard of 9 ppm for 8 hr *(13)*, for a typical person inhaling 5 m^3 air/8 hr *(7)*.

Residual CO levels in the 20 and 30% fat treatments peaked on Days 21 and 25, respectively. The a* values (Table 3-13) for those fat levels on Days 21 and 25, however, were significantly lower ($P < 0.05$) than those for previous days, suggesting that discoloration may occur even when the carboxymyoglobin complex is still present.

Residual levels of CO found in the meat are unlikely to present a health risk to consumers. If one consumed a large meat meal (8.8 oz; 0.25 kg), and all the CO in a typical CO-MAP package remained bound to the meat after cooking, one would consume only 3.5% of the EPA 8-hr maximum safe level *(7, 12, 13)*. In reality, one would consume even less CO per meal because it is known that only 15% of bound CO remains with the meat after cooking *(86)*. Also, it is unlikely that all ingested CO is absorbed. Thus, CO exposure from consumption of CO-packaged meat is well below EPA safety standards.

Table 4-1. Mean total aerobic counts for ground beef stored at 4 ± 1°C for 7 days in polyvinyl chloride overwrap

		Log_{10} CFU[1]/g				
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7
PVC[2]	10	$3.69^{a,xz}$	$3.64^{a,xy}$	$4.33^{a,xyz}$	$5.11^{a,z}$	$4.77^{a,xyz}$
	20	$2.98^{a,wx}$	$1.86^{b,x}$	$3.77^{a,wy}$	$5.03^{a,z}$	$4.83^{a,yz}$
	30	$3.60^{a,x}$	$3.92^{a,xy}$	$4.49^{a,xyz}$	$5.27^{a,yz}$	$4.46^{a,xyz}$

[1]Colony forming units per gram, [2]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-2. Mean total Gram negative counts for ground beef stored at 4 ± 1 °C for 7 days in polyvinyl chloride overwrap

		Log_{10} CFU[1]/g				
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7
PVC[2]	10	$2.51^{a,w}$	$2.32^{ab,wx}$	$3.31^{a,wxy}$	$4.65^{a,z}$	$3.87^{a,yz}$
	20	$2.36^{a,xy}$	$1.53^{a,x}$	$2.71^{a,y}$	$4.41^{a,z}$	$3.41^{a,yz}$
	30	$2.67^{a,x}$	$2.72^{b,xy}$	$3.54^{a,xyz}$	$4.51^{a,z}$	$3.83^{a,z}$

[1]Colony forming units per gram, [2]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-3. Mean total psychrotroph counts for ground beef stored at 4 ± 1°C for 7 days in polyvinyl chloride overwrap

		Log_{10} CFU[1]/g				
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7
PVC[2]	10	$3.33^{a,x}$	$4.99^{a,yz}$	$4.31^{a,xy}$	$5.86^{a,z}$	$5.99^{a,z}$
	20	$3.20^{a,w}$	$4.06^{a,wx}$	$4.23^{a,wxy}$	$6.14^{a,z}$	$5.29^{a,xyz}$
	30	$4.06^{a,y}$	$5.70^{b,z}$	$5.64^{a,z}$	$6.31^{a,z}$	$5.39^{a,yz}$

[1]Colony forming units per gram, [2]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-4. Mean pH values for ground beef stored at 4 ± 1°C for 7 days in polyvinyl chloride overwrap

		Storage Time				
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7
PVC[1]	10	$5.71^{a,x}$	$5.60^{a,y}$	$5.63^{a,xy}$	$5.53^{a,y}$	$6.04^{a,z}$
	20	$5.61^{a,y}$	$5.53^{a,y}$	$5.59^{a,y}$	$5.62^{a,y}$	$6.07^{a,z}$
	30	$5.67^{a,x}$	$5.57^{a,x}$	$5.55^{a,x}$	$5.95^{b,y}$	$6.17^{a,z}$

[1]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-5. Mean total aerobic counts for ground beef stored at 4 ± 1°C for 25 days

		Log_{10} CFU[1]/g					
Packaging	Fat %	Day 0	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[2]	10	$3.68^{a,xy}$	$3.52^{a,y}$	$4.85^{a,xz}$	$5.72^{a,z}$	$5.77^{a,z}$	$5.43^{a,z}$
	20	$2.98^{a,y}$	$3.35^{a,y}$	$4.80^{a,z}$	$5.50^{a,z}$	$5.41^{a,z}$	$5.29^{a,z}$
	30	$3.59^{a,y}$	$3.59^{a,y}$	$4.81^{a,z}$	$5.54^{a,z}$	$5.30^{a,z}$	$5.21^{a,z}$
VP[3]	10	$3.69^{a,x}$	$3.89^{a,x}$	$4.48^{a,xy}$	$5.58^{a,z}$	$5.63^{a,yz}$	$5.54^{a,yz}$
	20	$2.98^{a,x}$	$3.17^{a,x}$	$4.57^{a,yz}$	$5.51^{a,z}$	$5.28^{a,z}$	$5.46^{a,z}$
	30	$3.59^{a,x}$	$3.72^{a,x}$	$4.60^{a,xy}$	$5.53^{a,yz}$	$5.60^{a,yz}$	$6.11^{a,z}$
PVC[4]	10	$3.68^{a,yz}$	$3.64^{a,y}$	$4.77^{a,z}$			
	20	$2.98^{a,x}$	$1.86^{b,y}$	$4.83^{a,z}$			
	30	$3.60^{a,z}$	$3.92^{a,z}$	$4.46^{a,z}$			

[1]Colony forming units per gram, [2]Modified atmosphere, [3]Vacuum packaging, [4]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-6. Mean Gram negative counts for ground beef stored at 4 ± 1°C for 25 days

		Log_{10} CFU[1]/g					
Packaging	Fat %	Day 0	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[2]	10	$2.51^{a,yz}$	$2.34^{ab,xy}$	$1.62^{a,z}$	$2.20^{a,xyz}$	$2.60^{a,y}$	$2.38^{a,yz}$
	20	$2.35^{a,z}$	$2.01^{ab,z}$	$1.82^{a,z}$	$1.78^{a,z}$	$2.38^{a,z}$	$2.43^{a,z}$
	30	$2.67^{a,yz}$	$2.80^{a,yz}$	$2.58^{ac,yz}$	$2.29^{a,z}$	$3.13^{a,y}$	$2.75^{a,yz}$
VP[3]	10	$2.50^{a,z}$	$2.43^{ab,z}$	$1.83^{a,z}$	$2.11^{a,z}$	$2.12^{a,z}$	$2.14^{a,z}$
	20	$2.36^{a,z}$	$1.77^{ab,z}$	$2.36^{ae,z}$	$2.32^{a,z}$	$2.26^{a,z}$	$2.49^{a,z}$
	30	$2.67^{a,z}$	$2.68^{a,z}$	$2.45^{ae,z}$	$2.47^{a,z}$	$2.81^{a,z}$	$2.58^{a,z}$
PVC[4]	10	$2.51^{a,y}$	$2.32^{ab,y}$	$3.87^{bd,z}$			
	20	$2.36^{a,x}$	$1.53^{b,y}$	$3.41^{cde,z}$			
	30	$2.67^{a,y}$	$2.72^{a,y}$	$3.83^{bd,z}$			

[1]Colony forming units per gram, [2]Modified atmosphere, [3]Vacuum packaging, [4]Polyvinyl chloride. $^{a-e}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-7. Mean total lactic acid bacteria counts for ground beef stored at 4 ± 1°C for 25 days

		Log_{10} CFU[1]/g					
Packaging	Fat %	Day 0	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[2]	10	$2.06^{a,y}$	$2.97^{a,y}$	$5.06^{a,z}$	$5.63^{a,z}$	$5.43^{a,z}$	$5.47^{ab,z}$
	20	$1.99^{a,x}$	$3.15^{a,y}$	$5.18^{a,z}$	$5.45^{a,z}$	$5.50^{a,z}$	$5.45^{ab,z}$
	30	$1.98^{a,x}$	$3.07^{a,y}$	$4.97^{a,z}$	$5.35^{a,z}$	$5.32^{a,z}$	$5.25^{a,z}$
VP[3]	10	$2.05^{a,x}$	$3.32^{a,y}$	$4.60^{a,z}$	$5.45^{a,z}$	$5.51^{a,z}$	$5.38^{ab,z}$
	20	$1.98^{a,x}$	$3.49^{a,y}$	$5.08^{a,z}$	$5.37^{a,z}$	$5.41^{a,z}$	$5.62^{ab,z}$
	30	$1.98^{a,x}$	$3.09^{a,y}$	$4.77^{a,z}$	$5.35^{a,z}$	$5.43^{a,z}$	$5.68^{b,z}$

[1]Colony forming units per gram, [2]Modified atmosphere, [3]Vacuum packaging. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-8. Mean total psychrotroph counts for ground beef stored at 4 ± 1°C for 25 days

		Log_{10} CFU[1]/g					
Packaging	Fat %	Day 0	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[2]	10	$3.24^{a,x}$	$3.35^{a,x}$	$4.61^{abd,y}$	$5.31^{abc,yz}$	$6.09^{a,z}$	$5.64^{a,yz}$
	20	$3.14^{a,x}$	$4.52^{ab,yz}$	$4.17^{ad,xy}$	$5.76^{ad,z}$	$5.74^{a,z}$	$5.56^{a,yz}$
	30	$3.92^{a,y}$	$4.06^{ac,y}$	$4.06^{ad,y}$	$5.81^{ae,z}$	$5.34^{a,yz}$	$5.19^{a,yz}$
VP[3]	10	$3.01^{a,y}$	$4.92^{bce,xz}$	$4.06^{ad,xy}$	$4.14^{bc,xy}$	$5.59^{a,z}$	$5.62^{a,z}$
	20	$3.39^{a,y}$	$3.74^{aef,y}$	$3.68^{d,y}$	$4.38^{cde,yz}$	$5.47^{a,z}$	$5.64^{a,z}$
	30	$3.99^{a,yz}$	$4.47^{adef,yz}$	$4.01^{ad,z}$	$4.15^{bc,z}$	$5.46^{a,y}$	$4.58^{a,yz}$
PVC[4]	10	$3.33^{a,y}$	$4.99^{bcf,z}$	$5.99^{bc,z}$			
	20	$3.20^{a,y}$	$4.06^{ac,y}$	$5.29^{ac,z}$			
	30	$4.06^{a,y}$	$5.70^{bd,z}$	$5.39^{ac,yz}$			

[1]Colony forming units per gram, [2]Modified atmosphere, [3]Vacuum packaging, [4]Polyvinyl chloride. $^{a-f}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-9. Mean pH values for ground beef stored at 4 ± 1°C for 25 days

		Storage Time					
Packaging	Fat %	Day 0	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[1]	10	$5.63^{a,w}$	$5.58^{a,wx}$	$5.53^{a,wx}$	$5.15^{a,yz}$	$5.36^{a,xy}$	$5.07^{a,z}$
	20	$5.62^{a,x}$	$5.59^{a,x}$	$5.43^{a,xy}$	$5.29^{a,y}$	$5.07^{b,z}$	$5.11^{a,z}$
	30	$5.62^{a,y}$	$5.58^{a,y}$	$5.51^{a,y}$	$5.19^{a,z}$	$5.09^{b,z}$	$5.10^{a,z}$
VP[2]	10	$5.59^{a,xy}$	$5.64^{a,x}$	$5.42^{a,y}$	$5.14^{a,z}$	$5.00^{b,z}$	$5.01^{a,z}$
	20	$5.61^{a,y}$	$5.56^{a,y}$	$5.52^{a,y}$	$5.23^{a,z}$	$5.06^{b,z}$	$5.08^{a,z}$
	30	$5.68^{a,x}$	$5.66^{a,x}$	$5.42^{a,y}$	$5.21^{a,yz}$	$5.10^{ab,z}$	$5.06^{a,z}$
PVC[3]	10	$5.71^{a,y}$	$5.60^{a,y}$	$6.04^{b,z}$			
	20	$5.61^{a,y}$	$5.53^{a,y}$	$6.07^{b,z}$			
	30	$5.67^{a,y}$	$5.57^{a,y}$	$6.17^{b,z}$			

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-10. Mean thiobarbituric acid reactive substances values for ground beef stored at 4 ± 1°C for 25 days

		mg of malonaldehyde/kg sample									
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14	Day 21	Day 25
	10	$1.07^{ab,z}$	$0.85^{a,z}$	$1.10^{ab,z}$	$0.88^{ac,z}$	$0.89^{a,z}$	$0.77^{a,z}$	$0.93^{a,z}$	$0.88^{ab,z}$	$1.08^{ab,z}$	$0.94^{ac,z}$
MAP[1]	20	$1.21^{ab,yz}$	$1.34^{ab,yz}$	$1.25^{ab,yz}$	$1.41^{abc,yz}$	$1.25^{ab,yz}$	$1.12^{ab,yz}$	$0.85^{a,z}$	$1.38^{a,y}$	$1.35^{ab,y}$	$0.87^{ac,z}$
	30	$1.83^{a,w}$	$1.50^{bc,wyz}$	$1.23^{ab,yz}$	$1.21^{ace,wyz}$	$1.52^{bd,wxz}$	$1.39^{bd,wyz}$	$1.10^{a,xy}$	$0.99^{ab,y}$	$1.66^{b,wz}$	$1.75^{b,wz}$
	10	$0.96^{b,z}$	$1.01^{ac,z}$	$0.85^{bd,z}$	$0.81^{a,z}$	$0.94^{ad,z}$	$0.81^{ad,z}$	$0.78^{a,z}$	$0.63^{b,z}$	$0.80^{a,z}$	$0.68^{ac,z}$
VP[2]	20	$1.75^{a,y}$	$1.08^{ac,z}$	$1.02^{bd,z}$	$1.02^{acd,z}$	$1.10^{adf,z}$	$1.16^{ade,yz}$	$1.05^{a,z}$	$1.12^{ab,yz}$	$1.15^{ab,yz}$	$0.98^{cd,z}$
	30	$1.45^{ab,z}$	$1.38^{acd,z}$	$1.15^{ad,z}$	$1.28^{acd,z}$	$1.25^{ab,z}$	$1.23^{abe,z}$	$1.00^{a,z}$	$1.12^{ab,z}$	$1.05^{a,z}$	$1.18^{abd,z}$
	10	$1.10^{ab,xy}$	$1.05^{ac,x}$	$1.65^{ac,y}$	$1.45^{bc,xy}$	$2.38^{c,z}$	$2.42^{c,z}$				
PVC[3]	20	$1.47^{ab,y}$	$1.76^{bd,yz}$	$2.04^{c,yz}$	$1.93^{b,yz}$	$2.23^{ce,z}$	$2.25^{c,z}$				
	30	$1.79^{a,yz}$	$1.76^{bd,z}$	$1.30^{ab,y}$	$1.55^{bde,yz}$	$1.68^{bef,yz}$	$1.45^{be,yz}$				

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride. $^{a-f}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-11. Mean L* values for ground beef stored at 4 ± 1°C for 25 days

		Storage Time							
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14	Day 21	Day 25
	10	$50.42^{af,yz}$	$49.40^{ad,yz}$	$49.85^{ade,yz}$	$51.22^{ade,yz}$	$51.65^{ade,yz}$	$50.42^{a,yz}$	$49.62^{ae,y}$	$51.58^{ad,z}$
MAP[1]	20	$52.46^{ae,z}$	$52.46^{ab,z}$	$53.06^{ab,z}$	$52.61^{abe,z}$	$53.18^{ab,z}$	$53.76^{b,z}$	$52.71^{a,z}$	$52.87^{ad,z}$
	30	$54.49^{bde,z}$	$54.68^{b,z}$	$54.89^{bg,z}$	$55.81^{b,z}$	$55.15^{b,z}$	$55.00^{b,z}$	$56.08^{c,z}$	$56.46^{b,z}$
	10	$47.95^{f,x}$	$44.24^{c,yz}$	$43.48^{c,y}$	$45.17^{c,yz}$	$44.28^{c,y}$	$45.52^{c,xyz}$	$44.89^{d,y}$	$46.94^{ce,xz}$
VP[2]	20	$48.20^{f,y}$	$45.86^{ce,z}$	$46.81^{ef,yz}$	$45.80^{ch,yz}$	$46.27^{cf,yz}$	$47.15^{ac,yz}$	$46.43^{de,yz}$	$46.40^{c,yz}$
	30	$51.54^{ae,y}$	$48.89^{de,z}$	$49.25^{efh,yz}$	$49.04^{dfgh,yz}$	$48.68^{df,z}$	$50.03^{a,yz}$	$50.33^{ab,yz}$	$50.04^{de,yz}$
	10	$51.08^{af,y}$	$49.30^{ad,z}$	$48.65^{df,z}$	$49.53^{ef,yz}$	$49.98^{ad,yz}$			
PVC[3]	20	$52.61^{ae,z}$	$52.36^{ab,z}$	$51.61^{adgh,z}$	$51.31^{aeg,z}$	$51.97^{ab,z}$			
	30	$57.34^{d,x}$	$55.05^{b,yz}$	$55.09^{b,xy}$	$53.33^{ab,z}$	$53.32^{be,yz}$			

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride. $^{a-h}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-12. Mean b* values for ground beef stored at 4 ± 1°C for 25 days

		Storage Time							
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14	Day 21	Day 25
MAP[1]	10	$10.40^{ac,w}$	$7.16^{a,xz}$	$6.94^{a,z}$	$8.20^{a,x}$	$7.89^{ac,xz}$	$6.91^{a,z}$	$5.20^{a,y}$	$6.95^{a,xz}$
	20	$9.94^{ac,v}$	$6.78^{ad,wz}$	$7.82^{ab,xz}$	$8.44^{a,x}$	$8.18^{a,xy}$	$7.76^{a,wxy}$	$6.52^{b,z}$	$6.99^{a,yz}$
	30	$9.69^{ac,w}$	$7.55^{a,y}$	$8.74^{be,wz}$	$8.91^{a,wz}$	$8.20^{a,yz}$	$7.93^{a,yz}$	$7.88^{c,yz}$	$8.58^{b,wyz}$
VP[2]	10	$9.74^{a,y}$	$3.89^{b,z}$	$3.27^{c,z}$	$3.18^{b,z}$	$2.97^{b,z}$	$3.35^{c,z}$	$3.34^{d,z}$	$3.51^{c,z}$
	20	$10.14^{ac,x}$	$5.72^{d,y}$	$4.06^{cd,z}$	$3.94^{bc,z}$	$3.72^{b,z}$	$3.65^{c,z}$	$3.96^{ad,z}$	$3.86^{c,z}$
	30	$11.17^{cd,x}$	$6.31^{ad,y}$	$4.90^{d,z}$	$4.73^{c,z}$	$6.60^{c,y}$	$4.28^{c,z}$	$4.98^{a,z}$	$5.52^{d,yz}$
PVC[3]	10	$12.65^{b,x}$	$11.20^{c,y}$	$7.95^{ab,z}$	$8.17^{a,z}$	$8.01^{a,z}$			
	20	$12.45^{bd,x}$	$11.15^{c,y}$	$8.66^{be,z}$	$9.29^{a,z}$	$9.16^{a,z}$			
	30	$13.03^{b,y}$	$11.99^{c,y}$	$9.64^{e,z}$	$9.26^{a,z}$	$9.02^{a,z}$			

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride. $^{a-e}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{v-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-13. Mean a* values for ground beef stored at 4 ± 1°C for 25 days

		Storage Time							
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14	Day 21	Day 25
MAP[1]	10	$23.68^{ad,vx}$	$22.98^{a,x}$	$20.66^{ab,x}$	$27.52^{a,w}$	$26.41^{a,vw}$	$22.21^{a,x}$	$14.77^{a,y}$	$11.63^{a,z}$
	20	$21.61^{ab,w}$	$21.43^{a,w}$	$22.86^{bd,wy}$	$26.01^{a,x}$	$25.10^{ab,xy}$	$22.31^{a,wy}$	$16.15^{ab,z}$	$13.48^{a,z}$
	30	$18.88^{b,y}$	$22.52^{a,x}$	$24.12^{d,wx}$	$26.07^{a,w}$	$22.94^{b,x}$	$22.26^{a,x}$	$17.97^{bc,y}$	$12.78^{a,z}$
VP[2]	10	$21.49^{ab,y}$	$17.82^{b,z}$	$19.63^{a,yz}$	$19.73^{d,yz}$	$19.23^{c,yz}$	$19.65^{ac,yz}$	$19.87^{c,yz}$	$18.52^{b,yz}$
	20	$21.61^{ab,x}$	$16.47^{b,y}$	$18.60^{a,xyz}$	$20.20^{d,xz}$	$19.16^{c,xyz}$	$19.30^{ac,xyz}$	$19.30^{bc,xyz}$	$18.35^{b,yz}$
	30	$21.16^{ab,y}$	$17.44^{b,z}$	$19.20^{a,yz}$	$19.71^{d,yz}$	$18.87^{c,yz}$	$18.70^{bc,yz}$	$18.48^{bc,yz}$	$18.26^{b,yz}$
PVC[3]	10	$27.11^{c,x}$	$24.26^{a,x}$	$14.13^{c,y}$	$7.47^{b,z}$	$8.32^{d,z}$			
	20	$25.87^{cd,w}$	$22.51^{a,x}$	$15.20^{c,y}$	$7.51^{b,z}$	$8.21^{d,z}$			
	30	$24.33^{ac,y}$	$22.37^{a,y}$	$13.46^{c,z}$	$10.91^{c,z}$	$10.40^{d,z}$			

[1]Modified atmosphere, [2]Vacuum packaging, [3]Polyvinyl chloride. $^{a-d}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{v-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-14. Consumer sensory panel color scores for evaluating ground beef stored at 4 ± 1°C for 25 days

		Average score[1]							
Packaging	Fat %	Day 0	Day 1	Day 3	Day 7	Day 9	Day 14	Day 21	Day 25
MAP[2]	10	$4.69^{ac,xz}$	$5.36^{ab,xz}$	$4.58^{ab,xz}$	$6.09^{a,x}$	$5.90^{ac,xy}$	$5.84^{ab,xy}$	$3.77^{a,z}$	$4.17^{a,xyz}$
	20	$4.21^{ac,x}$	$6.28^{ac,yz}$	$6.94^{c,y}$	$6.20^{a,xyz}$	$6.14^{ac,xyz}$	$6.42^{a,y}$	$4.56^{ab,xz}$	$4.36^{a,xz}$
	30	$5.60^{ab,yz}$	$6.75^{a,y}$	$6.72^{ac,yz}$	$7.48^{a,y}$	$7.15^{a,y}$	$7.25^{a,y}$	$6.08^{b,yz}$	$4.69^{a,z}$
VP[3]	10	$3.38^{a,z}$	$3.79^{bd,xz}$	$5.42^{ac,y}$	$5.43^{a,xy}$	$3.99^{bcd,yz}$	$3.59^{b,yz}$	$2.68^{a,z}$	$4.16^{a,yz}$
	20	$3.88^{ac,z}$	$3.94^{bcd,z}$	$6.77^{ac,y}$	$5.46^{a,yz}$	$5.25^{ad,yz}$	$4.00^{b,z}$	$4.20^{ab,z}$	$4.07^{a,z}$
	30	$3.88^{ac,z}$	$5.32^{ad,yz}$	$6.15^{ac,y}$	$6.14^{a,y}$	$5.43^{ad,yz}$	$5.50^{ab,yz}$	$4.08^{ab,z}$	$4.63^{a,yz}$
PVC[4]	10	$5.99^{bc,y}$	$4.69^{ab,y}$	$2.29^{b,z}$	$2.40^{b,z}$	$1.97^{b,z}$			
	20	$4.98^{ac,y}$	$5.15^{ab,y}$	$2.72^{b,z}$	$2.44^{b,z}$	$2.26^{b,z}$			
	30	$7.23^{b,y}$	$6.28^{ac,y}$	$2.78^{b,z}$	$2.52^{b,z}$	$2.55^{b,z}$			

[1]Score Scale: 1 = extremely dark brown, 2 = very dark brown, 3 = dark brown, 4 = dark red, 5 = slightly dark red, 6 = cherry red, = 7 moderately light cherry-red and 8 = light cherry-red, [2]Modified atmosphere, [3]Vacuum packaging, [4]Polyvinyl chloride. $^{a-d}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{x-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4-15. Consumer sensory panel off-odor scores for evaluating ground beef stored at 4 ± 1°C for 25 days

		Average score[1]							
Packaging	Fat %	Day 0	Day 1	Day 3	Day 7	Day 9	Day 14	Day 21	Day 25
MAP[2]	10	$1.45^{a,xz}$	$1.22^{a,x}$	$1.92^{a,xz}$	$2.26^{a,z}$	$1.82^{ab,xz}$	$1.83^{ac,xz}$	$3.31^{ab,y}$	$3.65^{a,y}$
	20	$1.35^{a,yz}$	$1.25^{a,z}$	$1.74^{a,wyz}$	$2.21^{a,xy}$	$1.53^{a,yz}$	$1.75^{ac,wyz}$	$2.54^{a,wx}$	$2.94^{ab,x}$
	30	$1.55^{a,y}$	$1.44^{a,y}$	$1.74^{a,xy}$	$2.14^{a,xy}$	$1.78^{ab,xy}$	$1.67^{a,y}$	$3.31^{ab,z}$	$2.69^{b,xz}$
VP[3]	10	$1.40^{a,x}$	$1.86^{a,x}$	$1.71^{a,x}$	$2.96^{ac,z}$	$2.79^{b,z}$	$3.41^{b,yz}$	$3.93^{b,y}$	$3.04^{ab,yz}$
	20	$1.35^{a,w}$	$1.44^{a,w}$	$2.39^{ab,x}$	$2.50^{a,x}$	$2.27^{ab,wx}$	$2.67^{ab,xy}$	$3.57^{ab,z}$	$3.57^{ab,yz}$
	30	$1.35^{a,w}$	$1.25^{a,w}$	$2.03^{ab,wx}$	$2.86^{ac,xyz}$	$2.38^{ab,xy}$	$2.75^{bc,xyz}$	$3.35^{ab,z}$	$3.00^{ab,yz}$
PVC[4]	10	$1.55^{a,w}$	$1.48^{a,w}$	$2.70^{ab,x}$	$3.88^{cd,y}$	$5.80^{c,z}$			
	20	$1.40^{a,x}$	$1.71^{a,x}$	$3.08^{b,y}$	$3.92^{cd,y}$	$5.48^{c,z}$			
	30	$1.25^{a,w}$	$1.32^{a,w}$	$2.63^{ab,x}$	$4.22^{d,y}$	$5.78^{c,z}$			

[1]Score Scale: 1 = none, 2 = barely perceptible, 3 = perceptible, 4 = slightly strong, 5 = moderately strong, 6 = very strong and 7 = very strong, [2]Modified atmosphere, [3]Vacuum packaging, [4]Polyvinyl chloride. $^{a-d}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{w-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4-16. Mean headspace carbon monoxide concentration values for ground beef stored at 4 ± 1°C for 25 days

		ppm CO[1]				
Packaging	Fat %	Day 1	Day 7	Day 14	Day 21	Day 25
MAP[2]	10	$260411^{a,yz}$	$314171^{a,y}$	$244993^{a,yz}$	$182056^{a,z}$	$243944^{a,yz}$
	20	$362527^{b,y}$	$312111^{a,yz}$	$253937^{a,z}$	$238716^{a,z}$	$253379^{a,z}$
	30	$366731^{b,y}$	$305913^{a,yz}$	$278447^{a,yz}$	$254906^{a,z}$	$248263^{a,z}$

[1]Parts-per-million of carbon monoxide, [2]Modified atmosphere. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{y-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements.

Table 4-17. Mean residual carbon monoxide concentration values for ground beef stored at 4 ± 1°C for 25 days

		ppm CO/g[1]									
Packaging	Fat %	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14	Day 21	Day 25
MAP[2]	10	$0.38^{a,r}$	$0.94^{a,rs}$	$1.23^{a,st}$	$1.41^{a,stvw}$	$1.53^{a,svw}$	$2.09^{a,vwy}$	$2.87^{a,z}$	$2.79^{a,yz}$	$2.40^{a,yz}$	$2.40^{a,yz}$
	20	$0.22^{a,v}$	$0.69^{a,vw}$	$1.40^{a,wx}$	$1.43^{a,wxy}$	$1.73^{a,xyz}$	$2.06^{a,xyz}$	$1.74^{b,xyz}$	$1.85^{b,xyz}$	$2.44^{a,z}$	$2.40^{a,z}$
	30	$0.46^{a,w}$	$0.90^{a,wx}$	$1.22^{a,wxy}$	$1.41^{a,xyz}$	$1.84^{a,yz}$	$1.86^{a,yz}$	$2.01^{b,yz}$	$1.93^{b,yz}$	$1.88^{a,yz}$	$2.08^{a,z}$

[1]Parts-per-million of carbon monoxide per gram of beef, [2]Modified atmosphere. $^{a-b}$ means in the same column with different superscripts are significantly different ($P < 0.05$). $^{r-z}$ means in the same row with different superscripts are significantly different ($P < 0.05$). Each mean value represents 4 measurements

CHAPTER 5
SUMMARY AND CONCLUSIONS

The objectives of this study were to evaluate the effects of three packaging treatments (MAP containing 0.4% CO, VP and PVC overwrap) and three fat treatments (10, 20 and 30% fat) and storage time on pH, color, TBARS, microbiological and sensory characteristics of ground beef, and to determine the concentration of carbon monoxide in the headspace of the package and in the meat during 25-d storage at 4 ± 1ºC. Two trials were conducted, each of which consisted of two phases. Phase 1 consisted of formulating, blending, grinding, packaging and storage of the meat. Phase 2 involved the evaluation of the different parameters associated with the shelf life of ground beef and also with CO levels in both the meat and the headspace of the packages.

Results from this study suggested that MAP and VP had a bacteriostatic effect on Gram negative microorganisms. The growth of aerobic, psychrotrophic and lactic acid bacteria, on the other hand, was not inhibited by MAP or VP. Within the MAP (10% fat, Day 21) and VP (30% fat, Day 25) groups, two of the six treatments reached aerobic plate counts and total psychrotrophs counts above spoilage levels (> 6 log CFU/g). Consumer panel sensory color and off-odors results suggested that discoloration (compared to Day 14) and off-odors were detected in those treatments on those Days. Furthermore, packaging ground beef in PVC overwrap allowed for significant increases ($P < 0.05$) in Gram negative, total aerobes and psychrotrophic bacteria by Day 5 of storage. In addition, total psychrotroph counts for the 20 and 30% fat treatments in the PVC group reached spoilage levels (>6 log CFU/g) by Day 5 of storage. Consumer panel sensory color and off-odors results suggested that discoloration and off-odors were detected in those treatments as early as Day 3.

The absence of O_2 from the packaging system, as was the case in MAP and VP treatments, favored the growth of lactic acid-producing bacteria. This production of lactic acid may have led to the significant decrease in pH values observed in all MAP and VP treatments by Day 14.

The MAP and VP systems had an antioxidant effect on ground beef regardless of fat level as shown by the TBARS results. Significant increases ($P < 0.05$) in TBARS for the PVC group were observed for both the 10 and 20% fat treatments.

A significant decrease ($P < 0.05$) in a* values was observed in the 10 and 20% fat treatments within the MAP group by Day 21. Consumer panel color scores showed that the modified atmosphere used in this study was able to enhance the color of the meat until Day 14 of storage under the conditions used in this study. Furthermore, consumer panel off-odor scores showed that MAP treatments had significantly higher ($P < 0.05$) off-odor scores by Day 21.

Carbon monoxide concentrations in both the package and the meat were deemed harmless at the levels detected in this study. However, as shown by the consumer panel analyses, consumers should rely on “use by” dates on the package and not on the color of the meat as indicators of freshness.

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BIOGRAPHICAL SKETCH

Nicolas Armando Lavieri was born in 1984 in Maracay, Venezuela. The third of four children, he moved to the US at age 17. He earned his Bachelor of Science degree from the Department of Animal Sciences in December 2005 at the University of Florida. He began his master's research in 2006 after receiving a departmental graduate assistantship. He earned his Master of Science degree in May 2008. After graduation, Nicolas plans to pursue a Doctor of Philosophy degree at Iowa State University under Dr. Joseph Cordray's supervision.